Dental Age Estimation in Adults. An in-depth analysis of secondary dentine deposition in different populations and clinical conditions.

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This thesis is presented for the degree of Doctor of Philosophy of The University of Western Australia

School of Human Sciences

2017
THESIS DECLARATION

I, Talia Yolanda Marroquin Peñaloza, certify that:

This thesis has been substantially accomplished during enrolment in the degree.

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No part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and where applicable, any partner institution responsible for the joint-award of this degree.

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The work described in this thesis did not need funded of tertiary parties.

This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

Signature:

Date: 30th November, 2017.
SUMMARY

Dental age estimation has been an important issue for humanity since ancient times. In the same way, different methods have been proposed through history for young individuals and adults. Once forensic dentistry is an important branch of the forensic sciences, and with the introduction of radiology in dentistry, it has been possible to propose non-invasive methods for age estimation, based on the radiological analysis of the inverse relation of the root canal size with age. Age estimation of adult individuals has always been a challenge, as those methods based on the narrowing of the root canal, as a result of secondary dentine production have shown wide variability in their results. This thesis analyses some of the observed obstacles for dental age estimation in adults, and proposes new approaches, aimed to overcome them.

The published papers presented in this thesis follow some of the previously proposed methods for dental age estimation in adults, such as Kvaal et al.’s method, and those methods based on volume measurements of pulp and tooth, adapting, and testing them in diverse groups of populations. Measurements have also been taken in individuals with different dental characteristics, thereby challenging some of the established parameters for dental age estimation as it is the exclusive analysis of totally sound teeth, in samples with a unique ethnic background. Additionally, this thesis also includes what could be considered to be the first literature review in forensic dentistry for dental age estimation in adults; a study, with enormous contributions to this research field. This thesis also includes the analysis of the possible factors that affect the accuracy of the tested methods.
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Talia Yolanda Marroquin Peñaloza
AUTHORSHIP DECLARATION.

This thesis contains work that has been published, accepted for publication, and submitted for publication, and follows the format of my thesis by a series of papers, as stipulated by The University of Western Australia for the Degree of Doctor of Philosophy,

In this thesis, all seven papers (7 primary) listed have undergone peer review, 6 have been already published, and in all papers, the candidate was responsible for the work. The candidate collected and analyzed the data.

The data were processed, and this information enabled the candidate to scrutinize existing theories. With this knowledge, the candidate created new knowledge generated new concepts, methodologies and understandings. With permission, the candidate was given authority to submit the work for publication.

This thesis is comprised of a series of published papers in peer reviewed journals of which all of them have been co-authored.

PhD Candidate, Talia Yolanda Marroquin Peñaloza. 30th, November, 2017.

Coordinating Supervisor, Winthrop Professor Marc Tennant. 30th November, 2017.
PUBLICATIONS ARISING FROM THIS THESIS

For all the papers of this thesis the contribution of the first author was estimated to be at least 85% as co-author:

1. **Chapter 1**

   Marroquin, T. Y., S. Karkhanis, S., Kvaal, S. I., Vasudavan, S., Kruger, E., & Tennant, M.


2. **Chapter 2.**


3. **Chapter 3.**


4. **Chapter 4.**

age estimation on juveniles in a Western Australian population. *Australian Journal of Forensic Sciences*. Accepted for publication. March 2016.

5. **Chapter 5.**


6. **Chapter 6.**


7. **Chapter 7.**


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I, Marc Tennant certify that the student statements regarding their contribution to each of the works listed above are correct

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Date: 30th November, 2017.
GENERAL INTRODUCTION

Forensic science has been defined as the application of science to the law or legal scenarios. In the same way, forensic dentistry refers to the application of dentistry to the law to interpret dental and related evidence in criminalistics fields, having important value for identification of individuals [1]. More, specifically, forensic dentistry is a specialized branch of dentistry that uses specialised knowledge to collect, manage, interpret, evaluate and present dental evidence for legal proceedings. This is applicable to dental identification, sex determination, cheiloscopy and palatoscopy, molecular biomarkers, bite mark analysis, human abuse and neglect, dental malpractice and negligence, dental anthropology and archaeology, and the main scope of this thesis is concerned with age estimation [2].

Age is one of the most important features to establish the identity of any individual, next to sex, height, and ethnicity [3]. The need to confirm the age of different individuals has been a crucial topic in anthropological, legal, and later in forensic scenarios. One forensic case that has been stipulated as the first using dental age estimation with forensic purposes [1], was narrated in the book “L’Art Dentaire en medicine legale” by Dr. Oscar Amoedo in 1898 [4], the father of forensic dentistry. This is related to the story of Louis-Charles Capet, or Louis XVII of France, son of Louis XVI and Marie-Antoinette.

He passed away at the age of ten years two months, in prison, in 1795. However, the coffin with his remains could not been found. It was believed that he had been substituted with
other boy his age, and that the prince was still alive; with this, a number of individuals unsuccessfully claimed to be Louis XVII. In 1849, in the old cemetery of St Margarite a lead coffin was found, with a skeleton, believed to belong to the young prince.

The first examinations, by Dr. Milcent, determined that these were the remains of Louis XVII, but Dr. Recamier rejected this hypothesis, based on a dental exam, and claimed that the individual was 15 to 16 years old. This little story, that could have been a fragment of a fairy tale, is only one simple example that highlights the relevance of forensic dentistry and the role of the forensic dentist to confirm the age and identity of different individuals.

Up to date, many methods have been proposed for age estimation, using different indicators which vary somewhat, since the study of bone changes with maturation up to more invasive techniques, based on chemical analysis of different tissue samples. All these methods, have displayed a wide variability in their accuracy and reliability, which is even more questionable when it comes to the analysis of individuals that have reached their physical maturity.

Dentistry has contributed substantially to forensic sciences, in regards of age estimation, having a relevant and positive impact, helping in the analysis and resolution of many cases around the world. Among the different available methods for age estimation, those that are minimally invasive are of interest owing to the major advantage of not requiring tooth extraction, making them appropriate for the identification of unknown deceased
individuals, and also for the identification of living individuals with doubtful documents of identification.

Teeth analysis for age estimation and identification of individuals has different advantages over the analysis of osseous tissue, such as the high resistance of teeth to decomposition and taphonomic processes, which make them suitable for study long after the individual has deceased [3]. Furthermore, age-related changes in teeth can be observed and studied on dental radiographs, offering minimally invasive methods, which is not always possible with bone analysis.

The genetically controlled process of tooth formation provides reliable data for the age estimation of individuals, that have not completed tooth maturation. In this way, there are different methods to estimate the age of individuals, from the 14th week of gestation, up to the age of 21 years, where due to the analysis of third molars formation it is possible to establish if they are over or under the age of 18 years [5].

Nevertheless, age estimation in individuals with their dentition, totally formed, is still a challenge. Numerous methods have been proposed for dental age estimation in adults. Although, in certain cases, the accuracy and reliability could be questionable, each one has been an important step for the proposal of more modern, easier, and accurate methods.

In 1925, Bodecker [6] identified a dental change that is related with age and can be observed once the root development has finished. This is the apposition of secondary
dentine, on the walls of the root canal. The formation of secondary dentine commences once the tooth formation has finished, making the root canal or pulp chamber smaller [7]. In this way, a negative correlation between the age of the individual and the dimensions of the root canal may be established [8].

In 1950, Gustafson [9] proposed a method that included the analysis of dental attrition, periodontosis, cementum apposition, root resorption, root transparency, and the formation of secondary dentine, which for his study, had to be observed on microscopic sections. The methodology proposed a system of points depending on the status of the different age indicators.

The sum of points and the relation with age was recorded; this revealed an increase of points with age. With this, the author affirmed that it was possible to draw a regression line for the relationship between age and points; however, this system was not applicable for teeth in broken-down condition.

The author also recommended that those who chose to use this method had to create their own standard curve. Since then, the statistical rigor of methods has noticeably increased, as well as the number of participants in different studies. However, the use of linear regression models has remained constant and is still an important method for the development for age estimation in adults.
As the dimensions of the root canal can be measured in dental radiographs or dental tomography, different methods have been proposed. In 1995 Kvaal et al. [8], published a method based on linear measurements of tooth, root and pulp chamber length and the measurement of the width of the tooth, and root canal at three different levels on the roots. All these measures had to be taken from six different single rooted teeth in periapical radiographs.

The original method demanded the use of Vernier calipers to measure the length, and a stereomicroscope with a measuring eyepiece to the nearest 0.1mm with which to measure the width of pulp and root. It was proposed that the calculation of pulp/tooth ratio dimensions and the application of linear regression models were adequate to produce a formula for age estimation in individuals older than 20 years.

Up until now, this method has been tested in at least 29 studies in 12 different countries; some studies have modified or adapted the original method to the new emerging imaging technology in dentistry. In this thesis, this method was tested in new scenarios and adapted to new technologies.

Following the same principle of secondary dentine production with age and the negative correlation between root canal size and age, Cameriere et al. [9], proposed a method based on area measurements of root canal and tooth. As the available technology, at the time of the elaboration of this method, was different, this method requires the use of digital radiograph and the access to specific software.
This method has also been tested in at least 29 studies in 11 countries. It is also based on the negative correlation of the pulp/tooth dimension ratio with age, but requires area measurements. One of the last proposals, for age estimation in adults, is the calculation of pulp and root canal volume from computed tomography (CT) or cone beam computed tomography (CBCT).

This last method, is still under development. However, at least 16 studies from 7 countries have been published using different techniques and software. In this thesis, some of the published methodologies were analyzed, and a new method is proposed to estimate dental age in adults. The above-mentioned methods, besides being based on the production of secondary dentine with age, have another similar feature: the proposal of linear regression models and equations to estimate the age of the individuals.

The use of the pulp/tooth ratio calculation, not only helps the researchers to observe how the size of the root canal or pulp chamber changes with age, but also, as established by Kvaal et al., it is useful to diminish the effects of image magnification [8], and probably sex. On the other hand, the brief description of some of the different methods for dental age estimation testifies to the strong relation between forensic dentistry and radiology. The use of radiology in dentistry, and forensic dentistry dates back to 1895, when Wilhelm Conrad Röntgen discovered the X-rays. One year later, in 1896, there were significant advances for the application of radiology in dentistry.
Some of the most relevant contributors were Otto Walhoff, who obtained a radiograph of his own maxillary sinuses, and a dental radiograph using dried human skulls. Furthermore, Edmund Kells, who is recognized as a dental pioneer and scientific genius [10], introduced radiology into dentistry, obtaining images from living patients.

Also in 1896, it was reported that one of the first uses of radiology for forensic applications to confirm the presence of bullets inside the head of a woman, who had been attacked by her husband [11]. For those days, to obtain a radiographic record, it was necessary to expose the individual to X-radiation for up to 70 minutes [12]. During the following years, the technology and new techniques in radiology have not only reduced the exposure times, but also have provided forensic science with new and better techniques, to support the resolution of different legal cases.

Now, in a digital era, plus the easy access to the Internet, to obtain, analyze, duplicate and share images, it is faster than ever, promoting the proposal of new emergent methods for identification and age estimation. This has allowed forensic teams, and academic researchers to collaborate, using two and three-dimensional images. As it has been described above, in forensic dentistry and age estimation, there have been major developments since the time of the young prince, Louis XVII of France.

At this stage, it is important to clarify that those studies on dental age estimation for young and older individuals have been based essentially, on the analysis of totally sound teeth, and the vast majority of the published studies have obtained data from individuals who
belong to the same population. However, there are different challenges for dental age estimation in adults that should be considered.

Firstly, there is no available systematic review that compares the accuracy of the above mentioned three different methods for age estimation. This thesis presents the first systematic review in forensic dentistry for age estimation in adults that follows the parameters established by the Cochrane handbook for systematic reviews-methodology, in the absence of guidelines for forensic sciences.

Secondly, it has been overlooked that the production of secondary dentine can be affected by external factors, generating the production of tertiary dentine, the two of which are undistinguishable in dental radiographs. Furthermore, it has been suggested that for dental age estimation it is necessary to use only totally sound teeth, excluding those individuals who have received orthodontic treatment, a dental procedure that is becoming popular and that also alters pulp chamber size and tooth length, parameters used for the application of the Kvaal et al. method.

Thirdly, tooth formation finishes, in general, at the age of 14 years. For age estimation of older individuals (adolescents and sub-adults), the available methods are based on the radiological analysis of third molar root formation. However, there is a problem with some young individuals with agenesis of third molars.

Fourthly, despite the introduction of tomography in dentistry, there are no studies testing the Kvaal et al. method on CBCT, an analysis that is useful to perform, as it could provide
more tools for dental age estimation in adults, performing linear measurements not only in a coronal, but also a sagittal plane, inaccessible in routine dental radiographs.

Fifthly, although the introduction of tomography to dentistry and forensic dentistry has promoted the proposal of different methods based on volume reconstruction, no study has considered the different technological aspects, unique to each software and techniques used to undertake the respective volume measurement.

Finally, there are distinct advantages of available imaging techniques, which allow researchers to obtain volume measurements of any anatomic region, as well as the advantages that telecommunication brings to share images and data.

The above factors, could be considered as challenges when different non-invasive methods are used for dental age estimation in adults. The first aim of this thesis is to look for an answer to these challenges. To this end, a series of 7 papers are presented and their results are discussed in 4 different sections that this thesis encompasses.
REFERENCES


Preface

Being one of the main issues in forensic and anthropological sciences, age estimation in adults has been the target of researchers in the field. In the same way, the analysis of dental age-related changes in adults has been widely explored, based on the clinical observation of certain dental changes such as tooth attrition and periodontal recession, which are not reliable age indicators.

The aim of this thesis is the analysis of non-invasive dental age estimation in adults, based on the radiological measurement of the age-related dimensional change of the root canal, due to secondary dentine production through life. To this end, a systematic review was carried out, collecting all the available publications on linear measurements of tooth and pulp width and length, area measurements of tooth and pulp, and also pulp/tooth volume quantification from dental computed tomography.

The aim, methodological design and findings of this systematic review are presented in Chapter 1. This systematic review is one notable achievement from this thesis as, to date, there is no similar publication in forensic dentistry or in the anthropological science literature.
1. Chapter 1. Age estimation in adults by dental imaging assessment systematic review

This chapter was published as:

ABSTRACT

Importance: The need to rely on proper, simple, and accurate methods for age estimation in adults is still a world-wide issue. It has been well documented that teeth are more resistant than bones to the taphonomic processes, and that the use of methods for age estimation based on dental imaging assessment are not only less invasive than those based on osseous analysis, but also have shown similar or superior accuracy in adults.

Objectives: To summarise the results of some of the most recently cited methods for dental age estimation in adults, based on odontometric dental imaging analysis, to establish which methods are more accurate, accessible, and simple.

Evidence review: A literature search from several databases was conducted from January 1995 to July 2016 with previously defined inclusion criteria.

Conclusion: Based on the findings of this review, it could be possible to suggest pulp/tooth area ratio calculation from first, upper canines and other single rooted teeth (lower premolars, upper central incisors), and a specific statistical analysis that considers the non-linear production of secondary dentine with age, as a reliable, easy, fast, and predictable method for dental age estimation in adults. The second recommended method is the pulp/tooth width-length ratio calculation. The use of specific population formulae would be recommended, but to include data of individuals from different groups of populations in the same analysis is not discouraged. A minimum sample size of at least 120 participants is recommended to obtain more reliable results. Methods based on volume calculation are time consuming and still need improvement.

Key words: Forensic dentistry, adults, age estimation, dental imaging, systematic review.
INTRODUCTION

Age is one of the most important characteristics used to establish the identity of any individual in different legal, forensic, or anthropological research context [1]. To this end, forensic teams depend on osseous analysis based methods, which have acceptable results for young individuals or in their early adulthood [2], and dental development based methods, which are highly reliable in individuals under 21 years of age [3].

However, these methods have some disadvantages: the poor resistance of bones to the taphonomic process [4], and once the individual reaches the threshold of 21 years of age, and third molars development concludes [3], the currently available dental development based methods are not applicable. In individuals with the congenital absence of third molar teeth, this threshold is not applicable for individuals older than 14 – 15 years of age.

To respond to the need of an aging population, and with the evident resistance of teeth to the taphonomic process, alternative methods for dental age estimation in adults have been proposed. Primarily, these are based on the formation of secondary dentine, studied since 1950 [5], and the subsequent narrowing of the pulp cavity, which can be observed in dental radiographs, leading to the proposal of minimally invasive methods.

This systematic review focuses on three methods based on odontometric analysis of the pulp cavity, performing length and width measurements [6], area measurements [7], and lastly, volume calculation [8]. The objective of this review is to summarise the results of
these recently most cited methods for dental age estimation in adults, to establish which method is more accurate, accessible, and simple.

**Description of the problem**

Different methods have been published for dental age estimation in adults, based on the pulp/tooth dimensions’ ratios. Nevertheless, the results of the application of some of these methods for dental age estimation, in adults from different population groups, exceeds the accepted threshold in forensic sciences, which indicates that the standard deviation of a method for adult’s age estimation should preferably be below a standard deviation (SD) of years ±10 years [9].

**Description of the methods being investigated**

The methods for dental age estimation in adults, analysed in this paper, were selected based on their minimally invasive nature, not a requirement for the extraction of teeth to be performed, and pulp/tooth ratio calculation, which have been applied in individuals from different populations. The Kvaal et al. [6] method is based on the analysis of linear measurements of the pulp, tooth, and root length as well as root and pulp width measurements at three different root levels, initially applied on periapical radiographs, and later on panoramic radiographs and tomographs. The Cameriere et al. [7, 10] method is based on the analysis of pulp and tooth area measurements on periapical, and panoramic radiographs. Finally, different methods for dental age estimation in adults, based on pulp/tooth volume ratio from cone beam computer tomography [8, 11-14], were included in this systematic review.
How these methods might work

The methods in this study are based on a negative correlation between age and the pulp chamber size, as well as on the tooth/pulp ratio calculation, regardless of the nature of the measurements: length/width, area, or volume. In other words, all measurements evaluate the association of one age related phenomenon, as it is the formation of secondary dentine with the decrease of pulp chamber size with age. This has been accepted as an age indicator that is observable and measurable with different dental imaging techniques. Ideally, the accuracy of the methods should not exceed the threshold of a SD ±10 years [1, 4].

Why it is important to do this review

The relevance of this review is grounded on the need to recommend a method for dental age estimation with the following characteristics: simple, fast, non-invasive, non-expensive, and reproducible, and over all, accurate; that can be systematically used in different academic and forensic scenarios. The objective is to aid the reconstruction of identity profiles of unidentified deceased individuals or living individuals with doubtful identity documents.

METHODS

Criteria for considering studies for this review

Qualitative analysis of the original human studies reporting the use of any of the listed methods for dental age estimation, based on pulp/tooth ratio calculation (length/width,
area, volume) were included; in particular, those that preferably reported intra-inter
observer calibration, generating population specific formulae.

Studies with samples including individuals younger than 14 years of age were excluded, as
well as studies with small sample sizes (n<50), and those using extracted teeth. Also,
studies that did not report the use of specific population formulae were excluded for the
quantitative analysis.

**Search methods for identification of studies**

The information was searched through data-bases available at The University of Western
Australia, which included the collections of:

- Directory of open access journals (DOAJ), Medline/PubMed (NLM), OneFile (GALE),
- ProQuest, collection, (Web of Science), Science Direct Journals (Elsevier), Social Sciences
  Citation Index (Web of Science), Scopus (Elsevier), Social Sciences Citation Index (Web of
  Science), Wiley (Cross Referenced), Wiley Online Library. Also, Google Scholar, by papers
  that referenced the original study performed by Kvaal et al., Cameriere et al., and those
  methods for age estimation that referred to the use of CBCT and volume reconstruction of
  pulp chambers and teeth.

The search key words were as follows: Kvaal and dental age estimation, Cameriere and
dental age estimation, age, and tooth volume. The literature search included publications
by these key authors after the publication of their original papers up until July 2016. The
search was conducted during the years 2014 to 2016. This systematic review follows the Cochrane handbook for systematic reviews-methodology and, where possible, the RevMan software recommended by the Cochrane handbook. (Graphs 1.1-1.3)

DATA COLLECTION AND ANALYSIS

Selection of studies
The initial selection was based on the title and abstract. Papers with titles that referred the inclusion of minors only (children) were excluded. Studies reporting the use of third molars or developing teeth were excluded. Studies that included the use of other methods for dental age estimation in children were excluded (Demirjian), or any other invasive method for dental age estimation were excluded. (Graphs 1.1-1.3, Tables 1.1-1.3)

Data extraction and management
The data were organized in an Excel spreadsheet as follows: Author, year, country, number of participants (male and female), age, intra and inter-observer agreement assessment. Also recorded were: the imaging technique, measuring instrument, best correlation coefficient between age and the different age predictors, best result in terms of accuracy per individual tooth or per set of teeth, when possible, as well as the highest error recorded by a set of teeth or individual tooth (Table 1.4-1.6).

Assessment of risk of bias in included studies
To avoid bias in this systematic review, and to avoid false positive or false negative
Graph 1.1. Flow chart of the study selection for the Kvaal et al. method.

Records identified through database searching (n=402) -> Search Results (n=698) -> Articles screened on basis of title and abstract -> Excluded (n=669) -> Articles included in systematic review and qualitative analysis (n=29) -> Excluded (n=13) (Use of extracted teeth=2, Not possible to compare estimated age with chronological age=2, Inclusion of individuals under 14 years of age=1, No use of specific population formulae=1, Use of third molars plus Kvaal method=1, No full text available in English or Spanish=1, Sample used in another study=1, Sample size <50 individuals=1, Accuracy not clearly reported=3) -> Articles screened on basis of full text -> Articles included in meta-analysis (n=16)
Graph 1.2. Flow chart of the study selection for the Cameriere et al. method

- Records identified through database searching (n=321)
- Google Scholar (n=102)

Search results (n=423)

- Articles screened on basis on title and abstract
- Excluded (n=391)

- Articles Included in the systematic review and qualitative analysis (n=29)

Articles screened on basis of full text

- Excluded (n=17)
  - Use of extracted teeth=4
  - Inclusion of individuals under 14 years of age=2
  - Sample size <50 individuals=4
  - Accuracy not clearly reported=7

- Articles included in meta-analysis (12)
Graph 1.3. Flow chart of the study selection for methods based on volume calculation.
Table 1.1. Total studies reporting the use of the Cameriere et al. method.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Total*</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kvaal et al. [6]</td>
<td>1995</td>
<td>Norway</td>
<td>100</td>
<td>20-87</td>
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<tr>
<td>Kvaal et al. [16]</td>
<td>1999</td>
<td>Sweden</td>
<td>21</td>
<td>20-60</td>
</tr>
<tr>
<td>Willems et al. [9]</td>
<td>2002</td>
<td>Belgium</td>
<td>29</td>
<td>26-85</td>
</tr>
<tr>
<td>Soomer et al. [1]</td>
<td>2003</td>
<td>Caucasian</td>
<td>20</td>
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<tr>
<td>Bosmans et al. [20]</td>
<td>2005</td>
<td>Belgium</td>
<td>197</td>
<td>19-75</td>
</tr>
<tr>
<td>Paewinsky et al. [19]</td>
<td>2005</td>
<td>Germany</td>
<td>168</td>
<td>18-81</td>
</tr>
<tr>
<td>Meinl A. [31]</td>
<td>2007</td>
<td>Austria</td>
<td>44</td>
<td>13-24</td>
</tr>
<tr>
<td>Avendaño et al. [18]</td>
<td>2009</td>
<td>Colombia</td>
<td>107</td>
<td>21-50</td>
</tr>
<tr>
<td>Landa M. [32]</td>
<td>2009</td>
<td>Portugal</td>
<td>100</td>
<td>14-60</td>
</tr>
<tr>
<td>Shetty et al. [15]</td>
<td>2010</td>
<td>India</td>
<td>100</td>
<td>20-70</td>
</tr>
<tr>
<td>Sharma et al. [71]</td>
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<td>India</td>
<td>50</td>
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</tr>
<tr>
<td>Saxena. [30]</td>
<td>2011</td>
<td>India</td>
<td>120</td>
<td>21-60</td>
</tr>
<tr>
<td>Saxena, Tiwari [40]</td>
<td>2011</td>
<td>India</td>
<td>160</td>
<td>21-60</td>
</tr>
<tr>
<td>Chandramala et al. [21]</td>
<td>2012</td>
<td>India</td>
<td>100</td>
<td>20-70</td>
</tr>
<tr>
<td>Kanchan et al. [35]</td>
<td>2012</td>
<td>India</td>
<td>100</td>
<td>25-77</td>
</tr>
<tr>
<td>N. Agarwal. [17]</td>
<td>2012</td>
<td>India</td>
<td>50</td>
<td>20-70</td>
</tr>
<tr>
<td>Erbudak et al. [29]</td>
<td>2012</td>
<td>Turkey</td>
<td>123</td>
<td>15-57</td>
</tr>
<tr>
<td>Thevissen et al. [33]</td>
<td>2012</td>
<td>Belgium</td>
<td>450</td>
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<td>Limdiwala et al. [22]</td>
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<td>India</td>
<td>150</td>
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<tr>
<td>Parkin et al. [28]</td>
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<td>India</td>
<td>30</td>
<td>15-60</td>
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<td>Kostenko. [72]</td>
<td>2013</td>
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<td>64</td>
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<tr>
<td>Misrioglu et al. [25]</td>
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<td>Turkey</td>
<td>114</td>
<td>17-72</td>
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<td>Patil et al. [69]</td>
<td>2014</td>
<td>India</td>
<td>200</td>
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<td>Edward et al. [23]</td>
<td>2014</td>
<td>Sudan</td>
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<td>Muszynska et al. [34]</td>
<td>2015</td>
<td>Poland</td>
<td>3</td>
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<tr>
<td>Marroquin et al. [26]</td>
<td>2016</td>
<td>Australia</td>
<td>74</td>
<td>12-28</td>
</tr>
<tr>
<td>Mittal et al. [27]</td>
<td>2016</td>
<td>India</td>
<td>152</td>
<td>14-56</td>
</tr>
<tr>
<td>Rajpal et al. [70]</td>
<td>2016</td>
<td>India</td>
<td>50</td>
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</tr>
<tr>
<td>29 papers</td>
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<td>3254</td>
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</table>

*Number of individuals reported in each study. Sample size.
Table 1.2. Total studies reporting the use of the Cameriere et al. method.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Total</th>
<th>Age</th>
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<tbody>
<tr>
<td>Cameriere et al. [7]</td>
<td>2004</td>
<td>Italy</td>
<td>100</td>
<td>18-72</td>
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<tr>
<td>Cameriere et al. [52]</td>
<td>2006</td>
<td>Italy</td>
<td>33</td>
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<td>100</td>
<td>20-79</td>
</tr>
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<td>Italy</td>
<td>100</td>
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<td>2009</td>
<td>Portugal and Italy</td>
<td>229</td>
<td>20-84</td>
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<td>Singaraju et al. [38]</td>
<td>2009</td>
<td>India</td>
<td>200</td>
<td>18-72</td>
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<tr>
<td>Babshed. [4]</td>
<td>2010</td>
<td>India</td>
<td>178</td>
<td>20-70</td>
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<tr>
<td>De luca et al. [2]</td>
<td>2010</td>
<td>Spain – Italy</td>
<td>73</td>
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</tr>
<tr>
<td>Babshed et al [50]</td>
<td>2011</td>
<td>India</td>
<td>61</td>
<td>21-71</td>
</tr>
<tr>
<td>Cameriere et al [73]</td>
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<tr>
<td>Jeevan et al. [39]</td>
<td>2011</td>
<td>India</td>
<td>228</td>
<td>16-72</td>
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<tr>
<td>Saxena [30]</td>
<td>2011</td>
<td>India</td>
<td>120</td>
<td>21-60</td>
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<tr>
<td>Vodanovic et al. [65]</td>
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<td>2013</td>
<td>Portugal</td>
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<td>Charis et al. [43]</td>
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<td>120</td>
<td>20-70</td>
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<td>Cameriere et al. [46]</td>
<td>2013</td>
<td>Portugal</td>
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<tr>
<td>AC Azevedo. [44]</td>
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<td>81</td>
<td>19-74</td>
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<tr>
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<td>114</td>
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<tr>
<td>Azevedo et al. [44]</td>
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<td>Brazil</td>
<td>443</td>
<td>20-78</td>
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<td>Ravindra et al. [47]</td>
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<tr>
<td>Cameriere et al. [74]</td>
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<td>Iran</td>
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<tr>
<td>Torkian. [75]</td>
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<td>Iran</td>
<td>120</td>
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29 papers: 11 Countries, 4167 individuals, 12-79 age range.

*Number of individuals reported in each study: Sample size.
Table 1.3. Total studies reporting the use of volume calculation.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Total*</th>
<th>Age</th>
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<tr>
<td>Vandervoort et al. [8]</td>
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<td>52</td>
<td>24-66</td>
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<td>Japan</td>
<td>155</td>
<td>12-79</td>
</tr>
<tr>
<td>Agematsu et al. [61]</td>
<td>2010</td>
<td>Japan</td>
<td>258 teeth</td>
<td>20-79</td>
</tr>
<tr>
<td>Aboshi et al. [13]</td>
<td>2010</td>
<td>Japan</td>
<td>50</td>
<td>20-78</td>
</tr>
<tr>
<td>Star et al. [55]</td>
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<td>Belgium</td>
<td>111</td>
<td>10-65</td>
</tr>
<tr>
<td>Tardivo et al. [56]</td>
<td>2011</td>
<td>France</td>
<td>58</td>
<td>14-74</td>
</tr>
<tr>
<td>Jagannathan et al. [14]</td>
<td>2011</td>
<td>India</td>
<td>188</td>
<td>10-70</td>
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<tr>
<td>Sakuma et al. [53]</td>
<td>2013</td>
<td>Japan</td>
<td>136</td>
<td>14-79</td>
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<tr>
<td>Tardivo et al. [57]</td>
<td>2014</td>
<td>France</td>
<td>210</td>
<td>15-85</td>
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<td>Sasaki et al. [54]</td>
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<td>Japan</td>
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<td>72</td>
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<td>Ge et al. [62]</td>
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<td>China</td>
<td>403</td>
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<tr>
<td>De Angelis et al. [58]</td>
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<td>Italy</td>
<td>91</td>
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<tr>
<td>Pinchi et al. [59]</td>
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<td>Ge et al. [59]</td>
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</table>

*Number of individuals reported in each study: Sample size.
Table 1.4. The Kvaal et al. method: Studies included in the quantitative analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Measuring instrument</th>
<th>SEE ± years per tooth or group of teeth (FDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 teeth</td>
</tr>
<tr>
<td>Kvaal [6] 1995, Norway.</td>
<td>100 PER 20-87 years</td>
<td>Stereomicroscope and Vernier calipers.</td>
<td>8.6</td>
</tr>
<tr>
<td>Bosman [20] 2005, Belgium.</td>
<td>197 OPG 19-75 years</td>
<td>Adobe Photoshop software</td>
<td>9.5</td>
</tr>
<tr>
<td>Paewinsky [19] * 2005, Germany.</td>
<td>168 OPG 14-81 Years</td>
<td>Hipax program</td>
<td>5.6</td>
</tr>
<tr>
<td>Avendano [18] 2009, Colombia</td>
<td>107 PER 21-50 years</td>
<td>Scion Image software</td>
<td>8.6</td>
</tr>
<tr>
<td>Shetty [15] 2010, India.</td>
<td>100 PER 20-70 years</td>
<td>SV-4 mini slide viewer, digital Vernier calipers, stereomicroscope</td>
<td>10.5</td>
</tr>
<tr>
<td>Erbudak [29] 2012, Turkey.</td>
<td>123 OPG 14-57 years</td>
<td>Image J software</td>
<td>10.5</td>
</tr>
<tr>
<td>Saxena** [40] 2011, India.</td>
<td>120 OPG 21-60 years</td>
<td>Autocad2005 software</td>
<td>10.5</td>
</tr>
<tr>
<td>Kanchan [35] 2012, India.</td>
<td>Group A, 47 PER 25-75 years</td>
<td>Adobe Photoshop software</td>
<td>12</td>
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<tr>
<td></td>
<td>Group B, 43 PER 25-77 years</td>
<td>Adobe Photoshop software</td>
<td>11.9</td>
</tr>
<tr>
<td>Lindawala [22] 2013, India.</td>
<td>Group A, 100 PER 25-50 years</td>
<td>Kodak Dental Imaging Software</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Group B, 50 PER 25-50 years</td>
<td>Kodak Dental Imaging Software</td>
<td>9.4</td>
</tr>
<tr>
<td>Misirlioglu [25] 2014, Turkey.</td>
<td>144 OPG 17-72 years</td>
<td>Easy-Dent PC software</td>
<td>8.3</td>
</tr>
<tr>
<td>Patil [69] 2014, India.</td>
<td>200 PER 20-50 years</td>
<td>Image-Pro Plus II software</td>
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</tr>
<tr>
<td>Mittal [27] 2016, India.</td>
<td>152 OPG 14-60 years</td>
<td>Vistascan DBSWIN software</td>
<td>7.9</td>
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<tr>
<td>Rajpal [70] 2016, India.</td>
<td>50 PER 15-57 years</td>
<td>Kodak Dental Imaging Software</td>
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</table>

*Error reported as standard deviation. **Error reported as the difference between the chronological age and the estimated. PER=Periapical radiographs. OPG=Panoramic radiographs. Tooth numeration FDI.
Table 1.5. The Cameriere et al. method: Studies included in the quantitative analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Measuring instrument</th>
<th>SEE ± years per tooth (FDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameriere et al. [7]* 2004, Italy.</td>
<td>100 OPG 18-72 years</td>
<td>AutoCAD2000</td>
<td>32/42 11/21 12/22 31/41 32/42 33/43 34/44 35/45 13/23</td>
</tr>
<tr>
<td>Cameriere et al. [37] 2009, Portugal and Italy.</td>
<td>229 PER 20-84 years</td>
<td>Adobe Photoshop</td>
<td></td>
</tr>
<tr>
<td>Babshed et al. [50] 2011, India.</td>
<td>61 PER 21-71 years</td>
<td>Adobe Photoshop AutoCAD 2004</td>
<td>12.22</td>
</tr>
<tr>
<td>Jeevan. [39]* 2011, India.</td>
<td>228 PER 16-72 years</td>
<td>Adobe Photoshop</td>
<td></td>
</tr>
<tr>
<td>Zaher et al. [41] 2011, Egypt.</td>
<td>144 PER 12-60 years</td>
<td>AutoCAD2008</td>
<td>2.63</td>
</tr>
<tr>
<td>Cameriere et al. [42] 2012, Spain.</td>
<td>606 OPG 18-75 years</td>
<td>Adobe Photoshop</td>
<td></td>
</tr>
<tr>
<td>Cameriere et al. [46] Portugal. 2013</td>
<td>116 PER 18-74 years</td>
<td>Adobe Photoshop</td>
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<tr>
<td>Charis et al [43]* 2013, India.</td>
<td>120 PER 20-70 years</td>
<td>Jenoptic ProgRess Version ss2.7.</td>
<td></td>
</tr>
<tr>
<td>AC Azevedo [44]** 2014, Italy.</td>
<td>81 PER 19-74 years</td>
<td>Jenoptic ProgRess Version ss2.7.</td>
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</tr>
<tr>
<td>Misirlioglu et al. [25] 2014, Turkey.</td>
<td>114 PER 17-72 years</td>
<td>Adobe Photoshop</td>
<td></td>
</tr>
<tr>
<td>Azevedo et al. [44] 2015, Brazil.</td>
<td>443 PER 20-78 years</td>
<td>Adobe Photoshop</td>
<td>6.41</td>
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</tbody>
</table>

*Error reported as mean absolute error (MAE), **Error reported as the module of the differences between the chronological and estimated age, ***Error reported as mean error, PER=Periapical radiographs. OPG=Panoramic radiographs. Tooth numeration FDI (Federation Dentaire International)
### Table 1.6. Volume calculation based methods.

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample</th>
<th>Image segmentation method</th>
<th>Measuring instrument</th>
<th>Accuracy pert tooth (FDI)</th>
</tr>
</thead>
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<tr>
<td>Star et al. [55]</td>
<td>111 CBCT 10-65 years</td>
<td>automatic segmentation (threshold), and manual correction</td>
<td>Simplant®</td>
<td>11/21 SD=12.8 15/25 SD=8.4</td>
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<td></td>
<td></td>
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<td></td>
<td>13/23 SD=13.1</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Tardivo et al. [57]</td>
<td>210 CT 15-85 years</td>
<td>Semiautomatic</td>
<td>Mimics®</td>
<td>13/23 MAE=3.4 33/43 MAE=4.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De Angelis et al. [58]</td>
<td>91 CBCT 17-80 years</td>
<td>Manual</td>
<td>Osirix software</td>
<td>prediction interval = ±28 years</td>
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<td>Pinchi et al. [59]</td>
<td>148 CBCT 10-80 years</td>
<td>cone shape approximation</td>
<td>Osirix® software</td>
<td>SEE=±11.45</td>
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CBCT: Cone beam computed tomography. SD: Standard deviation. MAE: Mean absolute error. SEE: Standard error of estimation. Tooth numeration FDI (Federation Dentaire International)
conclusions, it was necessary to analyse the possibility of author bias. This was determined by the participation of the same authors in repeated publications. To this end, the results were analysed comparing individual papers, and then grouping them per author.

Additionally, in certain cases where there were doubts about the origin of the sample, which means the likelihood of finding different studies by the same authors that had used the same sample, the authors were contacted to confirm the origin of the sample. In case that two studies had the same sample, the authors were asked to suggest which study should be included in the meta-analysis. Likewise, to deal with missing data, the authors were contacted for more information. There was another possible source of bias observed in this study: the use of non-specific population regression models and equations, which could generate the wrong assumption on the accuracy of the analysed methods. To overcome this problem, only studies using specific population formulae were used in the quantitative analysis. However, there is not enough evidence to state that the production of secondary dentine varies among individuals of different population.

RESULTS.

Kvaal et al. [6] (29 papers, 3254 participants)

Qualitative analysis

The original paper on the Kvaal et al. [6] method was published in 1995. Since then, this method has been applied to a global sample of 3254 individuals, from 12 different
countries (Graph 1.1, Table 1.1). The initial methodology suggested the use of periapical radiographs from six different teeth, using Vernier calipers to measure the maximum tooth length, pulp length and root length, and a stereomicroscope with a measuring eyepiece to the nearest 0.1mm, to measure pulp and root width at three different levels previously described by Kvaal et al. [6].

