Age estimation from anterior cervical vertebral ring apophysis ossification in South Africans

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Abstract

Age estimation in living individuals around the age of 18 years remains a difficult challenge. In this study, the anterior inferior vertebral ring apophysis development of cervical vertebrae C2, C3, and C4 of 496 white and 478 black South African individuals aged between 15 and 22 years was assessed from cephalometric radiographs. Apophysis development was scored according to a four-stage scoring system. Ancestry and sex differences in apophysis maturation were assessed and likelihood values determined for individuals in each population group being 18 years, based on developmental stages. Regression equations were developed for each ancestry and sex group. The results indicated that the median ages for attainment of stages 0, 1, and 2 were below the 18-year threshold for all ancestry and sex groups. Additionally, WSA males and BSA females attained stage 3 for C2, and WSA females attained stage 3 for C2, C3, and C4, below the 18-year threshold. The presence of stage 0 for black and white males in all three observed vertebrae and stage 1 for black males for C2, C3, and C4, white females for C2 and C3, and white males for C4 indicates an age below 18 years (with a 95% or higher probability). The results indicate that anterior inferior apophysis ossification stages of C2, C3, and C4 can be used as a reliable indicator to determine the likelihood of being 18 years of age at a 95% confidence index level. Apophysis development provides a valuable addition to the methods that can be used to assess age in the adolescent years.

Keywords: Cervical vertebral ring apophysis ossification; Age estimation; Cephalometric radiographs

Introduction

Age estimation in living individuals is frequently required, and this estimation usually has to assess the probability that the individual has reached a specific age threshold. This is important for assessing age in unaccompanied minors, in criminal investigations, undocumented migrants, asylum seekers, and children involved in child labour [1]. The methods available for age estimation of living individuals around the critical age of 18 years are however limited. Methods assessing the development of tooth formation on radiographs are mostly used and compared with chosen developmental stages [2,3,4,5,6,7,8,9]. A

multifactorial approach is however recommended to give the most accurate assessment of age [10] and include combining skeletal and tooth development [11, 12]. The methods based on radiological assessment of dental and skeletal development should be used together to improve identification and diagnostic accuracy [12, 13].

Patterns of maturation of the dentition and skeleton tend to follow clear and distinct patterns for all populations. The rate of maturation, however, differs between ancestry groups and highlights the importance of population specific standards [5]. The rate of skeletal maturation is strongly associated with the socioeconomic status of a given population [14], but genetics also play a role.

Information regarding the maturation of the vertebral epiphyses is limited, and most anatomy and osteology text books merely state that vertebral ring epiphyses appear at puberty and complete their union by the age of 25 years [15, 16]. The vertebral ring apophysis is described as a thin cartilaginous mound that encircles the borders of the inferior and superior surfaces of the vertebrae. These rings develop outside of the epiphyseal plates [17]. The branching fibres of the longitudinal and intervertebral ligaments insert into the individual vertebrae at this site. Traction takes place at the point of insertion by these fibres. The development of the ring takes place within the peripheral osseous depression and cells concentrate within this area. The depression is only evident radiographically and histologically. Calcification of the circular ring takes place at about 6 years of age. Ossification of the ring begins at about age thirteen, and it is said to fuse with the vertebral body at about 17 years. In some studies, histological sections of the area showed that fusion was complete at age eighteen and that the ring could not be identified histologically at age twenty [17, 18]. As these vertebral rings fuse relatively late, they can provide valuable information regarding the age of young adults [19]. The fusion of the superior and inferior epiphyses of the thoracic and first lumbar vertebral centra, for example, has been shown to be practically usable to establish skeletal age in teenagers and young adults [19, 20].

Vertebral rings have been used in ageing young adult American males [21], and active union was found to occur between ages 19 and 21 years. Union was complete at age 24 years for all cases. This study was limited to males and ancestry differences were not mentioned [22]. Another study [19] found that females matured earlier than males for the thoracic and first two lumbar vertebral ring epiphyseal unions, but these differences were not statistically significant. These authors found that vertebral ring epiphyseal union was a good predictor of age in teenagers and young adults, and age could be calculated with 99.9% confidence within a range of $\pm$ 2.566 years. The cervical vertebrae were not included due to the internal and external damage to the neck region during autopsy [19].

Age estimation using stages of epiphyseal union in the presacral vertebrae was also assessed in a small sample of Portuguese individuals [23] aged 9 to 30 years. A three-stage scoring method was used to score the degree of fusion of the epiphyses: (1) no union, (2) partial union, and (3) completed union. For the cervical vertebrae C2, C3, and C4, stage 1 was present in individuals younger than 18 years of age. The age range for stage 2 was 14 to 21 years (C2), 14 to 21 years (C3), and 11 to 21 years (C4). Stage 3 for C2, C3, and C4 was only present in individuals older than 15 years. The sexes were pooled and no sex differences were observed for the cervical vertebrae. The conclusion was that data from vertebral ring fusion provide additional information that can be used in a variety of settings [23]. Other studies regarding the vertebrae, used skeletons, and the stages of epiphyseal ring union were not addressed [24, 25].

Cervical vertebral maturation has also been assessed to determine the connection with mandibular growth where the dimensional changes of cervical vertebrae (C2–C6) are related to peak mandibular growth [26,27,28,29]. It is also often used for routine assessment of skeletal maturation in orthodontic practice, along with radiographs of the wrist. However, their usability in estimating age especially around the critical age of 18 years has not been extensively explored. This is especially pertinent in the case of living individuals, where these changes are observable on radiographs.

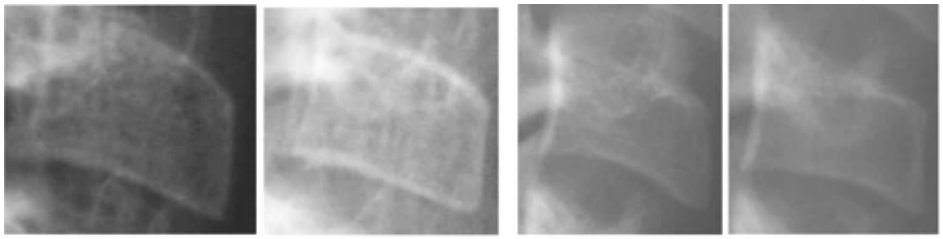
The aim of this study was to establish the relationship between the chronological age of South Africans and the timing of ossification and fusion of the anterior inferior vertebral ring apophysis of cervical vertebrae C1, C2, and C3. The likelihood of being 18 years of age at a specific stage of development was assessed, and differences between populations and sex groups were determined. Multiple regression equations to estimate age were also established for each population and sex group.

