Validation of Roberts' method using root canal width patterns as a mandibular maturity marker in determining the 18-year threshold.

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Abstract:

The South African Bill of Rights and the Children's Act defines a 'child' as a person under the age of 18 years. The age of 18 years is therefore significant for legal purposes in South Africa. The third molar is an important indicator in determining the age of 18-year threshold. Human biological growth markers are accepted indicators of a subject attaining the age of 18 years. A recent study suggested that the relative width of the distal root canals (RCW) of the lower left permanent molars (Fédération Dentaire Internationale notation 36, 37 and 38) as visualised on dental panoramic radiographs can be used as such a growth marker. This study aimed to validate this human biological growth marker in both black and white populations living in South Africa according to Roberts' method. The findings of this validation study were in agreement with this method which showed that individuals with Demirjian stage H left molars and category RCW-C were indeed over the 18-year threshold. Important aspects regarding the rationality and application of Roberts' method are discussed. The authors

conclude that this method should not be used in isolation but as an adjunct with other age estimation methods.

Keywords: Dental age estimation, root canal width patterns, 18-year threshold, forensic science.

Declarations

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Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Ethics approval

This study was approved by the Research Ethics Committee, Faculty of Health Sciences of the University of Pretoria. Protocol number 215/2018.

Consent to participate

Not applicable

Consent for publication

All authors consent for publication.

Availability of data and material (data transparency)

All data can be made available if required.

Author Contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Christy Davidson], [Chané Nel], [André Uys], [Herman Bernitz] and [Paul van Staden]. The first draft of the manuscript was written by [Christy Davidson] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Introduction

The South African Bill of Rights and the Children's Act defines a 'child' as a person under the age of 18 years [1]. In South Africa, an 18 year old individual has reached the critical age of criminal responsibility which has legal and social implications. It is of the utmost importance to assess whether an individual is younger or older than 18 years.

When ageing living individuals, a sufficiently accurate and peer-reviewed method is required [2]. Closure of the distal root apex of the third molar (Demirjians' stage H [3]), is frequently used to indicate that an individual is above the 18-year-old threshold [4-6]. Human Biological Growth Markers (HBGM) are accepted indicators in age estimation. Two examples of such indicators are the root pulp visibility (RPV) and periodontal ligament visibility (PLV) [7-9]. Studies on these indicators directed the identification of another HBGM, the relative widths of the distal root canals (RCW) [10]. In RCW, the lower left permanent molars (LL6, LL7, and LL8 [FDI dental notation 36, 37, and 38]) are assigned a category according to the pattern of the distal root canal widths as visualized on a panoramic radiograph. This RCW marker was proposed to indicate reliably if an individual is over the 18-year-old threshold. Since the validity of age estimation significantly depends on the classification system, the most appropriate and accurate method should be selected. The present work examines the validity, reliability and repeatability in using the RCW marker in both black and white populations living in South Africa.

Materials and Methods

Digital panoramic radiographs of 945 individuals of known age and sex were selected from the archives of the Diagnostic Imaging Unit, School of Dentistry, University of Pretoria, South Africa. The sample comprised of 467 white and 478 black South Africans aged between 16.00 and 23.99 years (Table 1). The sex and ancestry were divided according to their chronological age and each half-year age interval was calculated to two decimal points. For instance, the 16-year-olds included individuals ranging from 16.00 to 16.49 and from 16.50 to 16.99.

Individuals treated at the School of Dentistry included the entire socioeconomic spectrum. Allocation of ancestry was by self-classification. All panoramic radiographs formed part of the patients' routine dental treatment and no radiographs were exposed primarily for this research project.