Only a few studies have followed the original methodology, with slight adaptations [1, 6, 9, 15-18]. In 2005, this method was applied to panoramic radiographs [20], which became more popular [20-34]. The use of digital imaging in dentistry also introduced the use of different software to perform the odontometric measurements.

The most commonly used software programs are: Adobe Photoshop (different versions n=5), Image J (n=4) and Kodak dental imaging software (n=2). From those studies that tested the effect of sex on the accuracy of the age estimated: three found that sex does not affect the models [15, 19, 30]; and two that it does [18, 23]. In the original study, sex was included as a factor for the mandibular lateral incisor, indicating that pulp cavity size changes occurred faster in males, as females need another 6 years to get the same age as males for this tooth [6].

**Quantitative analysis**

From the total sample, only 16 papers met the inclusion criteria for the quantitative analysis. (Table 1.4). The most accurate result (SD=5.6, r=-0.95) was obtained from panoramic radiographs using the Hipax program (version 3.01) software, 168 participants
(77 female and 91 male) in a Caucasian group [19]. The largest error reported error was SEE >13 years $r^2=0.1$ [15, 35] obtained from the lower canine, following original Kvaal et al. methodology on periapical radiographs [35] and the Adobe Photoshop 6.0 software in panoramic radiographs [15].

Kvaal et al. measurements have been reported to have a high degree of intra and inter-observer agreement indicating the reproducibility of the measurements, even in those studies that did not follow the original methodology. Only one study reported significant differences in the root length and pulp width at the level B and root width at the level A measurement, although this study reported a SEE=±13.8 ($r^2=0.38$), the average error of age estimation was ±18-21 years using periapical radiographs. [35]

**Note:** The studies that did not meet the inclusion criteria for the quantitative analysis also reported high intra and inter-observer agreement [31-33]. One study reported that the lower lateral incisor produced lower intra-observer correlation [32], and one affirmed that the use of a stereomicroscope improved the accuracy of the age estimates [9].

**Cameriere et al. [7] (29 papers, 4167 participants)**

**Qualitative analysis**

The original paper documenting the Cameriere et al. method [7] was published in 2004. In the present systematic review, 29 papers using this method were studied, having a global sample of 4167 individuals from 11 countries (Graph 1.2, Table 1.2). This method was
initially applied to digital panoramic radiographs, using (AutoCAD2000, Install Shield3.0, 1997) and different versions of this software have been used in 11 studies. The most used software, to perform the pulp/tooth area measurements, was Adobe Photoshop (13 studies).

This method was designed to be applied to single rooted teeth, especially canines, but it has also been tested in premolars, central, and lateral incisors. From those studies that assessed the effect of sex on the regression models, the vast majority reported that sex does not affect the regression model [4, 7, 25, 30, 36-45]. Only 4 studies reported that sex affects the regression model [46-49].

Quantitative analysis

Twelve studies met the inclusion criteria for the quantitative analysis (Table 1.5). The use of this method has a standard error of estimation (SEE) from ±1.2 [41] to ±12-13 years (r=0.2-0.4) [50]. From these studies, two reported a median error <±5 years [7, 37], six reported an error from ±5 to ±8.5 years [25, 39, 41-43, 45], and three studies reported an error from ±10 to ±13 years [4, 46, 50]. From the studies included in the qualitative analysis, only one reported significant intra-observer differences in lateral incisors and first premolars (p<0.05) [50].

Note: All the studies that did not meet the inclusion criteria for the qualitative analysis and that included intra and inter-observer calibration, reported high intra/inter-observer agreement [36, 48, 51, 52].
Volume (16 papers, 2444 participants)

Qualitative analysis

Owing to the relatively few studies doing pulp/tooth volume reconstruction, in the qualitative analysis those studies using extracted teeth were included. The first study doing pulp/tooth volume reconstruction reported the use of micro-focus X-ray in 2004 [8]. In this current systematic review, 16 studies used the pulp/tooth volume reconstruction, by means of micro-focus computer tomography, CT scan or CBCT, and the use of different software to do manual or semiautomatic volume reconstruction, such as Tri/3D Bon, Mimics®, ITK-SNAP 2.4, Osirix, Amira among others, in a global sample of 2444 individuals from 7 countries (Graph 1.3, Table 1.3).

However, the methodology used by some studies, required the use of extracted teeth [8, 12, 13, 53, 54]. With regard to the effect of sex on the regression models, nine studies reported that sex has no effect [8, 14, 25, 53, 55-59]. Three reported that the models are more accurate in females than in males, having a higher determination coefficient for women than for men [12, 60, 61]. The comparative data are as follows: $r^2=0.6$ for males and $r^2=0.7$ for females [12], or $r^2=0.29$ for males and $r^2=0.15$ for males [60], and $r^2=0.67$ for males and $r^2=0.75$ for females when the mandibular central incisor is used, or $r^2=0.56$ for males and $r^2=0.58$ for females when the second premolar is used.

Two studies, which reconstructed only the pulp cavity volume, also reported significant differences between tooth type and sex [62, 63]. One of them found a significant
difference in the pulpal volume between both genders ($p=0.028 <0.05$), and a stronger relation between pulp chamber and age for females ($r^2=0.6$) than for males ($r^2=0.5$) [62]. The other one reported a significant difference in volume between the two genders for 12 types of teeth ($p=0.000$) [63].

**Quantitative analysis**

Four studies meet the inclusion criteria for the quantitative analysis. The error of these studies varies between $\pm 3.47$ years [57] to $\pm 28$ years [58]. A high intra-observer agreement was reported [59]. Among those studies that did not meet the inclusion criteria for the quantitative analysis, seven also reported high intra- and inter-observer agreement [8, 11-14, 56, 62, 63].

**DISCUSSION**

Age estimation in adults is a challenge in all forensic investigations, especially in cases that require the use of non-invasive methods. The formation of secondary dentine and the resultant non-linear narrowing of the root canal with age [11], is one age predictor measurable in dental radiographs and tomographs, leading to the proposal of different methods for age estimation in adults as an alternative to more invasive methods, and as a complement to osseous analysis [2, 16, 34, 64-67].

In the same way, many methods have been suggested for dental age estimation in adults. Nevertheless, there are few available studies that compare their accuracy [68].
In the lack of a consensus on a uniformly applicable method for age estimation in adults, the need to perform a systematic review was timely. This systematic review summarizes and compares the results of some of the most used methods for dental age estimation in adults.

In terms of the qualitative analysis, it has been reported that there are no significant differences between right or left teeth [14, 56]. Another aspect to test among the different papers was the effect of sex of the different methods. Although not all the included studies assessed the effect of sex on age estimation, those that did (n=38), 71% (n=27) affirm that sex has no statistically significant effect on age estimation, when pulp/tooth ratio is calculated. However, if only pulp volume is measured and used as age indicator, there is a significant difference between the accuracy for males and females [62, 63].

In the light of the above evidence one could suggest that ratio calculation not only diminishes the effects of tooth magnification in dental radiographs, as reported by Kvaal [6] et al., but also reduces the effect of sexual dimorphism related to tooth size. The age of the participants is also another relevant aspect to include.

In this systematic review, those studies including participants under 14 years of age were excluded for two main reasons. Firstly, there are other methods more reliable for aging teenagers and infants, and secondly, before 14 years of age, not all the teeth have completed the apex closure, which by definition, is a requirement for the formation of the secondary dentine [76].
For the qualitative analysis, characteristics related to sample size and inclusion criteria established in different studies were compared. As compared to previous efforts, no significant correlation between sample size and the accuracy of the method was found, when all the data were analysed together ($r^2=0.1$, p-value <0.05, sample size of the analysed studies n=50 to 604) or when the data were analysed individually for each method.

It is necessary to highlight that from the studies included in this current analysis (n=30) only 4 (13.3%) had a sample smaller than 100 individuals (n=50 to 91 individuals), which may suggest that in any study for age estimation the minimum sample should include data from at least 100 participants, or over 100 teeth from different individuals in those studies using only one tooth type. Some of the excluded studies, for the quantitative analysis, fail in this aspect [55, 56, 60, 61], including data from several teeth of the same individual and counting them as different samples, which may affect the reported results.

A previous study recommended a minimum of 120 participants in order to achieve 80% of power and 5% of significance [30]. One almost universal inclusion criterion was the use of only totally sound teeth. Only two studies did not follow this parameter, using teeth with lesions to do volume calculations [54], or using panoramic radiographs that did not meet the inclusion criteria suggested by Kvaal et al. [22]. Both studies found no significant effect of these situations on the accuracy of the results.
Another aspect to consider is the description of the different methods, in terms of the procedure to measure the different pulp/tooth dimensions, as well as the statistical processing of the data. In certain cases, it was necessary to re-read the papers several times to understand the proposed methodology and how the accuracy was reported.

In the quantitative analysis two main characteristics were compared among the studies: repeatability of the measurements, in terms of intra and inter-observer agreement; and their accuracy, reported as standard error of estimation (SEE), mean absolute error (MAE) or standard deviation (SD). Due to this variation in the approach to evaluate and to report the obtained accuracy, it was not possible to perform a deeper statistical analysis, as a meta-analysis to confirm that one method was superior to another. However, in this review the studies using pulp/tooth area ratio reported a lower error (table 1.4, 1.5 and 1.6).

All the studies reported significant intra- and inter-observer agreement, regardless of the method and the measuring instrument, which suggests that after adequate training any of these methods is reproducible. However, with regard to the simplicity of the technique, and the access to the required tool to measure and calculate the respective pulp/tooth ratio dimensions, Kvaal et al., and Cameriere et al. are more convenient.

By using periapical radiographs or panoramic radiographs, as the radiological technique does not affect the accuracy of the measurements, as long as the quality of the image
allows the observer to clearly observe the boundaries of the root canal and tooth surface [22].

The volumetric reconstruction of anatomic structures involves more technical and time-consuming training as well as the use of more complex software. These factors could be considered as obstacles for the larger scale application of some of these methods. Likewise, the user spends more time doing the volumetric reconstruction per tooth, which varies from 4 hours [8] to 15 minutes [60], when reported.

Another important aspect, with regard to the accuracy of the method, is the use of specific population formulae, which has been believed to improve the results of the age estimates, independently of the used method [14, 29, 41, 45, 63, 69]. As in those studies, using non-specific population formulae the reported error was notably larger (error > ±20 years) [31, 32]. But the most recent findings about age mimicry, explained in Chapter 7, could reveal that this assumption is called into question [77].

The use of data from different populations in the same statistical analysis, to generate a unique age estimation equation, is achievable [2, 20, 46]. Furthermore, it has been reported that the pulp chamber size variation can be detected only each 10 years, which opens a question about the reliability of those methods reporting a lower error.

In the specific analysis by the Kvaal et al. method, although requiring the inclusion of six different single rooted teeth per individual, the use of only one tooth [18], only upper
central incisor [69], canines [25], mandibular teeth [32], or different combinations of teeth to apply the odontometric analysis described by Kvaal et al [24, 26], has also been reported, with acceptable results (SEE < ±10 years).

It has also been reported that its accuracy depends on the quality of the image and on the precision of the measurements [22]. Several studies reported that tooth length is not strongly correlated with age [15, 17, 19, 22], as tooth length would depend on tooth wear, bruxism, and food habits, rather than a physiological aging related process [15].

In regard to the Cameriere et al method, it only requires the use of one tooth, and the majority of the studies report the use of canines. However, one study using three different lower teeth (lateral incisor, canines, first premolars) found that lower canines had the poorest correlation coefficient with age ($r^2=-0.2$) [50]. Another study found more accurate estimations from upper lateral incisors, claiming that the narrowing velocity in the pulp chamber of this tooth is twice as fast as in lower incisors [46].

The Cameriere et al. method reported the lowest error (total average ±5.6 years), and a sample of at least 120 individuals was reported as being adequate to obtain more accurate age estimates [30].

The combined use of the Kvaal et al. width ratios and Cameriere area ratios has been reported on canines measured from digital panoramic radiographs. More accurate age estimates were achieved when the pulp/tooth width ratio at the mid-root level (i.e. half-
way between the enamel-cementum junction, and the apex of the root level) and pulp/tooth area ratio were used together [30], or when the area ratio calculation from canines is used individually [25].

In this systematic review the following advantages about the use of pulp/tooth area calculation over other methods were noted: area measurements on digital radiographs with different software are faster; and the most recent study using this method proposes software for the automatic selection of pulp and tooth borders. These steps minimize the time taken to obtain the area of tooth and pulp chamber, and reduce the error associated with the observer, when performing the area selection [74].

Linear measurements can also be performed on digital radiographs, but, the observer needs to take nine measurements per tooth, in six teeth, and calculate several ratios. In the method using pulp/tooth area measurements, only one ratio needs to be calculated and allows the researcher to develop the respective statistical regression model. In the same way, the use of canines, especially upper canines, to calculate pulp/tooth area ratio has certain advantages, as facilitated by their longer survival, less wear, and the larger size of the pulp chamber in comparison with other teeth [58]. Also, as observed in table 1.5, there are not only more studies reporting the use of upper canines for pulp/tooth area calculations but also, these studies present acceptable accuracy.

This is in contrast to what was reported by Kvaal et al when doing pulp/tooth length/with ratio calculations; they observed that upper canines had the lowest correlation with age,
when using dental radiographs of extracted teeth [76], which was an exclusion criterion, in this current systematic review.

Finally, although the use of pulp/tooth volume ratio calculations from non-extracted teeth still requires more research. The initial results of the analysis of pulp volume from individuals of different age groups provide important and detailed information to understand the formation of secondary dentine.

It has been reported that, the pulp/tooth volume ratios in the cervical area, were more correlated with age, and that this correlation decreased towards the tooth apex [13]. Also, that the most marked reduction in volume ratio was observed between the second and the fifth decades of life in lower first and second premolars, and between the second and the third decades of life in lower first premolars [13]. These clear findings may enlighten the prospect of future and more accurate methods for age estimation in adults.

CONCLUSION

Narrowing of the root canal caused by the formation of secondary dentine is a well-accepted age indicator in adults. This systematic review observed that age estimation methods based on pulp/tooth area ratio calculation provided more accurate results, even when one tooth is analysed per individual. However certain conditions need to be fulfilled: a sample of minimum 120 individuals; older than 14 years of age; assessment of the
accuracy of the observer. The inclusion of data of individuals from different populations in the same analysis is not discouraged.

Future studies for dental age estimation in adults, must consider the non-linear deposition of secondary dentine through life in their statistical analysis, and also that the use of linear regression models would generate the over-estimation of age in young individuals and the under-estimation of age in older individuals [78]. This systematic review also recommends the use of dental age estimation methods: firstly pulp/tooth area ratio calculation of single rooted teeth: upper canines and other teeth (lower premolars, upper central incisors); and secondly pulp/tooth length/width ratio calculation, following the methodology proposed by Kvaal et al.

These factors need to be considered in combination with other methods that include diverse age indicators to produce more reliable age estimates. The author of this systematic review also recommends future studies to report their results in terms of standard deviation, mean absolute error and standard error of estimation simultaneously. Such a display of data will facilitate more meaningful meta-analyses in future reviews.
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SECTION TWO. Assessment of possible effect of orthodontic treatment on one of the available methods for age estimation in adults: The Kvaal et al. method.

Preface

Section two answers one of the main aims of this thesis; the effect of external factors on the accuracy of the Kvaal et al. method. This section presents two studies that tested this method in individuals who had received orthodontic treatment. For chapter 2, the Kvaal et al. method was tested on two different data sets: the first, collected from individuals that had not received orthodontic treatment; and the second from individuals in whom orthodontic treatment was completed.

Two sets of formulae to estimate the age of individuals were proposed: one for individuals who did not receive orthodontic treatment; and the second for those who had received orthodontic treatment; comparisons of the results were performed. It is necessary to highlight that this is the first study that applies one age estimation method for adults, in individuals whose dental conditions have been altered. This is in contrast to previous studies that used data from individuals with sound teeth or who had not been exposed to other dental procedures.

In Chapter 3, the statistical approach for the analysis of the data is innovative, as it follows the design of epidemiological studies to assess risk factors. As in the study presented in Chapter 2, it was observed that there was no statistically significant difference for the age
estimation from individuals with and without orthodontic treatment. Chapter 3 presents a detailed analysis, at the tooth level, of the effect of orthodontic treatment on the accuracy of the Kvaal et al. method testing formulae that were previously published for dental age estimation in adults.

The chapters presented in this section share the methodological design with regards to the inclusion and exclusion criteria of the participants: analyzed teeth, use of panoramic radiographs, and measurements collection as follows:

**Inclusion criteria**

All subjects who had a recorded panoramic radiograph of high quality with respect to factors of image brightness, contrast, and sharpness. In addition, all teeth included in the analysis were clinically sound, with completed root formation, and at the occlusal plane level.

**Exclusion criteria**

Any radiographs that did not meet the inclusion criteria, or had observable failings (Eg. image distortion, poor contrast, overlap or superposition of tooth structure, or improper positioning) were excluded. Teeth with rotations, incomplete root formation, dilacerations, pulpal pathologies and endodontic treatment, and/or restorations were excluded. Teeth with developmental abnormalities in size, shape and tooth structure, as well as previous pulpal and periodontal pathologies, and endodontic treatment were excluded. Teeth with
large areas of enamel overlap between neighboring teeth were excluded in the studies that are included in this section.

**Teeth Selection**

In accordance to Kvaal et al. [1], three single rooted upper teeth (with Federation Dentaire International (FDI) notation 11/21, 12/22, 15/35) and three single rooted lower teeth (FDI 32/42, 33/43, 34/44) were measured. Kvaal et al. [1] reported that there no significant differences between teeth from the left or the right side of the arch.

Consequently, in the absence of one of the teeth, the contralateral tooth was used, as long as it met the inclusion criteria. In accordance with Karkhanis et al. [2], panoramic radiographs that presented any combination of the required teeth were included. Both studies also shared the procedures to perform the measurements on digital panoramic radiographs, the calibration of the observer, and the methods to test the measurement precision as described below:

**Measurements**

All the panoramic radiographs were obtained in digital format, the measurements were performed using Image J software (version 1.48 19 April 2014 - National Institute of Health, USA). Following the methodology used by Kvaal et al. [1], the following measurements were recorded: tooth, pulp and root length, as well as the pulp and tooth width at three different levels: A (cemento-enamel junction in the mesial surface of the roots); B (midpoint between the points A and C); and C (midpoint between the cemento-enamel
junction and root apex). After the collection of the figures in an Excel (2013) spreadsheet, a series of ratios was calculated: (T) tooth/root lengths; (R) pulp/tooth length, (P) pulp/root length, and root width/ pulp width ratio at levels A, B and C.

The ratio calculation and use, was proposed by Kvaal et al. [1] with the aim to reduce the effect of magnification and angulation inherent in most radiographs [1, 3]. The different mean values calculated with these ratios provided the age estimation predictors. M: mean value all ratios, W: mean value width ratios B and C, L: mean value ratios P and R, W-L: difference between W-L).

In Chapter 2, the predictors M and W-L were later used to formulate the age estimation models by means of multiple regression analysis. In Chapter 3, the predictors M and W-L were later used to estimate the age of all the participants, as established by Kvaal et al. [1]. The measurements were completed by a single observer (TYM). All data were collected using Excel (version 2013 Microsoft Redmont, USA) and statistical analyses were completed using the program 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/).

**Calibration**

Intra and inter-observer calibration was estimated using five randomly selected panoramic radiographs from the final study sample. These panoramic radiographs were measured on five different days with at least one-day interval. With the aim to avoid the recall of the measurements and landmarks, five new panoramic radiographs were also measured on
each occasion. Data were recorded using the software Image J by TYM and SK. To determine intra-observer precision, three estimates of precision were calculated: the technical error of measurement (TEM <1.0), relative technical error of measurement (rTEM <5%) and coefficient of reliability was calculated to quantify the inter- and intra-observer measurement error [4].

**Measurement precision**

After the estimation of intra- and inter-observer error, the obtained values were as follows: intra-observer error: TEM <1.0 (0.92), rTEM<5% (2.99%) and R>0.75 (0.95). Inter-observer error (between SK and TYM) was: TEM<1.0 (0.80), rTEM<5 (2.37) and R>0.75 (0.98) showing comparable intra and inter-observer measurement precision; within acceptable standards for all the measurements.
REFERENCES


2. Chapter 2. Changes in age estimation standards secondary to full fixed edgewise orthodontic treatment

This chapter was published as:

ABSTRACT

Objective: The aim of this cohort study was to evaluate if the side effects of orthodontic treatment have any significant impact when the Kvaal et al. method is used for dental age estimation. The objective was to observe the potential effects of orthodontic treatment on the accuracy of the age estimation, as performed by using the Kvaal et al. standards when it was applied on individuals before and after orthodontic treatment.

Methods: Following the methodological approach of Kvaal et al., odontometric measurements were acquired and the data were statistically analyzed to develop age estimation regression models. The total number of radiographs analyzed was 182 (64%, n=58) female and 36% (n=33) male. The ages ranged from 12 to 50 years for females (mean age 22 years), and 12 to 52 for males (mean age 22 years), before starting the treatment. The average length of the treatment was 2.2 years for both females and males.

Results: The standard error of estimate (SEE) for the regression models did not change dramatically for the pre- and post-treatment data.

Conclusions: It is recommended that similar analyses be performed for other methods of dental age estimation, for example methods based on cone beam computer-tomography or micro-focus computer tomography. These novel approaches, although more accurate, are also potentially more sensitive to dental changes caused by orthodontic treatment.

Key words: Forensic sciences, dental age estimation, secondary dentine formation, adults, orthodontic treatment, root resorption.
INTRODUCTION

Kvaal et al. [1] developed a non-invasive method for the estimation of age in adults with measurement of dimensions of the pulp chamber and tooth length to formulate regression models for the estimation of age. Originally, this method was proposed to be used on periapical radiographs. However, more recent work has focused on the use of the Kvaal et al. [1] method in panoramic radiographs [2-4]. During tooth development, secondary dentine deposition commences once the root formation is complete, and the tooth erupts to the level of the occlusal plane [5].

Annually, the rate of secondary dentine formation has been documented to be 6.5 μm/year for the crown, and 10 μm/year for the root [6]. The rate of secondary dentine formation, is influenced by external stimuli such as occlusal forces, trauma and caries [7]. These external factors also generate the production of tertiary dentine [5], which is indistinguishable from secondary dentine on dental radiographs [8].

The estimation of dental age has been a valid tool in forensic and anthropological research [1, 9, 10]. For younger persons, including newborns, infants and adolescents, there are dental age estimation methods, generally based on tooth maturation, which are considered the most accurate to estimate of chronological age in sub-adults [3, 11-13].

In adults, and once the root formation is deemed complete, methods for dental age estimation are broadly based on the analysis of secondary dentine deposition and the
subsequent narrowing of the pulp chamber [1, 6, 14]. A potential confounding factor in the estimation of dental age is the high incidence of orthodontic treatment in developed countries [15]. The judicious application of forces is central to successful orthodontic treatment and is well known to induce biological changes in dental hard tissues, including the formation of secondary dentine [7] and root shortening [16]. The corollary being a change in the dental morphological features analysed in the estimation of dental age in adults [7, 17].

Given the potential of the development of tooth structure in adolescents and adult patients to be influenced by the application of orthodontic forces, and these structures being the foundation of age estimation methods, it is important to recognise and quantify such influences. The first aim of this study was to evaluate if the side effects of orthodontic treatment had any significant impact on dental age estimation when using the Kvaal et al. [1] method. The objective of this study was also to observe the potential effects of orthodontic treatment on the accuracy of age estimation, as performed by using the Kvaal et al. [1] standards when applied to individuals before and after orthodontic treatment.

**MATERIALS AND METHODS**

Ethics approval was obtained from the Human Research Ethics Committee of The University of Western Australia (Ref: RA/4/1/6797) prior to commencement. The design of the study, data collection and analysis has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.
Sample

This cohort study consisted of a series of subjects who consecutively presented for orthodontic treatment at a private specialist orthodontic clinic (SV). All panoramic radiographs were acquired from the same machine and in digital format, with the informed consent of the participants. All of the panoramic radiographs were unidentified, and the only information known was the sex and age of the participants at the starting and finishing time of the orthodontic treatment.

Sample population

This study analyzed a total of 182 pre-, and post-orthodontic treatment panoramic radiographs from 91 participants, 64% (n=58) female and 36% (n=33) male, from a Western Australian population. All participants presented for orthodontic treatment that, after diagnosis and treatment planning, was completed with fixed appliances. The ages ranged from 12 to 50 years for females (mean age 22) and 12 to 52 for males (mean age 22) before starting the treatment. The average length of treatment was 2.2 years for both females and males. Digital panoramic radiographs were taken as part of routine clinical orthodontic treatment to evaluate the initial condition of the participants and the final treatment results.

Statistical analysis

The strength of linear association between chronological age and the Kvaal et al. [1] dental ratios was determined by Pearson correlation coefficient ($r^2$). Following this stage, regression analysis was applied to evaluate the relationship between the ratios and the
chronological age. The final results were later used to generate the statistical models for age estimation. To quantify the predictive accuracy of the models, the standard error of estimation (SEE) was used in the cross-validation sample. Multivariable linear regression analyses were used to examine the association between the independent and outcome variables. The outcome variables included chronological age (dependent variable), and the predictors M and W-L (independent variables). Different equations were generated to estimate the chronological age, by individual teeth and by groups of teeth, as follows: Three maxillary teeth with M and W-L values (6 predictors) individually calculated for each tooth, and the average of M and W-L for the same teeth (2 predictors).

Three mandibular teeth with M and W-L values (6 predictors) individually calculated for each tooth, and the average of M and W-L for the same teeth (2 predictors). All six teeth, with M and W-L values (12 predictors) individually calculated for each tooth, and the average of M and W-L for the same teeth (2 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. The formula proposed by Preoteasa et al. [18] was used to calculate the percentages of teeth with root shortening.

RESULTS

Age distribution

The age distribution before orthodontic treatment for females was: 12-19 years (62%, n=36), 20-39 years (28%, n=16), >40 years (10%, n=6), and after treatment it was: 15-19
years (57%, n=33), 20-39 years (33%, n=19), >40 years (10%, n=6). For males, before orthodontic treatment was: 12-19 years (52%, n=17), 20-39 years (42%, n=14), and >40 years (6%, n=2), and after orthodontic treatment it was: 13-19 years (48%, n=16), 20 to 39 years (45%, n=15) and >40 years (6%, n=2).

The correlation coefficient between chronological age and the dental ratios was calculated for the data before and after orthodontic intervention (Table 2.1). In both cases, the correlation coefficient related with the width ratios (A, B and C) were more significant than those related with length (P, T, R and L). As established by Kvaal et al. [1], the predictors M and W-L are required to be included in the final equation for dental age estimation. Based on the Pearson correlation coefficients, it could be observed that after orthodontic treatment, the significance of the values for the predictor W-L decreased.

Individual tooth regression models, and multiple teeth regression models, were proposed for individuals that had not received orthodontic treatment (Tables 2.2 and 2.3 respectively), and for individuals after finishing the treatment (Table 2.3 and 2.4). In regard to the models for age estimation using individual teeth, the SEE tends to increase after orthodontic treatment, especially for the mandibular canine, where the increase was ±2 years. Furthermore, while the results for the SEE provided by the mandibular canine showed a lower SEE (± 8.26 years) in the data analysis before treatment, the same tooth showed the greatest value of SEE (±10.23 years) for individual tooth analysis, after orthodontic treatment.
Table 2.1. Correlation coefficients before (1) and after orthodontic treatment (2).

<table>
<thead>
<tr>
<th>ratio</th>
<th>Tooth number (FDI tooth numeration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11/21</td>
</tr>
<tr>
<td>$P_1$</td>
<td>0.15</td>
</tr>
<tr>
<td>$P_2$</td>
<td>-0.26</td>
</tr>
<tr>
<td>$T_1$</td>
<td>0.03</td>
</tr>
<tr>
<td>$T_2$</td>
<td>-0.16</td>
</tr>
<tr>
<td>$R_1$</td>
<td>0.16</td>
</tr>
<tr>
<td>$R_2$</td>
<td>-0.09</td>
</tr>
<tr>
<td>$A_1$</td>
<td>-0.44**</td>
</tr>
<tr>
<td>$A_2$</td>
<td>-0.31*</td>
</tr>
<tr>
<td>$B_1$</td>
<td>-0.43**</td>
</tr>
<tr>
<td>$B_2$</td>
<td>-0.44**</td>
</tr>
<tr>
<td>$C_1$</td>
<td>-0.34**</td>
</tr>
<tr>
<td>$C_2$</td>
<td>-0.34**</td>
</tr>
<tr>
<td>$M_1$</td>
<td>-0.13</td>
</tr>
<tr>
<td>$M_2$</td>
<td>-0.31*</td>
</tr>
<tr>
<td>$W_1$</td>
<td>-0.44**</td>
</tr>
<tr>
<td>$W_2$</td>
<td>-0.45**</td>
</tr>
<tr>
<td>$L_1$</td>
<td>0.18</td>
</tr>
<tr>
<td>$L_2$</td>
<td>-0.22*</td>
</tr>
<tr>
<td>$W-L_1$</td>
<td>-0.36</td>
</tr>
<tr>
<td>$W-L_2$</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Correlation coefficients between chronological age before (1) and after (2) starting orthodontic treatment and the Kvaal et al. [1] dental ratios (P: pulp length/root length, T: tooth length/root length, R: Pulp length/tooth length, A: pulp width/root width at level A, B: pulp width/root width at level B, C: pulp width/root width at level C) and predictors (M: mean value all ratios, W: mean value width ratios B and C, L: mean value ratios P and R, W-L: difference between W-L). *p<0.05** p<0.01 NS non-significant
Table 2.2. Multiple regressions for estimation of chronological age (in years) for individual maxillary and mandibular teeth in subjects before (1) and after (2) orthodontic treatment

<table>
<thead>
<tr>
<th>Teeth FDI</th>
<th>n</th>
<th>R</th>
<th>$R^2$</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/21(1)</td>
<td>83</td>
<td>0.22</td>
<td>0.21</td>
<td>Age = 39.87 - 76.79(M) - 51.75(W-L)</td>
<td>8.57</td>
</tr>
<tr>
<td>11/21(2)</td>
<td>83</td>
<td>0.14</td>
<td>0.12</td>
<td>Age = 54.50 - 73.67(M) - 30.64(W-L)</td>
<td>9.06</td>
</tr>
<tr>
<td>12/22(1)</td>
<td>81</td>
<td>0.18</td>
<td>0.16</td>
<td>Age = 68.21 - 99.54(M) - 33.52 (W-L)</td>
<td>8.92</td>
</tr>
<tr>
<td>12/22(2)</td>
<td>81</td>
<td>0.25</td>
<td>0.23</td>
<td>Age = 83.07 - 128.42(M) - 42.68(W-L)</td>
<td>8.48</td>
</tr>
<tr>
<td>15/25(1)</td>
<td>58</td>
<td>0.08</td>
<td>0.04</td>
<td>Age = 79.8105 - 81.5881(M) - 0.5444(W-L)</td>
<td>9.99</td>
</tr>
<tr>
<td>15/25(2)</td>
<td>58</td>
<td>0.21</td>
<td>0.18</td>
<td>Age = 90.22 - 129.66(M) - 18.62(W-L)</td>
<td>9.29</td>
</tr>
<tr>
<td>32/42(1)</td>
<td>87</td>
<td>0.12</td>
<td>0.10</td>
<td>Age = 15.40 - 37.51(M) - 43.05(W-L)</td>
<td>9.73</td>
</tr>
<tr>
<td>32/42(2)</td>
<td>87</td>
<td>0.09</td>
<td>0.74</td>
<td>Age = 49.10 - 77.55(M) - 37.65(W-L)</td>
<td>9.89</td>
</tr>
<tr>
<td>33/43(1)</td>
<td>63</td>
<td>0.47</td>
<td>0.45</td>
<td>Age = 111.80 - 238.35(M) - 132.7(W-L)</td>
<td>8.26</td>
</tr>
<tr>
<td>33/43(2)</td>
<td>63</td>
<td>0.19</td>
<td>0.16</td>
<td>Age = 76.60 - 159.75(M) - 105.79(W-L)</td>
<td>10.26</td>
</tr>
<tr>
<td>34/44(1)</td>
<td>68</td>
<td>0.35</td>
<td>0.33</td>
<td>Age = 21.67 - 69.84(M) - 69.59(W-L)</td>
<td>8.40</td>
</tr>
<tr>
<td>34/44(2)</td>
<td>68</td>
<td>0.31</td>
<td>0.29</td>
<td>73.52 -137.96(M) - 64.43(W-L)</td>
<td>8.71</td>
</tr>
</tbody>
</table>

Table 2.3. Multiple regressions for estimation of chronological age (in years) for the combination of different teeth before orthodontic treatment

<table>
<thead>
<tr>
<th>Teeth</th>
<th>N</th>
<th>R</th>
<th>R²</th>
<th>Equation (FDI tooth numeration)</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 max 2 pds</td>
<td>50</td>
<td>0.27</td>
<td>0.24</td>
<td>Age = 80.40 - 145.51(M) - 47.69 (W-L)</td>
<td>7.78</td>
</tr>
<tr>
<td>3 max 6 pds</td>
<td>50</td>
<td>0.31</td>
<td>0.21</td>
<td>Age = 63.704 - 6.412 (11/21M) - 17.216 (11/21W-L) - 32.812 (12/22M)-19.332 (12/22W-L) - 77.969 (15/25M) + 10.594 (15/25W-L)</td>
<td>7.93</td>
</tr>
<tr>
<td>3 mdb 2 pds</td>
<td>46</td>
<td>0.4</td>
<td>0.37</td>
<td>Age = 13.11-36.64 (M) - 19.83 (W-L)</td>
<td>9.12</td>
</tr>
<tr>
<td>3 mdb 6 pds</td>
<td>46</td>
<td>0.6</td>
<td>0.54</td>
<td>Age = 124.528 + 81.230 (32/42M) - 7.784 (32/42W-L) - 328.184 (33/43M) - 121.021 (33/43W-L) - 37.503 (34/44M) - 9.855(34/44W-L)</td>
<td>7.8</td>
</tr>
<tr>
<td>6 teeth 2 pds</td>
<td>36</td>
<td>0.45</td>
<td>0.41</td>
<td>Age = 133.86 - 238.98 (M) - 67.88 (W-L)</td>
<td>7.6</td>
</tr>
<tr>
<td>6 teeth 12 pds</td>
<td>36</td>
<td>0.72</td>
<td>0.58</td>
<td>Age = 130.727 + 15.058 (11/21M) + 23.906 (11/21W-L) + 61.249 (12/22M) - 29.091 (12/22W-L) - 100.481 (15/25M) - 2.441 (15/25W-L) + 54.517 (32/42M) + 3.089 (32/42W-L) - 268.438 (33/43M) - 89.805 (33/43W-L) - 41.009 (34/44M) - 25.32 (34/44W-L)</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Table 2.4. Multiple regressions for estimation of chronological age (in years) for the combination of different teeth after orthodontic treatment

<table>
<thead>
<tr>
<th>Teeth</th>
<th>n</th>
<th>R</th>
<th>R²</th>
<th>Equation (FDI tooth numeration)</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 max 2 pds</td>
<td>50</td>
<td>0.38</td>
<td>0.36</td>
<td>Age = 102.46 -102.16(M) -63.13(W-L)</td>
<td>7.21</td>
</tr>
<tr>
<td>3 max 6 pds</td>
<td>50</td>
<td>0.42</td>
<td>0.34</td>
<td>Age = 114.631 - 0.876 (11/21M) + 2.218 (11/21 W-L) + -115.748 (12/22M) - 38.699 (12/22W-L) - 91.406 (15/25M) - 20.963(15/25W-L)</td>
<td>7.31</td>
</tr>
<tr>
<td>3 mdb 2 pds</td>
<td>46</td>
<td>0.33</td>
<td>0.30</td>
<td>Age = 98.68-213.10 (M) - 105.05 (W-L)</td>
<td>9.63</td>
</tr>
<tr>
<td>3 mdb 6 pds</td>
<td>46</td>
<td>0.44</td>
<td>0.36</td>
<td>Age = 42.761 + 81.109 (32/42M) + 1.905 (32/42W-L) - 111.346 (33/43M) - 173.252(33/43W-L) - 151.769 (34/44M) - 43.162 (34/44W-L)</td>
<td>9.23</td>
</tr>
<tr>
<td>6 teeth 2 pds</td>
<td>36</td>
<td>0.49</td>
<td>0.46</td>
<td>Age = 174.12 -288.42 (M) - 59.22 (W-L)</td>
<td>7.37</td>
</tr>
<tr>
<td>6 teeth 12 pds</td>
<td>36</td>
<td>0.6</td>
<td>0.39</td>
<td>Age = 154.569+9.383(11/21M) + 19.718 (11/21W-L)-70.675 (12/22M) - 39.284(12/22W-L)-39.932(15/25M) - 4.881 (15/25W-L)+47.114 (32/42M) - 17.071 (32/42W-L)-58.568 (33/43M) - 29.183 (33/43W-L)-149.635(34/44M)-0.842 (34/44W-L)</td>
<td>7.8</td>
</tr>
</tbody>
</table>

When multiple regression models were formulated using different combinations of teeth, it is also noticeable that in a few cases the SEE decreased after orthodontic treatment: maxillary lateral incisor (±0.437), maxillary first premolar (±0.698), the multiple tooth regression models using three maxillary teeth with two (±0.574) and six (±0.613) predictors, and in the multiple regression models using six teeth and two predictors (±0.237): In both circumstances, the multiple regression models using the different teeth combinations improved the accuracy of estimation of chronological age, consistent with previous studies [2].

Root shortening was detected in in 62% of the upper central incisors, 53% of upper lateral incisors, 48% of upper second premolars, 48% of lower lateral incisors, 50% of lower canine and 51% of lower second premolars, with a formula previously proposed [18].

**DISCUSSION**

This is the first study specifically designed to apply the Kvaal et al. [1] method to a group of orthodontically treated subjects. Also, the first study evaluating the impact of the side effects of orthodontic treatment on one of the proposed methods for dental age estimation in adults based on the formation of secondary dentine and the pulp/tooth dimension ratios.

Previous investigations failed to disclose whether subjects had previously received orthodontic treatment. There are well known biological changes that occur secondarily to
the application of orthodontic forces, including apical root resorption and secondary dentine formation.