Materials and methods

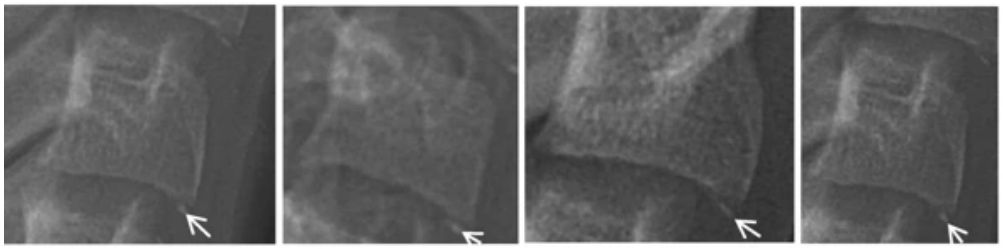
Cephalometric radiographs of 974 individuals with known age and sex were retrospectively selected using a quota sampling method. The cephalometric radiographs were obtained from the School of Dentistry, University of Pretoria, Sefako Makgatho Health Sciences University, and two private orthodontic practices situated in Pretoria, South Africa. All radiographs were taken between 2013 and 2018. The sample composed of 496 white individuals (WSA) (235 males and 261 females) and 478 black South African (BSA) (226 males and 252 females) individuals aged between 15 and 22 years (Table 1). The allocation of ancestry was made according to self-classification information present in the patient's hospital or practice records (Table 1). None of the cephalograms was specifically recorded for the purposes of this study, and all were sourced from patient files. Ethical clearance was obtained from the University of Pretoria, Faculty of Health Sciences (ethics reference number 263/2015).

Table 1 Age, ancestry, and sex distribution of the total sample (n= 974). Age 15 indicates all individuals aged 15.00–15.99 years, etc.

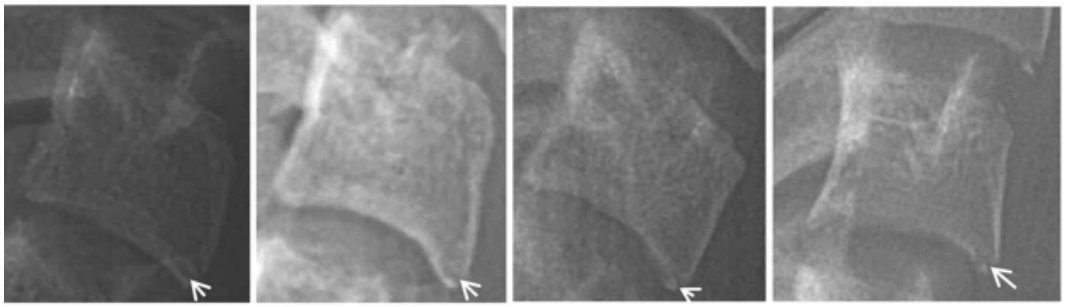
Age (last birthday)	Black South Africans		White South Africans	
	Males	Females	Males	Females
15	35	34	34	31
16	31	33	32	36
17	33	32	33	33
18	30	34	33	34
19	30	31	30	32
20	30	31	30	35
21	20	30	26	30
22	17	27	17	30
Total	226	252	235	261
Total	478		496	



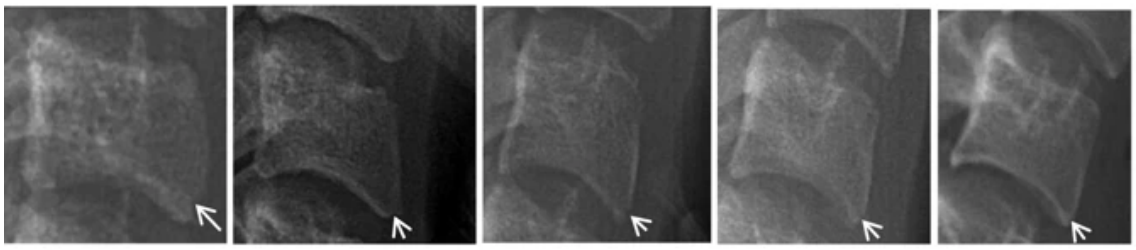
Stage 0- No ossification of the anterior inferior apophysis visible. The inferior surface of C2, C3 and C4 are flat or somewhat concave.



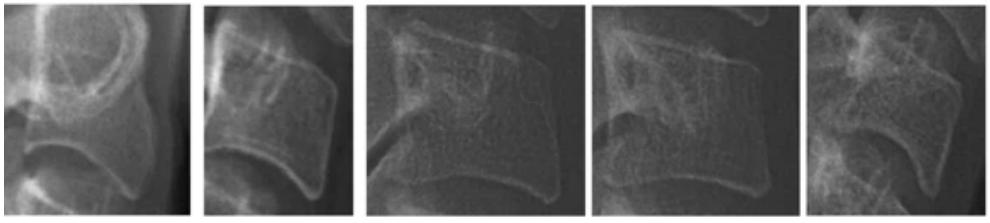
Stage 1- Ossification of the apophysis.No union between the ossification center and the inferior border of the veterbral body has taken place (indicated by the arrows).



Stage 2- The apophysis has begun to unite/fuse with the inferior vertebral body at the posterior end of the ossification center. A radiolucent opening/line is present between the ossification center and inferior vertebral body anterior(indicated by the arrows).



Stage 3- The apophysial ring has united with the vertebral body,but a notch is still present between the apophysis and the inferior vertebral body(indicated by the arrows).



Stage 4- Complete union with an intact and smooth cortical margin.

Fig. 1. Radiographic stages, progress, and description of the anterior inferior ring apophysis ossification of cervical vertebrae C2, C3, and C4

Exclusion criteria included the following: the presence of systemic diseases, presence of congenital anomalies, unclear cephalometric radiographs, and cephalometric radiographs that did not include C2, C3, and C4. The anterior inferior vertebral ring apophysis development of cervical vertebrae C2, C3, and C4 was scored according to a self-developed scoring system from stage "0" to "4." Scoring stages for the apophysis ossification and progress towards union were scored as follows, as also demonstrated in Fig. 1. Each individual vertebra was assessed according to the scoring system.