Each radiograph was allocated a number to maintain anonymity and confidentiality. Radiographs were included if they met the following criteria:

- Lower left first, second and third molar (LL6, LL7 and LL8) were all present
- Distal root apices of all the third molars were staged as Demirjian stage H (apex closed)

Dental panoramic radiographs were excluded if:

- Any of the molars had a single root
- The area of interest could not be clearly visualised on the panoramic radiograph

Two dentists with experience in Maxillofacial and Oral Radiology evaluated the panoramic radiographs using Cliniview (10.1. Instrumentarium Dental) and Sidexis software (XG 2.63 2016 Sirona Dental Systems). All radiographs were assessed using a calibrated monitor. The images were magnified and adjusted to optimize the visual conditions. Prior to the assigning of RCW categories, the two investigators underwent a calibration of the method as described by Roberts et al [10]. Together the investigators assigned RCW patterns to ten random panoramic radiographs that were not part of the study sample. Thereafter the two investigators independently assigned RCW categories to 5 additional panoramic radiographs until standardisation was achieved.

The two investigators independently assigned a category to the pattern of the distal root canals according to the criteria described by Roberts *et al.* [10]:

- **Root canal width A (RCW-A)**: The mesio-distal width of the distal root canal of the LL6 was narrower than that of the LL7, which in turn was narrower than the width of the LL8 (LL6 < LL7 < LL8). (Fig 1. a)
- **Root canal width B (RCW-B)**: The mesio-distal width of the distal root canal of the LL6 was equal to that of the LL7, which in turn was narrower than the width of the LL8 (LL6 = LL7 < LL8). (Fig 1. b)
- **Root canal width C (RCW-C)**: The mesio-distal width of the distal root canal of the LL6 was equal to that of the LL7, which in turn was equal to that of the LL8 (LL6 = LL7 = LL8). (Fig 1. c)

Fig. 1 (a) represents RCW–A, **(b)** represents RCW-B and **(c)** represents RCW-C.

An additional category was added to the pattern of the distal root canals.

Root canal width U (RCW-U): This group included all the individuals that met the inclusion criteria but did not fall into one of the pattern categories as described by Roberts *et al.* [10]. The investigators recorded all the different root canal patterns of the lower left molars encountered within this category.

The statistical and graphical analyses were carried out with Wolfram Mathematica, Version 12.0.0.0 (Wolfram Research, Champaign, IL, USA). The between observer agreement (BOA) was determined by the two investigators independently reviewing all 945 radiographs. The within observer agreement (WOA) was determined by re-examining 98 radiographs (10%) after three months. Cohen's kappa coefficient was determined to assess both BOA and WOA.

This study was approved by the Ethics Committee of the University of Pretoria (Ethics number 215/2018).

Results

The BOA was 81.07% [Kappa 0.632] excluding the RCW-U category and 71.53 % [Kappa 0.576] including RCW-U. The WOA was 88.73% [Kappa 0.767] when RCW-U was excluded and 88.88% [Kappa 0.830] when RCW-U was included.

Table 1 demonstrates the means and standard deviations (SD), the minimum values (0%), first quartile (25%), median (50%), third quartile (75%) and maximum values (100%), and the probability of an individual being 18 years of age based on the RCW pattern. The probability of an individual being older than 18 years was 100% for all population and sex groups when RCW-C is considered (Table 1). The mean value refers to the mean age of each ancestry and sex group in each RCW category. When a 95% probability is considered, RCW-A, B and U for black males and RCW-A for white females and males were below the age threshold.

Table 1: Summary data of the RCW pattern for each ancestry and sex group.

Summary data for each of the grades of RCW per ancestry and sex group									
Black female	**n**	**Mean**	**SD**	**0%**	**25%**	**50%**	**75%**	**100%**	**P (Age ≥ 18)**
RCW-U	64	21.2099	1.7196	16.1644	19.9644	21.1973	22.6575	23.8904	0.9688
RCW-A	120	21.0466	1.7008	16.8658	19.6493	21.1534	22.5246	23.7890	0.9750