External root resorption is one unavoidable, unpredictable, and undesirable sequela [19-21] of orthodontic treatment. The reported frequency of external root resorption is about 100% when it is examined under microscopy. When it is quantified on periapical or panoramic radiographs, the frequency falls to 70%, with a mean reported value of root shortening of 1.42 ±0.44mm, and 1% to 5% of the cases with a loss of 4mm or one third of root length [18].

In addition, maxillary teeth are more sensitive than mandibular teeth to root resorption. The most frequently affected teeth, according to severity, are the maxillary laterals, maxillary centrals, mandibular incisors, the distal root of mandibular first molars, second premolars, and maxillary second premolars [20, 22], and all these teeth have been used in different methods for dental age estimation in adults [1, 2, 23]. In this study, a higher percentage of root resorption for upper teeth was also observed.

Confirming the findings of Karkhanis et al. [2], the length ratios (P, T, R, and L ratios) in this study have a non-significant correlation coefficient with age, (p>0.05). Furthermore, the percentage of negative correlation coefficients (Table 2.1), closer to -1, is higher in the observed values after orthodontic treatment (23% before vs 66% after).
As the Kvaal et al. [1] method is based on the negative correlation between age and the above-mentioned ratios, it is possible to observe that after orthodontic treatment this negative correlation is more evident, but not statistically significant. In this study, there were no significant variations in the correlation coefficients of the calculated length ratios with age among the different teeth. Furthermore, as the Kvaal et al. [1] method is based on length ratio calculations, not the linear measurements per se, it is possible that the effect of root shortening on the estimated ages had been diminished when the ratios are calculated.

In terms of secondary dentin formation due to orthodontic treatment (a phenomenon that has not been as widely investigated as root resorption), there are reports of complete obliteration of the canals in maxillary incisors [24]. In one study analysing the tooth changes in terms of root length and pulp chamber within maxillary central incisors (tooth 11 and 21), statistically significant changes in the width of the pulp chamber at the midpoint of the dental root [25] were found, equivalent to the point C in the Kvaal et al. [1] method.

Another study, using cone beam computer tomography in maxillary teeth, found a statistically significant difference between the volumes before and after orthodontic treatment, with the highest mean volume loss observed in the upper left lateral incisor (3.86 mm³), and the least for the upper right central incisor (3.04 mm³) [7].
In this study, the correlation coefficients of the pulp/root width ratios (A, B, C, and W) maintained their negative correlation with age before and after the treatment, without showing the same variation observed for the length ratios. It would still be necessary to assess if the root shortening after orthodontic treatment, displaced the location of the reference points B and C towards the crown (where the pulp chamber is wider), and its relationship with the observed variation of the SEE before and after orthodontic treatment, which in both cases is acceptable in forensic terms (SEE=± 10 years).

CONCLUSION

This current investigation demonstrated an alteration in tooth and pulp chamber morphology and dimensions following orthodontic treatment. However, these changes did not serve to significantly influence the validity and accuracy (SEE) of the Kvaal et al. [1] method when applied in the assessment of panoramic radiographs to estimate chronological age. Furthermore, it is not possible to affirm that the instrument used, the dental panoramic is sensitive enough to detect such small changes in the pulp chamber dimensions. It is recommended that further analysis of other methods for dental age estimation in adults based on the formation of secondary dentine, such as developed by Cameriere et al. [3], or the more recently proposed methods based on volumetric reconstructions of the tooth and pulp chamber in CBCT [26, 27], which could be more susceptible to be affected by orthodontic treatment sequels.
REFERENCES


3. Chapter 3. Orthodontic treatment: Real risk for dental age estimation in adults?

This chapter was published as:

ABSTRACT

Dental age estimation becomes a challenge once the root formation has finished. In living adults, the most commonly used methods are based on the formation of secondary dentine. One of the side effects of orthodontic treatment is the formation of secondary dentine and root shortening.

The aim of this study was to establish if the secondary effects of orthodontic treatment generate a statistically significant difference in dental age estimations. The study sample included 34 pairs of pre- and post-orthodontic treatment panoramic radiographs, from different individuals with exactly the same age and sex distribution. Females (n=22, 65%) age range 15-50 years old, median 17.5, and males (n=12, 35%) age range 16-37 years old, median 22.5 were included.

After data collection, dental age was estimated per tooth using formulae previously published. The risk of obtaining over-estimation of age was calculated (RR=1.007). The changes caused by orthodontic treatment do not have any significant effect on age estimation when the Kvaal et al method is applied on panoramic radiographs.

Key words: Forensic science, dental age estimation, secondary dentine formation, adults, orthodontic treatment, root resorption.
INTRODUCTION

The analysis of teeth for age estimation has been scientifically reported since the early 1800s [1]. Methods based on tooth formation in juveniles have shown high reliability [2-4]. Once the root formation has finished, (generally at the age of 14 years) [5], dental age estimation becomes a challenge, partly as a result of third molar formation variability [6]. The most reported non-invasive methods for dental age estimation are based on the formation of secondary dentine and the decrease of pulp chamber dimensions.

These features are measurable in periapical radiographs [7, 8], panoramic radiographs [9, 10], micro-focus computed tomography [11], computed tomography [12], and cone beam computed tomography [13]. These methods proposed different formulae to be used in specific populations. An important characteristic of these studies is that in their analysis, they included only totally sound teeth.

It is well known that orthodontic forces generate irreversible changes on tooth structure, such as root shortening [14], and secondary dentine formation [15]. These two biological changes in tooth structure may directly affect the features used for dental age estimation in adults, especially the method proposed by Kvaal et al. [7].

This method is based on the measurement of tooth/pulp length, root canal and root width at different levels, followed by ratio calculations and linear regression analysis for dental age estimation. It would be expected that with the mentioned changes, secondary to
orthodontic treatment, age estimation in participants post orthodontic treatment, would show a higher estimation error and higher overestimation, compared to that of non-treated participants. If that were to be the case, it would be necessary to develop specific standards for orthodontically treated participants, not only for the Kvaal et al. [7] method, but for any method based on secondary dentine formation and the variation of pulp/tooth dimensions with age.

In the event of the results being different to the expected, it would mean that the Kvaal et al. method could be used despite the evidence of anatomic changes related to orthodontic treatment. The aim of this study was to establish if orthodontic treatment could generate changes in dental age estimations at the tooth level when the Kvaal et al. [7] method is applied per individual tooth.

**MATERIALS AND METHODS**

Ethics approval was obtained from the Human Research Ethics Committee of The University of Western Australia (Ref: RA/4/1/6797) prior to commencement.

**Panoramic radiographs, orthodontics, and dental age estimation**

Initially, the Kvaal et al. [7] method was proposed to be used on periapical radiographs. However, recent studies have also applied this method on panoramic radiographs [9, 10]. Panoramic radiographs have more image distortion than periapical radiographs, but it has been reported that when root resorption associated with orthodontic treatment is
measured on panoramic radiographs, it is significantly higher than when measured on periapical radiographs [16].

This is the first study to see the effect of orthodontic related changes on age estimation therefore, no power calculation was achievable. However, based on the size of previous research assessing pulpal changes associated to orthodontic treatment [17], a sample size in a similar range was deemed appropriate.

**Sample Selection**

The study cohort consisted of a series of participants who consecutively presented for orthodontic treatment at a private specialist orthodontic clinic (SV). The initial sample for this study was 91 participants, who had a pre, and post-treatment panoramic radiographs from a Western Australia population. From the initial sample, a total of 34 pre-, and 34 post-orthodontic treatment panoramic radiographs, were selected, resulting in a final sample of 34 pairs of panoramic radiographs (n=68).

Sample size reduction corresponded to the need of age and sex matching between the different individuals in each pair, one of them without orthodontic treatment and the other one with a concluded treatment. Females 65% (n=22, for both groups), age range 15-50 years old, median 17.5. Males 35% (n=12, for both groups) age range 16-37 years old, median 22.5. The minimum length treatment was 1.2 years, the maximum 3.6 years, median 2.1 years.
**Special characteristics of this study**

The purpose of this study was to assess the biologic variation effects of orthodontic treatment at the tooth level. Therefore, the tooth-by-tooth formulae for the Kvaal method were applied. [10, 18]. Measurements were taken before categorizing the sample into two groups: *pre* for panoramic radiographs obtained from non-treated participants, and *post* for treated participants, in order to avoid observation bias.

**Statistical analysis**

After the completion of the measurements, dental age estimation was calculated using the age estimation equations from individual teeth. In participants 30 years or older the equations used were those reported by Karkhanis et al. [10]. And for participants 29 years or younger, the equations used were obtained from a different group of non-treated participants (n=74 aged 12-28 years). Both set of equations were obtained from a Western Australian population. Then, the respective analysis for risk assessment was performed.

**RESULTS.**

After age estimation per individual tooth, there was a total of 189 age estimates for group *pre*, and 196 estimates for group *post*. The age estimates obtained were then compared between participants with exactly the same age and sex in group *pre*, and group *post*. In this way 183 pairs of age estimates were obtained and compared 1 to 1. For 79% (n=145) of the total observations, the fluctuation of the data was the same, regardless if there was
over or under estimation of the age. In terms of over-estimation, in 47% of these observations, the over estimation was higher after the orthodontic treatment.

Although the fluctuation of the data was the same in groups, pre-and post, there was a clear difference between the ages. Before the age of 25, the large majority of results showed a slight over estimation of age which did not exceed the reported SEE. In contrast, the majority of age estimation data obtained from older participants showed under estimation of the age that notably exceeded the reported SEE.

A contingency table (Table 3.1) was developed to test if the secondary effects of orthodontic treatment were a real risk to produce over-estimation of the age when the Kvaal et al. method is applied. The relative risk calculated was RR=1.0071 (Low risk).

The fluctuation of the data in regard to the chronological current age of the individual was analyzed per individual tooth (Table 3.2), showing that there was not a significant difference in the percentages of over estimation and under estimation between both groups (Pearson correlation coefficient=0.78).

Although the fluctuation of the data was the same in groups, pre-and post, there was a clear difference between the ages. Before the age of 25, the large majority of results showed a slight over estimation of age which did not exceed the reported SEE. In contrast, the majority of age estimation data obtained from older participants showed under estimation of the age that notably exceeded the reported SEE.
Table 3.1. Contingency table to estimate if orthodontic treatment was a causal of overestimation, when the Kvaal et al. method is used to age estimation.

<table>
<thead>
<tr>
<th>Orthodontic treatment</th>
<th>Over-estimation</th>
<th>Under-estimation</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>48%</td>
<td>52%</td>
<td>n=196</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>n=94</td>
<td>n=102</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>48%</td>
<td>52%</td>
<td>n=189</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>n=184</td>
<td>n=99</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>n=184</td>
<td>n=201</td>
<td>n=385</td>
</tr>
</tbody>
</table>

Incidence over-estimation group A=47.9%. Incidence under-estimation group B=47.6%. Relative risk of presenting overestimation owed to orthodontic treatment secondary effects when Kvaal et al. method is used for dental age estimation: RR=1.0071 (No or low risk).
<table>
<thead>
<tr>
<th>OVER ESTIMATION</th>
<th>TOOTH (FDI)</th>
<th>11/21</th>
<th>12/22</th>
<th>15/25</th>
<th>32/42</th>
<th>33/43</th>
<th>34/44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>39%</td>
<td>50%</td>
<td>52%</td>
<td>47%</td>
<td>54%</td>
<td>47%</td>
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</tr>
<tr>
<td>(n=13)</td>
<td>(n=16)</td>
<td>(n=17)</td>
<td>(n=16)</td>
<td>(n=13)</td>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>38%</td>
<td>45%</td>
<td>48%</td>
<td>44%</td>
<td>61%</td>
<td>52%</td>
<td></td>
</tr>
<tr>
<td>(n=13)</td>
<td>(n=15)</td>
<td>(n=16)</td>
<td>(n=15)</td>
<td>(n=20)</td>
<td>(n=15)</td>
<td></td>
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</tbody>
</table>

<table>
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<tr>
<th>UNDER ESTIMATION</th>
<th>TOOTH (FDI)</th>
<th>11/21</th>
<th>12/22</th>
<th>15/25</th>
<th>32/42</th>
<th>33/43</th>
<th>34/44</th>
</tr>
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<tbody>
<tr>
<td>Pre-treatment</td>
<td>61%</td>
<td>50%</td>
<td>48%</td>
<td>53%</td>
<td>46%</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>(n=20)</td>
<td>(n=16)</td>
<td>(n=16)</td>
<td>(n=18)</td>
<td>(n=11)</td>
<td>(n=17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>62%</td>
<td>55%</td>
<td>52%</td>
<td>56%</td>
<td>39%</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>(n=21)</td>
<td>(n=18)</td>
<td>(n=17)</td>
<td>(n=19)</td>
<td>(n=13)</td>
<td>(n=14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SEE ± years (&lt;30 years)</th>
<th>4.3</th>
<th>4.2</th>
<th>4.5</th>
<th>4.4</th>
<th>3.7</th>
<th>3.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEE ± years (&gt; 30 years)</td>
<td>9.3</td>
<td>9.6</td>
<td>9.5</td>
<td>10.2</td>
<td>10.9</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Reported standard estimation error (SEE) per individual tooth, before and after orthodontic treatment, for participants younger than 30 years (<30 years) and over 30 years of age (> 30 years).
DISCUSSION

The reliability of dental age estimation in adults, based on the formation of secondary dentine, have shown superior accuracy to other methods, based on the analysis of other age related dental or osseous changes [8, 10]. However, teeth are subjected to different changes through life, related with pathology, biological, chemical, or mechanical trauma or dental procedures.

It has been reported that orthodontic treatment causes mechanical trauma to the periodontal ligament and induces pulpal reactions [20]. The most frequently reported side effect is external root resorption, a process characterized by the destruction of root structure [20], with the subsequent diminution of root length (mean reported value of 1.42 ±0.44mm) [21].

Another side effect is the reduction of pulp chamber dimensions, owed to secondary dentine formation [15]. With these changes, it was expected to obtain significant differences between group pre, and post, with higher percentages of over estimates of age in treated participants.

Analysis of the obtained data facilitated the calculation of the potential risk of having over estimation in participants after finishing the orthodontic treatment (Table 3.1). After calculating the incidence of overestimation in groups pre, and post, there was no evidence of association between over-estimation of age and orthodontic treatment (RR=1.0071).
However, it is necessary to mention that in this study none of the tested teeth showed signs of severe apical root resorption.

Previous studies have found that, maxillary teeth are more affected by root shortening due to orthodontic treatment, especially, lateral and central incisors [22, 23]. In this study, they also presented the higher percentage of underestimates of age for both groups, followed by the mandibular lateral incisor. In the case of under estimation, it was observed in a higher percentage in lower canines for group pre, and group post, 54% and 61% respectively.

Previous studies using the Kvaal et al. method and their proposed formulae, reported a constant under-estimation of age, from 18 to 20 years [19] up to 47.10 years [24]. These studies did not use population specific formulae. In this study, the formulae used were obtained from the same Western Australian population. There was a clear cutline (24 years for both groups) where the estimates would notably exceed the reported SEE (1 to 5 years), having statistically significant under estimates.

It is necessary to determine in future studies if this finding could be related to the fact that the apposition of secondary dentine does not occur in a linear manner through life [24], causing a larger decrease of pulp dimensions between 20 to 40 years of age, than between 40 to 60 years of age [25]. A limiting factor to clearly examine this relation in the current study was the lack of individual over 40 years of age.
As the main objective of this study was to establish if orthodontic treatment would generate changes in dental age estimation, and as different teeth are affected with different severity degrees, in this study previously published formulae were used for dental age estimation for individual teeth rather than per set of teeth or per individual.

The use of the Kvaal et al [7] individual teeth formulae to estimate dental age has been reported on extracted teeth [26]. In the same way, there are other methods based on the assessment of a single tooth per individual to generate dental age estimation models with acceptable results in a forensic framework [5, 7, 13].

**CONCLUSION.**

In this current study, orthodontic treatment did not affect the final results when the Kvaal et al. method was used for dental age estimation. Although it has been previously established that, to use the pulp complex as a biomarker for general ageing, the analysed teeth have to fulfil the requirements of being in normal and functional occlusion, totally sound and free from dental procedures [7], the real effect of different dental conditions on methods based on the formation of secondary dentine, for dental age estimation, has not been tested. The results of this current study allow forensic dentists to use the Kvaal et al method in participants who have had previous orthodontic treatment, when there are no signs of severe apical root resorption.
REFERENCES


SECTION THREE: New uses of the Kvaal et al method for age estimation in young individuals and adults from different groups of population.

Preface

The studies presented in this section explore the use of the Kvaal et al method in two new scenarios. In Chapter 4, this method is applied on a sample that contains data from panoramic radiographs of young individuals (14 to 30 years of age) from a Western Australian Population. The objective was to address one of the issues faced by forensic dentists: individuals that present agenesis of third molars, which limits the use of methods based on tooth formation.

Chapter 5 presents a new proposal to implement the Kvaal et al method on computed tomography (CT) and cone beam computer tomography (CBCT). It also explores the pulp/tooth width ratio calculation not only in the coronal view of the tooth, observable in dental radiographs, but also in the sagittal view, which is only observable with this last imaging techniques. Simultaneously, in this chapter, one of the controversies, or previously stated principles of dental age estimation (that only sound teeth should be used), was tested by the use of teeth with a variety of different conditions.
4. Chapter 4. Determining the effectiveness of adult measures of standardized age estimation on juveniles in a Western Australian population.

This chapter was published as:

ABSTRACT

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 this investigation was twofold: 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. The sample size included panoramic radiographs from 74 subjects (aged 12-28 years).

A set of ratios was calculated and then used to apply different statistical models of linear regression, to generate a final formula to estimate age. The most accurate estimations were obtained from the models generated by the mandibular canine measurement (SEE ±3.708 years), and for the 3 mandibular teeth (SEE ±3.388 years). The results indicate that inclusion of juveniles did not affect the 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 forensic [1] 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 [4]. Saunders originally proposed the estimation of chronological age based on different stages of tooth eruption [5], since then several more methods have been suggested to estimate dental age in children.

Amongst these various methods, those that are based on the radiological examination of permanent teeth development, are 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 age [10]. The Demirjian et al. method has been adapted to 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 challenging [12], leading to the
development of alternative methods in adults. Bodecker [13] 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 viable [16]. 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 years [20].

The aim of the present study was 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. This also assessed 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**

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

**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 years for both genders. All panoramic radiographs were acquired from the same machine in digital format.

Analyses were 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 were 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 (eg. 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 at occlusal plane level. 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 neighboring 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 International (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: maximum tooth length (from the incisal border or cusp tip to the root apex), maximum pulp length (from the pulp chamber roof to the root apex), and 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 an initial training and benchmarking to an expert in the field (SK). Six of the 30 radiographs were measured on 6 different days, in addition to 4 randomly selected panoramic radiographs, thus resulting in the analysis of 10 panoramic radiographs per day. Based on this, intra- and inter-observer error calculations were performed [17].

The measurements were acquired from all the panoramic radiographs with a minimum of one day between each session to minimize the possibility of memorizing reference points and/or measurements in each observer. A second intra- and inter-observer calibration was performed using 5 randomly selected panoramic radiographs from the final study sample, and recording the measurements on 5 different days, also using 4 additional panoramic radiographs per day, to avoid measurements memorizing, with at least one day between each evaluation.
Statistical Analysis

Simple descriptive statistics (mean, standard deviation, and frequency distributions) were used to summarize 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 4.1). 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 4.1).

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 association of several factors with chronological age, multivariable linear regression analyses were used to look at the association between the independent and outcome variables. The outcome variables included chronological age. The predictor variable M and W-L were used as the independent variables. Age estimation models were developed individually for each tooth (Table 4.2), and the following tooth combinations (Table 4.3): Three maxillary teeth with the predictors M and W-L individually introduced for each tooth, (6 predictors), and the average of M and W-L (2 predictors).
Three mandibular teeth with the predictors M and W-L individually introduced for each tooth, having 6 predictors in the equation, and the average of M and W-L resulting in 2 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 (2 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 Bonferroni 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 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-
Table 4.1. Correlation coefficients between chronological age and Kvaal dental measurements and ratios, for individuals under 30 years of age.

<table>
<thead>
<tr>
<th>TOOTH NUMBER (FDI numbering system)</th>
<th>11/21</th>
<th>12/22</th>
<th>15/25</th>
<th>32/42</th>
<th>33/43</th>
<th>34/44</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.13&lt;sub:NS&lt;/sub&gt;</td>
<td>0.23&lt;sub:NS&lt;/sub&gt;</td>
<td>0.13&lt;sub:NS&lt;/sub&gt;</td>
<td>0.16&lt;sub:NS&lt;/sub&gt;</td>
<td>0.45&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.15&lt;sub:NS&lt;/sub&gt;</td>
</tr>
<tr>
<td>T</td>
<td>0.04&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.07&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.002&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.13&lt;sub:NS&lt;/sub&gt;</td>
<td>0.17&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.12&lt;sub:NS&lt;/sub&gt;</td>
</tr>
<tr>
<td>R</td>
<td>0.29&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.15&lt;sub:NS&lt;/sub&gt;</td>
<td>0.33&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.28&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>-0.13&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.28&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.004&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.26&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.25&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.14&lt;sub:NS&lt;/sub&gt;</td>
</tr>
<tr>
<td>B</td>
<td>-0.38&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.30&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.28&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.35&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.57&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.38&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>-0.34&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.38&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.01&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.09&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.61&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.5&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>M</td>
<td>-0.05&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.2&lt;sub:NS&lt;/sub&gt;</td>
<td>0.0001&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.12&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.11&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.26&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>W</td>
<td>-0.41&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.39&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.15&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.23&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.12&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.49&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>L</td>
<td>0.15&lt;sub:NS&lt;/sub&gt;</td>
<td>0.29&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.002&lt;sub:NS&lt;/sub&gt;</td>
<td>0.25&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.47&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>W-L</td>
<td>-0.31&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.42&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.05&lt;sub:NS&lt;/sub&gt;</td>
<td>-0.36&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.62&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.54&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* = p<0.05 ** = p<0.01 NS = Not significant

Abbreviations: P, ratio between length of pulp and root; T, ratio between length of tooth and root; R, ratio between length of pulp and root; A , ratio between width of pulp and root at CEJ (Level A); B, ratio between width of the pulp and root at mid-point between level C and A (level B); C, ratio between width of pulp and root at mid-root level (level C); M, mean value of all ratios (first predictor); W, mean value of width ratios from levels B and C; L mean value of the length ratios P and R; W-L, difference between W and L (second predictor)(1).
Graph 4.1. Scatter plot showing a positive association between the estimated age in years and the chronological age in years for the teeth 33/43

Table 4.2. Multiple regression for estimation of chronological age (in years) from individual maxillary and mandibular teeth from individuals under 30 years of age.

<table>
<thead>
<tr>
<th>Tooth (FDI)</th>
<th>n</th>
<th>R</th>
<th>R²</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/21</td>
<td>69</td>
<td>0.15</td>
<td>0.12</td>
<td>Age = 22.915-28.78 (M) -22.376 (W-L)</td>
<td>4.3</td>
</tr>
<tr>
<td>12/22</td>
<td>67</td>
<td>0.19</td>
<td>0.17</td>
<td>Age = 19.724-25.935 (M) – 23.631(W-L)</td>
<td>4.2</td>
</tr>
<tr>
<td>15/25</td>
<td>68</td>
<td>0.003</td>
<td>-0.27</td>
<td>Age = 17.78-3.962(M)-2.204(W-L)</td>
<td>4.5</td>
</tr>
<tr>
<td>32/42</td>
<td>71</td>
<td>0.13</td>
<td>0.10</td>
<td>Age = 4.644-5.363(M)-22.558(W-L)</td>
<td>4.4</td>
</tr>
<tr>
<td>33/43</td>
<td>48</td>
<td>0.41</td>
<td>0.39</td>
<td>Age = 11.26-41.33(M)-86.06(W-L)</td>
<td>3.7</td>
</tr>
<tr>
<td>34/44</td>
<td>71</td>
<td>0.32</td>
<td>0.31</td>
<td>Age = 10.513-27.075(M)-38.176(W-L)</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Note. *R², coefficient of determination. SEE, standard error of estimation in years. See table 4.1. for abbreviations.
Table 4.3. Multiple regressions for estimation of chronological age (in years) from the combined maxillary and mandibular teeth.

<table>
<thead>
<tr>
<th>Teeth (FDI)</th>
<th>n</th>
<th>R</th>
<th>R²</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>3mx (2pds)</td>
<td>60</td>
<td>0.15</td>
<td>0.12</td>
<td>Age= 28.702-46.338(M) - 24.233(W-L)</td>
<td>4.1</td>
</tr>
<tr>
<td>3mx(6pds)</td>
<td>60</td>
<td>0.23</td>
<td>0.15</td>
<td>Age=24.394-30.751 (11/21M) -12.090 (11/21W-L) - 29.2257 (12/22M)-12.894 (12/22W-L) + 2.611 (15/25M) - 0.631 (15/25 W-L)</td>
<td>4.1</td>
</tr>
<tr>
<td>3mdb(2pds)</td>
<td>45</td>
<td>0.41</td>
<td>0.38</td>
<td>Age= -27.44 + 10.48(M) – 63.82 (W-L)</td>
<td>3.6</td>
</tr>
<tr>
<td>3 mdb (6pds)</td>
<td>45</td>
<td>0.53</td>
<td>0.46</td>
<td>Age= 1.894 + 13.428 (32/42M) -5.931 (32/42W-L) - 51.067 (33/43M) -66.495 (33/43W-L) - 5.114 (34/44M) -16.806 (34/44W-L)</td>
<td>3.3</td>
</tr>
<tr>
<td>6 teeth (2 pds)</td>
<td>40</td>
<td>0.25</td>
<td>0.21</td>
<td>Age= 31.91 - 63.34 (M) - 63.34 (W-L)</td>
<td>4.1</td>
</tr>
<tr>
<td>6 teeth (12 pds)</td>
<td>30</td>
<td>0.54</td>
<td>0.33</td>
<td>Age= 2.627 -58.42 (11/21 M) + 4.526 (11/21 W-L) + 32.169 (12/22 M) -12.913 (12/22 W-L) + 25.460 (15/25M) + 1.098 (15/25 W-L) + 5.270 (32/42 M) + 0.996 (32/42 W-L) - 28.692 (33/43 M) + 1.686 (33/43 W-L) - 13.857 (34/44 M) - 49.336 (34/44 W-L)</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*R², coefficient of determination. SEE, standard error of estimation in years. See table 4.1 for abbreviations.
observer calibration (between TM and SK) showed comparable precision [21] (TEM 0.80, rTEM 2.37 and R 0.98). In this way, individual (Table 4.2) and multiple (Table 4.3) tooth regression models were obtained. The accuracy to predict the age was quantified by the standard error of estimation (SEE ± years). The most accurate age estimation model based on the analysis of individual tooth was for mandibular canines (SEE ±3.708 years) (Graph 4.1). Prediction accuracy improved with the combined analysis of teeth; it was highest for the model developed with the three mandibular teeth (6 predictors, SEE ± 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. Those methods, based on dental changes, are well accepted and have shown their potential for forensic and anthropological applications [22].

Once 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 are 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 numerous benefits such as its relatively non-invasive approach, applicability in live individuals, and low cost, among others. Previous research has applied this method to a Western Australian population [17], and showed significant results that are valid under forensic standards. Although the Kvaal et al. method [1] was initially developed to estimate age in individuals over 20 years old, previous studies have tested this method in younger populations [19].

The present study applied the Kvaal et al. method [1] 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.

To this end, regression models were developed based on the data acquired from individual teeth (Table 4.2) and a combination of teeth (Table 4.3). The accuracy to predict age was quantified by the standard error of estimation (SEE ± 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 4.3). In this study, the highest level of accuracy was obtained when the equation included 3 mandibular teeth and 6 predictors, (SEE ±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 6 teeth and 12 predictors (SEE ±7.963).

Age estimation in individuals older than 14 years is difficult, as all permanent teeth, except the third molars (when present), have completed their development [19]. Some examples of previous studies amongst people under 30 years of age, using the Kvaal et al. method [1] 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= ± 8.73 years at best, using the regression formulas of Paewinsky et al. [25] and Kvaal et al. [1].

Further such studies were published by: Landa et al. [20] in a Spanish population with an age range of 14 to 60 (n=100,) from which 40 individuals 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 Meinl 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 Paewinsky et al. [25] formula was applied. In contrast, the present study (n=74) shows that the Kvaal et al. [1] method provides acceptable results [2] 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 insightful to compare 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 Senn [10]. 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 population [26], 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 studies [27]. 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 determining 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 decrease of pulp chamber can be applied. This also presents the opportunity to examine the application of this method in other juvenile population groups.
REFERENCES


This chapter was published as:

**ABSTRACT**

Different non-invasive methods have been proposed for dental age estimation in adults, with the Kvaal et al. method as one of the more frequently tested in different populations.

The purpose of this study was to apply the Kvaal et al. method for dental age estimation on modern volumetric data from 3D digital systems. To this end, 101 CBCT images from a Malaysian population were used. Fifty-five per cent were female (n=55), and forty-five percent were male (n=46), with a median age of 31 years for both sexes. As tomography allows the observer to obtain a sagittal and coronal view of the teeth, the Kvaal pulp/root width measurements and ratios were calculated in the bucco-lingual and mesio-distal aspects of the tooth.

From these data, different linear regression models and formulae were built. The most accurate models for estimating age were obtained from a diverse combination of measurements (SEE ±10.58 years), and for the mesio-distal measurements of the central incisor at level A (SEE ±12.84 years). This accuracy, however is outside an acceptable range for forensic application (SEE ±10 years), and is also more time consuming than the original approach based on dental radiographs.

**Key words:** Adults, age estimation, tomography, Kvaal method.
INTRODUCTION

Currently, the need for developing more accurate and non-invasive methods for age estimation, as part of the identification of adult individuals in situations of forensic scenarios is increasing globally [1]. Gustafson first proposed a method for dental age estimation in adults, [2] since then, the analysis of dental changes in adults relative to chronological age has continued. The adult dentition is relatively resistant to environmental and chemical influences, and methods based on the assessment of teeth are considered advantageous for this reason [3, 4].

Furthermore, methods based on odontometric measurement of pulp and tooth structures from dental radiographs or tomographs are non-invasive/non-destructive and can be applied to both living or deceased individuals [5]. Kvaal et al, proposed a method for age estimation in adults which has been tested in different populations around the world since 1995, but all reported a high standard error (±8.5 to ±13 years) [1, 5, 6]. The basis of this method is the analysis of the narrowing of the pulp chamber with age, which is observable and measurable in dental radiographs. However, there is scope for improvement in the accuracy of the age estimations.

With the emergence of computer tomography (CT) and cone beam computer tomography (CBCT), new methods based on volumetric reconstruction of tooth and pulp volume and ratio calculations, have been proposed for dental age estimation in adults [7, 8]. However, those techniques have not provided better accuracy relative to the previously described
Kvaal et al. method, using dental radiographs. Moreover, pulp/tooth volume calculation is labor intensive, [9] and can require the use of complex, and often costly computer software [10]. Clearly, these more technical multi-dimensional systems provide considerably more data, at a substantially more detailed level, and with a reduction in many of the complications of simple plane film images.

The method described by Kvaal et al. [5] was initially proposed, with measuring dimensions on periapical radiographs with a microscope and a pair of calligraphers. The applications of this method have been expanded to include panoramic radiographs using different analytical software [6, 11]. The purpose of the present study is to apply the Kvaal et al. [5] method for dental age estimation using modern computer tomographic data from 3D digital systems. The null hypothesis is that the substantially increased quantity (and quality) of the data from these systems will provide greater accuracy of dental age estimation in adults.

MATERIALS AND METHODS

Sample
The study sample included a total of 101 tomographs obtained as part of normal treatment from the radiology department of the University of Keibangsaan Malaysia. Almost half (n=47) were obtained with a Kodak device, reference K9000-3D (exposure parameters: scanning time: 10.8 s, 3-15 mA, 60-85kVp, field of view FOV: 5.2 cm x 5.2cm to 5.5cm to 5.5cm, 180° rotation, slice thickness 0.076-0.2 mm), and 54 were obtained with an i-CAT
device (Dental Imaging Technologies Corporation. i-CAT® FLX™) (exposure parameters: scanning time 4s, 5 mA, 120 kVp, FOV: 16cm x 16cm, Slice thickness 0.3 mm to 0.4 mm). Of these tomographs, 55% were from female (n=55), and 45% were from male (n=46) patients, with a median age of 31 years for both sexes (Table 5.1).

All images were received in DICOM format, and analysed using the Osirix® software package (OnDemand 3D software CyberMed Inc, Seoul, South Korea). The study sample included teeth with very small coronal restorations, as long as they did not compromise the pulp chamber and secondary caries was not present. Teeth with shape abnormalities, active caries, root canal treatment, prosthetic restorations, signs of pulp or periapical pathologies, or open root apex, were duly excluded from the sample. Multi-rooted upper second premolars were also excluded.

**Teeth analysed**

Initially, Kvaal et al. [5] proposed a set of linear measurements, including the length of the tooth, pulp, and root, in addition to the width of the root and pulp chamber at different levels, in six different teeth: upper central and lateral incisors, and the second premolar; and the lower lateral incisor, canine and first premolar. In the present study, due to the unclear definition of the pulp chamber and tooth boundaries for the lower teeth (most likely related to their small dimensions) only upper teeth were included: central incisor, lateral incisor, canine, and second upper premolar (when possible). As the dental images are tomographs, it is possible to assess the teeth in sagittal and coronal views; accordingly, the root length, and pulp/tooth width measurements proposed by Kvaal et al. [5] were
acquired in the mesio-distal and bucco-lingual aspects of the teeth (see below). Significant differences between teeth of the left and right side of the maxilla have not been reported previously, [5] and therefore in this study, teeth from either the left or right side were measured.

**Measurements**

As proposed by Kvaal et al., [5] the mesio-distal measurements of the teeth (coronal view) were performed as follows: first, the level A, was located at the CEJ (cemento-enamel junction) on the mesial side of the tooth, then level C was located halfway between point A and the root apex, and finally point B was located halfway between point A and C.

As this is the first study applying the Kvaal et al. method exclusively using tomographs, bucco-lingual measurements were also acquired (sagittal view). Point A was located at the vestibular CEJ, point C halfway between the vestibular CEJ and the root apex, and point B, halfway between point A and point C. In this way, there were six pulp/tooth width measurements per tooth. All measurements were recorded by one observer (TYM) using the Osirix® software.

**Statistical analysis**

Intra-observer error was tested, four tomographs were randomly selected (two Kodak and two i-CAT) and all the measurements were recorded by one observer (TYM). Upper central, lateral and canine were measured on four separate occasions, with a minimum of one day between repetitions ensuring that the observer did not remember the previously
recorded measurements. Measurement error was assessed by calculating the technical error of measurement (TEM), coefficient of reliability (R) and relative technical error of measurement (rTEM) [12].

All data were collected in an Excel spreadsheet, 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/). Descriptive statistics were used to summarise the data (mean, standard deviation, and frequency distributions). Normality was also tested (skewness, kurtosis and Shapiro-Wilk) to establish whether parametric or non-parametric analysis was required.

Pearson correlation coefficient (r) was calculated to assess the relationship between the dental ratios and age, for data obtained from the Kodak CBCT and for data obtained from i-CAT CBCT individually, and also when both data sets were combined. This was done with the aim of testing if the difference between the resolutions of both devices could cause any statistically significant differences (Table 5.2).

Linear regression models were built with age designated as the dependent variable. Different age estimation equations were then formulated using different groupings of age predictors: 1) the tooth/pulp ratios at the levels A, B or C individually; 2) calculating the average of the pulp/tooth ratios of all the teeth at the different levels (A, B or C) obtaining a formula for each level; 3) calculating the average of the pulp/tooth ratios of the lateral (Lat)
and central (Cent) upper incisor at the different levels (A, B and C); 4). Averages of the pulp/tooth ratios from all the teeth at the levels A, B and C in the same formula (A+B+C); and 5) same as 4, but excluding the ratios obtained from the canines (Cent-Lat). A total of 17 linear regression models were thus generated per data-set: sagittal (s) view (bucco-lingual measurements); coronal (c) view; and the average of both (sc). The accuracy of the models is reported as the standard error of estimation (SEE) [11, 13].

RESULTS

Intra-observer error results were considered to be within acceptable range. (TEM <1.0 and the coefficient of reliability R>0.80%), regardless the source of the tomograph (Kodak or i-CAT) or whether the measurements were recorded in the bucco-lingual or mesio-distal view of the teeth. In terms of the relative technical error of measurement (rTEM), 72% of the observations scored <5% and 28% had an rTEM < 10%. It was also observed that the accuracy of the observer was better for the bucco-lingual measurements (sagittal view of the teeth) regardless the source of the tomography.

Pearson correlation coefficient test (r) demonstrated a stronger correlation between the pulp/tooth ratios and age for the bucco-lingual measurements (sagittal (s)) than for the mesio-distal measurements (coronal view (c)) (Table 5.2). Although there were differences between the correlation coefficients from the Kodak and i-CAT CBCT's, scatter-plots including the results of both sources do not show outliers associated with the specific use.
There was a clear and significant negative correlation between age and pulp/tooth ratios in all the data sets.

Table 5.1. Age and sex distribution for CBCT and i-CAT tomographs.