Scoring

- Stage 0—No ossification of the apophysis visible. A cervical vertebral maturation stage (CVMS) of I to IV is present [26]. The following features characterized stage 0: The inferior borders of all the cervical vertebrae are flat, or a slight concavity may be present at the inferior borders of C2 and C3. The superior borders are tapered from posterior to anterior.
- Stage 1—Ossification of the apophysis. No union between the ossification center and the inferior border of the vertebral body has taken place, but the apophysis is visible as a small radiodense area at the anterior border of the vertebral body.
- Stage 2—The apophysis has begun to unite/fuse with the inferior vertebral body at the posterior end of the ossification center. A radiolucent opening/line is present between the ossification center and the inferior vertebral body anteriorly.
- Stage 3—Union has taken place, but a notch is still present between the apophysis and the inferior vertebral body.
- Stage 4—Complete union with an intact and smooth cortical margin.

All the examinations were carried out by the first author (with more than 10 years of experience in Maxillofacial Radiology) who scored each vertebra separately and entered each value into an Excel spreadsheet. Fifty randomly selected cases were also reexamined by the second author (with more than 10 years of experience in Forensic Odontology) to determine the level of interexaminer reliability. The fist author calibrated the second author by presenting examples of each cervical vertebral stage until proficiency in identifying the apophysis stage was reached. Fleiss's kappa coefficient was determined to assess interobserver repeatability. Median, maximum, and minimum values, together with means and standard deviations, were calculated for ages at each stage of development for the different sex-ancestry groups. Since the residuals were not found to follow a normal distribution, a parametric one-way ANOVA was not suitable as indicated by the results of the Shapiro-Wilk test. Therefore, non-parametric tests were used. The Kruskal-Wallis rank sum test was used to determine if there were any statistically significant differences between the groups delineated by ancestry and sex. Dunn's test was then conducted to establish where the differences, if any, arose. The likelihood of an individual being at least 18 years at a specific apophysis stage was calculated. A similar method to Liversidge and Marsden [8] was used where the number of observed individuals, per category, older than age 18, was divided by the total number of observations in that category to establish probability estimates. The combined probability was estimated by the number of individuals who were classified jointly in both stages. The number of individuals older than 18 in that stage combination was then divided by the total number of individuals in that stage combination. Multiple linear regression analysis was considered for each population and sex group to determine age by using the stage classifications as independent variables. Straightforward linear regression fitted assumed that the stages are equally spaced and directly correlated to the magnitude of the numerical value associated with them. From each model, determination coefficients (R^2)

and root mean square errors (RMSE) were analysed. Alternatively, it was also assumed that the ordinal classification categories are independent of their numerical value and should therefore be coded. By creating dichotomous variables for each level of categorical variables as contrasted to the reference level (in this case, determined as the combined lowest levels for each category as observed or C2 stage = 1, C3 stage = 1, and C4 stage = 1), we derived alternative regression formulae for this dataset. Note that for males, the four observations with category zero classifications (2 for white males and 2 for black males) were excluded in this case. In the case of zero observations in practice, it should be assumed that the age is less than the intercept implied by the regression formula.

Results

The results for interobserver repeatability indicated that the agreement among raters exceeded what would be expected if all raters made their ratings completely randomly. Fleiss's kappa values for the stage classifications were 0.3730, 0.4090, and 0.5700 for C2, C3, and C4 respectively. The *p* value tested whether the estimated kappa was not due to chance and not the strength of the agreement. For stage C2, raters agreed in 60% of cases and differed by 1 stage in the remaining 40% of cases. For stage C3, raters agreed in 58% of cases, differed by 1 stage in 40% of cases and by more than 1 stage in 2% of cases. For stage 4, raters agreed in 70% of cases, differed by 1 stage in 26% of cases and by more than 1 stage in 4% of cases.

The data were separated into population and sex groups and further subdivided into each vertebra. Table 2 displays the mean and median ages of attainment for each stage of anterior inferior apophysis ossification for vertebrae C2, C3, and C4. The analysis began at stage 0. Stage 0 was only present in black and white males, demonstrating that the ossification of the apophysis has started in all females by age 15. Only one black male, aged 15 years, was still in stage 0 for C2, C3, and C4. One black male, aged 16 years, was also still in stage 0 for all three vertebrae. In white males, aged 15 years, stage 0 was achieved in one individual only for C2 and in two individuals for C3and C4. The median ages of attainment for stage 1 for BSA males were lower compared with WSA males. For stages 2, 3, and 4, the average ages were lower for WSA males relative to those of BSA males, suggesting that white South African males mature earlier than their black South African counterparts. All stages, except stage 2 for C2, were reached earlier in WSA females compared with BSA females. The largest difference between BSA and WSA males was for stage 1 for C2, with a 0.81-year difference and stage 3 for C4 with a 0.65-year difference. The largest difference between BSA and WSA females was for stage 3 for C3, with a 0.8-year difference, and stage 3 for C4, with a 0.96-year difference. Anterior inferior apophysis development of C2, C3, and C4 did not exceed a 1-year difference for any developmental stage between BSA and WSA males or for BSA and WSA females. Dunn's test was performed to investigate ancestry differences. Statistically significant differences were found between BSA- and WSA males for stage 1 for C2, stage 3 for C3. No statistically significant differences were found when BSA females were compared with WSA females. Larger median age differences were noted between males and females from the same ancestry compared with sexes from different ancestries.

The median ages for attainment of stages 0, 1, and 2 were below the 18-year threshold for all ancestry and sex groups. Additionally, WSA males and BSA females attained stage 3 for C2, and WSA females attained stage 3 for C2, C3, and C4 below the 18-year threshold. The maximum ages for attainment of stage 0 and stage 1 were below the 18-year threshold for BSA males for all vertebrae. White South African females also had a maximum age of

Table 2 Median, mean, minimum, and maximum ages and standard deviations (SD) of anterior inferior apophysis development at the given stages for Black South African (BSA) and White South African (WSA) individuals