RCW-B	34	21.5747	1.8255	16.6858	20.7781	21.5014	23.2466	23.9096	0.9706
RCW-C	9	22.3686	1.6005	18.7233	21.8521	22.2849	23.5808	23.9178	1.0000
Subtotal	**227**								
Black male	**n**	**Mean**	**SD**	**0%**	**25%**	**50%**	**75%**	**100%**	**P (Age ≥ 18)**
RCW-U	74	21.2559	1.8354	17.2630	19.8301	21.4658	22.7945	23.8575	0.9459*
RCW-A	136	20.4569	1.8877	16.0384	19.0904	20.5123	21.9096	23.9589	0.8750*
RCW-B	28	20.7150	1.8625	17.4849	18.9836	20.4438	22.1421	23.9479	0.9286*
RCW-C	13	21.8808	1.4764	19.1616	20.7151	22.4563	22.7295	23.9781	1.0000
Subtotal	**251**								
White female	**n**	**Mean**	**SD**	**0%**	**25%**	**50%**	**75%**	**100%**	**P (Age ≥ 18)**
RCW-U	77	21.0861	1.7474	17.2137	19.6667	21.1123	22.4164	23.9808	0.9870
RCW-A	93	20.9491	1.8073	16.8438	19.5918	21.0164	22.5137	23.9344	0.9355*
RCW-B	32	21.1348	1.4334	17.0137	20.2959	21.0136	21.9616	23.8630	0.9688
RCW-C	20	22.3442	1.1518	19.6466	21.3798	22.3904	23.4000	23.8521	1.0000
Subtotal	**222**								
White male	**n**	**Mean**	**SD**	**0%**	**25%**	**50%**	**75%**	**100%**	**P (Age ≥ 18)**
RCW-U	124	21.4156	1.7077	16.4918	20.1507	21.6753	22.8219	23.9123	0.9597
RCW-A	89	20.0527	1.7389	17.1068	18.7616	19.7240	21.3579	23.9014	0.8764*
RCW-B	19	21.2903	1.764	18.2904	19.7049	21.6438	23.0329	23.4356	1.0000
RCW-C	13	21.2806	1.4721	18.4959	20.7104	21.4767	22.8247	23.1753	1.0000
Subtotal	**245**								
Grand total	**945**								

The number of individuals in each RCW category is indicated by n. SD is the standard deviation for each RCW category. 0% indicates the minimum age, 25%, 50% and 75% indicate the 25th percentile, 50th percentile (median age) and 75th percentile of the ages respectively, and 100% indicates the maximum age for each RCW category. * P (Age ≥ 18) < 0.95

Histograms of the age distributions for the four ancestry and sex groups are displayed in Figure 2. On each histogram, horizontal lines for the range of ages from the minimum age (on the left) to the

maximum age (on the right) are superimposed for all four RCW categories respectively. Furthermore, a vertical line at the 18-year threshold was drawn on each histogram to show the RCW categories within each ancestry and sex group which contained ages less than 18 years. The addition of the range of ages for the RCW categories as well as the vertical line at the 18-year threshold on each histogram enables comparison with the corresponding figures from Roberts *et al.* [10] (although they did not indicate maximum ages in their figures).