<table>
<thead>
<tr>
<th>SEX</th>
<th>FEMALE</th>
<th>MALE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE RANGE (YEARS)</td>
<td>CBCT (n=23)</td>
<td>i-CAT (n=32)</td>
<td>CBCT (n=24)</td>
</tr>
<tr>
<td>15-20</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>20-29</td>
<td>12</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>30-39</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>40-49</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>50-59</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>60-75</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>55</td>
<td>46</td>
<td>101</td>
</tr>
</tbody>
</table>

Table 5.2. Correlation coefficient measurements CBCT

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>CBCT Coronal</th>
<th>CBCT Sagittal</th>
<th>i-CAT Coronal</th>
<th>i-CAT Sagittal</th>
<th>CBCT &amp; i-CAT Coronal</th>
<th>CBCT &amp; i-CAT Sagittal</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/21 A</td>
<td>-0.05 NS</td>
<td>-0.36*</td>
<td>-0.634**</td>
<td>-0.65**</td>
<td>-0.45**</td>
<td>-0.61**</td>
</tr>
<tr>
<td>11/21 B</td>
<td>-0.26 NS</td>
<td>-0.37*</td>
<td>-0.421**</td>
<td>-0.59**</td>
<td>-0.32*</td>
<td>-0.5**</td>
</tr>
<tr>
<td>11/21 C</td>
<td>-0.29 NS</td>
<td>-0.45**</td>
<td>-0.288*</td>
<td>-0.36*</td>
<td>-0.23*</td>
<td>-0.41**</td>
</tr>
<tr>
<td>12/22 A</td>
<td>-0.53**</td>
<td>-0.08 NS</td>
<td>-0.326*</td>
<td>-0.62**</td>
<td>-0.31*</td>
<td>-0.41**</td>
</tr>
<tr>
<td>12/22 B</td>
<td>-0.35*</td>
<td>-0.5**</td>
<td>-0.3*</td>
<td>-0.60**</td>
<td>-0.22*</td>
<td>-0.46**</td>
</tr>
<tr>
<td>12/22 C</td>
<td>-0.24 NS</td>
<td>-0.57**</td>
<td>-0.013 NS</td>
<td>-0.41**</td>
<td>-0.05 NS</td>
<td>-0.41**</td>
</tr>
<tr>
<td>13/23 A</td>
<td>-0.25 NS</td>
<td>-0.13 NS</td>
<td>-0.153 NS</td>
<td>-0.50**</td>
<td>-0.04 NS</td>
<td>-0.17 NS</td>
</tr>
<tr>
<td>13/23 B</td>
<td>-0.59**</td>
<td>-0.56**</td>
<td>-0.329*</td>
<td>-0.41*</td>
<td>-0.28**</td>
<td>-0.39**</td>
</tr>
<tr>
<td>13/23 C</td>
<td>-0.52**</td>
<td>-0.34**</td>
<td>-0.348*</td>
<td>-0.24 NS</td>
<td>-0.28**</td>
<td>-0.21*</td>
</tr>
</tbody>
</table>

*p< 0.05, ** p< 0.01, NS= non-significant
Table 5.3. Linear regression analysis, linear regression formulae, correlation coefficient (r) between age and the ratios of bucco-lingual and mesio-distal width measurements from CBCT & i-CAT

<table>
<thead>
<tr>
<th>Age predictors</th>
<th>n</th>
<th>Linear regression formulae</th>
<th>r</th>
<th>r^2</th>
<th>s</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/21 A sc</td>
<td>86</td>
<td>Age=99.74-72.07(11Ac)-161.63(11As)</td>
<td>0.4</td>
<td>0.4</td>
<td>12.2</td>
</tr>
<tr>
<td>11/21 B sc</td>
<td>86</td>
<td>Age=81.82-35.031(11Bc)-147.14(11As)</td>
<td>0.26</td>
<td>0.24</td>
<td>14</td>
</tr>
<tr>
<td>11/21 C sc</td>
<td>86</td>
<td>Age=71.92-29.16(11Cc)-134.69(11Cs)</td>
<td>0.17</td>
<td>0.15</td>
<td>14.9</td>
</tr>
<tr>
<td>12/22 A sc</td>
<td>89</td>
<td>Age=87.43-68.05(12Ac)-129.37(12As)</td>
<td>0.21</td>
<td>0.19</td>
<td>14.5</td>
</tr>
<tr>
<td>12/22 B sc</td>
<td>89</td>
<td>Age=73.98-24.34(12Cc)-127.46(12Bs)</td>
<td>0.21</td>
<td>0.19</td>
<td>14.5</td>
</tr>
<tr>
<td>12/22 C sc</td>
<td>89</td>
<td>Age=57.56+37.84(12Cc)-148.22(12Cs)</td>
<td>0.18</td>
<td>0.17</td>
<td>14.7</td>
</tr>
<tr>
<td>13/23 A sc</td>
<td>95</td>
<td>Age=51.14-5.4(13Ac)-47.192(13As)</td>
<td>0.03</td>
<td>0.01</td>
<td>15.4</td>
</tr>
<tr>
<td>13/23 B sc</td>
<td>95</td>
<td>Age=69.51-47.15(13Cc)-70.44(13Bs)</td>
<td>0.17</td>
<td>0.15</td>
<td>14.2</td>
</tr>
<tr>
<td>13/23 C sc</td>
<td>95</td>
<td>Age=60.81-78.34(13Cc)-34.37(13Sc)</td>
<td>0.09</td>
<td>0.08</td>
<td>14.9</td>
</tr>
<tr>
<td>A sc</td>
<td>78</td>
<td>Age=100.41-33.11(Ac)-206.40(As)</td>
<td>0.34</td>
<td>0.33</td>
<td>13.5</td>
</tr>
<tr>
<td>B sc</td>
<td>78</td>
<td>Age=89.22-20.54(Bc)-178.29(Bs)</td>
<td>0.31</td>
<td>0.29</td>
<td>13.9</td>
</tr>
<tr>
<td>C sc</td>
<td>78</td>
<td>Age=69.46+16.4(Cc)-174.42(Cs)</td>
<td>0.17</td>
<td>0.15</td>
<td>15.2</td>
</tr>
<tr>
<td>A sc Cent/Lat</td>
<td>83</td>
<td>Age=105.42-72.96(Ac1/2)-187.22(As1/2)</td>
<td>0.4</td>
<td>0.38</td>
<td>12.8</td>
</tr>
<tr>
<td>B sc Cent/Lat</td>
<td>83</td>
<td>Age=86.31-19.86(Bc1/2)-180.19(Bs1/2)</td>
<td>0.29</td>
<td>0.27</td>
<td>13.9</td>
</tr>
<tr>
<td>C sc Cent/Lat</td>
<td>83</td>
<td>Age=70.41+42.09(Cc1/2)-206.7(Cs1/2)</td>
<td>0.23</td>
<td>0.21</td>
<td>14.4</td>
</tr>
<tr>
<td>Asc+Bsc+Csc</td>
<td>74</td>
<td>Age= 104.09 -17.65 (Ac)-135.48 (As)-38.18(Bc) -105.54(Bs)+40.87(Cc)+10.93(Cs)</td>
<td>0.4</td>
<td>0.35</td>
<td>13.3</td>
</tr>
<tr>
<td>Asc+Bsc+Csc Lat/Cent</td>
<td>82</td>
<td>Age=106.25-180.14(Asc1/2)-135.83(Bsc1/2)+51.73(Ccs1/2)</td>
<td>0.4</td>
<td>0.39</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Abbreviations: Tooth number (11/21, 12/22 and 13/23) with Asc, Bsc or Csc= pulp/tooth ratio at the levels A, B or C in the sagittal and coronal view (sc) calculated per tooth. Asc, Bsc or Csc: average of the pulp/tooth ratio at the respective level, from all the teeth. Asc, Bsc or Csc with Lat/Cent: average of the pulp/tooth ratio only from central and lateral incisors. Asc+Bsc+Csc=average from the pulp/tooth ratio from all the teeth at the respective level. Asc+Bsc+Csc=average from the pulp/tooth ratio from all the teeth at the respective level including only lateral and central teeth.
Correlation coefficients were calculated as follows: i) for the measurements obtained from the Kodak tomographs; ii) for the measurements recorded from the i-CAT tomographs; and iii) for the combination of both. The i-CAT tomographs showed the strongest correlation to age, especially for the upper central incisor \((r = -0.65)\), followed by the combination of the Kodak & i-CAT data sets \((r = -0.61)\). The correlation coefficients obtained from the Kodak tomographs, although statistically significant, were lower than those obtained from the i-CAT and combined Kodak & i-CAT images (Table 5.2).

In relation to the accuracy of the different linear regression models, all estimates were over the threshold generally accepted for forensic application \((\text{SEE} \pm 10 \text{ years})\) [14]. However, the SEE is reduced when both data-sets are combined into a linear regression model (Table 5.3). There was only one equation with an acceptable level of predictive accuracy \((\text{SEE} 10.58 \text{ years} \ (n=72, r=0.59, r^2=0.56))\):

\[
\text{Age} = 101.46 + 734.74 \times (Ac) - 374.81 \times (Ac_{(\text{Cent-Lat})}) - 1075.18 \times (Csc) + 327.07 \times (Bc) + 310 \times (Cc_{(\text{Cent-Lat})}) - 159.25 \times (11/21 \text{ As})
\]

Abbreviations: Ac: average of the pulp/tooth ratio in the coronal view at level A from the three teeth. \(Ac_{(\text{Cent-Lat})}\): average of the pulp/tooth ratios from the lateral and central incisors. Csc: average of the pulp/tooth ratio at level C from the sagittal and coronal views (sc) from all teeth. Bc: average of the pulp tooth ratio at the level B from all the teeth in the coronal view. \(Cc_{(\text{Cent-Lat})}\): average of the pulp/tooth ratios at the level C from the lateral
and central incisors in the coronal view. 11/21 As: pulp/tooth ratio at the level A from the central incisor in the sagittal view.

The most accurate linear regression models obtained from the individual analysis of the bucco-lingual measurements were as follows: the central incisor at level A (Age= 84.059 – 191.003 (11/21 As), $r^2=0.37$, $SEE=±12.84$), the average of the bucco-lingual measurements from the central incisor and lateral incisor at the level A, B and C (Age= 102.08-157.94 (As) - 73.0 (Bs) -36.81 (Cs), $r^2=0.39$, $SEE=±12.73$ years).

When both data-sets were combined (Table 5.3) the highest accuracy was achieved from: the average of the bucco-lingual (s) plus the average of the mesio-distal (c) measurements at level A, (SEE ±12.17 years); the average of the bucco-lingual and mesio-distal measurements at level A from the central and lateral incisors, (SEE ±12.76); and the average of the bucco-lingual and mesio-distal measurements from the central and lateral incisors at level A, plus level B, plus level C, (SEE ±12.7). The linear regression models generated by the inclusion from only the mesio-distal (c) measurements did not result in an SEE lower than ±14 years, and therefore these results are not included.

DISCUSSION

This is the first study testing the Kvaal et al. method exclusively in tomographs, and also the first one applying Kvaal et al’s. pulp/tooth width ratio calculations in the bucco-lingual aspect of the teeth. Computer tomography is increasingly being used in dental practice
and provides three-dimensional information about any area of interest, in a relatively quick and cost-effective manner [15].

In this study, it was expected that application of the Kvaal et al. method using CBCT diagnostic images would increase the array of methods available for the forensic profiling of living and deceased individuals.

Until now, there has been only one other study proposing a similar method for adult dental age estimation, based on the assessment of the pulp/tooth bucco-lingual dimensions (area) in canines [16]. Although the results of the latter study were satisfactory (Mean prediction error, ME ±2.8 years at best), this method required extracted teeth, and thus has restricted application in a forensic context. The use of tomographs overcomes issues related to the requirement for tooth extraction, and also permits the analysis of multiple teeth with less radiation exposure.

Although, in this study, the number of individuals was small in certain age intervals, there are well documented biological age-related changes in the human dentition, which have allowed forensic dentists to propose and use different methods for dental age estimation [17].

In previous publications applying the Kvaal et al. [5] method for dental age estimation in adults, one of the principal limitations is the absence of a clear limit between the dentine and the pulp camber, [11] which is observed as a grey zone instead of a distinct line [6].
This affects the accuracy of the measurements and intra/inter-observer agreement.

In the present study, neither Kodak nor i-CAT tomographs were exempt from this issue. In certain cases, the border between the pulp chamber and dentine, or tooth and bone are also more blurred than in periapical radiographs.

It is noteworthy that dental Kodak tomographs may have higher resolution than i-CAT tomographs. However, in the present study the data obtained from the i-CAT had a higher correlation to age compared to the Kodak scans (Table 5.2). Similarly, the bucco-lingual width ratios had a higher correlation to age than the mesio-distal width ratios (with the latter measurable in periapical radiographs).

It has previously been reported that Kvaal et al. length measurements are both: difficult to register, and do not provide significant information to the final equation [5, 18]. It is important to note that in the tomographs analysed in the present study, it was even more difficult to observe the root apex compared to periapical radiographs.

The only length measurement registered was the root length from the CEJ towards the apex to locate points A, B, and C. In this way, only pulp/tooth width, and ratios were used as the only age predictors. The exclusive use of pulp/tooth ratios proposed by Kvaal et al. [5] has previously been documented [18] on panoramic radiographs, showing more accurate results (SEE=±10.02 years) than those obtained in this study. (SEE=±10.58 years at best).
In a previous study that analysed the odontometric age-related changes in different teeth, the rate of secondary dentine formation varied depending on tooth type: in canines, the increase of coronal dentinal thickness was not as high as for the incisor, and premolars [19].

In the present study, it was similarly observed that although the correlation coefficient between pulp/tooth ratios and age were significant, the exclusion of canine ratios when the linear regression models were generated, improved their accuracy.

On the other hand, this is also the first study documenting the inclusion of teeth with small crown fillings in the sample to generate linear regression formulae, in contrast to previous publications using the Kvaal et al. method, [5] where one of the inclusion criteria was to use only totally clinically sound teeth. There were no statistically significant differences caused by inclusion of these teeth. This may relate to the nature of the formation of tertiary dentine or reactionary dentine to external threats. Tertiary dentine formation is highly dependent on the stimulus, and that caries mediators induce a focal dentine production, [19-21] with a rate formation of about 3µm per day [22].

Regression analysis, has been chosen for age estimation due to its simplicity [23]. Nevertheless, it is necessary to note the limitations of the use of linear regression models for dental age estimation; for example, the questionable believe that higher standard deviations when the original formulae of Kvaal et al. are applied in populations of different ethnic backgrounds [24], are related to the population or origin of the individual rather than to the intrinsic characteristics of the sample. Also, it assumes that formation of secondary dentine is a linear process, when it is more similar to a curve [25]. Additionally,
although using tomography resulted in obtaining more odontometric data from the same teeth (mesio-distal and bucco-lingual measurements), this apparent advantage over the use of dental radiographs, did not improve the final outcome. Also, the need to properly align the teeth in the sagittal and coronal plane demands more time than the traditional Kvaal et al. approach in dental radiographs.

Finally, it is necessary to mention that CBCT is not exempt from artefacts intrinsic to the process of image acquisition [26]. This could explain, not only the high SEE obtained in this study, but also the poor accuracy of those methods based on pulp/tooth volume calculation, [9,10] when compared with more simple approaches such as the measurement of pulp/tooth area on dental radiographs (Cameriere et al method, with a SEE=±3.27 years) [16].

CONCLUSION

Radiological assessment of dental age-related changes is a valid tool for age estimation. It is the case that new diagnostic technologies provide practitioners and forensic researchers new opportunities to use and propose new methods for dental age estimation. However, the use of tomographs in this study, applying the Kvaal et al method, did not improve the results. It is recommended to also include teeth with small fillings in studies based on pulp/tooth dimension ratios, as long as the pulp chamber is not compromised. The aim of this is to further investigate the application of these different methods on those individuals who do not have totally sound dentitions.
REFERENCES


SECTION FOUR: New technologies in dental age estimation.

PREFACE

The main contribution of this section to the thesis is the study of those methods for age estimation based on volume reconstruction from dental CBCT. These methods do not surpass the results obtained from the analysis of dental radiographs, which means that although there are more advanced imaging techniques, they deserve to be deeply explored and studied for their application in forensic dentistry for dental age estimation in adults. Chapter 6 presents a study on three previously proposed methods for volume reconstruction in dental age estimation and the possible technological and methodological causes of variation on their results.

Chapter 7 presents an ingenious proposal for age estimation in adults using CBCT from individuals native to different countries. Such individuals had very different ancestry, and ethnic backgrounds, thereby challenging one of the established requirements for the creation of methods for dental age estimation that demand the use and elaboration of specific population studies and formulae.
Chapter 6. Reliability and repeatability of pulp volumetric reconstruction through three different volume calculations

This chapter was published as:

ABSTRACT

Dental age estimation by means of the analysis of dental regressive changes is still a valid tool in the forensic field. The latest methods are based on the analysis of CBCT and volumetric reduction of dental pulp with advancing age in adults. These methods do not show superior accuracy to those based on the analysis of conventional dental radiographs.

Objective: To test the variability of the volume measurements when different segmentation methods are applied in pulp volume reconstruction.

Materials and methods: Osirix® and ITK-SNAP software were used. Different segmentation methods (Part A) and volume approaches (Part B) were tested in a sample of 21 dental CBCTs from upper canines. Different combinations of the data set were also tested on one lower molar and one upper canine (Part C) to determine the variability of the results when automatic segmentation is performed.

Results: Although the results show correlation among them ($r>0.75$), there is no evidence that these methods are sensitive enough to detect small volumetric changes in structures such as the dental pulp canal (Part A and Part B). Automatic segmentation is highly susceptible to small variations in the setting parameters (Part C).

Conclusion: Although the volumetric reconstruction and pulp/tooth volume ratio has not shown better results than methods based on dental radiographs, it is advisable to persevere with the research in this area with new developments in imaging techniques.

Key words: Age estimation, pulp volume calculation, image segmentation.
INTRODUCTION

Since 1950 different methods have been proposed to estimate dental age in adults [1]. Most of them are based on the formation of secondary dentine and the decrease of the pulp chamber size with age [2, 3]. Methods that have an invasive approach, (for example aspartic acid racemization and cementum annulation), are not of preference, as they often require the physical damage of the sample [4]. With the evolution of different diagnostic imaging techniques, non-invasive methods for dental age estimation have become the preferred methods [5], starting with the use of periapical dental radiographs, [2, 3] then panoramic radiographs [6], and more recently cone beam computed tomography (CBCT) [7].

Micro-Computer Tomography (µCT) was introduced in the early 20th century [8] to determine age related three-dimensional changes in pulp cavities, from maxillary first premolar teeth. Consistent with early histological research, µCT findings indicate that a decrease in pulp volume is not linear. It demonstrates a quicker reduction between 20 and 40 years, and then it slows down thereafter. Further research correlating the ratio between pulp and tooth volume with the chronological age, using µCT and linear regression models to estimate dental age in adults, revealed promising outcomes [8].

However, in light of the high radiation doses associated with µCT, only extracted teeth can be measured in this way [9]. More recently, CBCT has been used for dental age estimation calculating the volumes from tooth and pulp chamber. Since then, volumetric
reconstruction from CBCT with different software has been reported in various studies for
dental age estimation in adults [10-13]. The most recent studies document the use of CBCT
from single- [5, 13] and multi-rooted teeth. [14]

These new methods have not shown superior accuracy to the methods based on dental
radiographs [2, 15]. It is evident that within each type of software there are sensitivity
settings that influence the mathematical approach to measure the baseline volumetric
data. These settings clearly have consequences for the outcome and may well be in part
the cause of the substantial variation. The primary aim of this study was to test the
variability of the volume measurements when different segmentation methods were
applied to pulp volume reconstruction. This study is designed to ensure that the
underpinning assumptions of a commonly used age estimation approach, based on pulp
volume calculation, are as accurate as possible.

MATERIALS AND METHODS

Ethics approval for this study was obtained from the Human Research Ethics Committee of
the University of Western Australia (Ref: RA/4/1/6797). This study has been performed in
accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Study sample

A total of 22 anonymized dental CBCTs were used in this study. All were recorded with
therapeutic purposes, with the consent of the participants, and there was no unnecessary
or repeated radiation exposure. All images were previously unidentified, and age and sex of each individual was the only known information. From these, 57% (n=12) were female, age range 17-63 years, mean age 33.7, and 43% (n=9) were male, age range=15-52 years, mean age 36.7.

The CBCTs were obtained from the Radiology Department of the University of Kebangsaan, Malaysia, and the research group INVIENDO from the National University of Colombia. In both cases, the images were obtained using the 9000 3D Extra-oral Imaging System (Carestream Dental, Atlanta, GA, USA) which had a 180° rotation and a field of view (FOV) of 50 mm by 37 mm. The radiation exposure parameters were 8-10 mA, 70 Kv, with a slice thickness of 0.076mm. Images were saved in DICOM format.

The selected tooth to apply the different segmentation approaches was the upper right canine, or the left when the right was absent (with Federation Dentaire International (FDI) notation 13 and 23) reconstructing one tooth per subject. Only sound teeth, free of any restorations with completed apex formation were included. The measurement software packages used were: ITK-SNAP version 3.4 (open source software, www.itksnap.org) and Osirix® (OnDemand 3D software CyberMed Inc, Seoul, South Korea).

All segmentations were completed by a single observer (TYM). All data were 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/.)
Description of the method

This study tested the comparative outcome of three different ways of estimating the pulp volume, dental age indicator used in adults [5, 13, 14]. The tests were carried out on the root pulp chamber of upper canines (n=21), to calculate the correlation between automatic [14], and manual segmentation [5] (Part A) (Table 6.1) and cone shape approach volume calculation [13] (Part B) (Table 6.2). The final part of the study (Part C) (Table 6.3) tested the variability of the results obtained with different setting parameter values combinations for automatic segmentation of the pulp canal of one multi-rooted tooth, as previously reported in the literature [14] and one single rooted tooth. (Graph 6.1)

Part A. Automatic and manual segmentation.

Automatic segmentation was performed using the software ITK-SNAP. (Graph 6.1. A). Manual segmentation used software Osirix® and its 2D viewer, (Graph 6.1. B), which allows the observer to go slice by slice and manually select the boundaries of the space to reconstruct, using the tool polygon. The automatic segmentation process in ITK-SNAP is called “seed region-growing”. To apply it, the observer sets up a “seed” inside the structure to reconstruct, in this case, the pulp chamber. This seed grows, and a volume is finally obtained.

Automatic segmentation of the pulp was generated using the same combination of setting parameters through the entire sample: Scale of Gaussian blurring: 3.0, edge transformation contrast: 0.2, edge transformation exponent: 4.0, expansion (balloon) force: 1.0, smoothing (curvature) force: 0.2, edge attraction (advection) force 2.0. In three cases, the edge...
transformation exponent needed to be adjusted to 0.05. Regression models were run to establish which of the setting parameters would have the most significant influence on the final results. Little changes in the Scale of Gaussian generated the bigger changes in the final values (p< 0.01).

With manual segmentation two approaches were used. Firstly, every fourth slice was analysed as for the published method. Secondly, the first and last slices were used as samples. To analyse the same length of root in the automatic and manual approaches, a length defined in the automatic approach was set for the manual approach.

Part B. Cone shape geometric approximation

The geometric approximation of the root canal to cone is a simple conservative method, and has been used for dental age estimation in adults. Using Osirix®, ovals of best fit were formed in the root canal at the cemento-enamel junction (CEJ) level, following the reported method [13]. Secondly, instead of best fit ovals, the boundaries of the pulp canal were drawn using the tool polygon (Graph 6.1, C). The root length was measured from the CEJ to the apex, (Graph 6.1, D), and finally two different volumes of cones were calculated per tooth with a mathematical formula.

Part C. Automatic segmentation and different setting parameters combinations

As observed in part A, it is not possible to apply the same combination of setting parameters values to all teeth with automatic segmentation. Considering the advantage of this automatic process, [16] twenty combinations of different values for all the setting
parameters (Table 6.3) were used to reconstruct the pulp chamber of one lower first molar (FDI 36, Male 23-year-old Malaysian origin, slice thickness 0.2mm) and an upper canine (FDI 23, Male 31-year-old Colombian origin). The volumetric reconstruction with each combination was completed twice per tooth. The variation among the volume from the same tooth was calculated.

**Statistical analysis**

Shapiro Wilk normality test, mean, standard deviation (SD), paired $t$-Test and Pearson correlation coefficient, were calculated to compare the results obtained using the different segmentation methods (Part A), cone shape volume approach (Part B), and the obtained results when different parameters are used to do the automatic segmentation of the same tooth (Part C).

**RESULTS**

When automatic and manual segmentation using each fourth slice were compared, the Pearson’s correlation coefficient ($r^2=0.83$), shows a greater correlation between them, than between automatic and manual segmentation using only the first and the last slices ($r^2=0.75$) or between the two manual segmentation methods ($r^2=0.79$).

However, this does not mean that manual segmentation using every fourth slice and automatic segmentation produces comparable results. The paired $t$ test shows that there is a statistically significant difference between the means of the results of both manual
segments and automatic segmentation ($p<0.01$).

When comparing the volumes, the manual segmentation produced volumes are larger than those obtained with automatic segmentation. To facilitate the understanding of this difference, a simple mathematical analysis was used, taking the obtained volume with automatic segmentation as 100% of the volume. Then the difference between the volumes obtained with automatic segmentation and both manual segmentation, were used in a rule of three to calculate the percentage this difference represents.

**Part A. Automatic and manual segmentation**

Three data-sets were obtained from the volumetric reconstruction of the root canal of the canines using the different methods (Table 6.1). The results of this calculation showed that the volumes obtained using every fourth slice are on average 25% (1% to 53%) larger than those obtained with automatic segmentation.

Also, using the first and the last slices are on average 57% (12% to 210%) larger than those obtained with automatic segmentation. The average of the volumes from both manual segmentation methods is in general 41% larger than automatic segmentation. The volumes obtained doing manual segmentation of each fourth slice were on average 28% larger than those obtained using only the first and the last slice. However, some of them were on average 16% smaller ($p>0.05$). The mathematical analysis was like the previously described, but in this case, the volume obtained with manual segmentation using only the first and last slice was taken as 100% in the rule of three.
Graph 6.1. Volumetric reconstructions, upper right canine using different methods.

Volumetric reconstruction of tooth 13 from a female individual, 36 years old, using the different methodologies mentioned in this paper. A. Semiautomatic reconstrution using ITK-SNAP software. B. Manual segmentation using Osirix ® software. C and D. Cone-shape geometric approximation, following the methodology reported by the author doing area measurement of the root canal at the level of the CEJ (D) and length measurement of the root from the CEJ up to the root apex (C), using Osirix ® software.
Table 6.1. Volume results of using automatic segmentation (Vol 1), manual segmentation using just the first and last slice (Vol 2), and manual segmentation each forth slice (Vol3), of cone beam computer tomography from upper canines. (FDI 13, 23)

<table>
<thead>
<tr>
<th>Individual</th>
<th>Vol 1 (mm³)</th>
<th>Vol 2 (mm³)</th>
<th>Vol 3 (mm³)</th>
</tr>
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<tr>
<td>1</td>
<td>10.87</td>
<td>10.6</td>
<td>13.2</td>
</tr>
<tr>
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<td>16.03</td>
<td>17</td>
<td>19.7</td>
</tr>
<tr>
<td>3</td>
<td>16.4</td>
<td>20.9</td>
<td>19.8</td>
</tr>
<tr>
<td>4</td>
<td>9.309</td>
<td>12.9</td>
<td>14.8</td>
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<td>Mean</td>
<td>14.68</td>
<td>20.18</td>
<td>22.41</td>
</tr>
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</table>
Table 6.2. Cone shape approach results using the tool polygon (Pol) and oval (Oval).
Pulp area (in mm²) at the CEJ level and volume (in mm³) calculation with the measured root length (in mm).

<table>
<thead>
<tr>
<th>area</th>
<th>Area Pol</th>
<th>Area Oval</th>
<th>Root length</th>
<th>Vol Pol</th>
<th>Vol Oval</th>
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Table 6.3. Setting parameter combinations used in part C.

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<th>Repetition</th>
<th>Scale of Gaussian</th>
<th>Edge contrast</th>
<th>Edge transformation exponent</th>
<th>Smoothing force</th>
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<th>Average canine volume mm³</th>
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<tr>
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<td>4</td>
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<td>SD</td>
<td>0.53</td>
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<td>0.59</td>
<td>0.07</td>
<td>5.74</td>
<td>7.12</td>
</tr>
<tr>
<td>Variance</td>
<td>0.28</td>
<td>0.01</td>
<td>0.35</td>
<td>0.005</td>
<td>32.94</td>
<td>50.75</td>
</tr>
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</table>

Note: There are 3 parameters available in ITK-SNAP software that are not listed, as they were constant for all the reconstructions: Edge attraction force (=2) and step size (=1) and smoothing force which was changed from 1 to 0.5 only for the 18th combination.
Part B. Cone shape geometric approximation

It was expected to obtain similar area and volume values when using ovals of best fit and the tool polygon following the outline of the pulp chamber. Unexpectedly, with the tool polygon the areas and volumes were noticeably larger (50%) than with the ovals (table 6.2). However, there is a strong correlation coefficient between both measured areas using the tools oval and polygon ($r=0.84$) at the CEJ and the volumes ($r=0.88$).

Repeatability of the automatic segmentation for the first and the second repetition for both teeth was evaluated with the paired $t$ test ($r^2=0.7$, $p=0.99$), showing that there is no statistically difference between both repetitions with a 95% level of confidence. The average between the obtained volumes in both repetitions was calculated.

The volumes for the molar pulp chamber (FDI 36), ranged from 31.7 mm$^3$ to 53.74 mm$^3$, with a SD of 5.7 and a variance of 33. For the volumetric reconstruction of the canine (FDI 23), the volume value ranged between 29.8 mm$^3$ to 50.8 mm$^3$, SD=7.12 and variance of 50.75. The SD for the different parameters was not larger than 0.6 and the sample variance was not greater than 0.3.

Part C. Automatic segmentation and different setting parameters combinations

When the areas using the two different tools are compared the data show a larger variability and a high SD; this effect is diminished once the volume is calculated. In this way, the length of the root is what really determines the final volume.
DISCUSSION

With the introduction of computer vision techniques for medical imaging and their application in dentistry and forensic sciences, the possibilities to propose methods for age and sex estimation are almost unlimited. Owing to the apposition of secondary dentine, the anthropometric assessment of the variation in the ratio between size of the pulp chamber and size of the tooth is still an acceptable dental age predictor [14].

The aim of this study was to test the variability of the volume measurements when different segmentation methods are applied in pulp volume reconstruction. This is because although it would be expected that with volume measurements the results for age estimation were more reliable, the literature shows the opposite. To find out an explanation to the lower accuracy of the methods based on volume reconstruction, in this study, we tested three different methods doing only pulp volume reconstruction.

The methods employed in this thesis, using CBCT, do not seem to be more accurate than those based on radiographs for dental age estimation in adults, based on the analysis of the available literature. This is regardless of the sample size of the study, which ranges from 19 individuals, analysing only 12 canines [7] to 403 individuals analysing only the pulp chamber of first molars [14]. This is clear when the reported accuracy of the different methods is compared. The mean absolute error using automatic segmentation of the first molar’s pulp chamber is 6.26 years at best [14]. The cone shape geometric approach of pulp and tooth
has a standard error of estimation of $\pm$ 11.45 years, [13] and finally, manual segmentation of pulp and tooth, has a prediction interval of $\pm$ 12 years [5].

Other methods also using automatic segmentation of canines reported a prediction interval of 3.47 years at best [17]. When these results are compared with some of the most commonly used adult age estimation methods using dental radiographs, such as Cameriere et al. [3] (with reported mean predictor errors of 2.37 years [18] to 11.01 years [19] (tooth/pulp area ratio)), or the method developed by Kvaal et al. [2] (pulp/tooth-linear measurements ratios with reported standard estimation errors of $\pm$9.367 years [20]), it is possible to observe that the use of CBCT and dental structures volume reconstruction, do not improve the final results for adult age estimation. The relation between the lack of the accuracy of the above mentioned methods for age estimation [5, 13, 14], and the different methods for volume reconstruction are presented as follows:

**Part A.** Manual and automatic segmentation may produce similar results, as observed in this study ($r^2=0.83$). Manual segmentation is time consuming, difficult to perform and prone to be influenced by the subjectivity of the observer, and personnel trained in this aspect is necessary [21]. Additionally, it is not accurate enough to estimate the variation of the volume in small anatomic structures, such as the root canal, as observed in this study.

Although there are different methods for automatic image segmentation, the first problem faced in the bio-medical area, is the exclusive dependence on gradient or intensity analysis without using anatomical information [21]. In the case of automatic segmentation, with
ITK-SNAP software, although the software chooses which pixels will be included in the segmentation, the observer finally decides how to adjust the parameters to control the algorithms in the segmentation. This makes this method prone to a certain degree of subjectivity from the observers. These parameters may also change from one CBCT to another.

Unfortunately, in the previous study using this software, the values of the setting parameters were not mentioned [14]. In this study, it was also observed that although all the CBCTs were obtained from machines with the same reference, and under similar exposure conditions (slice thickness, Kv, mA, and time of exposure) and the same parameters for automatic segmentation, the interaction of the seed region-growing with the boundaries of the structure to reconstruct may also differ from one CBCT to another. For this reason, it is not possible to follow the recommendations of the ITK-SNAP manufacturer, who recommends keeping the same parameters among different CBCT, and it is neither possible to warrantee the uniformity of the segmentations obtained [14].

In this study, it was also observed that small changes in the values of the setting parameters can generate significant variation in the final volumes with automatic segmentation, even though the segmentation was made on the same CBCT, same tooth, and same anatomic region. The significance of this is that the final volume will be always the same under the same parameters, in the same CBCT, and anatomic region, but these parameters generally need to be modified from one CBCT to another, and in the same way, small changes in the parameters alter the final volume.
Part B. With the aim of simplifying the measurement of the tooth structure volume, a cone shape geometric approach was proposed (Part B) [13]. The developers of this method were aware of the main disadvantages of this proposal: measurements inaccuracy and subjectivity. They also reported that this method tends to underestimate the real volume of the tooth structures (56% to 67%), which in theory would not affect the final results after pulp/tooth ratio that was calculated to estimate the age.

To test this method, the current study used two differ approaches for doing the volume calculation. The first, following the published methodology [13] displaying ovals at the CEJ, and a second, selecting manually the outline of the root canal at the CEJ, using the tool polygon. As observed in the results section, despite the difference between both area measurements, at the CEJ, once the volume is calculated the difference decreases.

None of these approaches is recommended to do pulp volume measurements, firstly, because of the subjectivity of the observer, who only counts with the naked eye to display one or another, and secondly because both bring a distorted representation of the root canal, ignoring the different variations in its internal shape.

Part C. In this study, there are different parameters that affect the final volumetric reconstruction of any anatomical region. The scale of Gaussian, or blurrier factor [22] was the most relevant when doing automatic segmentation. To increase the value of the Gaussian scale, allows the observer to eliminate certain noises in the image, which affect the “growing of the seed”. However, the bigger the value the Gaussian scale is, the more
principal structures, and details will be lost [23]. This would mean that the initial volume of the pulp chamber will be magnified [16].

Although automatic segmentation is less prone to the subjectivity of the observer than manual segmentation, in this study it was observed that it is not possible to keep the same volume reconstruction parameters for all the CBCT, and that minimum changes may produce significant variations, even though they are done on the same tooth. The volume obtained with this tool must be understood as an approximation to the real volume of the structure to measure.

Taking into consideration the reported rate of secondary dentine formation is 6.5 μm/year for the crown, and 10 μm/year for the root [24], the methods in this study are not sensitive enough to detect such small dimension changes, generating a big variation of the measurements, which could explain the greater error of pulp/tooth volume based methods for age estimation when compared with simpler methods based on linear [2] or area measurements [3] on dental radiographs.

Previous research in the biomedical area has highlighted the problems related to semiautomatic segmentation, and specifically, to the process that the software uses to select which pixels or voxels to be included in the final volume reconstruction. To overcome this problem, the probabilistic anatomic atlas has been elaborated, which facilitates the segmentation of an organ of interest [21]. When doing the semiautomatic segmentation of teeth, and pulp chambers, different obstacles were observed.
Firstly, owing to the similar density of dentine and bone, and the irregularity of the periodontal ligament, it was not possible to obtain a volumetric reconstruction of the tooth as it is for the pulp chamber. This is the reason why the automatic tooth volume reconstruction was not analysed. Secondly, owing to the large variety in the pulp chamber shapes in the apical third of the tooth, there was always a leak of the seed region growing into the dentine.

To create a probabilistic atlas for dentistry could help to overcome these issues, and help the dentist, and forensic dentist to implement more accurate, and easier methods for automatic segmentation, and volume reconstruction. Thirdly, although the automatic reconstruction of the pulp chamber of molars may be understood as a simple process, it may be more reliable to use other methods for age estimation when using molars.

**CONCLUSIONS**

Based on the results of this study, it is possible to affirm that the volume measurements with any segmentation method must be understood as an approximation of the measured structure, and not as the real volume. In the same way, manual segmentation could produce volume values that are even more inaccurate, and should be avoided in future studies.

Although the volumetric reconstruction and P/T volume ratio has not shown better results than methods based on dental radiographs, it is worth to persevere with the research in
this area with an interdisciplinary team to build software that can reliably reconstruct pulp, and tooth volumes for forensic purposes.
REFERENCES


In press version, under revision.

ABSTRACT

Objectives: The formation of secondary dentine on the walls of the root canal has been used as an age indicator in adults. The aims of this study are: firstly, to propose a method for dental age estimation in adults, regardless of their nationality or ethnicity; and secondly to test if by volume reconstruction of only a fraction of the tooth it is possible to obtain linear regression models and a unique equation for dental age estimation.

Materials and Method: Eighty-one anonymized cone beam computed tomography images obtained from two different population groups were used. Only sex and age information were kept. One group had a Latin-American background (Colombian individuals aged 23 to 71 years), and the other had an Asian background (Malaysian individuals aged 15 to 58 years). The tooth analyzed was the upper canine. The software used was ITK-SNAP version 3.4 (open source software, www.itksnap.org), to do automatic volume reconstruction of the cervical third of root canal, and root. The images were shared via online under the strictest security parameters to guarantee the integrity and safety of the data.

Results: The correlation coefficient between pulp/pulp+tooth volume ratio increased when data from individuals of both populations were included in the same statistical analysis ($r^2=0.42$). It is possible to propose a formula for age estimation regardless the origin of the individual: Age=67.104+(-434.741 x p/pt) (Standard error of estimation=±11.4 years).

Conclusion: It has been established that methods for age estimation must be population specific. In this study is has been demonstrated that meaningful data may be obtained from individuals that are ethnically different and widely separated geographically.

Key words: Computed tomography, secondary dentine, age estimation, ethnic variation.
INTRODUCTION

Much has been said in biological sciences about race and ethnicity since 1775, when Johann Friedric Blumenbach defined five different races: Caucasians (whites), Mongolians (East Asians) Malayans (South Asians), Negroids (Black Africans), and Americans (First Nations) [1]. Since then, different studies followed this structure to analyze, interpret data, and present results; a phenomenon that has been named “scientific racism” [2].

However, owing to the different nomadic and migration processes that humanity has experienced across history, this racial classification is less accurate and practical [2]. Nevertheless, in forensic sciences it is still stipulated that the ethnic background of an individual is of paramount relevance, to formulate a biological profile and to clarify the identity of living or deceased individuals, next to sex, stature, and age [3].