Stages		0			1			2			3			4		
Vertebra		C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4
Males																
BSA	Mean	15.59	15.59	15.59	15.53	15.68	15.80	16.74	16.85	17.07	18.08	19.20	19.57	20.25	20.64	20.62
	Median	15.59	15.59	15.59	15.28	15.45	15.73	16.60	16.61	17.10	18.17	19.22	19.67	20.44	20.77	20.77
	SD	0.72	0.72	0.72	0.72	0.76	0.74	1.08	1.16	1.24	1.66	1.42	1.60	1.78	1.59	1.53
	LCI 95%	− 6.02	− 6.02	− 6.02	7.8	9.22	9.75	12.27	13.12	13.45	13.52	14.17	13.82	16.18	15.84	15.76
	UCI 95%	37.2	37.2	37.2	23.26	22.13	21.84	21.21	20.59	20.68	22.64	24.23	25.32	24.32	25.44	25.48
	Min	15.08	15.08	15.08	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.27	15.00	15.27	16.32	16.59
	Max	16.10	16.10	16.10	17.76	17.76	17.76	19.84	19.85	19.85	21.94	22.43	22.82	22.96	22.96	22.96
WSA	Mean	15.18	15.39	15.39	16.31	16.01	16.14	16.50	16.89	17.02	17.89	18.42	18.98	20.24	20.70	20.79
	Median	15.18	15.39	15.39	16.09	15.81	15.98	16.42	16.95	16.97	17.76	18.84	19.02	20.56	20.92	21.01
	SD	0	0.29	0.29	1.05	0.98	0.93	1.17	1.08	1.22	1.50	1.56	1.39	1.68	1.39	1.42
	LCI 95%	− 14.57	− 5.94	− 5.94	10.82	10.54	11.31	10.81	12.19	12.49	13.24	13.76	14.13	16.47	16.35	16.16
	UCI 95%	44.93	36.71	36.71	21.8	21.49	20.98	22.19	21.59	21.55	22.54	23.07	23.83	24.01	25.05	25.42
	Min	15.18	15.18	15.18	15.00	15.00	15.00	15.00	15.02	15.00	15.17	15.17	15.17	16.01	16.51	16.27
	Max	15.18	15.59	15.59	18.43	18.43	18.43	19.18	19.77	19.92	20.85	21.76	21.76	22.92	22.92	22.92
Median difference in months (years)					− 9.72 (− 0.81*)	− 4.32 (− 0.36)	− 3.00 (− 0.25)	2.16 (0.18)	− 4.08 (− 0.34)	1.56 (0.13)	4.92 (0.41)	4.56 (0.38*)	7.80 (0.65)	1.44 (− 0.12)	1.8 (− 0.15)	2.88 (− 0.24)
Females																
BSA	Mean				16.61	16.40	16.49	16.47	16.73	16.81	18.05	18.17	18.74	19.75	20.05	20.12
	Median				16.51	16.36	16.17	16.03	16.44	16.59	17.76	18.31	18.48	19.89	20.29	20.33
	SD				0.77	0.97	1.5	1.42	1.31	1.35	1.73	1.82	1.98	2.05	1.95	1.88
	LCI 95%				6.85	8.12	9.03	11.5	12.12	12.46	12.47	12.99	13.82	16.69	16.69	16.54
	UCI 95%				26.37	24.68	23.96	21.43	21.33	21.15	23.62	23.35	23.67	22.81	23.41	23.70
	Min				15.58	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.02	15.00
	Max				18.51	18.51	21.6	21.6	21.6	20.36	21.58	21.61	22.59	22.93	22.93	22.93
WSA	Mean				16.06	16.08	16.33	16.63	16.4	16.39	17.52	17.79	18.04	19.49	19.9	20.04
	Median				16.17	15.94	16.14	16.52	16.18	16.23	17.33	17.51	17.52	19.77	20.06	20.14
	SD				0.81	0.72	0.95	1.12	1.19	1.07	1.72	1.78	1.82	2.2	1.94	1.88
	LCI 95%				6.5	9.26	9.87	9.56	10.88	11.36	10.73	11.19	11.43	16.78	16.97	17.01
	UCI 95%				25.61	22.89	22.78	23.7	21.92	21.42	24.32	24.4	24.65	22.21	22.83	23.06
	Min				15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.67	15.00	15.43	15.00
	Max				17.18	17.76	18.00	19.36	19.68	19.68	21.76	21.92	21.92	22.92	22.92	22.92
Median difference in months (years)					4.08 (0.34)	5.04 (0.42)	0.36 (0.03)	5.88 (− 0.49)	3.12 (0.26)	4.32 (0.36)	5.16 (0.43)	9.6 (0.8)	11.52 (0.96)	1.44 (0.12)	2.76 (0.23)	2.28 (0.19)

* Statistically significant at $p < 0.05$

Table 3 Median ages and median difference comparison of anterior inferior apophysis ossification at the given stages of development for males and females

Stages	0			1			2			3			4		
Vertebra	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4
BSA															
Males	15.59	15.59	15.59	15.28	15.45	15.73	16.60	16.61	17.10	18.17	19.22	19.67	20.44	20.77	20.77
Females				16.51	16.36	16.17	16.03	16.44	16.59	17.76	18.31	18.48	19.89	20.29	20.33
Median difference in months(years)				− 14.76 (− 1.23*)	− 10.92 (− 0.91)	− 5.28 (− 0.44)	6.84 (0.57)	2.04 (0.17)	6.12 (0.51)	4.92 (0.41)	10.92 (0.91*)	14.28 (1.19*)	6.60 (0.55)	5.76 (0.48)	5.28 (0.44)
WSA															
Males	15.18	15.39	15.39	16.09	15.81	15.98	16.42	16.95	16.97	17.76	18.84	19.02	20.56	20.92	21.01
Females				16.17	15.94	16.14	16.52	16.18	16.23	17.33	17.51	17.52	19.77	20.06	20.14
Median difference in months (years)				− 0.96 (− 0.08)	− 1.56 (− 0.13)	− 1.92 (− 0.16)	− 1.2 (− 0.1)	9.24 (0.77)	8.88 (0.74)	5.16 (0.43)	15.96 (1.33)	18.00 (1.5)	9.84 (0.79*)	10.32 (0.86*)	10.44 (0.87*)

Black South African (BSA) and White South African (WSA) individuals

* Statistically significant at $p < 0.05$

attainment below 18 years for stage 1 for C2, C3, and C4. This suggests that the ossification of the apophysis can provide valuable information on ageing around the age of 18 years.

Black South African (BSA) males achieved stage 1 earlier for C2, C3, and C4 compared with BSA females (Table 3). However, after that, BSA females achieved stages 2, 3, and 4 earlier for all vertebrae compared with BSA males. Similarly, white South African (WSA) males achieved stage 1 earlier for C2, C3, and C4 and stage 2 for C2 compared with females. Thereafter, stage 2 for C3 and C4 and stages 3 and 4 were achieved earlier for C2, C3, and C4 by females compared with males (Table 3). Median differences exceeding 1 year between BSA males and females were noted for stage 1 for C2 and stage 3 for C4. Stage 3 for C3 and C4 exceeded a 1-year median difference between WSA males and females.