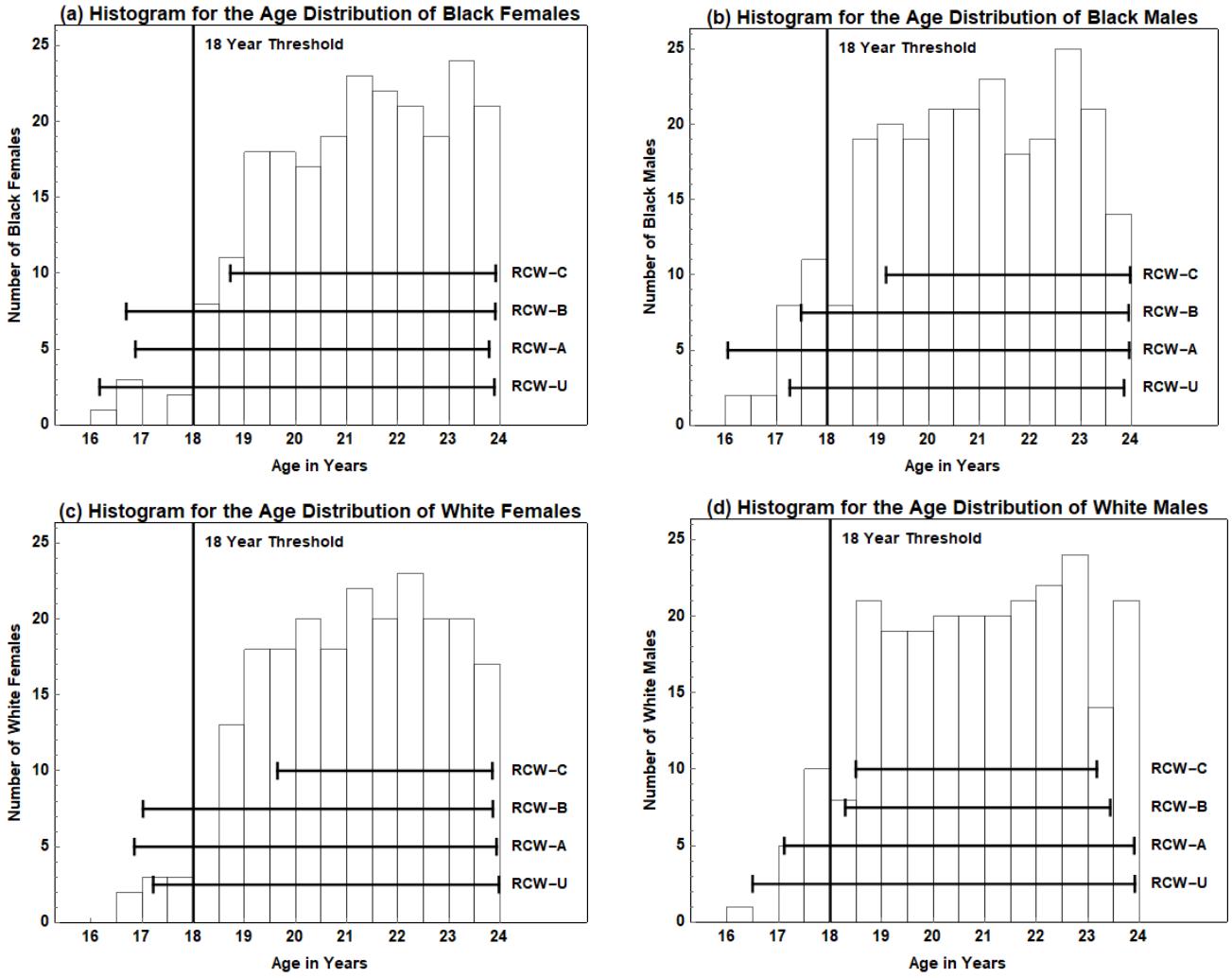
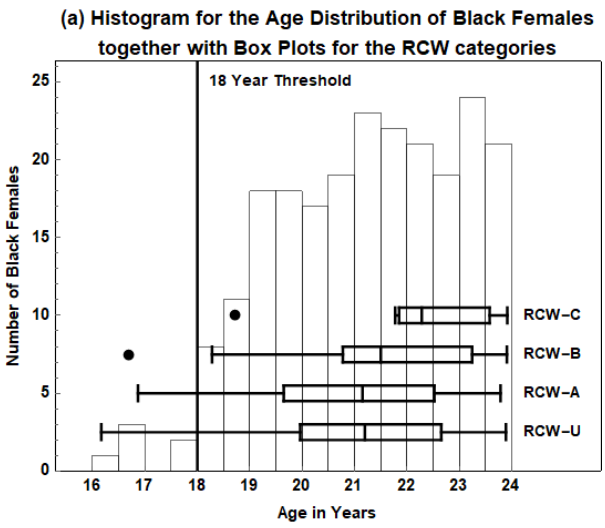
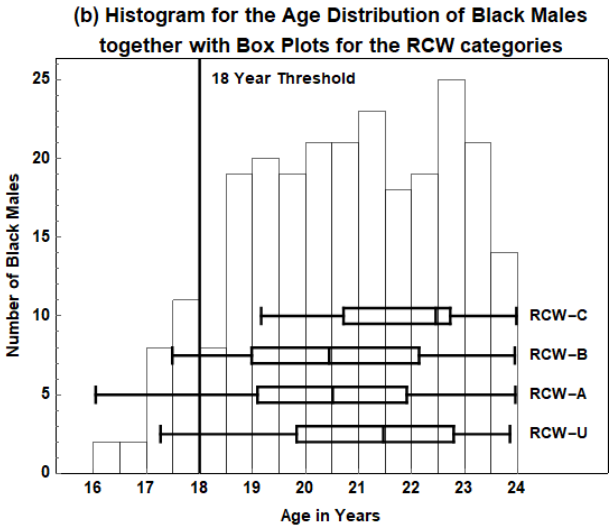