In terms of age estimation, many methods have been proposed for individuals of different ages, based on bone and tooth formation or deterioration with age [4]. Methods based on the radiological analysis of dental development, have high accuracy, due to the genetically controlled process of tooth formation, which seem to be similar for individuals from the same group of population [5].

However, the most recent findings suggest that their results may be incurring on a selection bias called “age mimicry”, [6, 7], which has caused the erroneous need to generate specific population formulae for age estimation.
Simultaneously, age estimation in adults, by means of non-invasive analysis, is still a challenge for scientists of different areas, especially, those who are involved in forensic investigations. Although different methods have been proposed, there is no consensus about a unique method to be applied to individuals from different countries, and it has been claimed that methods for age estimation must be population specific [8] without testing the use of mixed samples. Additionally, the few changes that bones and teeth have after-body maturation, are a setback for the accuracy of the existing methods.

One of the first methods for dental age estimation in adults established that the formation of secondary dentine on the wall of the root canal could be considered an age predictor in adults [9]. Based on the negative correlation of pulp/tooth ratio size with age, different non-invasive methods were proposed: linear measurements [10]; area measurements; in dental radiographs [11]; and lastly volume measurements from dental tomography [12].

The aims of this study are: firstly, to propose a method for dental age estimation in adults, regardless of their nationality or ethnic background; and secondly to test if by reconstructing only a fraction of the tooth it is possible to obtain linear regression models and an equation for age estimation.

MATERIALS AND METHODS

The sample for this cohort study encompasses 81 cone-beam computed tomography (CBCT) obtained from two different population groups. Latin-American (Colombian
individuals aged 23 to 71 years, 60% female (n=23) and 40% male (n=15)) and Asian (Malaysian sample individuals aged 15 to 58 years 46% female (n=20) and 54% male (n=23)) previously collected for different dental diagnosis and treatment procedures, with informed consent. All the images were anonymized keeping information about sex and age.

Table 7.1 shows the age and country of origin distribution. Although the CBCT were obtained in different institutions, in both cases the reference of the device was the same: Kodak device, reference K9000-3D (exposure parameters: scanning time: 10.8 s, 3-15 mA, 60-85kVp, field of view FOV: 5.2 cm x 5.2cm to 5.5cm to 5.5cm, 180° rotation, slice thickness 0.076-0.2 mm).

The tooth analyzed was the upper canine, left or right, one per individual, as previous studies have shown no right/left difference in tooth development [13]. The reconstructed root fraction was in all cases 5 mm from the highest point of the vestibular cemento-enamel junction, towards the root apex, with the aim to include only the cervical third of the root [12].

Two different proceedings were tested to obtain the pulp/tooth ratio. Firstly, by dividing the volume of the pulp by the volume of the tooth, and secondly, dividing the volume of the pulp by the sum of the volume of the tooth and pulp.
Inclusion criteria

The included roots portions were from sound teeth, teeth with no evidence of physical trauma, free of active caries, free of evidence of periapical disease, without signs of pulp pathology, and free of cervical lesion of any nature. The analysis also included teeth with small coronal fillings, with at least 2 mm of dentine between the tooth restoration and the pulp chamber, which is assumed to have negative effect on the root portion.

The software used was ITK-SNAP version 3.4 (open source, www.itksnap.org), consistent with previous studies, doing automatic reconstruction of the root canal and root. In certain cases, it was necessary to manually correct the semiautomatic root segmentation, as the software included portions of bone, that were required to be manually deleted. The measurements were collected by only one observer, who had been previously trained in the use of this software.

All the data were collected in an Excel spreadsheet, using Excel (version 2013 Microsoft Redmont, USA), before and after manual correction of the image segmentation. Data analysis was performed with 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

Pearson correlation coefficient tests were calculated to observe if there was any difference between the volume values obtained, for pulps and teeth doing automatic segmentation, and after manual correction slide by slide. There was no significant difference between both measurements ($r^2=0.98$). The two established proceedings to obtain the pulp tooth ratio were compared. The second approach, dividing the volume of the pulp by the sum of the volume of the tooth and pulp, generated a higher correlation coefficient ($r^2=0.34$ vs $r^2=0.42$).

In the same order, the determination coefficient between the pulp/pulp+tooth ratio ($p/pt$) was calculated for the individuals of each group separately ($r^2=0.26$ for the Colombian sample and $r^2=0.38$ for the Malaysian sample) and for the total sample using the data of both groups in the same analysis, obtaining a higher correlation coefficient when all the data are included in the analysis ($r^2=0.42$). (Graph 7.1)

In regard to tooth condition, teeth with small crown fillings, that did not involve the root portion, as were the used teeth, did not generate any outlier that could affect the linear regression model (Graph 7.1). Linear regression models were generated to produce a formula for dental age estimation. The final formula is:

$$\text{Age}=67.104+(-434.741 \times p/pt)$$
where \( p/pt \) is the ratio between the volume of the pulp divided by the sum of the volume of pulp and tooth. The standard error of estimation (SEE) is \( \pm 11.4 \) years \( (r^2=0.42) \).

It is necessary to mention, that owed to the age distribution of the sample used for this study, this formula could not estimate the age if individuals over 67 years of age.

**DISCUSSION**

It is undeniable that dentistry has made a valuable contribution to anthropological sciences, bringing insights about human evolution. In the same way, dental analysis has become an important tool for human identification in forensic sciences. In both fields, anthropology and forensics, the concepts about racial and ethnic differences have deep roots and a significant influence in research [14].

However, in a changing world, where day by day the biological boundaries between individuals of different backgrounds are constantly vanishing, the proposal of methods for age estimation that are racially or ethnically focused must be re-evaluated.

The last systematic review about Demirjian’s developmental stages of third molars [6] and the Greulich and Pyle’s bone atlas [7] for age estimation in children and adolescents, exposes a selection bias called *age mimicry*, present in numerous publications. This bias explains the variation, in the accuracy of different studies, which has been wrongly associated to the population origin and ethnicity of the participants, rather than to the intrinsic characteristic of the used sample.
Table 7.1. Distribution of the individuals for age in years and country; Malaysia (Mal) and Colombia (Col) used for the image segmentation and volume calculation in this study.

<table>
<thead>
<tr>
<th>Age range</th>
<th>Mal</th>
<th>Col</th>
<th>Total</th>
<th>%</th>
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<td>0</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>21-30</td>
<td>13</td>
<td>2</td>
<td>15</td>
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<tr>
<td>31-40</td>
<td>6</td>
<td>11</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>41-50</td>
<td>9</td>
<td>12</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>51-60</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>12.3</td>
</tr>
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<td>61-71</td>
<td>0</td>
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<td>6</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>38</td>
<td>81</td>
<td>100</td>
</tr>
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</table>

Graph 7.1. Scatter plot showing the correlation between age and the pulp/pulp+tooth volume ratio. (P/PT ratio) $R^2=0.42$
To avoid age mimicry, studies must have a study population with an even age distribution, large even samples, same number of individuals in each age group, appropriate upper and lower age limits facilitating the observation of the different age-related changes. Furthermore, and ideally samples that include data of individuals from different regions, to conclude whether regional differences are important for age estimation in children [6] and probably in adults. Subsequently, it is necessary to perform multi population studies in adults.

The research in this thesis makes a contribution to disclose the population related differences in age estimation in adults when secondary dentine production is analyzed as an age predictor.

In terms of age mimicry in this study, it is possible to note that the age distribution is not even when both samples are analysed separately: 58% (n=25) of the Malaysian sample belongs to the age range of 15 to 30 years, while for the Colombian sample, 60% (n=23) of the sample belongs to the age range 31 to 50 years. Once both samples are summed, the age distribution is more even, as well as the general increase of the total data used to perform the linear regression analysis, which could explain why the determination coefficient is higher.

In this study, the production of secondary dentine and the narrowing of the root canal with age, which is one of the used parameters for dental age estimation in adults, has a similar behavior in individuals that are not only ethnically different but also geographically
separated. Also, that the separate generation of regression models, for each one of the population groups, would have generated age mimicry, owed to the unequal age distribution of both samples, as in the Malaysian group the younger group dominates, while for the Colombian group the middle age dominates (Table 7.1).

By adding both samples it is possible to obtain a more even age distribution and also a higher correlation coefficient between age and p/pt ratio ($R^2=0.42$). However, for future studies it is recommended to have a sample with the same number of individuals for the different populations to analyse per age group, to obtain more reliable results. To follow up on the current work, further studies will be necessary, to test the proposed formula, and also, to perform a similar analysis in a much larger sample, that follows the established requirements to avoid age mimicry (7).

Up to now different methods and formulae have been published to estimate dental age in adults [15]. Nevertheless, few studies have tested the proposed formulae in groups of populations different to those included in the original studies. In the few studies that have done this analysis, it has been suggested that local formulae produce more accurate results [15].

With the results of the present study it could be possible to affirm that it is necessary to include the data of individuals of different populations in the same statistical analysis. This would enable the observation that there is no significant difference between populations
or ethnical groups with regards to the production of secondary dentine, and to generate an equation that can be used regardless the origin of the individual.

Furthermore, there are other factors that can affect the length, area, and the total volume of the tooth, such as bruxism and attrition [17], among others, that also affect the production of dentine in the crown and apical third of the root. These factors are not ethnically related but individual dependent. By analyzing sound cervical root third of canines, or any other teeth, the problems related to tooth attrition are diminished.

In this study, the maxillary canine was selected as the tooth to analyze, owing to the reported longer survival, compared to other teeth [18]. It was also preferable to measure the volume of only the cervical third, as it has been reported that pulp/tooth ratios, had a higher correlation with age at the cervical root level and that the correlation with age decreased towards the apex, for different types of teeth, when doing linear [19] or volume [20] measurements.

In the first studies, that analyzed the production of secondary dentine with age [9], the methods required not only the use of extracted teeth but also the destruction of the sample, which is unethical in a modern context. The use of different radiological techniques has allowed researchers to analyze age indicators in a non-invasive and more conservative way. The use of CBCT to analyze and obtain the volume of any anatomical structure is much easier, cheaper and faster, and has great potential for applications in anthropological and forensic studies.
In this study, this radiological technique allowed the observer to separate only a fraction of the tooth, calculate its volume, and guarantee not only the integrity of the individual but also the uniformity of the samples size. In terms of accuracy, although the error exceeds the threshold that must accomplish a method for age estimation in adults (±10) [21] the results of this study (SEE=±11.4 years) are not so different to previous studies that used data of individuals from the same country or belonging to the same ethnic group (±12.5 years at best) [22].

In the same way, other studies also based on volume reconstruction has been published. Never the less, some of them are based only on pulp volume reconstruction [23, 24], or on a geometrical approach of rooth canal and root [25]. As their methods are not based on a clear mesurement of the real volume of the root canal and root, and a pulp/tooth ratio calculation, their results can not be compared with thos obtained from the present study.

Finally, confirming the tooth selection in our study, the latest study on pulp/tooth volume ratio calculation for dental age estimation in adults, confirms that the upper canine is one of the teeth, (next to upper central incsor), that presents a better correlation between age and pulp/tooth ratio, which encourages future studies based on utilisation of this specific tooth [26].

CONCLUSION

It has been claimed that in the modern world would testify the end of ethnicity, and that it changes according to circumstances [13]. This study proposes the use of data from individuals not only from different countries but also have been historically catalogued as
members of different racial and ethnical groups. By calculating the pulp/tooth+pulp volume of only one part of the tooth there is no significant difference between these two populations and that is possible to generate a formula to estimate the age for adult individuals regardless their origin.
REFERENCES


GENERAL DISCUSSION.

This thesis emerges as an answer to some observed challenges faced by forensic dentistry for dental age estimation in adults. The age indicator to do this analysis was the formation of secondary dentine on the walls of the root canal, which generates a negative correlation between pulp/tooth ratio with age. As there is a demand for non-invasive methods, this thesis analyses radiological-based methods for dental age estimation in adults as those proposed by Kvaal et al., (linear measurements) Cameriere et al., (area measurements), and the different approaches using volume calculation from cone beam computed tomography (CBCT).

The above-mentioned methods have been tested in many studies. However, there is no available systematic review which compares their results and helps forensic dentists to select a method that can be systematically applied, which was the first observed challenge in this thesis. To face this challenge, Chapter 1 presents what can be considered the first systematic review for dental age estimation in adults, following the established parameters in the Cochrane handbook.

This systematic review discloses some of the weaknesses observed in previous studies as the lack of consensus for minimum standards for the methodological design of different studies regarding the materials, methods, minimum sample size, statistical analysis, and data analysis. Based on this fact, it is possible to state that there is a need to generate proper methodological guidelines to be followed by the international community, taking in
account the different requirements to avoid age mimicry, and the over-estimation of age for young individuals or the under-estimation of age for older individuals, when regression models are applied, which would guarantee the proposal of more accurate methods as well as the uniform use of the existent ones. In this way, a proper meta-analysis could be performed in future studies.

The second observed challenge was the need to test the available methods on individuals whose dental conditions were different to “totally sound teeth”; to this end, one of the mentioned methods was used to estimate the age in individuals whose dental conditions had been affected by the same external factor.

The selected method to do this analysis was that proposed by Kvaal et al. in 1995, owing to the linear measurements of length and width of tooth and root canal that this method demands, and the external factor was orthodontic treatment which affects tooth length as well as the production of secondary dentine. This analysis was performed in two papers following different methodological designs (Chapters 1 and 2) presented in section one.

Section one also defies one of the established parameters for the use and proposal of dental age estimation methods: the exclusive inclusion of sound teeth, a status that changes once the individual is exposed to orthodontic treatment, because even though, these teeth are free from other pathologies, such as caries or periodontal disease, their integrity is altered once the orthodontic treatment starts.
No previous study in forensic dentistry, had considered the evaluation of the effects that orthodontic treatment has on dental anatomy and how these associated dental changes could affect the accuracy of the available methods.

The hypothesis for this section was that in individuals with orthodontic treatment, the age estimates would show a considerable over-estimation of age, owing to the root shortening and the production of dentine associated to this treatment. Before obtaining the results, it was believed that it would be necessary to propose different standards for dental age estimation in individuals with orthodontic treatment.

In Chapter 2 different linear regression models were proposed for individuals before and after orthodontic treatment. The methodological design of this chapter facilitated the comparison of correlation coefficients of different pulp/tooth ratios, and the standard error of estimation (SEE) obtained from linear regression models obtained before and after orthodontic treatment.

Although, for some teeth a higher SEE after orthodontic treatment, in general terms, there was no significant difference between both observations (Pearson correlation test r=0.58), with a difference that ranged between -0.69 to 2 years. The results of this chapter also reassure some previous concepts that are important for the following chapters of this thesis, as the non-significant correlation between age and the length ratios (Chapter 2 table 2.1), as well as the concept that the more teeth are included to estimate the age of the individual, the better the results are going to be (Chapter 2, tables 2.3 and 2.4).
Alike previous studies, that evaluated the root resorption associated to orthodontic treatment, Chapter 2 also demonstrated that this dental change is evident in dental radiographs, and affects in a higher percentage upper teeth. Contrary to the expected, although there are some changes in the morphology of tooth and pulp chamber, these changes do not alter the accuracy of the linear regression models.

However, Chapter 2 had a debatable point in its methodology: this was a longitudinal study that observed the same group of individuals before and after orthodontic treatment. To do a more detailed analysis, that confirmed the findings of Chapter 2, the study presented in Chapter 3 was designed following the methodology of epidemiological studies for risk assessment, to test whether orthodontic treatment could be considered a risk factor able to affect the accuracy of the Kvaal et al method, causing more over-estimation of age.

This methodological design has no precedents in forensic dentistry, making this chapter a relevant piece for research of dental age estimation in adults. This analysis included data from different individuals, who were grouped in two cohorts, with the same chronological age and sex distribution. After age estimation using previously published formulae, a comparison between age estimation obtained from individuals from both groups, distributed in equal pairs, was done at the tooth level.

After obtaining the different age estimates, and compare the percentage of observations where the result showed over- or under-estimation, no significant difference was observed between both groups (Chapter 3, table 3.1). The respective contingency table was
elaborated (Chapter 3, table 3.2) revealing that orthodontic treatment could not be considered as a real risk factor for obtaining over-estimation of age, when the Kvaal et al. method is used for age estimation in adults (RR=1.0071). It is necessary to mention that none of the teeth analyzed in this chapter presented severe apical root resorption.

The analysis presented in Chapter 3, is an outstanding contribution to dental age estimation in adults, as it presents a study design that could be applied to analyze the effect of other dental conditions on different methods for age estimation. This methodology could be replicated, including teeth with and without cavities, dental fillings, or periodontal diseases, to establish whether those teeth are non-suitable for age estimation of adults.

The fact that this section did not find significant differences among both groups, could represent two different scenarios: 1. the Kvaal method could be used regardless the history of orthodontic treatment exposure, or 2. the instrument used to measure the tooth and pulp dimensions, as well as the statistical analysis are not sensitive enough to detect the differences between both groups. To clarify which one of the two scenarios is the applicable, it would be necessary to perform a similar analysis using periapical radiographs, and data from a larger sample.

Nevertheless, there are some obstacles: the use of periapical radiographs for orthodontic treatment is not as popular as the use of panoramic radiographs, as they would provide limited information about the different anatomic structures, for example maxillary sinuses,
and osseous structures. For that reason, the analysis presented in this section used only panoramic radiographs.

As the third listed challenge for dental age estimation in this thesis, was the lack of methods for age estimation in young individuals with absence of third molars, the Kvaal et al method was tested in a younger population, as presented in Chapter 4. For the analyzed sample, the Kvaal et al method seems to be a useful tool for younger individuals. However, owing to the SEE (±4.5 years), it would be necessary to analyse other age indicators, to establish whether an individual is an adult, using methods with higher accuracy.

To cover the fourth listed challenge in this thesis, Chapter 5 presents a study that explores new uses and applications of one the existent methods for age estimation in adults. This chapter presents the first adaptation of the Kvaal et al method on CBCT. For this case, width measurements only were collected, in sagittal and coronal views. Although the exclusive analysis of width measurements of root and pulp canal on CBCT also showed negative correlation between age and pulp/tooth ratio, the accuracy (SEE>10 years) does not surpass the analysis of dental radiographs with mesio-distal pulp/tooth width measurements.

Even though the results of the papers presented in Chapters 4 and 5 (section 3), are not as accurate as expected, the analysis was deemed necessary to be done, exploring different applications of this method for dental age estimation, especially in the case of the use of CBCT doing linear measurements. It is necessary to mention that for future studies
following the presented methodology, it would be necessary to analyze larger samples and test different statistical methods, not only linear regression models as it was done for these studies.

On the other hand, the constant advances in imaging analysis and telecommunications, demand forensic dentistry to evolve and to adapt to new sources of information and emerging technologies. In this way, Chapters 6 and 7 are clear examples of the application of one the most recent programs for imaging segmentation on dental CBCT, and illustrate, how, counting with the support of the international academic community it is possible to design innovative studies.

The use of CBCT and volume reconstruction of different anatomical structures has led to the proposal of different methods for identification of individuals. However, when it comes to their use in forensic dentistry for dental age estimation in adults, the results are limited. The analysis is more complex and demands more time, than when using dental radiographs, aspects that could be considered as disadvantages of previously proposed methods.

To help forensic dentists to face the fifth listed challenge in this thesis; the need to understand the different technological aspects that affect those methods based on pulp/tooth volume reconstruction, the study presented in Chapter 6 compares the previously proposed methodologies, and analyses the possible circumstances that affect their accuracy.
Chapter 6, demonstrated that although there are different approaches available to quantify the volume of root canal, it is difficult to obtain the real volume of this space, also that regardless the technique used to obtain volume measurements from pulp chamber, there are more implicit aspects in the software that forensic dentists need to understand and be aware of, to maintain the objectivity of the proposed methods. In this study, it was also observed that the method which proved to be more reliable to measure volume was semiautomatic segmentation using the software ITK-SNAP version 3.4 (open source software, www.itksnap.org), as it was less likely to be affected by the subjectivity of the observer.

Based on the findings of the study presented in Chapter 6, the software was used to analyze a sample that included data from two population groups with different ethnic backgrounds.

The paper presented in Chapter 7, is a homage to the international community that collaborated in this research, and an example of how the use of telecommunications is promoting the generation of innovative methods for forensic sciences, helping researchers to overcome the geographical barriers, that previously limited the design of studies using large scale radiological data from different population groups.

This chapter also discusses one of the most recent findings in forensic dentistry as it is a selection bias called age mimicry, which probably has affected the results of previous studies for age estimation. This selection bias could be the explanation to the wrong
assumption that there are population and ethnic differences that affect the accuracy of past studies.

To avoid age mimicry, the sample needs to meet rigorous conditions that guarantee the reliability of the results. Although, the paper presented in Chapter 7 could not fulfill all the required conditions, it presents the pathway forward in proposing a model for inter-population studies for age estimation in adults.

**GENERAL CONCLUSIONS**

This thesis confronts some established paradigms for the use of methods for dental age estimation in adults. Likewise, each one of the chapters that this thesis encompasses, reveals new concepts, and presents new proposals that might be useful for future studies, which makes each one of the sections and chapters of this thesis a valuable contribution for forensic dentistry.

Likely, the existent literature has elaborated some parameters for the design and application of methods for age estimation. These parameters specify: the study of individuals from the same population; and the exclusion of teeth with pathologies or dental procedures. Evidence presented in this thesis challenges these rather limited parameters which are too restrictive to be used as guidelines. Rather it has been shown that samples from widely different populations and from teeth which are in various conditions may be used to generate meaningful data in forensic analyses.
Despite, the similarities of the study design of previous publications, that followed or slightly modified the three mentioned methods in this thesis, it was possible to observe that there are no established protocols for data analysis and the report of the results. More importantly, there are no parameters that consider the characteristics of the individuals included in the study as well as a sample size, which might be big enough, to simultaneously include individuals with different dental conditions and the uniqueness of each subject.

Under this frame, it is possible that the main conclusion from section one is that an ideal research scenario, the study design should ensure the inclusion of individuals with a dental status different to “totally sound teeth” representing the variability existent in any population, taking in account the parameters mentioned in Chapter 7 to avoid age mimicry bias.

Section two sends some clear messages:

1. It is necessary to test the available methods for dental age estimation in adults in individuals with different dental characteristics.

2. Orthodontic treatment is not an obstacle to the application of the Kvaal et al method.

3. It is necessary to perform similar analyses using periapical radiographs.
4. The use of CBCT for dental age estimation needs to be thoroughly tested, for linear, area and volume measurements, and if necessary, discourage its use, due to the simplicity of those methods using dental radiographs.

5. It is recommended to conduct studies that include individuals from different groups of population.

After analyzing the results of Section three it is possible to state:

1. The Kvaal et al. method is a validated tool for age estimation, and could be an alternative for those individuals with agenesis of third molars. However, for the determination of the age of majority of an individual, it would be advisable to use the Kvaal et al. method in association with an analysis of other age indicators, such as skeletal age-related changes.

2. Minor dental changes are not an obstacle to the use of the Kvaal et al method, as long as they do not affect the root of the tooth.

3. Although CBCT provides more information, the accuracy for age estimation in adults using the Kvaal et al. method is not better than that provided by dental radiographs.

The main conclusions from Section four are:
1. Before proposing a method for dental age estimation using volume measurements, it is necessary to understand the functioning of the software to be used.

2. Although there are different approaches for tooth volume measurements from CBCT, the more reliable are those based on semi-automatic or automatic segmentation.

3. With the new technological advances in telecommunications and imaging analysis, it is possible to do studies which include data from individuals with different ethnic backgrounds.

4. Future studies need to follow the stated conditions to avoid the selection bias called age mimicry, which are:
   a. Even number of participants in each age category.
   b. Large samples.
   c. Well established age ranges.
   d. Equal numbers of individuals per age range.
   e. Individuals from different populations.

5. It is recommended to test the methodology proposed in Chapter 7 in a larger sample, which includes at least two groups of individuals with different ethnic backgrounds but, with the same age and sex distribution, to ensure the reliability of the results.
6. To do a reliable meta-analysis in the future, it is necessary to establish appropriate standards for the publication of the results and accuracy of different studies.

And finally, the different new approaches and statements presented by this thesis deserved to be tested, debated, and confronted by future researchers, using innovative techniques and more developed tools that will be available in the coming years for forensic dentistry.

Final note: Some of the chapters have been slightly modified after the publication of the most recent findings about age mimicry (Chapter 1, Chapter 5, Chapter 7).
APPENDIX 1. Age estimation in adults by dental imaging assessment systematic review.

Age estimation in adults by dental imaging assessment systematic review

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Abstract

Importance: The need to rely on proper, simple, and accurate methods for age estimation in adults is still a world-wide issue. It has been well documented that teeth are more resistant than bones to the taphonomic processes, and that the use of methods for age estimation based on dental imaging assessment are not only less invasive than those based on osseous analysis, but also have shown similar or superior accuracy in adults.

Objectives: To summarise the results of some of the recently most recently cited methods for dental age estimation in adults, based on odontometric dental imaging analysis, to establish which is more accurate, accessible, and simple.

Evidence review: A literature search from several databases was conducted from January 1995 to July 2016 with previously defined inclusion criteria.

Conclusion: Based on the findings of this review, it could be possible to suggest pulp/tooth area ratio calculation from first, upper canines and other single rooted teeth (lower premolars, upper central incisors), and a specific statistical analysis that considers the non-linear production of secondary dentine with age, as a reliable, easy, faster, and predictable method for dental age estimation in adults. The second recommended method is the pulp/tooth width–length ratio calculation. The use of specific population formulae is recommended, but to include data of individuals from different groups of population in the same analysis is not discouraged. A minimum sample size of at least 120 participants is recommended to obtain more reliable results. Methods based on volume calculation are time consuming and still need improvement.

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1. Introduction

Age estimation is one of the most important characteristics used to establish the identity of any individual in different legal, forensic, or anthropological research context [1]. To this end, forensic teams depend on osseous analysis based methods, which have acceptable results for young individuals or in their early adulthood [2], and dental development based methods, which are highly reliable in individuals under 21 years of age [3]. However, these methods have some disadvantages: the poor resistance of bones to the taphonomic process [4], and once the individual reaches the threshold of 21 years of age, and the third molars development concludes [3], the currently available dental development based methods are no applicable. In individuals with the congenital absence of third molar teeth, this threshold falls down up to 14–15 years of age.

To respond to the need of an ageing population, and with the evident resistance of teeth to the taphonomic process, alternative methods for dental age estimation in adults have been proposed. Primarily, these are based on the formation of secondary dentine, studied since 1950 [5] and the subsequent narrowing of the pulp cavity, which can be observed in dental radiographs, leading to the proposal of minimally invasive methods. This systematic review focuses on three methods based on odontometric analysis of the pulp cavity, performing length and with measurements [6], area measurements [7] and lastly volume calculation [8]. The objective of this review is to summarise the results of these recently most cited methods for dental age estimation in adults, to establish which method is more accurate, accessible, and simple.

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1.1. Description of the problem or issue

Different methods have been published for dental age estimation in adults, based on the pulp/tooth dimensions’ ratios. Nevertheless, the obtained results of the application of some of these methods for dental age estimation, in adults from different population groups, surpass the accepted threshold in forensic sciences which says that the standard deviation of a method for adult’s age estimation should preferable be below a standard deviation (SD) of years ±10 years [9].

1.2. Description of the methods being investigated

The methods for dental age estimation in adults analysed in this paper were selected based on their minimally invasive nature, not requirement for the extraction of teeth to be performed, and pulp/tooth ratio calculation which have been applied in individuals from different populations. Kvaal et al. [6] method is based on the analysis of linear measurements of the pulp, tooth, and root length as well as root and pulp width measurements at three different root levels, initially applied on periapical radiographs and later on panoramic radiographs and tomographs. Cameriere et al. [7,10] method is based on the analysis of pulp and tooth area measurements on periapical and panoramic radiographs. Finally, the different methods for dental age estimation in adults based on pulp/tooth volume ratio from cone beam computer tomography [8,11–14] were also included in this systematic review.

1.3. How these methods might work

The included methods in this study are based on a negative correlation between age and the pulp chamber size, as well as on the tooth/pulp ratio calculation, regardless the nature of the used measurements: length/width, area, or volume. In other words, all of them look at the association of one age related phenomenon, as it is the formation of secondary dentine and the decrease of pulp chamber size with age, which has been accepted as an age indicator, observable and measurable with different dental imaging techniques. As an ideal, the accuracy of the studied methods should not exceed the threshold of a SD ±10 years [4,1].

1.4. Why it is important to do this review

The relevance of this review is grounded on the need to recommend a method for dental age estimation with the follow characteristics: simple, fast, non-invasive, non-expensive, reproducible and over all, accurate, that can be systematically used in different academic and forensic scenarios. Helping the reconstruction of identity profiles of unidentified deceased individuals or alive individuals with doubtful identity documents.

2. Methods

2.1. Criteria for considering studies for this review

Qualitative analysis of the information: original studies, in humans, reporting the use of any of the listed methods for dental age estimation, based on pulp/tooth ratio calculation (length/width, area, volume) that preferably reported intra-inter observer calibration, generating population specific formulae or in case that did not, that reported if the obtained results were obtained by using the method’s author’s original formulae. English or Spanish language that expressed the results in terms of accuracy for dental age estimation.

Quantitative analysis of the information: same criteria than qualitative analysis plus the exclusion of studies which sample included individuals younger than 14 years of age, studies with small samples (n < 50), studies using extracted teeth, and studies that did not report the use of specific population formulae.

2.2. Search methods for identification of studies

The information was searched though the data-base available at the University of Western Australia which included the collections of:

- Directory of open access journals (DOAJ), Medline/Pubmed (NLM), OneFile (GALE), ProQuest, collection, (Web of Science), Science Direct Journals (Elsevier), Social Sciences Citation Index (Web of Science, Scopus (Elsevier)), SocialSciences Citation Index (Web of Science), Wiley (CrossRef), Wiley Online Library. Also, google scholar, by looking at the papers that reference the original study performed by Kvaal et al. Cameriere et al., and those methods for age estimation that referred in their methodology the use of CBCT and volume reconstruction of pulp chamber and tooth.

The search key words were as follow: Kvaal and dental age estimation; Cameriere and dental age estimation; age; and tooth volume.

The literature search included papers published after the publication of the original papers of the authors to July 2016, the search was conducted during the years 2014 to 2016. In the lack of a manual for systematic review in forensic dentistry, this systematic review follows the Cochrane handbook for systematic reviews-methodology review, and when possible the RevMan software recommended by the Cochrane handbook (Figs. 1–3).

2.3. Data collection and analysis

2.3.1. Selection of studies

The initial selection was based on the title and then abstract. Papers with titles that referred the inclusion of only minors were excluded (children). Studies reporting also the use of third molars or developing teeth, were excluded. Studies that included in the title also the use of other methods for dental age estimation in children were excluded (Demirjian) or any other invasive method for dental age estimation were excluded (Diagrams 1–3, Tables 1–3).

2.3.2. Data extraction and management

The collected information was organized in an excel spreadsheet as follow: Author, year, country, number of participants (male and female), age, intra and inter-observer agreement assessment, imaging technique to obtain the images, measuring instrument, best correlation coefficient between age and the different age predictors, best result in terms of accuracy per individual tooth or per set of teeth, when possible, as well as the highest error recorded by set of teeth or individual tooth (Tables 4–6).

2.3.3. Assessment of risk of bias in included studies

To avoid bias in this systematic review, and to avoid false positive (declare that a method is more accurate than other when it is not) or false negative conclusions (declare that a method is less accurate than other when it is not), it was necessary to analyse the possibility of author bias. This owed to the participation of the same authors in repeated publications. To this end, the results were analysed comparing individual papers, and then grouping them per author. Additionally, in certain cases were there doubts in regards to the origin of the sample, which means the likelihood to find studies that had used the same sample, the authors were contacted to confirm the origin of the sample, and in case that two studies had the same sample, the authors were asked to suggest which study should be included in
the meta-analysis, in case that the study met the criteria for the quantitative analysis. In the same way, to deal with missing data, the authors were contacted via e-mail.

There was another possible source of bias observed in this study: the use of non-specific population regression models and equations, which could cause the judgement of a method as no or less accurate. To overcome this issue, only studies using specific population formulae were used in the quantitative analysis.

3. Results


3.1.1. Qualitative analysis

The original paper about Kvaal et al. [6] method was published in 1995. Since then, this method has been applied to a global sample of 3254 individuals from 12 different countries (Graph 1, Table 1). The initial methodology suggested the use of periapical radiographs from six different teeth, using vernier callipers to measure the maximum tooth length, the pulp length and root length, and a stereomicroscope with a measuring eyepiece to the nearest 0.1 mm to measure pulp and root width at three different levels previously described by the author of this method [6]. Just few studies have followed the original methodology with slight adaptations [16,9,16–19]. In 2005, this method is applied on panoramic radiographs (20), which became popular [21–35]. The use of digital imaging in dentistry also introduced the use of different software to perform the odontometric measurements. The most commonly used software are: Adobe Photoshop (different versions n = 5), Image J (n = 4) and Kodak dental imaging software (n = 2). From those studies that tested the effect of sex on the accuracy of the age estimated, 3 found that sex does not affect the models [16,20,31] and 2 that it does [19,24]. In the original
study, sex was included as a factor for the mandibular lateral incisor, indicating that pulp cavity size changes occurred faster in males, as females need another 6 years to get the same age as females for this tooth [6].

3.1.2. Quantitative analysis

From the total sample, only 16 papers met the inclusion criteria for the quantitative analysis (Table 4). The most accurate result (SD = 5.6, r = 0.95) was obtained from panoramic radiographs using Hipax program (version 3.01) software, 168 participants (95 female and 102 male) in a Caucasian group [20] The larger error reported was SEE > 13 years r² = 0.1 [16,36] obtained from the lower canine, following original Kvaal methodology on periapical radiographs [36] and the Adobe Photoshop 6.0 software in panoramic radiographs [16].

Kvaal et al. measurements have been reported to have a high degree of intra and inter-observer agreement, indicating the reproducibility of the measurements, even in those studies that did not follow the original methodology. Only one study reported significant differences in regards to the root length and pulp width at the level B and root width at the level A measurement, although this study reported a SEE = ±13.8 [r² = 0.38], the average error of age estimation was ±18–21 years using periapical radiographs [36].

Note: the studies that did not meet the inclusion criteria for the quantitative analysis also reported high intra and inter-observer agreement [32–34]. One study reported that the lower lateral incisor produced lower intra-observer correlation [33], and one of them affirmed that the use of stereomicroscope improve the accuracy of the age estimates [9].

### Table 1

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<tr>
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<td>India</td>
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</table>

* Number of individuals reported in each study. Sample size.

#### Table 2

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<td>India</td>
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<td>120</td>
<td>&gt;12</td>
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</table>

* Number of individuals reported in each study. Sample size.
Table 3
Total studies reporting the use of volume calculation.

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<th>Country</th>
<th>Total*</th>
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<td>Japan</td>
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<td>Japan</td>
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</tr>
<tr>
<td>Abosi et al. [13]</td>
<td>2010</td>
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<td>20–78</td>
</tr>
<tr>
<td>Star et al. [56]</td>
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<td>Belgium</td>
<td>111</td>
<td>10–65</td>
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<tr>
<td>Tardivo et al. [57]</td>
<td>2011</td>
<td>France</td>
<td>58</td>
<td>14–74</td>
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<tr>
<td>Jagannathan et al. [14]</td>
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<td>Sakuma et al. [54]</td>
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<td>Japan</td>
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<td>Tardivo et al. [58]</td>
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<td>210</td>
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<td>Sasaki et al. [55]</td>
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<td>Mendonca et al. [61]</td>
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<td>Brazil</td>
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<td>Ge et al. [63]</td>
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<td>Ge et al. [64]</td>
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</table>

* Number of individuals reported in each study. Sample size.

3.2. Cameriere et al. [7] (29 papers n = 4167)

3.2.1. Qualitative analysis

The original paper documenting Cameriere et al. method [7] was published in 2004. In this systematic review, 29 papers using this method were found, having a global sample of 4167 individuals from 11 countries (Graph 2, Table 2). This method was initially applied to digital panoramic radiographs, using (AutoCAD2000, Install Shield3.0, 1997) and different versions of this software have been used in 11 studies. The most used software to perform the pulp/tooth area measurements is Adobe Photoshop (13 studies). This method was designed to be applied in single rooted teeth, especially canines, but it has also been tested in premolars and central and lateral incisors. From those studies that assessed the effect of sex on the regression models, the vast majority reported that sex does not affect the regression model [4,7,26,31,37-46]. Only 4 studies reported that sex affect the regression model [47-50].

3.2.2. Quantitative analysis

Twelve studies met the inclusion criteria for the quantitative analysis (Table 5). The use of this method has shown a standard error of estimation (SEE) from ±1.2 [42] to ±12–13 years (r = 0.2–0.4) [51]. From these studies, 2 reported a median error < ±5 years [7,38], 6 studies reported an error from ±5 to ±8.5 years [26,40,42-44,46], and three studies reported an error from ±10 to ±13 years [4,47,51]. From the included studies in the qualitative analysis, only one reported significant intra-observer differences in lateral incisors and first premolars [p < 0.05] [51].

Note: all the studies that did not meet the inclusion criteria for the qualitative analysis and that did intra and inter-observer calibration, reported high intra inter-observer agreement [37,49,52,53].

3.3. Volume (16 papers n = 2444)

3.3.1. Qualitative analysis

Owing to the low number of studies doing pulp/tooth volume reconstruction, in the qualitative analysis those studies using extracted teeth were included. The first study doing pulp/tooth volume reconstruction reported the use of micro-focus X-ray in 2004 [8]. In this systematic review, 16 studies were found using the pulp/tooth volume reconstruction, by means of micro-focus computer tomography, CT scan or CBCT, and the use of different software to do manual or semi-automatic volume reconstruction, such as Tri 3D Bon, Mimics®️, ITK-SNAP 2.4, OsiriX, Amira among others, in a global sample of 2444 individuals from 7 countries (Graph 3, Table 3). However, the methodology of some of them require the use of extracted teeth [8,12,13,54,55]. In regards to the effect of sex on the regression models, 9 studies reported that sex has no effect [8,14,26,54,56–60]. 3 studies reported that the models are more accurate in female than in male, having a higher determination coefficient for women than men [12,61,62], as follow: R2 0.6 for males and R2 0.7 for females [12], or R2 = 0.29 for males and R2 = 0.15 for men [61] and R2 = 0.67 for male and R2 = 0.75 for female when the mandibular central incisor is used or R2 = 0.56 for male and R2 = 0.58 for female when the second premolar is used. Two studies, which only reconstructed the pulp cavity volume, also reported significant difference between tooth type and sex [63,64]. One of them found a significant difference in the volume between the volume of both genders (p = 0.028 < 0.05) and a stronger relation between pulp chamber and age for female (R2 0.6) than for male (R2 0.5) [63]. The other one reported a significant difference in volume between genders for 12 types of teeth (p = 0.0000) [64].