Table 4 demonstrates the likelihood of an individual being 18 years of age or older based on the anterior inferior ossification of the apophysis for C2, C3, and C4, respectively. When a 95% probability is considered for an individual to be younger than 18 years, the following stages are below that level for stage 1: black males for C2, C3, and C4, white females for C2 and C3, and white males for C4. When a 95% probability is considered for an individual to be older than 18 years, the following stages are above that level for stage 4: white males for C3 and C4. Figure 2 shows the age dispersion for each ossification stage for each of the vertebrae.

Table 4 Likelihood of an individual being at least 18 years of age based on the anterior inferior apophysis ossification stage for C2, C3, and C4

Probability of being age 18 last birthday					
Ancestry, sex, vertebra	Stage				
	0	1	2	3	4
Black, female, C2		0.091	0.125	0.462	0.796
Black, female, C3		0.0667	0.1429	0.5833	0.8357
Black, female, C4		0.0556	0.2143	0.6296	0.8548
Black, male, C2	0.000	0.000	0.132	0.557	0.887
Black, male, C3	0.000	0.000	0.158	0.875	0.917
Black, male, C4	0.000	0.000	0.244	0.889	0.943
White, female, C2		0.000	0.190	0.360	0.725
White, female, C3		0.000	0.121	0.333	0.822
White, female, C4		0.083	0.100	0.444	0.841
White, male, C2	0.000	0.091	0.156	0.446	0.904
White, male, C3	0.000	0.094	0.120	0.635	0.978
White, male, C4	0.000	0.049	0.204	0.780	0.975

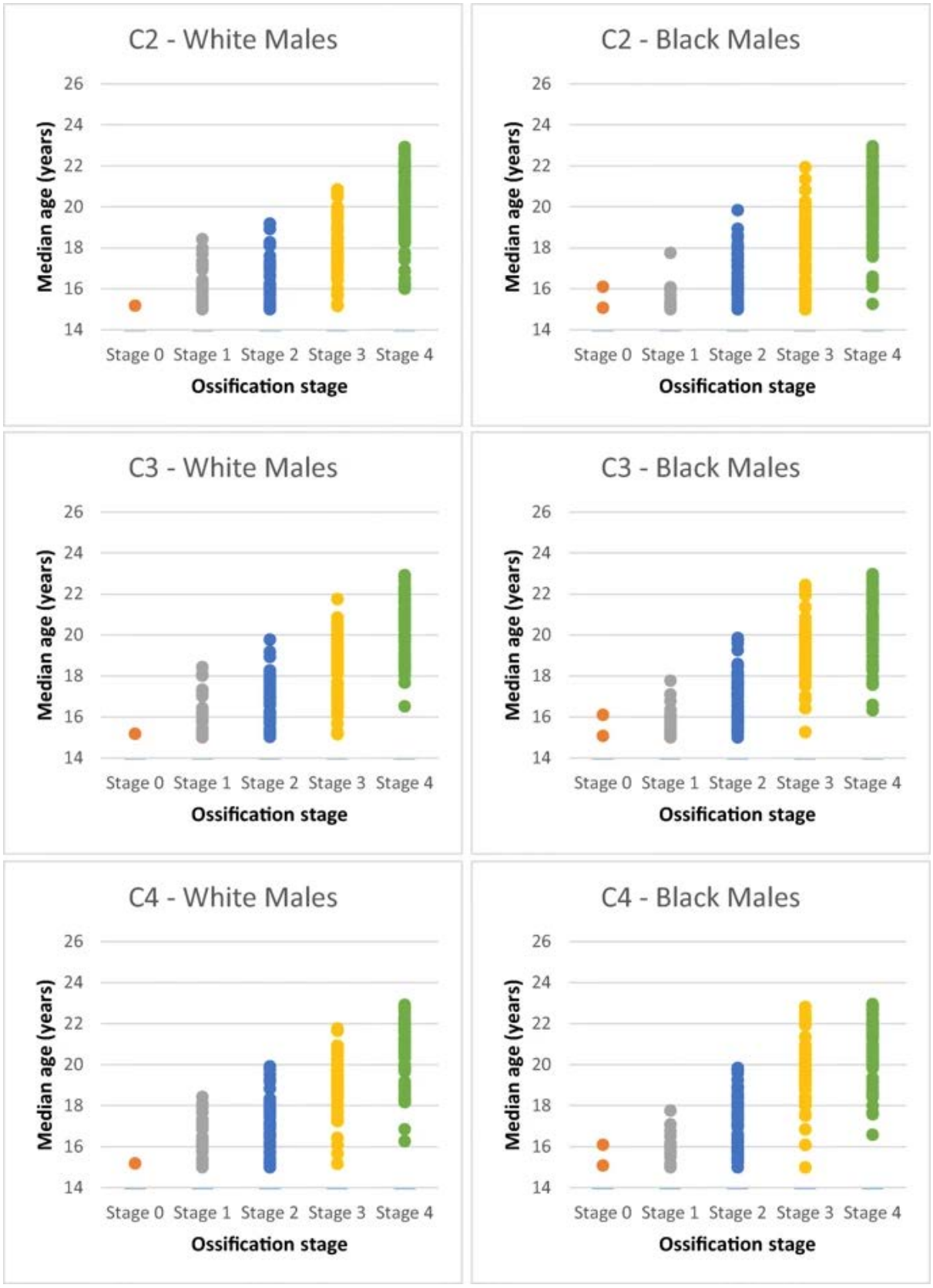


Fig. 2.Dot plots of the median ages at which ossification stages 0 through 4 were attained for the anterior inferior apophysis for C2, C3, and C4