Fig. 2 (a-d) Histograms showing the age distributions for RCW-A, RCW-B, RCW-C and RCW-U for each ancestry and sex group. Horizontal lines indicating the range of ages from minimum age on the left to maximum age on the right are added on each histogram for each of the RCW categories respectively. A vertical line at the 18-year threshold is drawn on each histogram indicating individuals younger than 18 years.

For black and white females and for black males, the minimum age for RCW-A, RCW-B and RCW-U show that an individual with stage H in these categories could be under 18 years of age (Fig.2 a - c). However, the minimum age for RCW-B in white males was greater than the 18-year threshold indicating that these individuals were over the age of 18 years (Fig. 2 d). If RCW-C is present, the minimum age is greater than 18 years for all ancestry and sex groups.

Although Fig. 2 clearly indicates which RCW categories within each ancestry and sex group contain ages less than 18 years, the distribution of ages in each individual RCW category within each ancestry and sex group is not evident. Therefore the histograms of the age distributions for the four RCW groups are given in Fig. 3. Here box plots have been superimposed over the histograms to show, together with the minimum and maximum ages already presented in Fig. 2, the median age (50th percentile), the interquartile range for the ages (i.e., the range between the 25th and 75th percentiles) as well as the outliers (indicated by the dots). For black and white females, when the outliers were excluded, the ages of all the other individuals for RCW-B were found to be above the 18-year threshold (Fig. 3 a and c). The median ages for all RCW groups were above 18 years. For the additional RCW-U group all ancestry and sex groups showed a minimum age of below 18 years. (Fig. 3 a-d).