3.3.2. Quantitative analysis

Four studies meet the inclusion criteria for the quantitative analysis. The error of these studies varies between ±3.47 years [58] to ±28 years [59]. It was reported high intra-inter-observer agreement [60]. Among those studies that did not meet the inclusion criteria for the quantitative analysis, seven studies also reported high intra-inter-observer agreement [8,11–14,57,63,64].

4. Discussion

Age estimation in adults is a challenge in all forensic contexts, especially in cases that require the use of non-invasive methods. The formation of secondary dentine and the non-linear narrowing of the root canal with age [11], is one age predictor measurable in dental radiographs and tomographs, leading to the proposal of different methods for age estimation in adults as an alternative to more invasive methods and as a complement to osseous analysis [2,17,35,65–68].

In the same way, many methods have been suggested for dental age estimation in adults. Nevertheless, there are few available studies that compare their accuracy [69]. In the lack of a consensus to uniformly apply a method for age estimation in adults, the need to perform a systematic review seemed to be evident. This systematic review summarises and compares the results of some of the most used methods for dental age estimation in adults, performing a qualitative and quantitative analysis.

In terms of the qualitative analysis, it has been reported that there are no significant differences between right or left teeth [14,57]. Another aspect to test among the different papers was the effect of sex of the different methods. Although not all the included studies assessed the effect of sex on age estimation, those that did (n = 38), 71% (n = 27) affirm that sex has not statistically significant effect on age estimation, when pulp/tooth ratio is calculated. However, if only pulp volume is measured and used as age indicator, there is a significant difference between the accuracy for males and females [63,64].

In the light of the evidence one could suggest that ratio calculation not only diminishes the effects of tooth magnification in dental radiographs, as reported by Kvaal et al. [6], but also reduces the effect of sexual dimorphism related to tooth size. The age of the participants is also another relevant aspect to include, in this systematic review those studies including participants under 14 years of age where excluded for two main reasons. First, there are other methods more reliable for aging teenagers and infants, and second, before 14 years of age, not all the teeth have completed
Table 4
Kvaal et al. method. Studies included in the quantitative analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Measuring instrument</th>
<th>SEE ± years per tooth or group of teeth (FDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>stereomicroscope and Vernier callipers</td>
<td>8.6 8.9 9.4 9.5 10 11 10.5 11.5 11.5</td>
</tr>
<tr>
<td>Kvaal et al. [6] 1995, Norway</td>
<td>100 PER 20–87 years</td>
<td>-</td>
<td><strong>[1]</strong></td>
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<td>Bosman et al. [21] 2005, Belgium</td>
<td>197 OPG 19–75 years</td>
<td>Adobe Photoshop software</td>
<td>9.5 9.2 9.9 9.7 9.8 9.3 11.6 8.2 8.1</td>
</tr>
<tr>
<td>Paewinsky et al. [20]* 2005, Germany</td>
<td>168 OPG 14–81 years</td>
<td>Hipax program</td>
<td>5.6 6.4</td>
</tr>
<tr>
<td>Garcia et al. [19] 2009, Colombia</td>
<td>107 PER 21–50 years</td>
<td>Scion Image software</td>
<td>7.1</td>
</tr>
<tr>
<td>Ranjani et al. [16] 2010, India</td>
<td>100 PER 20–70 years</td>
<td>SV- 4 mini slide viewer, digital Vernier callipers, Stereomicroscope</td>
<td>10.5 10.2 11.8 11.5 12.4 11.8 12.7 13.3 11.8</td>
</tr>
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<td>Kanchan-Talreja et al. [36] 2012, India</td>
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<td>Adobe Photoshop software</td>
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<td>Limdawala and Shah [23] 2013, India</td>
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<td>Misirlioglu et al. [26] 2014, Turkey</td>
<td>Group A 100 PER 25–50 years</td>
<td>Kodak Dental Imaging Software</td>
<td>8.3 8.21 9.09</td>
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<tr>
<td>Patil et al. [70] 2014, India</td>
<td>Group B 50 PER 25–50 years</td>
<td>-</td>
<td><strong>[3]</strong></td>
</tr>
<tr>
<td>Mittal et al. [28] 2016, India</td>
<td>152 OPG 14–60 years</td>
<td>Vistaview DBSWIN software</td>
<td>7.97 8.59 7.51 8.15 8.53 7.89 8.85 7.95 7.58</td>
</tr>
<tr>
<td>Rajpal et al. [71] 2016, India</td>
<td>50 PER 15–57 years</td>
<td>Kodak Dental Imaging Software</td>
<td>6.42 7.3 7.84</td>
</tr>
</tbody>
</table>

PER = periapical radiographs. OPG = panoramic radiographs. Tooth numeration FDI (Federation Dentaire International).

* Error reported as standard deviation.

a Error reported as the difference between the chronological age and the estimated.

the apex closure, which by definition, is a requirement for the formation of the secondary dentine [77].

For the qualitative analysis, characteristics related with sample size and inclusion criteria established in different studies were compared. As compared to previous efforts, we did not find a significant correlation between sample size and the accuracy of the method, when all the data were analysed together ($r^2 = 0.1$, p-value <0.05, sample size of the analysed studies $n = 50–604$) or when the data were analysed individually for each method. It is necessary to highlight that from the studies included to perform this analysis ($n = 30$) only 4 (13.3%) had a sample smaller than 100 individuals ($n = 50–91$ individuals), which may suggest that in any study for age estimation the minimum sample should include data from at least 100 participants, or over 100 teeth from different individuals in those studies using only one tooth type. Some of the excluded studies for the quantitative analysis, fail in this aspect [56,57,61,62], including data from several teeth of the same individual and counting them as different samples, which may affect the reported results.

A previous study recommended a minimum of 120 participants to achieve 80% of power and 5% of significance [31]. One almost universal inclusion criteria, among the papers, was the use of only totally sound teeth. Only two studies did not follow this parameter, using teeth with cervical lesions to do volume calculation [55], or using panoramic radiographs that did not meet the inclusion criteria suggested by Limdawala et al. [23]. Both of them found not significant effect of these situations on the accuracy of the results.

Another aspect to consider, is the description of the different methods, in terms of the procedure to measure the different pulp/tooth dimensions, as well as the statistical processing of the data. In certain cases, it was necessary to re-read the papers several times to understand the proposed methodology and how the accuracy was reported.

In the quantitative analysis two main characteristics were compared among the studies: repeatability of the measurements, in terms of intra and inter-observer agreement and their accuracy, reported as standard error of estimation (SEE), mean absolute error (MAE) or standard deviation (SD). It is necessary to mention that owed to this variety in the approach to evaluate and to report the obtained accuracy, it was not possible to perform a deeper statistical analysis, a meta-analysis, to confirm that one method was superior to another, however in this review we found that in average, the studies using pulp/tooth area ratio reported a lower error (Tables 4–6).

All the studies reported significant intra- and inter-observer agreement, regardless the used method and the measuring instrument, which suggests that after adequate training any of these methods is reproducible. However, in regards of the
simplicity of the technique, and the access to the required tool to measure and to calculate the respective pulp/tooth ratio dimensions, Kvaal et al. and Cameriere et al. are more convenient, using periapical radiographs or panoramic radiographs, as the radiological technique does not affect the accuracy of the measurements, as long as the quality of the image allows the observer to clearly observe the boundaries on the root canal and tooth surface [23]. The volumetric reconstruction of anatomic structures involves a more technical and time consuming training as well as the use of more complex software, that are not always free access. This could be considered as an obstacle for the big scale application of some of these methods. Likewise, the user spends more time doing the volumetric reconstruction per tooth, which varies from 4 h [8] to 15 min [61], when reported.

Another important aspect in regards of accuracy, is the use of the specific population formulae, which also improves the results of the age estimates, independently of the used method [14,30,42,46,64,70]. In those studies, using non-specific population formulae the reported error was notably larger (error > ±20 years) [32,33]. However, the use of data from different population in the same statistical analysis, to generate a unique age estimation equation, is not discouraged [2,21,47]. Furthermore, it has been reported that the pulp chamber size variation can be detected only each 10 years, which opens a question mark about the reliability of those methods reporting a lower error.

In the specific analysis of Kvaal et al. method, although this method required the inclusion of six different single rooted teeth per individual, the use of only one tooth [19], only upper central incisor [70], canines [26], mandibular teeth [33], or different combination of teeth to apply the odontometric analysis described by Kvaal and co-workers [25,27], has also been reported, with acceptable results (SEE < ±10 years). It has also been reported that its accuracy depends on the quality of the image and on the precision of the measurements [23]. Several studies reported that tooth length is not strongly correlated with age [16,18,20,23] as tooth length would depend on tooth wear, bruxism, and food habits, rather than a physiological aging related process [16].

In regards to Cameriere et al. method, it only requires the use of one teeth, and the majority of the studies report the use of canines. However, one study using three different lower teeth (Lateral incisor, canines, first premolars) found that lower canine had the poorest correlation coefficient with age ($r = -0.2$) [51]. Another study found more accurate estimations from upper lateral incisors, claiming that the narrowing velocity in the pulp chamber of this tooth is twice faster than in the lower incisors [47].

### Table 5
Cameriere et al. method. Studies included in the quantitative analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Measuring instrument</th>
<th>SEE ± years per tooth (FDI)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cameriere et al. [7]</td>
<td>100 OPG</td>
<td>AutoCAD2000</td>
<td>3.27</td>
</tr>
<tr>
<td>Italy, 2004</td>
<td>18–72 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cameriere et al. [38]</td>
<td>229 PER</td>
<td>Adobe Photoshop</td>
<td>4.33</td>
</tr>
<tr>
<td>Portugal and Italy, 2009</td>
<td>20–84 years</td>
<td></td>
<td></td>
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<tr>
<td>Babshet et al. [4]</td>
<td>178 PER</td>
<td>Adobe Photoshop</td>
<td>10</td>
</tr>
<tr>
<td>India, 2010</td>
<td>20–70 years</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Babshet et al. [51]</td>
<td>61 PER</td>
<td>Adobe Photoshop</td>
<td>12.22</td>
</tr>
<tr>
<td>India, 2011</td>
<td>21–71 years</td>
<td></td>
<td>12.28</td>
</tr>
<tr>
<td>Jeevan et al. [40]</td>
<td>228 PER</td>
<td>Adobe Photoshop</td>
<td>6.39</td>
</tr>
<tr>
<td>India, 2011</td>
<td>16–72 years</td>
<td></td>
<td>4.28</td>
</tr>
<tr>
<td>Zaher et al. [42]</td>
<td>144 PER</td>
<td>AutoCAD2008</td>
<td>2.63</td>
</tr>
<tr>
<td>Egypt, 2011</td>
<td>12–60 years</td>
<td></td>
<td>1.94</td>
</tr>
<tr>
<td>Cameriere et al. [43]</td>
<td>606 OPG</td>
<td>Adobe Photoshop</td>
<td>6.38</td>
</tr>
<tr>
<td>Spain, 2012</td>
<td>18–75 years</td>
<td></td>
<td>5.75</td>
</tr>
<tr>
<td>Cameriere et al. [47]</td>
<td>116 PER</td>
<td>Adobe Photoshop</td>
<td>7.03</td>
</tr>
<tr>
<td>Portugal, 2013</td>
<td>18–74 years</td>
<td></td>
<td>6.64</td>
</tr>
<tr>
<td>Charsis et al. [44]</td>
<td>120 PER</td>
<td>Jenoptik ProgRess</td>
<td>5.4</td>
</tr>
<tr>
<td>India, 2013</td>
<td>20–70 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azevedo et al. [45]</td>
<td>81 PER</td>
<td>Jenoptik ProgRess</td>
<td>3.05</td>
</tr>
<tr>
<td>Brazil, 2015</td>
<td>20–78 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missirlioglu et al. [26]</td>
<td>114 PER</td>
<td>Adobe Photoshop</td>
<td>6.75</td>
</tr>
<tr>
<td>Italy, 2014</td>
<td>19–74 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azevedo et al. [45]</td>
<td>209 PER</td>
<td>Adobe Photoshop</td>
<td>6.41</td>
</tr>
<tr>
<td>Brazil, 2015</td>
<td>17–72 years</td>
<td></td>
<td>5.79</td>
</tr>
</tbody>
</table>

PER = periapical radiographs. OPG = panoramic radiographs. Tooth numeration FDI (Federation Dentaire International).

a Error reported as mean error.
b Error reported as mean absolute error (MAE).
c Error reported as the module of the differences between the chronological and estimated age.

### Table 6
Volume calculation based methods.

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample</th>
<th>Image segmentation method</th>
<th>Measuring instrument</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Star et al. [56]</td>
<td>111 CBCT</td>
<td>Automatic segmentation (threshold), and manual correction</td>
<td>Simplant™</td>
<td>11/21 SD = 12.8</td>
</tr>
<tr>
<td>2011, Belgium</td>
<td>10–65 years</td>
<td></td>
<td>and manual correction</td>
<td>13/23 SD = 13.1</td>
</tr>
<tr>
<td>Tardivo et al. [58]</td>
<td>210 CT</td>
<td>Semiautomatic</td>
<td>Mimics™</td>
<td>13/23 MAE = 3.4</td>
</tr>
<tr>
<td>2014, France</td>
<td>15–85 years</td>
<td></td>
<td>and manual correction</td>
<td>33/43 MAE = 4.6</td>
</tr>
<tr>
<td>De Angelis et al. [59]</td>
<td>91 CBCT</td>
<td>Manual</td>
<td>Osiris software</td>
<td>prediction interval = ±28 years</td>
</tr>
<tr>
<td>2015, Italy</td>
<td>17–80 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinchi et al. [60]</td>
<td>148 CBCT</td>
<td>Cone shape approximation</td>
<td>Osiris® software</td>
<td>SEE = ±11.45</td>
</tr>
<tr>
<td>2015, Italy</td>
<td>10–80 years</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CBCT: cone beam computed tomography; SD: standard deviation; MAE: mean absolute error; SEE: standard error of estimation.
Although, the majority of the studies reporting the use of this method also mention its creator as part of the authors, which could generate certain bias in the results. It was observed that in those studies that did not count with his participation, the results in terms of the repeatability of the method and accuracy were similar [26]. In average, Cameriere et al. method reported the lowest error (total average ±5.6 years), and a sample of at least 120 individuals was observed as adequate to obtain more accurate age estimates [31].

The combined use of Kvaal et al. width ratios and Cameriere area ratios has been reported on canines from digital panoramic radiographs, finding more accurate age estimates when the pulp/tooth width ratio at level C and pulp/tooth area ratio were using together [31] or when area ratio calculation from canines is used individually [26].

In this systematic review we found the following advantages about the use of pulp/tooth area calculation over other methods: area measurement on digital radiographs with different software is faster, and the most recent study using this method proposes a software to do and automatic selection of the borders of the pulp and tooth, which minimizes the required time to obtain the area of tooth and pulp chamber, and reduces the error associated with the observer, when performing the area selection [75]. Linear measurements can also be performed on digital radiographs, but, the observer needs to take nine measurements per tooth and six teeth, and calculate several ratios. In the method using pulp/tooth area measurements, only one ratio needs to be calculated and allows the researcher to develop the respective statistical regression model. In the same way, the use of canines, especially upper canines to calculate pulp/tooth area ratio has certain advantages, as their longer survival, in comparison with other teeth, less wear, and the big size of the pulp chamber [59]. Also, as observed in Table 5, there are not only more studies reporting the use of upper canines to do pulp/tooth area calculation but also, these studies present an acceptable accuracy, in contrast to what was reported by Kvaal and Solheim, when doing pulp/tooth length/ with ratio calculation who observed that upper canines had the lowest correlation with age, when using dental radiographs of extracted teeth [77], which was an exclusion criteria in this systematic review.

Finally, although the use of pulp/tooth volume ratio calculation from non-extracted teeth still requires more research. The initial results of the analysis of pulp volume among individuals of different age groups, bring important and detailed information to understand of the formation of secondary dentine. It has been reported that the pulp/tooth volume ratios in the cervical area were more correlated with age, and that this correlation decrease towards the apex [13]. Also, that the most marked reduction in volume ratio was observed between the second and the fifth decades of life in lower first and second premolars, and between the second and the third decades of life in lower first premolars. [13] This clear findings may enlighten the proposal of future and more accurate methods for age estimation in adults.

5. Conclusion

The narrowing of root canal caused by the formation of secondary dentine is a well-accepted age indicator in adults. This systematic review observed that age estimation methods based on pulp/tooth area ratio calculation reported more accurate results, even when one tooth is analysed per individual. However certain conditions need to be fulfilled: a sample of minimum 120 individuals, older than 14 years of age, assessment of the accuracy of the observer, and generation of specific population equation. The inclusion of data of individuals from different population group in the same analysis is not discouraged. These studies must consider in their statistical analysis the non-linear deposition of secondary dentine through life. This systematic review also recommends the use of dental age estimation methods, firstly pulp/tooth area ratio calculation of single first, upper canines and other single rooted teeth (lower premolars, upper central incisors) and secondly pulp/tooth length/with ratio calculation, as reported by Kvaal et al., in combination with other methods that include diverse age indicators to produce a more reliable age estimates. The authors of this systematic review also recommend future studies to report their results in terms of standard deviation, mean absolute error and standard error of estimation simultaneously, with this, there will be more be more homogeneity. Thereby, to perform a proper meta-analysis will be more likely.

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Changes in age estimation standards secondary to full fixed edgewise orthodontic treatment

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ABSTRACT

Objective: The aim of this cohort study was to evaluate if the side effects of orthodontic treatment have any significant impact when the Kvaal et al., method is used for dental age estimation. The objective of this study was to observe the potential effects of orthodontic treatment on the accuracy of the age estimation, as performed using the Kvaal et al. Standards when it was applied on individuals before and after orthodontic treatment. Materials and Methods: Following the methodological approach of Kvaal et al., odontometric measurements were acquired, and the data were statistically analyzed to develop age estimation regression models. The total number of radiographs analyzed was 182 (64%, n = 58) female and 36% (n = 33) male. The ages ranged from 12 to 50 years for females (mean age 22 years) and 12-52 years for males (mean age 22 years) before starting the treatment. The average length of the treatment was 2.2 years for both females and males. Results: It was observed that the standard error of estimate for the regression models did not change dramatically for the pre- and post-treatment data. Conclusions: It is recommended that similar analyses be performed for other methods of dental age estimation, for example, methods based on cone-beam computer tomography or microfocus computer tomography. These novel approaches, though more accurate, are also potentially more sensitive to dental changes caused by orthodontic treatment.

KEY WORDS: Forensic sciences, forensic odontolgy, adults, dental age estimation, orthodontic treatment, root resorption, secondary dentin formation

INTRODUCTION

Kvaal et al. [1] developed a non-invasive method for the estimation of age in adults with the measurement of dimensions of the pulp chamber and tooth length to formulate regression models in the estimation of age. Originally, this method was proposed to be used on periapical radiographs. However, more recent work has focused on the use of the Kvaal et al. [1] method in panoramic radiographs [2-4]. During tooth development, secondary dentin deposition commences once the root formation is complete, and the tooth erupts to the level of the occlusal plane [5]. Annually, the rate of secondary dentin formation has been documented to be 6.5 μm/year for the crown and 10 μm/year for the root [6]. The rate of secondary dentin formation is influenced by external stimuli such as occlusal forces, trauma, and caries [7]. These external factors also generate the production of tertiary dentin [5], which is indistinguishable to secondary dentin on dental radiographs [8].

The estimation of dental age has been a valid tool in forensic and anthropological research [1,9,10]. For younger persons, including newborns, infants, and adolescents, there are dental age estimation methods, generally based on tooth maturation, which are considered the most accurate to estimate the chronological age in subadults [3,11-13]. In adults, once the root formation is deemed complete, methods for dental age estimation are broadly based on the analysis of secondary dentin deposition and the subsequent narrowing of the pulp chamber [1,6,14]. A potential confounding factor in the estimation of dental age is the high incidence of orthodontic treatment in developed countries [15]. The judicious application of forces is central to successful orthodontic treatment and is well known to induce biological changes in dental hard tissues, including the formation of secondary dentin [7] and root shortening [16]. The corollary being a change in the dental morphological features analyzed in the estimation of dental age in adults [7,17].

Given the potential of the development of tooth structure in adolescents and adult patients to be influenced by the application of orthodontic force, and these structures being the foundation of age estimation methods, it is important...
to recognize and quantify the effect. The first aim of this study was to evaluate if the side effects of orthodontic treatment had any significant impact on dental age estimation when using the Kvaal et al. [1] method. The objective of this study was to observe the potential effects of orthodontic treatment on the accuracy of the age estimation as performed using the Kvaal et al. [1] standards when applied on individuals before and after orthodontic treatment.

MATERIALS AND METHODS

Ethics approval was obtained from the Human Research Ethics Committee of The University of Western Australia (Ref: RA/4/1/6797) before commencement. In the design of the study, data collection and analysis have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Sample

This cohort study consisted of a series of subjects who consecutively presented for orthodontic treatment at a private specialist orthodontic clinic (SV). All panoramic radiographs were acquired from the same machine and in digital format, with the informed consent of the participants. All of the panoramic radiographs were unidentified, and the only information known was the sex and age of the participants at the starting and finishing time of the orthodontic treatment.

Inclusion Criteria

All subjects who had a recorded panoramic radiograph of high quality with respect to factors of image brightness, contrast, and sharpness were included. In addition, all the analyzed teeth were clinically sound with completed root formation and in functional occlusion.

Exclusion Criteria

Any radiographs that did not meet the inclusion criteria or had observable failings (e.g., image distortion, poor contrast, overlap of tooth structure, or improper positioning) were excluded. Teeth with rotations, incomplete root formation, dilacerations, pulpal pathologies, and endodontic treatment and/or restorations were excluded. Teeth with developmental abnormalities in size, shape, and tooth structure, as well as previous pulpal and periodontal pathologies and endodontic treatment, were excluded. Teeth with large areas of enamel overlap between neighboring teeth were excluded in this study.

Sample Population

This study analyzed a total of 182 pre- and post-orthodontic treatment panoramic radiographs from 91 participants, 64% (n = 58) female and 36% (n = 33) male, from a Western Australian population. All the participants presented for orthodontic treatment that, after diagnosis and treatment planning, was completed with fixed appliances. The ages ranged from 12 to 50 years for females (mean age 22) and 12-52 years for males (mean age 22) before starting the treatment. The average length of treatment was 2.2 years for both females and males. Digital panoramic radiographs were taken as part of routine clinical orthodontic treatment to evaluate the initial condition of the participants and the final treatment results.

Teeth Selection

As established by Kvaal et al. [1], three single rooted upper teeth (with Federation Dentaire International [FDI] notation 11/21, 12/22, 15/35) and three single rooted lower teeth (FDI 32/42, 33/43, 34/44) were measured. Kvaal et al. [1] reported that there are not significant differences between teeth from the left or the right side of the arch. In accordance with this, if one tooth was absent, the contralateral was used, as long as this was observable in a straight position.

Measurement

The measurements were performed using Image J software (version 1.48 19 April 2014 - National Institute of Health, USA). As proposed by Kvaal et al [1], different odontometric measurements were recorded: tooth length, pulp length and root length as well as pulp width and tooth width at three different levels: A (cemento-enamel junction in the mesial surface of the roots); B (midpoint between the points A and C); C (midpoint between the cemento-enamel junction and root apex). After the collection of the figures in an Excel (2013) spreadsheet, a serial of ratios was calculated: (T) tooth/root length, (R) pulp/tooth length, (P) pulp/root length, and root width/pulp width ratio at levels A, B, and C. The ratio calculation and use were proposed by Kvaal et al. [1] with the aim to reduce the effect of magnification and angulation inherent in most radiographs [1,4]. The different mean values calculated with these ratios provided the age estimation predictors (M: Mean value all ratios, W: Mean value width ratios B and C, L: Mean value ratios P and R, W-L: Difference between W-L). The predictors M and W-L were later used to formulate the age estimation models using multiple regression analysis as established by Kvaal et al. [1]. The measurements were completed by a single observer (TYM). All data were collected using Excel (Version 2013 Microsoft Redmont, USA), and statistical analysis was completed using the program R Core Team version 3.1.3 (2015) (R: A language and environment for statistical computing and R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/).

Calibration

Intra- and inter-observer calibration was estimated using five randomly selected panoramic radiographs from the final study sample. These panoramic radiographs were measured on five different days with at least 1 day interval. With the aim to avoid the recall of the measurements and landmarks, five new panoramic radiographs were also measured on each occasion. Data were recorded using the software Image J by TYM and SK. To determine intra-observer precision, three estimates of
precision were calculated: The technical error of measurement (TEM <1.0), relative TEM (rTEM <5%), and coefficient of reliability were calculated to quantify the inter- and intra-observer measurement error [18].

**Statistical Analysis**

The strength of linear association between chronological age and the Kvaal et al. [1] dental ratios was determined by Pearson correlation coefficient (r). Following this stage, regression analysis was applied to evaluate the relationship between the ratios and the chronological age. The final results were later used to generate the statistical models for age estimation. To quantify the predictive accuracy of the models, the standard error of estimation (SEE) was used in the cross-validation sample. Multivariable linear regression analyses were used to examine the association between the independent and outcome variables. The outcome variables included chronological age (dependent variable) and the predictors M and W-L (independent variables). Different equations were generated to estimate the chronological age, by individual tooth and by group of teeth, as follows: Three maxillary teeth with M and W-L values (6 predictors) individually calculated for each tooth, and the average of M and W-L for the same teeth (2 predictors). Three mandibular teeth with M and W-L values (6 predictors) individually calculated for each tooth, and the average of M and W-L for the same teeth (2 predictors). All six teeth, with M and W-L values (12 predictors) individually calculated for each tooth, and the average of M and W-L for the same teeth (2 predictors). All regression models were built using the ordinary least squares approach. R² values were computed to examine the amount of variance explained by the predictor variables. The formula proposed by Preoteasa et al. [19] was used to calculate the percentage of teeth with root shortening.

**RESULTS**

**Measurement Precision**

After the estimation of intra- and inter-observer error, the obtained values were as follow: Intra-observer error: TEM <1.0 (0.92), rTEM<5% (2.99%), and R > 0.75 (0.95). Inter-observer error (between SK and TYM) was: TEM <1.0 (0.80), rTEM <5 (2.37), and R > 0.75 (0.98) showing comparable intra- and inter-observer measurement precision.

**Age Distribution**

The age distribution before orthodontic treatment for females was: 12-19 years (62%, n = 36), 20-39 years (28%, n = 16), >40 years (10%, n = 6), and after treatment, it was: 15-19 years (57%, n = 33), 20-39 years (15%, n = 19), and >40 years (10%, n = 6). For males, before orthodontic treatment was: 12-19 years (52%, n = 17), 20-39 years (42%, n = 14), and >40 years (6%, n = 2), and after orthodontic treatment, it was: 13-19 years (48%, n = 16), 20-39 years (45%, n = 15), and >40 years (6%, n = 2).

The correlation coefficient between chronological age and the dental ratios was calculated for the data before and after orthodontic intervention [Table 1]. In both cases, the correlation coefficient related with the width ratios (A, B, and C) was more significant than those related with length (P, T, R, and L). As established by Kvaal et al. [1], the predictors M and W-L are required to be included in the final equation for dental age estimation. Based on the Pearson correlation coefficients, it could be observed that after the orthodontic treatment, the significance of the values for the predictor W-L decreased.

Individual tooth regression models and multiple teeth regression models were proposed for individuals that had not received orthodontic treatment [Tables 2 and 3, respectively] and for individuals after finishing the treatment [Tables 3 and 4]. In regards to the models for age estimation using individual teeth, the SEE tends to increase after orthodontic treatment, especially for the mandibular canine, where the increase was ±2 years. Furthermore, while the results for the SEE provided by the mandibular canine showed a lower SEE (±8.26 years) in the data analysis before treatment, the same tooth showed the greatest value of SEE (±10.23 years) for individual tooth analysis after orthodontic treatment.

When multiple regression models were formulated using different combinations of teeth, it is also noticeable that in a few

<table>
<thead>
<tr>
<th>Table 1: Correlation coefficients between chronological age before and after starting orthodontic treatment and the Kvaal et al.[1] dental ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ratio</strong></td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>P₁</td>
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<tr>
<td>P₂</td>
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<td>T₁</td>
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<tr>
<td>W₁</td>
</tr>
<tr>
<td>W₂</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.001, NS non-significant. P: Pulp length/root length, T: Tooth length/root length, R: Pulp length/tooth length, A: Pulp width/root width at level A, B: Pulp width/root width at level B, C: Pulp width/root width at level C and predictors, M: Mean value all ratios, W: Mean value width ratios B and C, L: Mean value ratios P and R, W-L: Difference between W-L.
cases the SEE decreased after orthodontic treatment: Maxillary lateral incisor (±0.437), maxillary first premolar (±0.698), the multiple tooth regression models using three maxillary teeth with two (±0.437) and six (±0.613) predictors, and in the multiple regression models using six teeth and two predictors (±0.237). In both circumstances, the multiple regression models using the different teeth combinations improved the accuracy of estimation of chronological age, consistent with previous studies [2].

Root shortening was detected in 62% of the upper central incisors, 53% of upper lateral incisors, 48% of upper second premolars, 48% of lower lateral incisors, 50% of lower canine, and 51% of lower second premolars, with a formula previously proposed [19].

### DISCUSSION

This is the first study to specifically apply the Kvaal et al. method to a group of orthodontically treated subjects, and also the first evaluating the impact of the side effects of orthodontic treatment on one of the proposed methods for dental age estimation in adults based on the formation of secondary dentine and the pulp/tooth dimension ratios. Previous

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**Table 2:** Multiple regression for estimation of chronological age (in years) for individual maxillary and mandibular teeth in subjects before (1) and after (2) orthodontic treatment

<table>
<thead>
<tr>
<th>Teeth FDI</th>
<th>n</th>
<th>R</th>
<th>$R^2$</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/21 (M)</td>
<td>83</td>
<td>0.229</td>
<td>0.21</td>
<td>Age = 39.87 − 76.79(M) − 51.75(W-L)</td>
<td>8.574</td>
</tr>
<tr>
<td>12/22 (M)</td>
<td>81</td>
<td>0.186</td>
<td>0.165</td>
<td>Age = 68.21 − 99.54(M) − 33.52(W-L)</td>
<td>8.923</td>
</tr>
<tr>
<td>15/25 (M)</td>
<td>58</td>
<td>0.08</td>
<td>0.046</td>
<td>Age = 79.8105 − 81.5881(M) − 0.5444(W-L)</td>
<td>9.993</td>
</tr>
<tr>
<td>32/42 (M)</td>
<td>87</td>
<td>0.124</td>
<td>0.103</td>
<td>Age = 90.22 − 129.66(M) − 18.62(W-L)</td>
<td>9.295</td>
</tr>
<tr>
<td>33/43 (M)</td>
<td>63</td>
<td>0.476</td>
<td>0.458</td>
<td>Age = 111.80 − 238.35(M) − 132.7(W-L)</td>
<td>8.260</td>
</tr>
<tr>
<td>34/44 (M)</td>
<td>68</td>
<td>0.356</td>
<td>0.336</td>
<td>Age = 21.67 − 69.84(M) − 69.59(W-L)</td>
<td>8.404</td>
</tr>
<tr>
<td>11/21 (W)</td>
<td>83</td>
<td>0.143</td>
<td>0.122</td>
<td>Age = 83.07 − 128.42(M) − 42.68(W-L)</td>
<td>8.486</td>
</tr>
<tr>
<td>12/22 (W)</td>
<td>81</td>
<td>0.255</td>
<td>0.236</td>
<td>Age = 80.40 − 145.51(M) − 47.69(W-L)</td>
<td>8.905</td>
</tr>
<tr>
<td>15/25 (W)</td>
<td>58</td>
<td>0.213</td>
<td>0.184</td>
<td>Age = 76.60 − 159.75(M) − 105.79(W-L)</td>
<td>10.26</td>
</tr>
<tr>
<td>32/42 (W)</td>
<td>87</td>
<td>0.095</td>
<td>0.074</td>
<td>Age = 49.10 − 77.55(M) − 37.65(W-L)</td>
<td>8.983</td>
</tr>
<tr>
<td>33/43 (W)</td>
<td>63</td>
<td>0.196</td>
<td>0.169</td>
<td>Age = 76.60 − 159.75(M) − 105.79(W-L)</td>
<td>10.26</td>
</tr>
<tr>
<td>34/44 (W)</td>
<td>68</td>
<td>0.312</td>
<td>0.291</td>
<td>Age = 21.67 − 69.84(M) − 69.59(W-L)</td>
<td>8.404</td>
</tr>
</tbody>
</table>

**Table 3:** Multiple regression for estimation of chronological age (in years) for the combination of different teeth before orthodontic treatment

<table>
<thead>
<tr>
<th>Teeth FDI</th>
<th>n</th>
<th>R</th>
<th>$R^2$</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 max 2 pds</td>
<td>50</td>
<td>0.278</td>
<td>0.247</td>
<td>Age = 60.40 − 145.51(M) − 47.69(W-L)</td>
<td>7.789</td>
</tr>
<tr>
<td>3 max 6 pds</td>
<td>50</td>
<td>0.314</td>
<td>0.291</td>
<td>Age = 63.704 − 6.412(11/21M) − 32.812(12/22M) − 19.332(12/22W-L) − 7.969(15/25M) + 10.594(15/25W-L)</td>
<td>7.932</td>
</tr>
<tr>
<td>3 md 6 pds</td>
<td>46</td>
<td>0.4</td>
<td>0.372</td>
<td>Age = 13.11 − 36.64(M) − 19.83(W-L)</td>
<td>9.124</td>
</tr>
<tr>
<td>3 md 6 pds</td>
<td>46</td>
<td>0.601</td>
<td>0.54</td>
<td>Age = 124.528 + 81.230(32/42M) − 7.784(32/42W-L) − 328.184(33/43M) − 121.021(33/43W-L) − 37.503(34/44M) − 9.855(34/44W-L)</td>
<td>7.805</td>
</tr>
<tr>
<td>6 teeth 2 pds</td>
<td>36</td>
<td>0.45</td>
<td>0.417</td>
<td>Age = 133.86 − 238.98(M) − 67.88(W-L)</td>
<td>7.609</td>
</tr>
<tr>
<td>6 teeth 12 pds</td>
<td>36</td>
<td>0.729</td>
<td>0.5889</td>
<td>Age = 130.727 + (15.058(11/21M) + 23.906(11/21W-L) + 61.249(12/22M) − 29.091(12/22W-L) − 100.481(15/25M) − 2.441(15/25W-L) + 54.517(32/42M) + 3.089(32/42W-L) − 268.438(33/43M) − 89.805(33/43W-L) − 41.009(34/44M) − 25.52(34/44W-L)</td>
<td>6.392</td>
</tr>
</tbody>
</table>

**Table 4:** Multiple regression for estimation of chronological age (in years) for the combination of different teeth after orthodontic treatment

<table>
<thead>
<tr>
<th>Teeth FDI</th>
<th>n</th>
<th>R</th>
<th>$R^2$</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 max 2 pds</td>
<td>50</td>
<td>0.389</td>
<td>0.363</td>
<td>Age = 102.46 − 100.481(15/25M) − 2.441(15/25W-L) + 54.517(32/42M) + 3.089(32/42W-L) − 268.438(33/43M) − 89.805(33/43W-L) − 41.009(34/44M) − 25.52(34/44W-L)</td>
<td>7.215</td>
</tr>
<tr>
<td>3 max 6 pds</td>
<td>50</td>
<td>0.425</td>
<td>0.344</td>
<td>Age = 114.631 + (−0.876(11/21M) + 2.218(11/21W-L)) + (−115.748(12/22M)) + (−38.699(12/22W-L)) + (−91.406(15/25M)) + (−20.963(15/25W-L))</td>
<td>7.319</td>
</tr>
<tr>
<td>3 md 6 pds</td>
<td>46</td>
<td>0.337</td>
<td>0.306</td>
<td>Age = 98.68 − 213.10(M) − 105.05(W-L)</td>
<td>9.632</td>
</tr>
<tr>
<td>3 md 6 pds</td>
<td>46</td>
<td>0.447</td>
<td>0.362</td>
<td>Age = 42.761(81.109(32/42M) + 1.905(32/42W-L) + (−111.346(33/43M) + (−173.252(33/43W-L) + (−151.769(34/44M) + (−43.162(34/44W-L)</td>
<td>9.232</td>
</tr>
<tr>
<td>6 teeth 2 pds</td>
<td>36</td>
<td>0.490</td>
<td>0.460</td>
<td>Age = 174.12 − 288.42(M) − 51.75(W-L)</td>
<td>7.372</td>
</tr>
<tr>
<td>6 teeth 12 pds</td>
<td>36</td>
<td>0.602</td>
<td>0.395</td>
<td>Age = 154.569 + 9.383(11/21M) + 19.718(11/21W-L) − 70.675(12/22M) − 39.284(12/22W-L) − 39.932(15/25M) − 4.881(15/25W-L) + 47.114(32/42M) − 17.071(32/42W-L) − 58.568(33/43M) − 29.183(33/43W-L) − 149.635(34/44M) − 0.842(34/44W-L)</td>
<td>7.803</td>
</tr>
</tbody>
</table>

$R^2$: Coefficient of determination. SEE: Standard error of estimation in years. See Table 1 for abbreviations.
investigations failed to disclose whether subjects had previously received orthodontic treatment. There are well-known biological changes that occur secondary to the application of orthodontic forces including apical root resorption and secondary dentin formation.

External root resorption is one unavoidable, unpredictable, and undesirable sequela [20-22] of orthodontic treatment. The reported frequency of external root resorption is about 100% when it is examined under microscopy. When it is quantified on periapical or panoramic radiographs, the frequency falls to 70%, with a mean reported value of root shortening of 1.42 ± 0.44 mm and 1% to 5% of the cases with a loss of 4 mm or one-third of root length [19]. In addition, maxillary teeth are more sensitive than mandibular teeth to root resorption. The most frequently affected teeth, according to severity, are the maxillary laterals, maxillary centrals, mandibular incisors, the distal root of mandibular first molars, second premolars, and maxillary second premolars [21,23], and all these teeth have been used in different methods for dental age estimation in adults [1,2,24]. In this study, a higher percentage of root resorption for upper teeth was also observed.