Cropped lateral cephalometric radiograph of C2, C3 and C4	Stage	Ancestry, sex	Chronological age	Age determined with regression equations: 1= Multiple linear regression analysis *2= Dichotomous indicator variable analysis*
	C2 stage = 1 C3 stage = 1 C4 stage = 1	White, male	15.59 years	1: Age =13.6065+0.1376 (C2 stage) + 0.7078 (C3 stage) + 0 .9344 (C4 stage) =13.6065+0.1376 (1) + 0.7078 (1) + 0.9344 (1) =15.35 ± 1.22 years *2: Age = 15.9375 – 0.1695 I(C2 Stage 2) - 0.1004 I(C2 Stage 3) + 0.5747 I(C2 Stage 4) + 0.4142 I(C3 Stage 2) + 1.0593 I(C3 Stage 3) + 1.9955 I(C3 Stage 4) + 0.4828 I(C4 Stage 2) + 1.5515 I(C4 Stage 3) + 2.4435 I(C4 Stage 4)* *= 15.9375 ± 1.19 years*
	C2 stage = 2 C3 stage = 2 C4 stage = 2	Black, female	17.02 years	1: Age = 12.6790 + 0.5171(C2 stage) + 0.6442(C3 stage) + 0.7371(C4 stage) = 12.6790 + 1.0342 + 1.2884 + 1.4742 = 16.48 ± 1.65 years *2: Age = 16.1144 – 0.0304 I(C2 Stage 2) + 0.6903 I(C2 Stage 3) + 1.4348 I(C2 Stage 4) – 0.0065 I(C3 Stage 2) + 0.0485 I(C3 Stage 3) + 1.4075 I(C3 Stage 4) – 0.22591 I(C4 Stage 2) + 1.0089 I(C4 Stage 3) + 1.4919 I(C4 Stage 4)* *= 16.1144 – 0.0304(1) - 0.0065(1) – 0.22591(1)* *= 15.8516 ± 1.6 years*
	C2 stage = 3 C3 stage = 3 C4 stage = 3	Black, male	19.45 years	1: Age = 13.2284+0.3617 (C2 stage) + 0.8018(C3 stage) + 0.7300 (C4 stage) = 13.2284+ 0.3617 (3) + 0.8018 (3) + 0.7300 (3) = 18.91± 1.30 years *2: Age = 15.2034 + 0.6460 I(C2 Stage 2) + 0.8826 I(C2 Stage 3) + 1.3062 I(C2 Stage 4) + 0.7031 I(C3 Stage 2) + 1.6965 I(C3 Stage 3) + 2.3734 I(C3 Stage 4) +0.1834 I(C4 Stage 2) + 1.5664 I(C4 Stage 3) + 1.8673 I(C4 Stage 4)* *= 15.2034 + 0.8826 (1) + 1.6965 (1) + 1.5664 (1)* *= 19.3489 ± 1.27 years*
	C2 stage = 4 C3 stage = 4 C4 stage = 4	White, female	21.25 years	1: Age =12.3657 + 0.3717 (C2 stage) + 0.6602(C3 stage) + 0.8972(C4 stage) = 12.3657 + 0.3717 (4) + 0.6602(4) + 0.8972(4) = 20.08± 1.64 years *2: Age = 15.6341 – 0.5256 I(C2 Stage 2) - 0.2254 I(C2 Stage 3) + 0.5555 I(C2 Stage 4)+ 0.1953 I(C3 Stage 2) + 0.9296 I(C3 Stage 3) + 1.614 I(C3 Stage 4)+ 0.1195 I(C4 Stage 2) + 1.2230 I(C4 Stage 3) + 2.3748 I(C4 Stage 4)* *=15.6341+ 0.5555 (1) + 1.614 (1)+ 2.3748(1)* *=20.1784 ± 1.62 years*

Fig. 3. Cropped lateral cephalometric radiographs of four individuals to illustrate the practical use of the regression equations with one example of each population and sex group (please take note that the cases were specifically selected for illustration purposes and with the same apophysis development stage for each cervical vertebra. The anterior cervical vertebral ring apophysis ossification development is not necessarily at the same stage for each cervical vertebra)

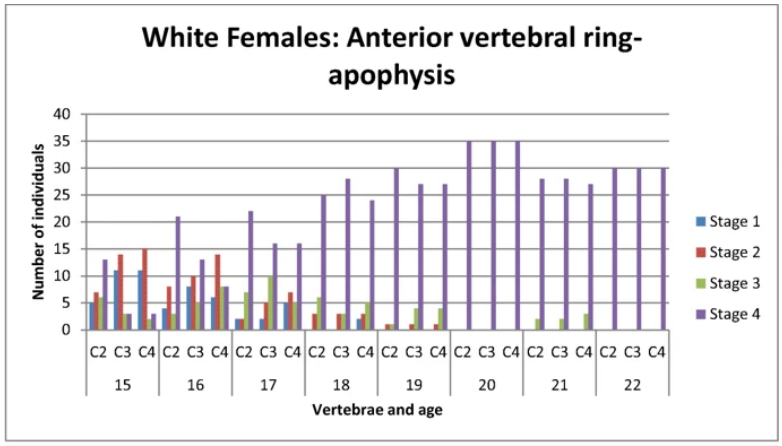
Table 5 Regression equations, correlation coefficients (R), coefficients of determination (R^2), and standard errors of estimate of multiple regression analyses, with age as the dependent variable and apophysis stage development changes as independent variables, for vertebrae C2, C3, and C4

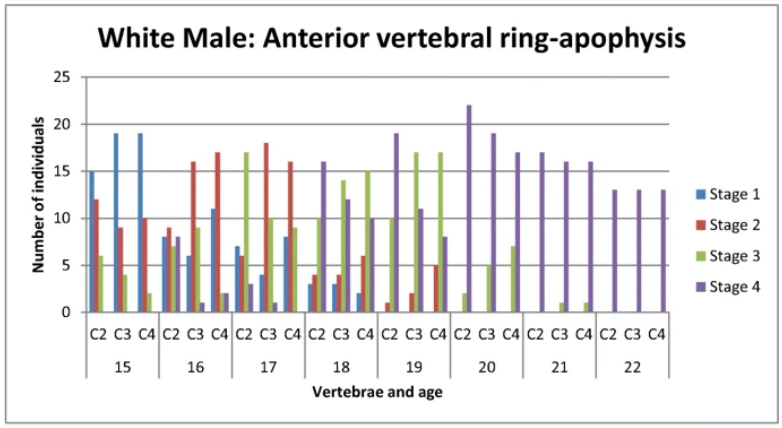
Ancestry, sex	Formula	R	R^2	Standard error
Black, males	Age = 13.2284 + 0.3617 (C2 stage) + 0.8018(C3 stage) + 0.7300 (C4 stage)	0.82	0.67	1.30
White, males	Age = 13.6065 + 0.1376 (C2 stage) + 0.7078 (C3 stage) + 0.9344 (C4 stage)	0.84	0.70	1.22
Black, females	Age = 12.6790 + 0.5171(C2 stage) + 0.6442(C3 stage) + 0.7371(C4 stage)	0.70	0.49	1.65
White, females	Age = 12.3657 + 0.3717 (C2 stage) + 0.6602(C3 stage) + 0.8972(C4 stage)	0.71	0.51	1.64