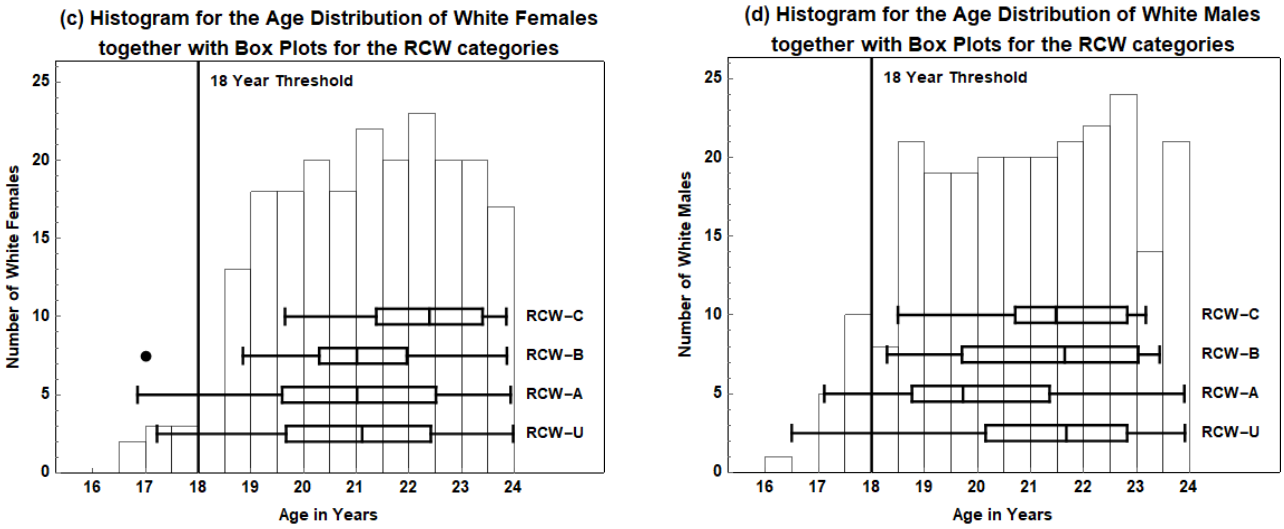


Fig. 3 (a-d) Histograms showing the age distribution of the four ancestry and sex groups. Box plots indicating the minimum age, the 25th percentile of the ages, the median age, the 75th percentile of the ages and the maximum age for RCW-A, RCW-B, RCW-C and RCW-U respectively were superimposed on the histograms. Where present in an RCW category, outliers are indicated by dots.

A total of 339/945 (35.87%) individuals were included in the RCW-U category. This category comprised of ten different RCW patterns that did not fall within the categories as described by Roberts *et al* [10] (Table 2).

Table 2: Frequencies, percentages[(a)] and medians[(b)] of the RCW-U patterns for each ancestry and sex group.

Frequencies, percentages[(a)] and medians[(b)] for each of the RCW-U patterns per ancestry and sex group										
	Black females		**Black males**		**White females**		**White males**		**All individuals**	
RCW-U pattern	**n (%)**	**Median**	**n (%)**	**Median**	**n (%)**	**Median**	**n (%)**	**Median**	**n (%)**	**Median**
LL6 < LL7 = LL8	41 (64.06)	20.5781	51 (68.92%)	21.4672	47 (61.04)	20.7896	69 (55.65)	20.9262	208 (61.36)	20.9425
LL6 < LL8 < LL7	11 (17.19)	22.2521	9 (12.16)	21.3014	8 (10.39)	22.5006	17 (13.71)	22.4877	45 (13.27)	22.2521
LL6 = LL8 < LL7	5 (7.81)	22.6575	4 (5.41)	–	11 (14.29)	22.4164	29 (23.39)	22.2213	49 (14.45)	22.4164
LL7 < LL6 < LL8	3 (4.69)	–	7 (9.46)	20.3014	4 (5.19)	–	2 (1.61)	–	16 (4.72)	20.8767
LL7 < LL6 = LL8	1 (1.56)	-–	0 (0.00)	–	3 (3.90)	–	0 (0.00)	–	4 (1.18)	–
LL8 < LL6 < LL7	2 (3.13)	–	3 (4.05)	–	2 (2.60)	–	5 (4.03)	22.0904	12 (3.54)	21.1658
LL8 < LL6 = LL7	1 (1.56)	–	0 (0.00)	–	0 (0.00)	–	1 (0.81)	–	2 (0.59)	–

LL7 < LL8 < LL6	0 (0.00)	–	0 (0.00)	–	0 (0.00)	–	1 (0.81)	–	1 (0.29)	–
LL7 = LL8 < LL6	0 (0.00)	–	0 (0.00)	–	1 (1.30)	–	0 (0.00)	–	1 (0.29)	–
LL8 < LL7 < LL6	0 (0.00)	–	0 (0.00)	–	1 (1.30)	–	0 (0.00)	–	1 (0.29)	–
Total	64	21.1973	74	21.4658	77	21.1123	124	21.6753	339	21.4645

(a) Percentages are calculated by 100 x n / Total, where n is the number of individuals. (b) Medians are only given where frequencies are 5 or more.

Discussion

Third molar development has been found to be a reliable method to determine the likelihood of being 18 years of age if the probability and 95% confidence interval are considered for Demirjian's stage H. [6]. Roberts *et al.* [10] further refined the method by introducing the concept of evaluating patterns of RCW of lower left molars in conjunction with using Demirjian stage H. The current study aimed to validate Roberts' method as a HBGM in a South African population.