Confirming the findings of Karkhanis et al. [2], the length ratios (P, T, R, and L ratios) in this study have a non-significant correlation coefficient with age (P > 0.05), and the percentage of negative correlation coefficients [Table 1], closer to −1, is higher in the observed values after orthodontic treatment (23% before vs. 66% after). As the Kvaal et al. method is based on the negative correlation between age and the mentioned ratios, it is possible to observe that after the orthodontic treatment, this negative correlation is more evident but not statistically significant. In this study, there was no significant variation in the correlation coefficients of the calculated length ratios with age among the different teeth. Furthermore, as the Kvaal et al. method is based on length ratio calculations, not the linear measurements per se, it is possible that the effect of root shortening on the estimated ages had been diminished when the ratios are calculated.

In terms of secondary dentin formation owed to orthodontic treatment (a phenomenon that has not been as widely investigated as root resorption), there are reports of complete obliteration of the canals in maxillary incisors [25], and in one study analyzing, the tooth changes in terms of root length and pulp chamber within maxillary central incisors (tooth 11 and 21), statistically significant changes in the width of the pulp chamber at the midpoint of the dental root [26] were found, equivalent to the point C in the Kvaal et al. method. [1] Another study using cone-beam computer tomography (CBCT) in maxillary teeth found a statistically significant difference between the volumes before and after orthodontic treatment, with the highest mean volume loss observed in the upper left lateral incisor (3.86 mm³) and the least for the upper right central incisor (3.04 mm³) [7].

In this study, the correlation coefficients of the pulp/root width ratios (A, B, C, and W) maintained their negative correlation with age before and after the treatment, without showing the same variation observed for the length ratios. It would still be necessary to assess if the root shortening after orthodontic treatment, displaced the location of the reference points B and C toward the crown (where the pulp chamber is wider), and its relation with the observed variation of the SEE before and after orthodontic treatment, which in both cases is acceptable in forensic terms (SEE = ±10 years).

**CONCLUSION**

Our investigation demonstrated an alteration in tooth and pulp chamber morphology and dimension following orthodontic treatment. However, these changes did not serve to significantly influence the validity and accuracy (SEE) of the Kvaal et al. [1] method when applied in the assessment of panoramic radiographs to estimate chronological age. We recommend further analysis of other methods for dental age estimation in adults based on the formation of secondary dentin, such as Cameriere et al. [3], or the more recently proposed methods based on volumetric reconstructions of the tooth and pulp chamber in CBCT [27,28] which could be more susceptible to be affected by orthodontic treatment sequel.

**REFERENCES**

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APPENDIX 3. Orthodontic treatment: Real risk for dental age estimation in adults?

Orthodontic Treatment: Real Risk for Dental Age Estimation in Adults?

ABSTRACT: Dental age estimation becomes a challenge once the root formation is concluded. In living adults, one dental age indicator is the formation of secondary dentine, also associated with orthodontic treatment as well as root shortening. The aim of this study was to establish whether these secondary effects of orthodontic treatment could generate a statistically significant difference in dental age estimations when using Kvaal’s method. The study sample included 34 pairs of pre- and postorthodontic panoramic radiographs, from different individuals with exactly the same age and sex distribution. Females 65%, median age 17.5 years, and males 35%, median age 22.5 years, were included. After data collection, dental age was estimated per tooth using formulae previously published. The risk of obtaining over-estimation of age was calculated. (RR = 1.007). The changes caused by orthodontic treatment do not have any significant effect on age estimation when Kvaal et al.’s method is applied on panoramic radiographs.

KEYWORDS: forensic science, dental age estimation, secondary dentin formation, adults, orthodontic treatment, root resorption

The analysis of teeth for age estimation has been scientifically reported since the early 1800’s (1). Methods based on tooth formation in juveniles have shown high reliability (2–4). Once the root formation has finished (generally at the age of 14 years, excluding the third molar) (5), dental age estimation becomes a challenge, partly as a result greater variability in the development of third molar (6). The most reported noninvasive methods for dental age estimation are based on the formation of secondary dentine and the decrease in pulp chamber dimensions. These features are measurable in periapical radiographs (7,8), panoramic radiographs (9,10), micro-focus-computed tomographs (11), computed tomographs (12), and cone beam-computed tomographs (13). These methods proposed different formulae to be used in specific populations. An important characteristic of these studies is that in their analysis, they only included totally sound teeth.

It is well known that orthodontic forces generate irreversible changes on tooth structure, such as root shortening (14) and secondary dentine formation (15). These two biological changes in tooth structure may directly affect the features used for dental age estimation in adults, especially the method proposed by Kvaal et al (7). This method is based on the measurement of tooth/pulp length, root canal, and root width at different levels, followed by ratio calculations and linear regression analysis for dental age estimation. It would be expected that with the mentioned changes, secondary to orthodontic treatment, age estimation in participants’ postorthodontic treatment would show a higher estimation error and higher over-estimation, compared to that of nontreated participants. If that were to be the case, it would be necessary to develop specific standards for orthodontically treated participants, not only for the Kvaal et al.’s method (7), but for any method based on secondary dentine formation and the variation of pulp/tooth dimensions with age. In the event of the results being different to the expected, it would mean that the Kvaal’s method could be used despite the evidence of anatomic changes related to orthodontic treatment. The aim of this study was to establish whether orthodontic treatment would generate changes in dental age estimations at the tooth level when the Kvaal et al.’s (7) method is applied per individual tooth.

Materials and Methods

Ethics approval was obtained from the Human Research Ethics Committee of The University of Western Australia (Ref: RA/4/1/6797) prior to commencement.

Panoramic Radiographs, Orthodontics, and Dental Age Estimation

Initially, the Kvaal et al.’s (7) method was proposed to be used on periapical radiographs. However, recent studies have also applied this method on panoramic radiographs (9,10). Panoramic radiographs have more image distortion than periapical radiographs, but it has been reported that when root resorption associated with orthodontic treatment is measured on panoramic radiographs, it is significantly higher than when measured on periapical radiographs (16). This study presents the first
study to see the effect of orthodontic-related changes on age estimation, and therefore, no power calculation was achievable. However, based on the size of previous research assessing pulpal changes associated with orthodontic treatment (17), a sample size in a similar range was deemed appropriate.

Sample Selection

The study cohort consisted of a series of participants who consecutively presented for orthodontic treatment at a private specialist orthodontic clinic (SV). The initial sample for this study was 91 participants from a Western Australia population, who had a pre- and post-treatment panoramic radiograph. From the initial sample, a total of 34 pre- and 34 postorthodontic treatment panoramic radiographs were selected, resulting in a final sample of 34 pairs of panoramic radiographs (n = 68). Sample size reduction corresponded to the need of age and sex matching between the different individuals in each pair, one of them without orthodontic treatment and the other one with a concluded treatment. Female 65% (n = 22, for both groups), age range 15–50 years old, median 17.5. Male 35% (n = 12, for both groups) age range 16–37 years old, median 22.5. The minimum length treatment was 1.2 years, the maximum 3.6 years, and median 2.1 years.

Intra-observer calibration was performed to test the repeatability and reliability of the main observer (TM). All the measurements were completed by a single observer (TM), and the analysis was completed with Image J software (version 1.48 19 April 2014—National Institute of Health, Bethesda, Maryland). All data were collated using Excel (version 2013 Microsoft, Redmond, WA), and statistical analysis was completed using the program 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/).

Inclusion Criteria

All participants who had a recorded panoramic radiograph of high quality with respect to factors of image brightness, contrast, and sharpness were included. In addition, all teeth included in the analysis were clinically sound with completed root formation and in functional occlusion.

Exclusion Criteria

Radiographs with observable failings (image distortion, poor contrast, superposition of tooth structure, or improper positioning) were excluded. Teeth with pulpal or periodontal pathologies and endodontic treatment and/or restorations, incomplete root formation, dilacerations, or rotations were excluded. Teeth with developmental abnormalities in size, shape and tooth structure, or large areas of enamel overlap between neighboring teeth were also excluded in this study.

Teeth Analyzed

The purpose of this study was to assess the biologic variation effects of orthodontic treatment at the tooth level. Therefore, the tooth-by-tooth formulae for the Kvaal method were applied (10,18).

Following the parameters as provided by Kvaal et al. (7), three upper and three lower single-rooted teeth were included: one maxillary central incisor (with Federation Dentaire Internationale (FDI) notation 11 or 21), one maxillary lateral incisors (FDI notation 12 or 22), and one maxillary second premolar (FDI notation 15 or 25), mandibular lateral incisors (FDI notation 32 or 42), one mandibular canine tooth (FDI notation 33 or 43), and one first premolar teeth (FDI notation 34 or 44).

In accordance with the study by Karkhanis et al. (10), panoramic radiographs that presented any combination of the required teeth were included. There is no evidence claiming that the accuracy of the results had varied depending on the use of left or right teeth. Consequently, in the absence of one of the teeth the contralateral was used, as long as they met the inclusion criteria.

Measurements

All the panoramic radiographs were obtained in digital format, and the measurements were performed using the software Image J software (developed by the National Institute of Health, USA). According to Kvaal et al. (7), the following measurements were recorded: tooth, pulp, and root length, as well as the pulp and tooth width at three different levels: A (cemento–enamel junction on the mesial surface of the roots), B (midpoint between the points A and C), and C (midpoint between the cemento–enamel junction and root apex). All the measurements were recorded before the sample was categorized into two groups: group pre for panoramic radiographs obtained from nontreated participants and group post for treated participants, to avoid observation bias. Following the recording of measurements, a series of length and width ratios were calculated: tooth/root length; pulp/tooth length, and root width/pulp width ratio at levels A, B, and C. These ratio calculations were proposed by Kvaal et al. (7) as it reduces the effect of magnification and angulation inherent in most radiographs (7,19). The different mean values from the ratios were used to obtain age estimation predictors. (M: mean value all five ratios, W: mean value of width ratios at level B and C, L: mean value of length ratios P and R, W-L: difference between W-L) (7). The predictors M and W-L were later used to estimate the age of all the participants.

Five randomly selected panoramic radiographs from the final study sample were used to estimate intra-observer calibration. These panoramic radiographs were measured on five different days with at least one-day interval. With the aim to avoid the recall of the measurements and landmarks, five new panoramic radiographs were also measured on each occasion. Three estimates of precision were calculated to quantify intra-observer measurement error and precision: the technical error of measurement (TEM), relative technical error of measurement (rTEM), and coefficient of reliability (20).

Statistical Analysis

After the completion of the measurements, dental age estimations were calculated using the age estimation equations from individual teeth. In participants aged 30 years or older, the equations used were those reported by Karkhanis et al. (10) And for participants 29 years or younger, the used equations were obtained from a different group of nontreated participants (n = 74 aged 12–28 years). Both sets of equations were obtained from a Western Australian population.

Results

For the estimation of intra-observer error, the obtained values were within acceptable standards for all the measurements. The
intra-observer results were as follows: TEM < 1.0 (0.92), rTEM < 5% (2.99%), and R > 0.75 (0.95).

After age estimation per individual tooth, there were a total of 189 age estimates for group pre and 196 estimates for group post. The age estimates obtained were then compared between participants with exactly the same age and sex in group pre and group post. In this way, 183 pairs of age estimates were obtained and compared 1 to 1.

For 79% (n = 145) of the total observations, the fluctuation of the data was the same, regardless whether there was over- or under-estimation of the age. In terms of over-estimation, in 47% of these observations, the over-estimation was higher after the orthodontic treatment.

The fluctuation of the data with regard to the chronological age of the individual was analyzed per individual tooth (Table 1), showing that there was no significant difference in the percentages of over- and under-estimation between both groups (Pearson’s correlation coefficient r = 0.78).

Although the fluctuation of the data was the same in both groups, pre and post, there was a clear difference between the ages. It was observed that before the age of 25, the large majority of results showed a slight over-estimation of age which did not exceed the reported SEE. In contrast, the majority of age estimation data obtained from older participants showed under-estimation of the age that notably exceeded the reported SEE.

A contingency table (Table 2) was developed to test whether the secondary effects of orthodontic treatment were a real risk to produce over-estimation of the age when the Kvaal et al.’s method is applied. The relative risk calculated was RR = 1.0071 (low risk).

Discussion

The reliability of dental age estimation in adults, based on the formation of secondary dentine, has shown superior accuracy to other methods, based on the analysis of other age-related dental or osseous changes (8,10). However, teeth are subjected to different changes through life related to pathology as well as biological, chemical, or mechanical trauma or dental procedures.

It has been reported that orthodontic treatment causes mechanical trauma to the periodontal ligament and induces pulpal reactions (21). The most frequently reported side effect is external root resorption, a process characterized by the destruction of root structure (21), with the subsequent diminution of root length (mean reported value of 1.42 ± 0.44 mm) (22). Another side effect is the reduction in pulp chamber dimensions, owing to secondary dentine formation (15). With these changes, it was expected to obtain significant differences between group pre and post, with higher percentages of over-estimates of age in treated participants. The analysis of the obtained data facilitated the calculation of the potential risk of having over-estimation in participants after finishing the orthodontic treatment (Table 1). After calculating the incidence of over-estimation in groups pre and post, there was no evidence of association between over-estimation of age and orthodontic treatment (RR = 1.0071). However, it is necessary to mention that in this study none of the tested teeth showed signs of severe apical root resorption.

According to previous studies, it was found that maxillary teeth are more affected by root shortening due to orthodontic treatment, specially lateral and central incisors (23,24). In this study, they also presented the higher percentage of under-estimates of age for both groups, followed by the mandibular lateral incisor. In the case of under-estimation, it was observed in a higher percentage in lower canines for group pre and group post, 54% and 61%, respectively.

Previous studies using the Kvaal et al.’s method and her proposed formulae reported a constant under-estimation of age, from 18 to 20 years (19) up to 47.10 years (25). These studies did not use population-specific formulae. In this study, the formulae used were obtained from the same Western Australian population. There was a clear-cut line (24 years for both groups) from 18 to 20 years (19) up to 47.10 years (25). These studies did not use population-specific formulae. In this study, the formulae used were obtained from the same Western Australian population. There was a clear-cut line (24 years for both groups) from 18 to 20 years (19) up to 47.10 years (25). These studies did not use population-specific formulae. In this study, the formulae used were obtained from the same Western Australian population. There was a clear-cut line (24 years for both groups) from 18 to 20 years (19) up to 47.10 years (25). These studies did not use population-specific formulae. In this study, the formulae used were obtained from the same Western Australian population. There was a clear-cut line (24 years for both groups) from 18 to 20 years (19) up to 47.10 years (25). These studies did not use population-specific formulae. In this study, the formulae used were obtained from the same Western Australian population. There was a clear-cut line (24 years for both groups) from 18 to 20 years (19) up to 47.10 years (25). These studies did not use population-specific formulae. In this study, the formulae used were obtained from the same Western Australian population. There was a clear-cut line (24 years for both groups) from 18 to 20 years (19) up to 47.10 years (25).

As the main objective of this study was to establish whether orthodontic treatment would generate changes in dental age estimation, and as each type of tooth is affected by different degrees of severity, in this study we used previously published formulae for dental age estimation for individual teeth rather than per set of teeth or per individual. The use of Kvaal et al.’s (7) individual teeth formulae to estimate dental age has been reported on extracted teeth (27). In the same way, there are other methods based on the assessment of a single tooth per individual to
generate dental age estimation models with acceptable results in a forensic framework (5, 7, 13).

Conclusion

In our study, orthodontic treatment did not affect the final results when the Kvaal et al.’s method was used for dental age estimation. Although it has been previously established that, to use the pulp complex as a biomarker for general aging, the analyzed teeth have to fulfill the requirements of being in normal and functional occlusion, totally sound and free from dental procedures (7), the real effect of this conditions on methods based on the formation of secondary dentine, for dental age estimation, has not been tested. The results of our study allow forensic dentists to use the Kvaal et al.’s method in participants who have had previous orthodontic treatment, when there are no signs of severe apical root resorption.

References


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APPENDIX 4. Determining the effectiveness of adult measures of standardized age estimation on juveniles in a Western Australian population.

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

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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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The accurate approximation of chronological age, secondary to sex determination, is an integral factor in constructing a biological profile for forensic and anthropological purposes, including the confirmation of identification at times of mass disasters, crimes, accidents and of unknown remains2-4. 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 accurate6,7. 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
different populations, showing a mean difference between the chronological age and the dental age of around one year in different studies\textsuperscript{5,9,11}.

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 challenging\textsuperscript{12}, leading to the development of alternative methods of dental age estimation in adults. Bodecker\textsuperscript{13}, 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\textsuperscript{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 viable\textsuperscript{16}. The method developed by Kvaal et al.\textsuperscript{1} is a relatively non-invasive method and has been validated in diverse populations \textsuperscript{17–20}. Karkhanis et al.\textsuperscript{17} applied the Kvaal et al.\textsuperscript{1} method in a Western Australian population to develop age estimation standards with acceptable results in a forensic framework (±10 years). Originally, the Kvaal et al.\textsuperscript{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 years\textsuperscript{20}.

The aim of the present study is to validate the applicability of the Kvaal et al.\textsuperscript{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 \texttt{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 International (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 research¹,¹⁷,¹⁹ 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 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 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 Bonferroni 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.

<table>
<thead>
<tr>
<th>Tooth number (FDI numbering system)</th>
<th>Ratio 11/21</th>
<th>12/22</th>
<th>15/25</th>
<th>32/42</th>
<th>33/43</th>
<th>34/44</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P)</td>
<td>0.129(^{NS})</td>
<td>0.236 (^{NS})</td>
<td>0.133 (^{NS})</td>
<td>0.164 (^{NS})</td>
<td>0.455(^{*})</td>
<td>0.152 (^{NS})</td>
</tr>
<tr>
<td>(T)</td>
<td>0.041 (^{NS})</td>
<td>–0.071 (^{NS})</td>
<td>–0.002 (^{NS})</td>
<td>–0.130 (^{NS})</td>
<td>0.171 (^{NS})</td>
<td>–0.117 (^{NS})</td>
</tr>
<tr>
<td>(R)</td>
<td>0.287(^{*})</td>
<td>0.287(^{*})</td>
<td>0.151 (^{NS})</td>
<td>0.327(^{*})</td>
<td>0.279(^{*})</td>
<td>0.397(^{**})</td>
</tr>
<tr>
<td>(A)</td>
<td>–0.130 (^{NS})</td>
<td>–0.281(^{*})</td>
<td>–0.004 (^{NS})</td>
<td>–0.263(^{*})</td>
<td>–0.254 (^{NS})</td>
<td>–0.137 (^{NS})</td>
</tr>
<tr>
<td>(B)</td>
<td>–0.378(^{*})</td>
<td>–0.305(^{*})</td>
<td>–0.280(^{*})</td>
<td>–0.354(^{*})</td>
<td>–0.570(^{**})</td>
<td>–0.381(^{*})</td>
</tr>
<tr>
<td>(C)</td>
<td>–0.345(^{*})</td>
<td>–0.377(^{*})</td>
<td>0.009 (^{NS})</td>
<td>–0.088 (^{NS})</td>
<td>–0.605(^{**})</td>
<td>–0.504(^{**})</td>
</tr>
<tr>
<td>(M)</td>
<td>–0.049 (^{NS})</td>
<td>–0.202 (^{NS})</td>
<td>0.001 (^{NS})</td>
<td>–0.123 (^{NS})</td>
<td>–0.108 (^{NS})</td>
<td>–0.265(^{**})</td>
</tr>
<tr>
<td>(W)</td>
<td>–0.415(^{***})</td>
<td>–0.397(^{**})</td>
<td>–0.150 (^{NS})</td>
<td>–0.237(^{*})</td>
<td>–0.123 (^{NS})</td>
<td>–0.497(^{**})</td>
</tr>
<tr>
<td>(L)</td>
<td>0.151 (^{NS})</td>
<td>0.295(^{*})</td>
<td>–0.002 (^{NS})</td>
<td>0.255(^{*})</td>
<td>0.467(^{**})</td>
<td>0.297(^{*})</td>
</tr>
<tr>
<td>(W - L)</td>
<td>–0.310(^{*})</td>
<td>–0.421(^{**})</td>
<td>–0.052 (^{NS})</td>
<td>–0.360(^{NS})</td>
<td>–0.625(^{**})</td>
<td>–0.547(^{**})</td>
</tr>
</tbody>
</table>

\(^{*}\)= \(p<0.05\); \(^{**}\)= \(p<0.001\).

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) \(^1\).
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 precision (TEM 0.80, rTEM 2.37 and R 0.98).

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

<table>
<thead>
<tr>
<th>Tooth (FDI)</th>
<th>n</th>
<th>R</th>
<th>R²</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/21</td>
<td>69</td>
<td>0.152</td>
<td>0.1271</td>
<td>Age= 22.915 – 28.78 (M) – 22.376 (W – L)</td>
<td>4.344</td>
</tr>
<tr>
<td>12/22</td>
<td>67</td>
<td>0.197</td>
<td>0.1727</td>
<td>Age= 19.724 – 25.935 (M) – 23.631 (W – L)</td>
<td>4.258</td>
</tr>
<tr>
<td>15/25</td>
<td>68</td>
<td>0.003</td>
<td>-0.273</td>
<td>Age= 17.78 – 3.962 (M) – 2.204 (W – L)</td>
<td>4.536</td>
</tr>
<tr>
<td>32/42</td>
<td>71</td>
<td>0.131</td>
<td>0.106</td>
<td>Age= 4.644 – 5.363 (M) – 22.558 (W – L)</td>
<td>4.465</td>
</tr>
<tr>
<td>33/43</td>
<td>48</td>
<td>0.417</td>
<td>0.391</td>
<td>Age= 11.26 – 41.33 (M) – 86.06 (W – L)</td>
<td>3.708</td>
</tr>
<tr>
<td>34/44</td>
<td>71</td>
<td>0.327</td>
<td>0.307</td>
<td>Age= 10.513 – 27.075 (M) – 38.176 (W – L)</td>
<td>3.709</td>
</tr>
</tbody>
</table>

Note. *R², 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.

<table>
<thead>
<tr>
<th>Teeth (FDI)</th>
<th>n</th>
<th>R</th>
<th>R²</th>
<th>Equation</th>
<th>SEE ± years</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mx (2 pds)</td>
<td>60</td>
<td>0.154</td>
<td>0.125</td>
<td>Age= 28.702 – 46.338 (M) – 24.233 (W – L)</td>
<td>4.141</td>
</tr>
<tr>
<td>3 mx (6 pds)</td>
<td>60</td>
<td>0.237</td>
<td>0.150</td>
<td>Age= 24.394 – 30.751 (11/21 M) – 12.090 (11/21 W – L) – 29.2257 (12/22 M) – 12.894 (12/22 W – L) + 2.611 (15/25 M) – 0.631 (15/25 W – L)</td>
<td>4.08</td>
</tr>
<tr>
<td>3 mdb (2 pds)</td>
<td>45</td>
<td>0.409</td>
<td>0.381</td>
<td>Age= -27.44 + 10.48(M) – 63.82 (W – L)</td>
<td>3.648</td>
</tr>
<tr>
<td>3 mdb (6 pds)</td>
<td>45</td>
<td>0.539</td>
<td>0.466</td>
<td>Age= 1.894 + 13.428 (32/42 M) – 5.931 (32/42 W – L) – 51.067 (33/43 M) – 66.495 (33/43 W – L) – 5.114 (34/44 M) – 16.806 (34/44 W – L)</td>
<td>3.388</td>
</tr>
<tr>
<td>6 teeth (2 pds)</td>
<td>40</td>
<td>0.252</td>
<td>0.211</td>
<td>Age= 31.91 – 63.34 (M) – 63.34 (W – L)</td>
<td>4.138</td>
</tr>
</tbody>
</table>

*R², coefficient of determination. SEE, standard error of estimation in years. See Table 1 for abbreviations.
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 ± years). The most accurate age estimation model based on the analysis of individual tooth was for mandibular canines (SEE ± 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 ± 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 applications. 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. and Cameriere et al.

The Kvaal et al. 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 population and has shown significant results that are valid under forensic standards. Although the Kvaal et al. method was initially developed to estimate age in individuals over 20 years old, previous studies have tested this method in younger populations. The present study applied the Kvaal et al. method 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 ± 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.\textsuperscript{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 ± 3.388 years), in comparison with the study of Karkhanis et al.\textsuperscript{17}, where the most accurate model was for the combined analysis of the six teeth and 12 predictors (SEE ± 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 development\textsuperscript{19}. Some examples of previous studies amongst people under 30 years of age, using the Kvaal et al. method\textsuperscript{1} on panoramic radiographs, are: Erbudak et al.\textsuperscript{24} in a Turkish population, including in their sample 75 participants between 14 to 35 years of age, obtaining a SEE= ± 8.73 years at best, using the regression formulas of Paewinsky et al.\textsuperscript{25} and Kvaal et al.\textsuperscript{1}; Landa et al.\textsuperscript{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.\textsuperscript{1} and Paewinsky et al.\textsuperscript{25} and a standard deviation of 12.53 at best when the Kvaal et al.\textsuperscript{1} regression equation was used. The last example is the study of Meinl et al.\textsuperscript{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.\textsuperscript{1} regression models were applied, or a mean overestimation of 20.88 years when the Paewinsky et al.\textsuperscript{25} formula was applied. In contrast, the present study (n=74) shows that the Kvaal et al.\textsuperscript{1} method provides acceptable results\textsuperscript{2} 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 Senn\textsuperscript{10}. 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 population\textsuperscript{26}, 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 studies\textsuperscript{27}. The Kvaal et al.\textsuperscript{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.\textsuperscript{1} method was initially purported 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.\textsuperscript{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.

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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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References
APPENDIX 5. Application of the Kvaal method for adult dental age estimation using Cone Beam Computed Tomography (CBCT).

Application of the Kvaal method for adult dental age estimation using Cone Beam Computed Tomography (CBCT)

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1. Introduction

Currently, the need for developing more accurate and non-invasive methods for age estimation, as part of the identification of adult individuals in situations of forensic scenarios is increasing globally. Gustafson first proposed a method for dental age estimation in adults, since then, the analysis of dental changes in adults relative to chronological age has continued. The adult dentition is relatively resistant to environmental and chemical influences, and methods based on the assessment of teeth are considered advantageous for this reason. Furthermore, methods based on odontometric measurement of pulp and tooth structures from dental radiographs or tomographs are non-invasive/non-destructive and can be applied to both living or deceased individuals. Kvaal et al., proposed a method for age estimation in adults which has been tested in different populations around the world since 1995, but all reported a high standard error (±8.5 to ±13 years). The basis of this method is the analysis of the narrowing of the pulp chamber with age, which is observable and measurable in dental radiographs. However, there is scope for improvement in the accuracy of the age estimations.

With the emergence of computer tomography (CT) and cone beam computer tomography (CBCT), new methods based on volumetric reconstruction of tooth and pulp volume and ratio calculations have been proposed for dental age estimation in adults. However, those techniques have not provided better accuracy relative to the previously described Kvaal method, using dental radiographs. Moreover, pulp/tooth volume calculation is labour intensive, and can require the use of complex, and often costly computer software. Clearly, these more technical multi-
dimensional systems provide considerably more data, at a substantially more detailed level, and with a reduction in many of the complications of simple plane film images. The method described by Kvaal et al. was initially proposed, with measuring dimensions on periapical radiographs with a microscope and a pair of calligraphers. The applications of this method have been expanded to include panoramic radiographs using different analytical software. The purpose of the present study is to apply the Kvaal et al. method for dental age estimation using modern computer tomographic data from 3D digital systems. The null hypothesis is that the substantially increased quantity (and quality) of the data from these systems will provide greater accuracy of dental age estimation in adults.

2. Materials and methods

2.1. Sample

The study sample included a total of 101 tomographs obtained as part of normal treatment from the radiology department of the University of Keibangsaa Malaysia. Almost half (n = 47) were obtained with a Kodak device, reference K9000-3D (exposure parameters: scanning time: 10.8 s, 3–15 mA, 60–85 kVp, field of view FOV: 5.2 cm × 5.2 cm–5.5 cm to 5.5 cm, 180° rotation, slice thickness 0.076–0.2 mm), and 54 were obtained with an i-CAT device (Dental Imaging Technologies Corporation, i-CAT® FLX®) (exposure parameters: scanning time 4 s, 5 mA, 120 kVp, FOV: 16 cm × 16 cm, Slice thickness 0.3 mm–0.4 mm). Of these tomographs, 55% were from female (n = 55), and 45% were from male (n = 46) patients, with a median age of 31 years for both sexes (Table 1). All the images were received in DICOM format, and analysed using the Osirix® software package (OnDemand 3D software CyberMed Inc. Seoul, South Korea). The study sample included teeth with very small coronal restorations, as long as they did not compromise the pulp chamber and secondary caries was not present. Teeth with shape abnormalities, active caries, root canal treatment, prosthetic restorations, signs of pulp or periapical complications of simple plane radiographs, 55% were from female (n = 46) patients, with a median age of 31 years for both sexes.

2.2. Teeth analysed

Initially, Kvaal et al. proposed a set of linear measurements, including the length of the tooth, pulp and root, in addition to the width of the root and pulp chamber at different levels, in six different teeth: upper central and lateral incisors, and the second premolar; and the lower lateral incisor, canine and first premolar. In the present study, due to the unclear definition of the pulp chamber and tooth boundaries for the lower teeth (most likely related to their small dimensions) only upper teeth were included: central incisor, lateral incisor, canine, and second upper premolar (when possible). As the dental images are tomographs, it is possible to assess the teeth in sagittal and coronal views; accordingly, the root length, and pulp/tooth width measurements proposed by Kvaal et al. were acquired in the mesio-distal and bucco-lingual aspects of the teeth (see below). Significant differences between teeth of the left and right side of the maxilla have not been reported previously, and therefore in this study, teeth from either the left or right side was measured.

2.3. Measurements

As proposed by Kvaal et al., the mesio-distal measurements of the teeth (coronal view) were performed as follows: first, the level A, was located at the CEJ (cemento-enamel junction) on the mesial side of the tooth, then level C was located halfway between point A and the root apex, and finally point B was located halfway between point A and C. As this is the first study applying the Kvaal et al. method exclusively in tomographs, bucco-lingual measurements were also acquired (sagittal view). Point A was located at the vestibular CEJ, point C halfway between the vestibular CEJ and the root apex, and point B, halfway between point A and point C. In this way, there were six pulp/tooth width measurements per tooth. All the measurements were recorded by one observer (TYM) using the Osirix® software.

2.4. Statistical analysis

Intra-observer error was tested, four tomographs were randomly selected (two Kodak and two i-CAT) and all the measurements were recorded by one observer (TYM). Upper central, lateral and canine were measured on four separate occasions, with a minimum of one day between repetitions ensuring that the observer did not remember the previously recorded measurements. Measurement error was assessed by calculating the technical error of measurement (TEM), coefficient of reliability (R) and relative technical error of measurement (rTEM).

All data were collected in an Excel spreadsheet, 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/). Descriptive statistics were used to summarise the data (mean, standard deviation, and frequency distributions). Normality was also tested (skewness, kurtosis and Shapiro-Wilk) to establish whether parametric or non-parametric analysis was required. Pearson correlation coefficient (r) was calculated to assess the relationship between the dental ratios and age, for the data obtained from the Kodak CBCT and for the data obtained from i-CAT CBCT individually, and also when both data sets were combined. This was done with the aim of testing if the difference between the resolutions of both devices could cause any statistically significant differences (Table 2). Linear regression models were built with age designated as the dependant variable. Different age estimation equations were then formulated using different groupings of age predictors: 1) the tooth/pulp ratios at the levels A, B or C individually; 2) the average of the pulp/tooth ratios of all the teeth at the different levels (A, B or C) obtaining a formula for each level; 3) the average of the pulp/tooth ratios of the lateral (Lat) and central (Cent) upper incisor at the different levels (A, B and C); 4) the average of the pulp/tooth ratios of all the teeth at the different levels (A, B or C) obtaining a formula for each level; 5) the average of the pulp/tooth ratios of the lateral (Lat) and central (Cent) upper incisor at the different levels (A, B and C); 6) the average of the pulp/tooth ratios of all the teeth at the different levels (A, B and C) in the same formula (A + B + C); and 5) same as 4, but excluding the ratios obtained from the canines (Cent-Lat). A total of 17 linear regression models were thus generated per data set: sagittal (s) view (bucco-lingual measurements); coronal (c) view; and the average of both (sc). The accuracy of the models is

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Age and sex distribution for CBCT and i-CAT tomographs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>CBCT Female</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>n = 32</td>
</tr>
<tr>
<td>15–20</td>
<td>2</td>
</tr>
<tr>
<td>20–29</td>
<td>12</td>
</tr>
<tr>
<td>30–39</td>
<td>3</td>
</tr>
<tr>
<td>40–49</td>
<td>4</td>
</tr>
<tr>
<td>50–59</td>
<td>2</td>
</tr>
<tr>
<td>60–75</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>55</td>
</tr>
</tbody>
</table>
reported as the standard error of estimation (SEE)\textsuperscript{11,13}

3. Results

Intra-observer error results were considered to be within acceptable range. (TEM <1.0 and the coefficient of reliability R>0.80%), regardless the tomograph source (Kodak or i-CAT) or whether the measurements were recorded in the bucco-lingual or mesio-distal view of the teeth. In terms of the relative technical error of measurement (rTEM), 72% of the observations scored <5% and 28% had a rTEM < 10%. It was also observed that the accuracy of the observer was better for the bucco-lingual measurements (sagittal view of the teeth) regardless the source of the tomography.

Pearson correlation coefficient test (r) demonstrated a stronger correlation between the pulp/tooth ratios and age for the bucco-lingual measurements (sagittal (s)) than for the mesio-distal measurements (coronal view (c)) (Table 2). Although there were differences between the correlation coefficients from the Kodak and i-CAT CBCT’s, scatter-plots including the results of both sources do not show outliers associated with the specific use. There was a clear and significant negative correlation between age and pulp/tooth ratios in all the data sets. Correlation coefficient were calculated as follows: i) for the measurements obtained from the Kodak tomographs; ii) for the measurements recorded from the i-CAT tomographs; and iii) for the combination of both. The i-CAT tomographs showed the strongest correlation to age, especially for the upper central incisor (r = −0.65), followed by the combination of the Kodak & i-CAT data sets (r = −0.61). The correlation coefficients obtained from the Kodak, although statistically significant, were lower than that obtained from the i-CAT and combined Kodak & i-CAT (Table 2).

In relation to the accuracy of the different linear regression models, it was observed that all the estimates were over the threshold generally accepted for forensic application (SEE ± 10 years).\textsuperscript{14} However the SEE is reduced when both data-sets are combined into a linear regression model (Table 3). There was only one equation with an acceptable level of predictive accuracy (SEE < 10%). It was also observed that the accuracy of the tooth ratios at the level C from the lateral and central incisor in the coronal view. 11/21 As: pulp/tooth ratio at the level A from the central incisor in the sagittal view.

The most accurate linear regression models obtained from the individual analysis of the bucco-lingual measurements were as follows: the central incisor at level A (Age = 84.059 – 191.003 (11/21 As), \( r^2 = 0.37, \text{SEE} = ±12.84 \)), the average of the bucco-lingual measurements from the central incisor and lateral incisor at the level A, B and C (Age = 102.08 – 157.94 (As) – 73.0 (Bs) – 36.81 (Cs), \( r^2 = 0.39, \text{SEE} = ±12.73 \) years).

When both data-sets were combined (Table 3) the highest accuracy was achieved from: the average of the bucco-lingual (s) plus the average of the mesio-distal (c) measurements at level A, (SEE ± 12.17 years); the average of the bucco-lingual and mesio-distal measurements at level A from the central and lateral incisors, (SEE ±12.76); and the average of the bucco-lingual and mesio-distal measurements from the central and lateral incisors at level A, plus level B, plus level C, (SEE ±12.7). The linear regression models generated by the inclusion from only the mesio-distal (c) measurements did not result in an SEE lower than ±14 years, and therefore these results are not included.

4. Discussion

This is the first study testing the Kvaal et al. method exclusively in tomographs, and also the first one applying Kvaal et al.’s. pulp/tooth width ratio calculations in the bucco-lingual aspect of the teeth. Computer tomography is increasingly being used in dental practice and provides three-dimensional information about any area of interest, in a relatively quick and cost-effective manner.\textsuperscript{15} In this study it was expected that application of the Kvaal et al. method using CBCT diagnostic images would increase the array of methods available for the forensic profiling of living and deceased individuals. Until now, there has only been one other study proposing a similar method for adult dental age estimation, based on the assessment of the pulp/tooth bucco-lingual dimensions (area) in canines.\textsuperscript{16} Although the results of the latter study were satisfactory (Mean prediction error, ME ±2.8 years at best), this method required extracted teeth, and thus has restricted application in a forensic context. The use of tomographs overcomes issues related to the requirement for tooth extraction, and also permits the analysis of multiple teeth with less radiation exposure.

Although, in this study, the number of individuals was small in certain age intervals, there are well documented biological age related changes in the human dentition, which have allowed forensic dentists to propose and use different methods for dental age estimation.\textsuperscript{11} In previous publications applying the Kvaal et al. method for dental age estimation in adults, it has been noted that one of the principal limitations is the absence of a clear limit between the dentine and the pulp camber,\textsuperscript{11} which is observed as a
grey zone instead of a distinct line. This affects the accuracy of the measurements and intra/inter-observer agreement. In the present study it was observed that neither Kodak nor i-CAT tomographs were exempt from this issue. In certain cases, the border between the pulp chamber and dentine, or tooth and bone are also more blurred than in periapical radiographs. It is true that dental Kodak tomographs may have higher resolution than i-CAT tomographs. However, in the present study it was observed that the data obtained from the i-CAT had a higher correlation to age compared to the Kodak scans (Table 2). Similarly, the bucco-lingual width ratios had a higher correlation to age than the mesio-distal width ratios (with the latter measurable in periapical radiographs).