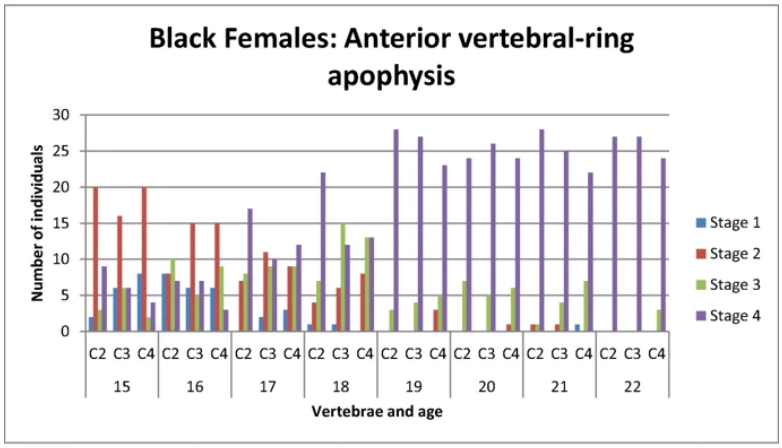
Table 6 Regression equations using dichotomous indicator variables for different stage classifications

Ancestry, sex	Formula	Adjusted R^2	Standard error
Black, males	Age = 15.2034 + 0.6460 I(C2 stage 2) + 0.8826 I(C2 stage 3) + 1.3062 I(C2 stage 4) + 0.7031 I(C3 stage 2) + 1.6965 I(C3 stage 3) + 2.3734 I(C3 stage 4) + 0.1834 I(C4 stage 2) + 1.5664 I(C4 stage 3) + 1.8673 I(C4 stage 4)	0.6819	1.27
White, males	Age = 15.9375–0.1695 I(C2 stage 2) - 0.1004 I(C2 stage 3) + 0.5747 I(C2 stage 4) + 0.4142 I(C3 stage 2) + 1.0593 I(C3 stage 3) + 1.9955 I(C3 stage 4) + 0.4828 I(C4 stage 2) + 1.5515 I(C4 stage 3) + 2.4435 I(C4 stage 4)	0.7171	1.19
Black, females	Age = 16.1144–0.0304 I(C2 stage 2) + 0.6903 I(C2 stage 3) + 1.4348 I(C2 stage 4) − 0.0065 I(C3 stage 2) + 0.0485 I(C3 stage 3) + 1.4075 I(C3 stage 4) − 0.22591 I(C4 stage 2) + 1.0089 I(C4 stage 3) + 1.4919 I(C4 stage 4)	0.5119	1.60
White, females	Age = 15.6341–0.5256 I(C2 stage 2) - 0.2254 I(C2 stage 3) + 0.5555 I(C2 stage 4) + 0.1953 I(C3 stage 2) + 0.9296 I(C3 stage 3) + 1.614 I(C3 stage 4) + 0.1195 I(C4 stage 2) + 1.2230 I(C4 stage 3) + 2.3748 I(C4 stage 4)	0.5119	1.62

I(Cy(cervical vertebrae C2, C3, or C4) stage x(2, 3, or 4) = 1 if Cy stage is present. If the stage is absent, a zero (0) gets allocated to the stage







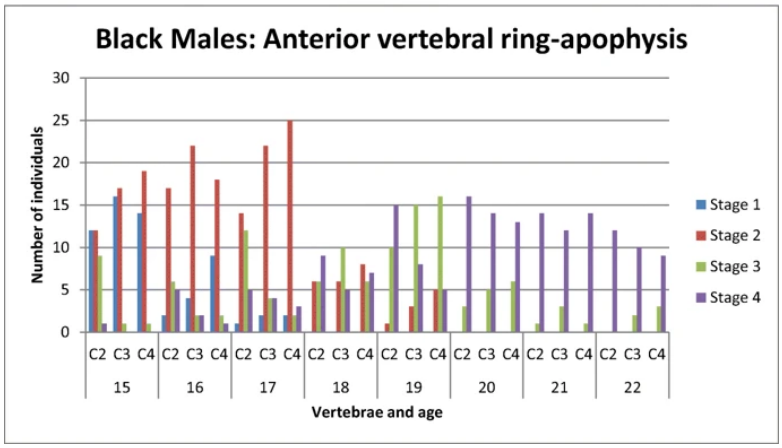


Fig. 4. Graphs display the development of the anterior inferior apophysis for C2, C3, and C4 and the relationship of development according to age for each ancestry and sex group

Table 5 shows the results of the multiple regression analysis for each population and sex group. The data are used to actually give a point estimate of the age of an unknown individual. From this table, it can be seen that the R^2 values ranged between 0.49 and 0.70, with standard errors from 1.22 years in white males to 1.65 years in black females. The practical use of these formulae, with examples, is shown in Fig. 3. By age 22.96 years, all apophyses are completely ossified, and the formulae can obviously not be used beyond this age. Table 6 demonstrates the results of the regression formulae using dichotomous indicator variables. The adjusted R^2 values ranged between 0.5119 and 0.7171, with standard errors from 1.19 to 1.60.

Figure 4 illustrates the development pattern for each vertebral ring apophysis stage according to age. The graph provides insight into the relationship of development among the vertebrae.

Discussion

The ability to ascertain whether a person has reached 18 years of age is critical in cases of unaccompanied minors, criminal investigations, illegal immigration, asylum seekers, and children involved in child labour. Estimating a living individual's age after 14 years of age is challenging, and only a few age indicators are available. Accurate and reliable methods are therefore required as the outcomes of such estimations have far-reaching consequences. These methods should be practical, accurate, and easy to use. Approaches combining dental observations, such as third molar development with age-related skeletal indicators, have been suggested to reduce wide age estimation predication intervals [11]. Cervical vertebrae growth increments of C1, C2, C3, and C4 have been studied before, and the characteristic morphological changes of the six stages of cervical vertebral maturation (CVM) related to growth changes have been suggested as an alternative method to determine skeletal maturation [30,31,32,33]. To our knowledge, however, the present study is the first to investigate the question of whether it is possible to estimate the age and probability of an individual being younger/older than 18 years based on cervical vertebral apophysis ossification.