The results of the validation study showed that all males and females from both ancestries with stage H left molars and category RCW-C were over the 18-year threshold. This is in agreement with the results published by Roberts *et al.* [10]. Forensic dentists giving expert witness in age estimation cases or writing age estimation reports can now confidently use the stage H plus RCW-C as a reliable HBGM indicator of the 18-year threshold. This validation study showed that categories RCW-U and RCW-A in all males and females from both ancestries could in fact be younger than 18 years. Additionally, for black males and females and for white females, RCW-B could also indicate an age under the 18-year threshold. In the study by Roberts *et al.* [10] they too showed that categories RCW-A and RCW-B in females and RCW–A in males could be younger than 18 years.

The validation study was able to improve on Roberts' method by the addition of category RCW-U where the patterns of root canal width do not fall into the categories of Roberts *et al*. [10]. Any individual falling within the RCW-U category, could be younger than 18. This forms an important finding, as expert witnesses in criminal cases involving age estimations are often asked if the possibility exists of the individual being younger than 18 with stage H root development. It is thus important to assess the HBGM's as a quality control measure in age estimation cases, taking into account the possibility of a stage H root development case having a category RCW-A, RCW-B or RCW-U patterns in the respective sex and ancestry groupings.

To draw conclusions between ancestry and sex groups the maximum age value was determined for each group. When stage H is used it must be kept in mind that the upper border is unbounded and that censored data for stage H will not be normally distributed [11,12]. The first quartile (25% percentile) showed an increase in age from RCW-A to RCW-B to RCW-C for white males and in white and black females. Black males did not demonstrate a similar pattern and the age decreased for the first quartile from RCW-A to RCW-B. The median ages differed between all ancestry and sex groups. The median age for RCW-B was higher than RCW-C for white males. The data showed population and sex variability for root canal width development when an RCW pattern is assigned.

The assessment criteria, as described by Roberts *et al.* [10], renders a visual comparison of the relative widths of the distal root canals of the lower left molars (LL6, LL7 and LL8). The pattern for each RCW category is based on the assumption that as age increases the mesiodistal diameter of the root canal decreases and a clear mesiodistal gradient is present for LL6, LL7 and LL8 [7,9]. Secondary dentine is secreted throughout life and results in the narrowing of the root canal chamber and canals with age [10]. The LL7 erupts later than the LL6 and therefore the LL7 canal can be predicted to be larger due to less secondary dentine deposition.

In this study, it was not possible to categorise all individual RCW to the patterns described by Roberts *et al*. [10]. In these cases, individuals were assigned to an additional category, RCW-U. This category comprised all of the other possible patterns for the width of the distal root canals (Table 2). More than a third of the total individuals (339/945, 35.87%) included in the study were placed in the RCW-U category. It must be noted that a large proportion (124/245, 50.61%) of the white males in this study, could not be classified according to the RCW categories of Roberts' method. Furthermore, 208/339 (61.36%) individuals in the RCW-U category presented with the RCW pattern LL6 < LL7 = LL8 (Fig.4). This suggests that an additional category should be considered when using the method described by Roberts *et al*. [10].