It has previously been reported that Kvaal et al. length measurements are both difficult to register and do not provide significant information to the final equation. It is important to note that in the tomographs analysed in the present study, it was even more difficult to observe the root apex compared to periapical radiographs. The only length measurement registered was the root length from the CEJ towards the apex to locate points A, B and C. In this way only pulp/tooth width and ratios were used as the only age predictors. The exclusive use of pulp/tooth ratios proposed by Kvaal et al. has previously been documented on panoramic radiographs, showing more accurate results than those obtained in this study. (SEE = ±10.02 years) than those obtained in this study. (SEE = ±10.58 years at best). In a previous study that analysed the odontometric age-related changes in different teeth, it was found that the rate of secondary dentine formation varied depending on tooth type: in canines the increase in coronal dentinal thickness was not as high as for the incisors and premolars. In the present study it was similarly observed that although the correlation coefficient between pulp/tooth ratios and age were significant, the exclusion of canine ratios when the linear regression models were generated, improved their accuracy.

On the other hand, this is also the first study documenting the inclusion of teeth with small crown fillings in the sample to generate linear regression formulae, in contrast to previous publications using the Kvaal et al. method, where one of the inclusion criteria was to use only totally clinically sound teeth. It was observed that there were no statistically significant differences caused by inclusion of these teeth. This may relate to the nature of the formation of tertiary dentine or reactionary dentine to external threats. It has been reported that tertiary dentine formation is highly dependent on the stimulus, and that caries mediators induce a focal dentine production, with a rate formation of about 3 μm per day.

Regression analysis, has been chosen for age estimation due to its simplicity. Nevertheless, it is necessary to note the limitations of the use of linear regression models for dental age estimation; for example, the higher standard deviations when the original formulae of Kvaal et al. are applied in populations of different ethnic backgrounds. The latter necessitates the need for population specific formulae. Also, it assumes that formation of secondary dentine is a linear process, when it is more similar to a curve.

Additionally, it was observed that, although using tomography result in obtaining more odontometric data from the same teeth (mesio-distal and bucco-lingual measurements), this apparent advantage over the use of dental radiographs, did not improve the final outcome. Also, the need to properly align the teeth in the sagittal and coronal plane demands more time than the traditional Kvaal et al. approach in dental radiographs.

Finally, it is necessary to mention that CBCT is not exempt of artefacts intrinsic to the process of image acquisition. This could explain, not only the high SEE obtained in this study, but also the poor accuracy of those methods based on pulp/tooth volume calculation, when compared with more simple approaches such as the measurement of pulp/tooth area on dental radiographs

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
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<tbody>
<tr>
<td>Linear regression analysis, linear regression formulae, correlation coefficient (r) between age and the ratios of bucco-lingual and mesio-distal width measurements from CBCT &amp; i-CAT.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sagittal and coronal</th>
<th>Age predictors</th>
<th>n</th>
<th>Linear regression formulae</th>
<th>r</th>
<th>r²</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/21 A sc</td>
<td>86</td>
<td>Age = 99.74 – 72.07/11Ac – 161.63(11As)</td>
<td>0.4</td>
<td>0.4</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>11/21 B sc</td>
<td>86</td>
<td>Age = 81.82 – 35.03/11Bc – 147.14(11As)</td>
<td>0.26</td>
<td>0.24</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>11/21 C sc</td>
<td>86</td>
<td>Age = 71.92 – 29.16/11Cc – 134.69(11Cs)</td>
<td>0.17</td>
<td>0.15</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>12/22 A sc</td>
<td>89</td>
<td>Age = 87.43 – 68.05/12Ac – 129.37(12As)</td>
<td>0.21</td>
<td>0.19</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>12/22 B sc</td>
<td>89</td>
<td>Age = 73.98 – 24.34/12Bc – 127.46(12Bs)</td>
<td>0.21</td>
<td>0.19</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>12/22 C sc</td>
<td>89</td>
<td>Age = 57.56 – 37.84/12Cc – 148.22(12 cc)</td>
<td>0.18</td>
<td>0.17</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>13/23 A sc</td>
<td>95</td>
<td>Age = 51.14 – 5.4/13Ac – 47.192(13As)</td>
<td>0.03</td>
<td>0.01</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>13/23 B sc</td>
<td>95</td>
<td>Age = 69.51 – 47.15/13Bc – 70.44(13Bs)</td>
<td>0.17</td>
<td>0.15</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>13/23 C sc</td>
<td>95</td>
<td>Age = 60.81 – 78.34/13Cc – 34.37(13Sc)</td>
<td>0.09</td>
<td>0.08</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>A sc</td>
<td>78</td>
<td>Age = 100.41 – 33.11(1Ac) – 206.40(1As)</td>
<td>0.34</td>
<td>0.33</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>B sc</td>
<td>78</td>
<td>Age = 89.22 – 20.54/1Bc – 178.29(1Bs)</td>
<td>0.31</td>
<td>0.29</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>C sc</td>
<td>78</td>
<td>Age = 69.46 + 16.44(1Cc) – 174.42(Sc)</td>
<td>0.17</td>
<td>0.15</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>A sc Cent/Lat</td>
<td>83</td>
<td>Age = 105.42 – 72.96/1Ac1/2 – 187.22(As1/2)</td>
<td>0.4</td>
<td>0.38</td>
<td>12.8</td>
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</tr>
<tr>
<td>B sc Cent/Lat</td>
<td>83</td>
<td>Age = 86.51 – 19.86/1Bc1/2 – 180.19(1B1/2)</td>
<td>0.29</td>
<td>0.27</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>C sc Cent/Lat</td>
<td>83</td>
<td>Age = 70.41 + 42.09(1Cc1/2) – 206.7(Cs1/2)</td>
<td>0.23</td>
<td>0.21</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Asc + Bsc + Csc</td>
<td>74</td>
<td>Age = 104.09 – 17.65/1Ac’ – 135.48(1As) – 38.18(1Bc) – 105.54(1Bs) – 40.87(1Cc) – 10.93(1Cs)</td>
<td>0.4</td>
<td>0.35</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Asc + Bsc + Csc Lat/Cent</td>
<td>82</td>
<td>Age = 106.25 – 180.14/1Ac1/2 – 135.83(1Bc1/2) + 51.73(1Cs1/2)</td>
<td>0.4</td>
<td>0.39</td>
<td>12.7</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Tooth number (11/22, 12/22 and 13/23) with Asc, Bsc or Csc (with the latter measurable in periapical radiographs).
sound dentitions.

Conflict of interest

None of the authors have any conflict of interest associated with this study.

Funding

None.

Ethics approval

Ethics approval for this study was obtained from the Human Research Ethics Committee of the University of Western Australia (Ref: RA/4/1/6797). Permission was also granted by the University of Keibangsaan Malaysia. The design of the study, data collection, and analyses have been performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki.

Acknowledgments

The authors would like to thank the department of radiology of the dental faculty of the University of Keibangsaan Malaysia, and to Dr. Shahida Mohd Said also to Ambika Flavel, from the Centre for the dental faculty of the University of Keibangsaan Malaysia.

References

APPENDIX 6. Reliability and repeatability of pulp volumetric reconstruction through three different volume calculations

Reliability and repeatability of pulp volume reconstruction through three different volume calculations

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The authors declare that they have no conflict of interest.

ABSTRACT
Objective: To test the variability of the volume measurements when different segmentation methods are applied in pulp volume reconstruction. Materials and methods: Osirix® and ITK-SNAP software were used. Different segmentation methods (Part A) and volume approaches (Part B) were tested in a sample of 21 dental CBCT’s from upper canines. Different combinations of the data set were also tested on one lower molar and one upper canine (Part C) to determine the variability of the results when automatic segmentation is performed. Results: Although the obtained results show correlation among them (r>0.75), there is no evidence that these methods are sensitive enough to detect small volume changes in structures such as the dental pulp canal (Part A and Part B). Automatic segmentation is highly susceptible to be affected by small variations in the setting parameters (Part C). Conclusions: Although the volumetric reconstruction and pulp/tooth volume ratio has not shown better results than methods based on dental radiographs, it is worth to persevere with the research in this area with new development in imaging techniques.

KEYWORDS: age estimation, pulp volume calculation, image segmentation.
INTRODUCTION
Since 1950 different methods have been proposed to estimate dental age in adults. Most of them are based on the formation of secondary dentine and the decrease of the pulp chamber size with age. Methods that have an invasive approach, (for example aspartic acid racemization and cementum annulation), are not of preference, as they often require the physical damage of the sample. With the evolution of different diagnostic imaging techniques, non-invasive methods for dental age estimation have become the preferred methods, starting with the use of periapical dental radiographs, then panoramic radiographs and more recently cone beam computed tomography (CBCT). Micro-Computer Tomography (µCT) was introduced in the early 20th century to determine age related three-dimensional changes in pulp cavities, from maxillary first premolar teeth. Consistent with early histological research, µCT study’s findings indicate that decrease in pulp volume is not linear. It demonstrates a quicker reduction between the 20 and 40 years and then it slows down thereafter. Further research correlating the ratio between pulp and tooth volume with the chronological age, using µCT and linear regression models to estimate dental age in adults, found promising outcomes. However, in light of the high radiation doses associated with µCT, only extracted teeth can be measured in this way. More recently CBCT has been used for dental age estimation calculating the volumes from tooth and pulp chamber. Since then, volumetric reconstruction from CBCT with different software have been reported in various studies for dental age estimation in adults. The most recent studies document the use of CBCT from single- and multi-rooted teeth. However, these new methods have not shown superior accuracy to the methods based on dental radiographs. It is evident that within each type of software there are a series of sensitivity settings that influences the mathematical approach to measure the baseline volumetric data. These settings clearly have consequences for the outcome and may well be in part the cause of the substantial variation. The primary aim of this study was to test the variability of the volume measurements when different segmentation methods were applied to pulp volume reconstruction. This study is designed to ensure that the underpinning assumptions of a commonly used age estimation approach based on pulp volume calculation are as accurate as possible.

MATERIALS AND METHODS
Ethics approval for this study was obtained from the Human Research Ethics Committee of the University of Western Australia (Ref: RA/4/1/6797). This study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Study sample
A total of 22 anonymized dental CBCT’s were used in this study. All of them were recorded with therapeutic purposes, with the consent of the participants, and there was no unnecessary or repeated radiation exposure. All the images were previously unidentified, and age and sex of each individual was the only known information. From these, 57% (n=12) were female, age range 17-63 years, mean age 33.7, and 43% (n=9) were male, age range=15-52 years, mean age 36.7. The CBCT’s were obtained from the radiology department of the University of Kebangsaan, Malaysia, and the research group INVIENDO from the National University of Colombia. In both cases the images were obtained using the 9000 3D Extraoral Imaging System (Carestream...
Dental, Atlanta, GA, USA) which had a 180° rotation and a field of view (FOV) of 50 mm by 37 mm. The radiation exposure parameters were 8-10 mA, 70 Kv, with a slice thickness of 0.076mm. The images were saved in DICOM format. The selected tooth to apply the different segmentation approaches was the upper right canine, or the left when the right was absent (with Federation Dentaire International (FDI) notation 13 and 23) reconstructing one tooth per subject. Only sound teeth, free of any restorations with completed apex formation were included.

Measurement software ITK-SNAP version 3.4 (open source software, www.itksnap.org) and Osirix® (OnDemand 3D software CyberMed Inc, Seoul, South Korea) were used in this study. All the segmentations were completed by a single observer (TYM). 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/.)

**Description of the method**
This study tested the comparative outcome of three different ways of estimating the pulp volume,\(^5,13,14\) dental age indicator used in adults. The tests were carried out on the root pulp chamber of upper canines (n=21), to calculate the correlation between automatic\(^14\) and manual segmentation\(^5\) (Part A)(Table 1) and cone shape approach volume calculation\(^13\) (Part B)(Table 2). The final part of the study (Part C)(Table 3) tested the variability of the results obtained with different setting parameter values combinations for automatic segmentation of the pulp canal of one multi-rooted tooth, as previously reported in the literature\(^14\) and one single rooted tooth. (Figure 1)

**Part A. Automatic and manual segmentation**
Automatic segmentation was performed using the software ITK-SNAP. (Figure 1. A). Manual segmentation used software Osirix® and its 2D viewer, (Figure 1. B) which allows the observer to go slice by slice and manually select the boundaries of the space to reconstruct, using the tool polygon. The automatic segmentation process in ITK-SNAP is called seed region-growing. To apply it, the observer sets up a “seed” inside the structure to reconstruct, in this case the pulp chamber. This seed grows and a volume is finally obtained.

Automatic segmentation of the pulp was generated using the same combination of setting parameters through the entire sample: Scale of Gaussian blurring: 3.0, edge transformation contrast: 0.2, edge transformation exponent: 4.0, expansion (balloon) force: 1.0, smoothing (curvature) force: 0.2, edge attraction (advection) force 2.0. In three cases the edge transformation exponent needed to be adjusted to 0.05.

Regression models were run to establish which of the setting parameters would have the most significant influence on the final results. Little changes in the Scale of Gaussian generated the bigger changes in the final obtained values (p< 0.001).

With manual segmentation two approaches were used. Firstly, every fourth slice was analysed as for the published method. Secondly, the first and last slice was used as the sample. To analyse the same length of root in the automatic and manual approaches, a length defined in the automatic approach was set for the manual approach.

**Part B. Cone shape geometric approximation**
The geometric approximation of the root canal to cone is a simple conservative method and has been used for dental age estimation in adults. Using Osirix®, ovals of best fit were formed in the root canal at the cemento-enamel junction (CEJ) level, following the reported method.\textsuperscript{13} Secondly, instead of best fit ovals, the boundaries of the pulp canal were drawn using the tool polygon. (Figure 1, C) The root length was measured from the CEJ to the apex, (Figure 1, D) and finally two different volumes of cones were calculated per tooth with a mathematical formula.

**Part C. Automatic segmentation and different setting parameters combinations**

As observed in part A, it is not possible to apply the same combination of setting parameters values to all teeth with automatic segmentation. Taking into account the advantage of this automatic process\textsuperscript{16} twenty combinations of different values for all the setting parameters (Table 3) were used to reconstruct the pulp chamber of one lower first molar (FDI 36, Male 23 year old Malaysian origin, slice thickness 0.2mm) and an upper canine (FDI 23, Male 31 year old Colombian origin). The volumetric reconstruction with each combination was completed twice per tooth. The variation among the obtained volume from the same tooth was calculated.

**Statistical analysis**

Shapiro Wilk normality test, mean, standard deviation (SD), paired \( t \)-Test and Pearson correlation coefficient, were calculated to compare the results obtained using the different segmentation methods (Part A), cone shape volume approach (Part B), and the obtained results when different parameters are used to do the automatic segmentation of the same tooth (Part C).

**Fig.1:** Volumetric reconstructions, upper right canine using different methods

*Volumetric reconstruction of tooth 13 from a female individual, 36 years old, using the different methodologies mentioned in this paper. A: Semiautomatic reconstruction using ITK-SNAP software. B: Manual segmentation using Osirix® software. C and D: Cone shape geometric approximation, following the methodology reported by the author doing area measurement of he root canal at the level of the CEJ (D) and length measurement of the root from the CEJ up to the root apex (C), using Osirix® software.*

**RESULTS**

**Part A. Automatic and manual segmentation**

Three data-sets were obtained from the volumetric reconstruction of the root canal.
of the canines using the different methods (Table 1).

When automatic and manual segmentation using each fourth slice were compared, the Pearson’s correlation coefficient ($r=0.83$), shows a greater correlation between them, than between automatic and manual segmentation using only the first and the last slice ($r=0.75$) or between the two manual segmentation methods ($r=0.79$). However, this does not mean that manual segmentation using every fourth slice and automatic segmentation produce comparable results. The paired $t$ test shows that there is a statistically significant difference between the means of the results of both manual segmentations and automatic segmentation ($p<0.001$).

When comparing the obtained volumes, it was observed that manual segmentation produced volumes that are larger than those obtained with automatic segmentation. To facilitate the understanding of this difference, a simple mathematical analysis was used, taking the obtained volume with automatic segmentation as 100% of the volume. Then the difference between the volumes obtained with automatic segmentation and both manual segmentation, were used in a rule of three to calculate the percentage this difference represents.

The results of this calculation showed that the volumes obtained using every fourth slice are in average 25% (1%-53%) larger than those obtained with automatic segmentation. Also, that using the first and the last slice are in average 57% (12% to 210%) larger than those obtained with automatic segmentation. The average of the obtained volumes from both manual segmentation methods is in general 41% larger than automatic segmentation.

The volumes obtained doing manual segmentation of each fourth slice were in average 28% larger than those obtained using only the first and the last slice. However, some of them were in average 16% smaller ($p>0.05$). The mathematical analysis was like the previously described, but in this case, the volume obtained with manual segmentation using only the first and last slice was taken as 100% in the rule of three.

**Part B. Cone shape geometric approximation**

It was expected to obtain similar area and volume values when using ovals of best fit and the tool polygon following the outline of the pulp chamber. Different than expected, it was observed that with the tool polygon the obtained areas and volumes were noticeably larger (50%) than with the ovals (Table 2). However, there is a strong correlation coefficient between both measured areas using the tools oval and polygon ($r=0.84$) at the CEJ and the obtained volumes ($r=0.88$).

When the obtained areas using the two different tools are compared the data show a big variability and a high SD, this effect is diminished once the volume is calculated. In this way, the length of the root is what really determines the final obtained volume.

**Part C. Automatic segmentation and different setting parameters combinations**

Repeatability of the automatic segmentation for the first and the second repetition for both teeth was evaluated with the paired $t$ test ($r=0.7, p=0.99$) showing that there is no statistically difference between both repetitions with a 95% level of confidence. The average between the obtained volumes in both
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repetitions was calculated. The volumes obtained for the molar pulp chamber (FDI 36), ranged from 31.7 mm$^3$ to 53.74 mm$^3$, with a SD of 5.7 and a variance of 33.

For the volumetric reconstruction of the canine (FDI 23), the volume value ranged between 29.8 mm$^3$ to 50.8 mm$^3$, SD=7.12.

Table 1. Volume results of using automatic segmentation (Vol 1), manual segmentation using just the first and last slice (Vol 2) and manual segmentation each forth slice (Vol 3), of cone beam computer tomography from upper canines (FDI 13, 23)

<table>
<thead>
<tr>
<th>Individual</th>
<th>Vol 1 (mm$^3$)</th>
<th>Vol 2 (mm$^3$)</th>
<th>Vol 3 (mm$^3$)</th>
</tr>
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<td>13.2</td>
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<tr>
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<tr>
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<td>7.1385</td>
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</tr>
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<tr>
<td>Mean</td>
<td>14.68</td>
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</table>

**DISCUSSION**

With the introduction of computer vision techniques for medical imaging and their application in dentistry and forensic sciences, the possibilities to propose methods for age and sex estimation are almost unlimited. Owed to the apposition of secondary dentine, the anthropometric assessment of the variation in the ratio between size of the pulp chamber and size
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Aim of the study:

The aim of this study was to test the variability of the volume measurements when different segmentation methods are applied in pulp volume reconstruction, because although it would be expected that with volume measurements the results for age estimation were more reliable, the literature shows the opposite. To find out an explanation to the lower accuracy of the methods based on volume reconstruction, in this study we tested three different methods doing only pulp volume reconstruction.

Table 2. Cone shape approach results using the tool polygon (Pol) and oval (Oval). Pulp area (in mm$^2$) at the CEJ level and volume (in mm$^3$) calculation with the measured root length (in mm).

<table>
<thead>
<tr>
<th>Area</th>
<th>Area Pol</th>
<th>Area Oval</th>
<th>Root length</th>
<th>Vol Pol</th>
<th>Vol Oval</th>
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</table>

The methodology analysed in this study, using CBCT, seems to be no more accurate than those methods based on radiographs for dental age estimation in adults, regardless the sample size of the study which ranges from 19 individuals, analysing only 12 canines$^7$ to 403 individuals analysing only the pulp chamber of first molars.$^{14}$ This is clear when the reported accuracy of the different
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methods is compared. The reported mean absolute error using automatic segmentation of the first molar’s pulp chamber is 6.26 years at best.\textsuperscript{14} The cone shape geometric approach of pulp and tooth has a standard error of estimation of ± 11.45 years,\textsuperscript{13} and finally, manual segmentation of pulp and tooth, has a prediction interval of ± 12 years.\textsuperscript{5}

Other methods also using automatic segmentation of canines reported a prediction interval of 3.47 years at best.\textsuperscript{17} When these reported results are compared with some of the most commonly used adult age estimation methods utilising dental radiographs, such as Cameriere et al.\textsuperscript{3} (with reported mean predictor errors of 2.37 years\textsuperscript{18} to 11.01 years\textsuperscript{19} (tooth/pulp area ratio)), or the method developed by Kvaal et al.\textsuperscript{2} (pulp/tooth-linear measurements ratios with reported standard estimation errors of ±9.367 years,\textsuperscript{20}) it is possible to observe that the

Table 3. Setting parameter combinations used in part C

<table>
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<tr>
<th>Repetition</th>
<th>Scale of Gaussian</th>
<th>Edge contrast</th>
<th>Edge transformation exponent</th>
<th>Smoothing force</th>
<th>Average molar volume mm(^3)</th>
<th>Average canine volume mm(^3)</th>
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<td>0.07</td>
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<td>0.35</td>
<td>0.005</td>
<td>32.94</td>
<td>50.75</td>
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</table>

There are 3 parameters available in ITK-SNAP software that are not listed, as they were constant for all the reconstructions: Edge attraction force (=2) and step size (=1) and smoothing force which was changed from 1 to 0.5 only for the 18\textsuperscript{th} combination.
use of CBCT and dental structures volume reconstruction, do not improve the final results for adult age estimation. The relation between the lack of the accuracy of the mentioned methods for age estimation and the different methods for volume reconstruction are presented as follow:

Part A. Manual and automatic segmentation may produce similar results, as observed in this study (r=0.83). Manual segmentation is time consuming, difficult to perform and prone to be influenced by the subjectivity of the observer, and personnel trained in this aspect is necessary. Additionally, it is not accurate enough to estimate the variation of the volume in small anatomic structures, such as the root canal, as observed in this study. Although there are different methods for automatic image segmentation, the first problem faced in the bio-medical area, is the exclusive dependence on gradient or intensity analysis without using anatomical information. In the case of automatic segmentation, with ITK-SNAP software, although the software chooses which pixels will be included in the segmentation, the observer finally decides how to adjust the parameters to control the algorithms in the segmentation, making this method prone to a certain degree of subjectivity from the observers. These parameters may change from one CBCT to another. Unfortunately, in the previous study using this software the values of the setting parameters were not mentioned. In this study it was also observed that although all the CBCT were obtained from machines with the same reference, and under similar exposure conditions (slice thickness, Kv, mA and time of exposure), and the same parameters for automatic segmentation, the interaction of the seed region-growing with the boundaries of the structure to reconstruct may also differ from one CBCT to another. For this reason, it is not possible to follow the recommendations of the ITK-SNAP manufacturer, who recommends to keep the same parameters among different CBCT, and it is neither possible to guarantee the uniformity of the segmentations obtained.

In this study, we also observe that small changes in the values of the setting parameters can generate significant variation in the final obtained volumes with automatic segmentation, even though the segmentation was made on the same CBCT, same tooth and same anatomic region.

Which means that the final volume will be always the same under the same parameters in the same CBCT and anatomic region, but these parameters generally need to be modified from one CBCT to another, and in the same way, small changes in the parameters alter the final volume.

Part B. With the aim of simplifying the measurement of the tooth structure volume, a cone shape geometric approach was proposed (Part B). The developers of this method were aware of the main disadvantages of this proposal: measurements inaccuracy and subjectivity. They also reported that this method tends to underestimate the real volume of the tooth structures (56% to 67%), which in theory would not affect the final pulp/tooth ratio that was calculated to estimate the age.

To test this method, we used two different approaches for doing the volume calculation. The first, following the published methodology displaying ovals
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at the CEJ, and a second one selecting manually the outline of the root canal at the CEJ, using the tool polygon. As observed in the results section, despite the difference between both area measurements, at the CEJ, once the volume is calculated the difference decreases. Neither of these two approaches is recommended to carry out pulp volume measurements, firstly because of the subjectivity of the observer, who only counts with the naked eye to display one or another and secondly, because both bring a distorted representation of the root canal, ignoring the different variations in its internal shape.

**Part C.** In this study, it has been shown, that there are different parameters that affect the final volumetric reconstruction of any anatomical region. The scale of Gaussian, or blurrier factor was found to be the most relevant when doing automatic segmentation. To increase the value of the Gaussian scale, allows the observer to eliminate certain noises in the image, which affect the “growing of the seed”. However, the bigger the value the Gaussian scale is, the more principal structures and details will be lost. This would mean that the initial volume of the pulp chamber will be magnified.

Although automatic segmentation is less prone to the subjectivity of the observer than manual segmentation, in this study we observed that it is not possible to keep the same volume reconstruction parameters for all the CBCT, and that minimum changes may produce significant variations even though they are done on the same tooth. The volume obtained with this tool must be understood as an approximation to the real volume of the structure being measured.

Taking into consideration the reported rate of secondary dentin formation is 6.5 μm/year for the crown and 10 μm/year for the root, the analysed methods in this study are not sensitive enough to detect such small dimension changes, generating a big variation of the measurements, which could explain the greater error of pulp/tooth volume based methods for age estimation when compared with simpler methods based on linear or area measurements on dental radiographs.

Previous research in the biomedical area have highlighted the problems related to semi-automatic segmentation, and specifically, to the process that the software uses to select which pixels or voxels to be included in the final volume reconstruction. To overcome this problem, they have elaborated the probabilistic anatomic atlas, which facilitates the segmentation of an organ of interest. When doing the semi-autographic segmentation of teeth and pulp chambers, different obstacles were observed. Firstly, owing to the similar density of dentine and bone and the irregularity of the periodontal ligament, it was not possible to obtain a volumetric reconstruction of the tooth as it is in for the pulp chamber. This is the reason why the automatic tooth volume reconstruction was not analysed. Secondly, owing to the large variety in the shape of the pulp chamber in the apical third of the tooth, there was always a leak of the seed region -growing in to the dentine. To create a probabilistic atlas for dentistry could help to overcome these issues, and help the dentist and forensic dentist to implement more accurate and easier methods for automatic segmentation and volume reconstruction. Thirdly, although the automatic reconstruction of the pulp chamber of molars may be understood as a simple process, it may be
CONCLUSIONS
Based on the results of this study, it is possible to affirm that the obtained volume measurements with any segmentation method must be understood as an approximation of the measured structure and not as the real volume. In the same way, manual segmentation could produce volume values that are even more inaccurate, and should be avoided in future studies.

Although the volumetric reconstruction and P/T volume ratio has not shown better results than methods based on dental radiographs, it is worth to persevere with the research in this area with an interdisciplinary team to build software that can reliably reconstruct pulp and tooth volumes for forensic purposes.

ACKNOWLEDGEMENTS
The authors of the study want to thank to Nurul Firdaus, Dr Shahida Mohd Said and Dr Shalini Kanagasiningam from the Radiology Unit, Dental Faculty University of Kebangsaan Malaysia. To the Forensic Science Centre from The University of Western Australia and to the research group INVIENDO from the National University of Colombia.

REFERENCES
Reliability and repeatability of pulp volume reconstruction through three different volume calculations.

Penaloza et al.


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APPENDIX 7. A new proposal to overtake population group differences for dental age estimation in adults through pulp/tooth volume calculation. Pilot Study

In press version. Under revision.

A new proposal to overtake population group differences for dental age estimation in adults through pulp/tooth volume calculation.

Abstract

Objectives.
The formation of secondary dentine on the walls of the root canal, has been used as an age indicator in adults. The aim of this study is to propose a model for dental age estimation in adults, regardless of their nationality or ethnicity by volume reconstruction of only a fraction of the tooth to obtain linear regression models and an equation for dental age estimation.

Materials and Methods
Eighty-one anonymized cone beam computed tomography images obtained from two different population groups were used. Only sex and age information was known. One group had a Latin-American background (Colombian individuals aged 23 to 71 years) and the other had an Asian background (Malaysian individuals aged 15 to 58 years) The analyzed tooth was the maxillary canine. The used software was ITK-SNAP version 3.4 (open source software, www.itksnap.org), to do automatic volume reconstruction of the cervical third of root and root canal.

Results
Analysis of the samples showed that the ages were unequally distributed in the two samples, but by combining them a more equal age distribution was obtained. The correlation coefficient between pulp/pulp+tooth volume ratio increased when data from individuals of both populations were included in the same statistical analysis ($R^2=0.42$). A formula for age estimation regardless the origin of the individual was proposed: $\text{Age}=67.104+(-434.741 \times \text{p/pt})$ (Standard error of estimation=±11.4 years)

Conclusion
It has been established that methods for age estimation must be population specific. This study presents an analysis that included data from individuals that are ethnically different and geographically separated, obtaining promising results.

Key words:Computed tomography, secondary dentine, age estimation, ethnic variation, Forensic Anthropology, Population Data
Introduction

Much has been said in biological sciences about race and ethnicity since 1775 when Johann Friedric Blumenbach defined five different races: Caucasians (whites), Mongolians (East Asians) Malayans (South Asians), Negroids (Black Africans) and Americans (First Nations) (1), since then, different studies followed this structure to analyze and interpret data and present results, phenomena that has been named as scientific racism (2). Owed to the different nomadic and migration processes in human history, this racial classification is day by day less accurate and practical (2). Nevertheless, in forensic sciences it is still stipulated that the ethnic background of an individual is of paramount relevance, to formulate a biological profile, and to clarify the identity of living or deceased individuals, next to sex, stature and age (3). In terms of age estimation, many methods have been proposed for individuals of different ages, based on bone and tooth formation or degenerative changes with age (4).

Methods founded on the radiological analysis of dental development, have shown acceptable accuracy, owed to the genetically controlled process of tooth formation, which seems to be similar for individuals from different group of populations (5). The most recent systematic review about age estimation based on tooth and bone formation (6,7) have stated that those studies claiming that there are differences among group of populations may be incurring on a selection bias inducing a phenomenon called age mimicry (6, 7), which has caused the erroneous need to generate specific population formulae for age estimation, fact that could be also affecting the results of studies for dental age estimation in adults.

Simultaneously, age estimation in adults, by means of non-invasive analysis, is still a challenge for scientists of different areas, specially, those who are involved in forensic investigations. Although different methods have been proposed, there is no consensus about a unique method to be applied to individuals from different countries, and even more, it has been claimed that methods for age estimation must be population specific (8). Additionally, the few changes that bones and teeth have after body maturation, are a setback for the accuracy of the existing methods.
One of the first methods for dental age estimation in adults, established that the formation of secondary dentine on the wall of the root canal could be considered an age predictor in adults (9). Based on the negative correlation of pulp/tooth ratio size with age, different non-invasive methods were proposed, doing first, linear measurements (10), then area measurements, in dental radiographs (11) and lastly volume measurements from dental tomography (12).

The aim of this study is to generate a model for dental age estimation in adults, regardless their nationality or ethnic background, by reconstructing only a fraction of the tooth to obtain linear regression models and an equation for age estimation.

**Materials and methods.**

The sample for this cohort study encompasses 81 cone beam computed tomography (CBCT) obtained from two different population groups. Latin-American (Colombian individuals aged 23 to 71 years, 60% female (n=23) and 40% male (n=15)) and Asian (Malaysian sample individuals aged 15 to 58 years 46% female (n=20) and 54% male (n=23)) previously collected for different dental diagnosis and treatment procedures, with the respective informed consent. All the images were anonymized keeping information about sex and age.

Table 1 shows the age and country distribution. Although the CBCT were obtained in different institutions, in both cases the reference of the used device was the same: Kodak device, reference K9000-3D (exposure parameters: scanning time: 10.8 s, 3-15 mA, 60-85kVp, field of view FOV: 5.2 cm x 5.2cm to 5.5cm to 5.5cm, 180° rotation, slice thickness 0.076-0.2 mm) The images were shared via online under the strictest security parameters to warrantee the integrity and safety of the data.

The analyzed tooth was the maxillary canine, left or right, one per individual, as previous studies have shown no right/left difference in tooth secondary dentine production (13). The
reconstructed root fraction was in all the cases 5 mm from the highest point of the vestibular cemento-enamel junction, towards the root apex, with the aim to include only the cervical third of the root.

Inclusion criteria: The included roots portions were from sound teeth, teeth with no evidence of physical trauma, free of active caries, free of evidence of periapical disease, without signs of pulp pathology and free of cervical lesion of any nature. The analysis also included teeth with small coronal fillings, with at least 2 mm of dentine between the tooth restoration and the pulp chamber, which is assumed to have negligible effect on the root portion.

The used software was ITK-SNAP version 3.4 (open source software, www.itksnap.org), doing automatic reconstruction of the root canal and root. In certain cases, it was necessary to manually correct the root segmentation. The measurements were collected by only one observer, who had been previously trained in the use of this software. All the data were collected in an excel spreadsheet, using Excel (version 2013 Microsoft Redmont, USA), before and after manual correction of the image segmentation. Data analysis was performed with 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

Pearson correlation coefficient test was calculated to observe if there was any difference between the volume values obtained for pulp and tooth doing automatic segmentation and after manual correction slide by slide. It was found that there is not significant difference between both measurements (r=0.98). Two different proceedings were tested to obtain the pup/tooth ratio. First dividing the volume of the pulp by the volume of the tooth, and second, dividing the volume of the pulp by the sum of the volume of the tooth and pulp. It was observed that the second approach generated a higher correlation coefficient (R²=0.34 vs R²=0.42). The determination coefficient between age and the pulp/pulp+tooth ratio (p/pt) for the individuals
of each group was $R^2=0.26$ for the Colombian sample and $R^2=0.38$ for the Malaysian sample and for the total sample using the data of both groups in the same analysis, $R^2=0.42$.

In regards to tooth condition, it was observed that those teeth with small fillings did not generate any outlier that could affect the linear regression model (Figure 1).

The linear regression models were generated to produce a formula for dental age estimation from the combined sample. The formula was:

$$\text{Age}=67.104+(-434.741 \times \text{p/pt})$$

where p/pt is the ratio between the volume of the pulp divided by the sum of the volume of pulp and tooth. The standard error of estimation (SEE) is ±11.4 years ($R^2=0.42$)

**Discussion**

It is undeniable that dentistry has done a valuable contribution to anthropological sciences bringing insights about human evolution. In the same way, dental analysis has become an important tool for human identification in forensic sciences, in both fields, anthropology and forensics, the concepts about racial and ethnic differences have deep roots and a significant influence in research (14). However, in a changing world, where day by day the biological boundaries between individuals of different backgrounds are constantly vanishing, to think about methods for age estimation, that are regionally, racially, or ethnically focused must be re-evaluated.

The last systematic review about Demirjian’s developmental stages of third molars (6) and the Greulich and Pyle’s bone atlas (7) for age estimation in children and adolescents, exposes a selection bias called *age mimicry*, present in numerous publications. This bias explains the observed results variation, which has been wrongly associated to the population origin and ethnicity of the participants, instead to the intrinsic characteristic of the used sample.
To avoid age mimicry, studies must have a study population with an even age distribution, large samples, same number of individuals in each age group, appropriate upper and lower age limits facilitating the observation of the different age related changes, and ideally samples that include data of individuals from different regions, to really conclude whether regional differences are important for age estimation in children (6) and probably in adults. Subsequently, it is necessary to perform multi-population studies in adults. This research, is a good start to disclose the population related differences in age estimation in adults when secondary dentine production is analyzed as an age predictor.

In this study, it was found that the production of secondary dentine and the narrowing of the root canal with age, which is one of the used parameters for dental age estimation in adults, has a similar behavior in individuals that are not only ethnically different but also geographically separated. Also, that the separate generation of regression models, for each one of the population groups, would have generated age mimicry, owed to the unequal age distribution of both samples, as in the Malaysian group the younger group dominates, while for the Colombian group the middle age dominates (Table 1). By adding both samples it is possible to obtain a more even age distribution and also a higher correlation coefficient between age and p/pt ratio ($R^2=0.42$). However, for future studies it is recommended to have a larger sample with the same age distribution for the different populations to analyse, and same number of individuals among the different age groups, avoiding age mimicry, which would generate more reliable results.

Up to now different methods and formulae have been published to estimate dental age in adults (15). Nevertheless, few studies have tested the proposed formulae in groups of population different from those included in the original studies. In the few studies that have done this analysis, it has been suggested that local formulae bring more accurate results. (16) With the results of the present study it could be possible to affirm that it is necessary to include the data of individuals of different populations with equal age distribution in the same statistical analysis.
to observe that there is not significant difference between population or ethничal groups, in regards to the production of secondary dentine, and also to generate an equation that can be used regardless the origin of the individual.

Furthermore, there are other factors that can affect the length, area and the total volume of the tooth, as bruxism and attrition (17), among others, that also affect the production of dentine in the crown and apical third of the root. These factors are not ethничally related but individual dependent. By analyzing sound cervical root third of canines, or any other teeth, the problems related with tooth attrition are diminished. In this study, the maxillary canine was selected as the tooth to analyze, owed to the reported longer survival, compared to other teeth (18). It was also selected to measure only the volume of the cervical third, as it has been reported that pulp/tooth ratios, had a higher correlation with age at the cervical root level and that the correlation with age decreased towards the apex, for different types of teeth, when doing linear (19) or volume (20) measurements.

In the first studies, that analyzed the production of secondary dentine with age, (9) the proposed methods required not only the use of extracted teeth but also the destruction of the sample, which is unethical in a modern context. The use of different radiological techniques has allowed researchers to analyze age indicators in a non-invasive and more conservative way. The use of CBCT to analyze and obtain the volume of any anatomical structure is much easier, cheaper, faster and has great potential for applications in anthropological and forensic studies. In this study, this radiological technique allowed the observer to separate only a fraction of the tooth, calculate its volume, and warrantee not only the integrity of the individual but also the uniformity of the tooth sample size.

In terms of accuracy, although the obtained error exceeds the threshold that must accomplish a method for age estimation in adults (±10 years) (21), the results of this study (SEE=±11.4 years)
are not so different to previous studies that used data of individuals from the same country or belonging to the ethnic group (±12.5 years at best). (22)

**Conclusion**

It has been claimed that the modern world would testify the end of ethnicity, and also that it changes according to circumstances, (14) This study proposes the use of data of individuals not only from different countries but that also have been historically cataloged as members of different racial and ethnical groups. It was found that by calculating the pulp/tooth+pulp volume of only one part of the tooth it is possible propose a formula to estimate the age for adult individuals regardless their origin.

**Ethics**

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

**References**


Figure 1. Scatter plot showing the correlation between age and the pulp/pulp+tooth (p/pt) volume ratio. (P/PT ratio) $R^2=0.42$. 
Table 1. Distribution of the individuals for age in years and country; Malaysia (Mal) and Colombia (Col)

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