In this study, we propose a new approach, in which the ossifications of the anterior inferior vertebral ring apophysis of cervical vertebrae C2, C3, and C4 are categorised into stages 0 through 4. The regions of interest are clearly visible on cephalometric radiographs used in routine orthodontic evaluations, and it is thus not necessary to expose a patient to additional radiation. Another advantage is that the radiation dose needed for a cephalometric radiograph varies between 2 to 3 microsievert (μSv) compared with panoramic radiographs with effective radiation doses of between 3.85 to 30 μSv [34]. The classification system proved to be easy to use and is usable to discern between stages. The simple classification system eliminates various doubts as to which stage an individual belongs to. This was also reflected in the acceptable rates of interobserver repeatability.

The results of this study indicate that the median ages for attainment of stages 0, 1, and 2 were below the 18-year threshold for all ancestry and sex groups. Additionally, WSA males and BSA females attained stage 3 for C2, and WSA females attained stage 3 for C2, C3, and C4 below the 18-year threshold. The maximum ages of stage 0 and stage 1 for C2, C3, and C4 in BSA males, stage 0 in WSA males, and stage 1 in WSA females were also below the 18-year threshold. These stages are important to determine whether an individual is below 18 years of age.

Black males achieved stage 1 for C2, C3, and C4 and stage 2 for C3 earlier than WSA males. All the other stages were achieved earlier by WSA males, suggesting that they have faster bone maturation. This is in contrast to the development of third molars where BSA males reached stage H earlier by 6.90 months in the maxilla and 9.06 months in the mandible compared with WSA males [5]. All stages, except for stage 2 for C2, were achieved earlier by WSA females compared with BSA females, again suggesting earlier skeletal maturation in white as opposed to black individuals. BSA females reached 11 out of 14 evaluated third molar development stages before WSA females [5]. These results indicate that ancestry and sex differences are present and that skeletal and tooth development takes place at different rates in the same population. Several factors have been suggested to explain accelerated maturation in individuals which include a stable calorie intake or a reduced calorie expenditure, an increase in calcium intake, an increase in the uptake of processed sugars and fats, reduced physical activity, and an improved socioeconomic and health status [35]. Socioeconomic factors are often indicated as the most important variable in maturation differences [35, 36]. The individuals from the School of Dentistry, University of Pretoria, and Sefako Makgatho Health Sciences University consist of different social groupings and include individuals living in the city and from the surrounding rural areas. The sample from the private practices is mainly composed of individuals living in the city. The sample as a whole includes the entire socioeconomic spectrum, and therefore, it is not possible to attribute observed differences to varying socioeconomic circumstances. These differences are more likely to be attributable to genetic factors. Black males achieved stage 1 for C2, C3, and C4 earlier compared with BSA females. The BSA females achieved all the other stages earlier. This is in contrast to the results for third molar development where development terminated earlier in BSA males compared with BSA females [5]. Dunn's test indicted that statistically significant differences were present between BSA males and females for stage 1 for C2 and stage 3 for C3 and C4. White males achieved stage 1 for C2, C3, and C4 and stage 2 for C2 earlier compared with WSA females. Statistically significant differences were found between WSA males and females for stage 4 for C2, C3, and C4. The final stage (stage 4) for anterior inferior apophysis development was reached 9.84, 10.32, and 10.44 months later for C2, C3, and C4, respectively, in WSA males compared with WSA females. Compared with third molar development, WSA males reached the final stage (stage H) an average of 9.0 months earlier in the maxilla and 10.02 months earlier in the mandible [5]. In both ancestry groups, the patterns of development between the males and females were similar. These results indicate that sexual differences for both ancestry groups are present. Earlier sexual maturation is found in industrialized populations [36, 37]. Sexual maturation is closely linked to skeletal maturation, and pubertal onset may play an important role in skeletal maturation [38]. It has been suggested that skeletal maturation does not depend on ethnicity but rather on optimum environmental conditions (i.e., high socioeconomic status). Conversely, lower socioeconomic status may lead to retardation of skeletal maturation [39]. However, there are many contributing factors to the differences observed in skeletal and dental maturation, and the exact cause is difficult to pinpoint.

In this study, the likelihood of an individual being older or younger than 18 years based on the ossification of the anterior inferior apophysis was determined. When a 95% probability is considered for an individual to be younger than 18 years, a few stages can be considered to be a reliable indicator. The presence of stage 0 for black and white males in all three observed vertebrae and stage 1 for black males for C2, C3, and C4, white females for C2 and C3, and white males for C4 indicates an age below 18 years (with a 95% or higher probability). When a 95% probability is considered for an individual to be older than 18 years, only two stages were found to be above that level: stage 4 for white males for C3 and C4. The cervical

vertebral ring apophysis likelihood data can thus considerably strengthen age estimation methods. The use of all skeletal markers for age estimation analysis provides the most accurate age estimation [20].

In this study, stepwise multiple regression analysis and dichotomous indicator regression formulae were additionally used to develop a model based on the anterior inferior ossification of the apophysis to estimate age of an individual. The progressive apophysis changes which correlate significantly with age were used to determine the corresponding correlation coefficients. The calculated *R* values were all above 0.71, with SEEs ranging from 1.2 to 1.7 years. The regression equations calculated in the present study can be used for the estimation of age of living individuals in a South African population. Regression model is an easy and useful method to practically estimate age for a specific individual. However, such an approach should be used with caution in a legal setting where it may be more prudent to provide probabilities rather than actual estimates which may be misinterpreted by uninformed individuals. The authors recommend that both methods be used to determine a range of possible age estimates. The results obtained from the different models are similar and can be used to construct an argument combined with other considerations such as probability values to provide an expert opinion.

Conclusion

Cephalometric radiographs of a large sample of individuals with known age, sex, and ancestral origin were studied to determine the ossification rates in sex and ancestral groups of South Africans. The new classification system proved to be valuable in staging the anterior inferior apophysis of the cervical vertebrae. The likelihood of being younger or older than 18 years, at a given ossification stage, was determined using these stages. The results indicate that anterior inferior apophysis ossification stages of C2, C3, and C4 can be used as a reliable indicator to determine the likelihood of being 18 years of age at a 95% confidence index level. Ancestral and sex differences were found which emphasize the need to use ancestry and sex specific data. Currently, no completely accurate non-invasive method to discern whether an individual is younger or older than 18 years of age is available and is unlikely to exist due to inherent individual biological variation. To achieve the most accurate age estimation, it is recommended that the results from this study are combined with other age estimation indicators. Evaluation of the anterior inferior apophysis could prove to be a valuable additional tool to discriminate the age of a living individual. Therefore, further studies are needed to support our results.

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