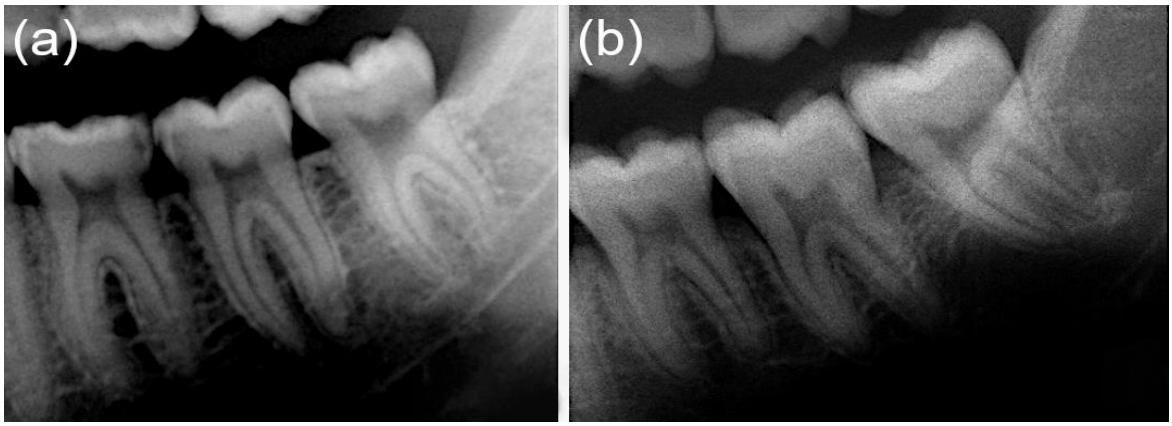


Fig. 4 Two examples (a and b) showing the RCW pattern LL6 < LL7 = LL8 which made up 61.36% of the RCW-U category.

Anatomical variations in tooth and root canal morphology need to be considered when using root canal widths as a maturity marker at the 18-year threshold. For example, the width of the distal root canal of a tooth with one large distal root cannot be compared with a tooth that has two separate distal roots and therefore two smaller distal canals. However, a 2007 study showed that 78% of second mandibular molars have two separate flat roots (one mesial and one distal), whilst 10% are C-shaped. [13]. Another study showed that 6 % of mandibular second molars had two canals, 54% three canals, 34% four canals, 3% a single canal and 3% were C-shaped [14]. Mandibular third molars commonly have three canals (two mesial canals and one distal canal) [15]. Two separate smaller root canals cannot always be visualised on a panoramic radiograph due to superimposition. Additionally, during panoramic radiographic acquisition, projection geometry and the focal trough can influence the image characteristics of the root canal width of the third molars. These factors might lead to the incorrect assignment of an RCW pattern and furthermore increase the difficulty in assigning a specific pattern according to the criteria by Roberts *et al*. [10].

The presence of dental anomalies such as taurodontism, would also affect the assignment of an RCW pattern. A South African based study found that although the prevalence of taurodontism in molars was low (0.73%), the mandibular second molars were the most commonly affected [16]. Additionally, individuals of Zulu and Khoisan ancestry were shown within their molar teeth to have the highest prevalence of taurodontism of their third molars as compared to the lowest prevalence of taurodontism in their first molars [17]. Both the anatomical variations of the roots and root canal morphologies would influence the assignment of a RCW pattern. These factors would negate the use of Roberts' method as a maturity marker [18].

Black South African males and females are considered to have advanced dental development with earlier eruption times [6,19]. The third molar was shown to erupt four years earlier in a South African population when compared with the London atlas [20]. Furthermore, additional factors such as craniofacial morphology, hormonal disturbances and systemic disease could all influence the rate of development and eruption of the teeth [21]. Population and sexual dimorphism differences could influence the use of this marker and should be considered when using this method in different population groups [22].

The presence of caries, attrition and dental restorations will influence the RCW pattern as an upregulation of dentine deposition will influence the root canal width. These factors were not mentioned as exclusion criteria in the study by Roberts *et al.* [23-25]. Conversely, if a tooth is necrotic, secondary dentine deposition would halt, also influencing the RCW pattern. As Roberts *et al.* [10] did not mention caries, attrition or dental restorations as an exclusion criterion in their study, we did not exclude such cases if all the other inclusion criteria were met.

Conclusion

The findings of this validation study were in agreement with the study done by Roberts *et al*. [10] who showed that individuals with stage H left molars and category RCW-C were indeed over the 18-year threshold. It also confirmed that stage H lower left molars with a RCW-A category in all males and females from both ancestries could in fact be younger than 18 years. The inclusion of category RCW-U further improved the discriminatory potential of this method. There are however certain problematic factors when analysing the dental panoramic radiographs which need to be addressed. The authors believe that the RCW method should not be used as a stand-alone technique but part of a battery of techniques when estimating age.

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