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Discriminatory ability of cervical vertebral maturation stages in predicting attainment of the legal age threshold of 14 years: A pilot study using lateral cephalograms

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ABSTRACT

Purpose: In India, the age of 14 years is the legal age threshold for child labour. Therefore, in suspected instances of child labour, age assessment plays a crucial role in determining whether a violation of the law on the employment of children has occurred. The aim of this retrospective cross-sectional study was to assess the discriminatory ability of stages of cervical vertebral maturation (CVM) in predicting the legal age threshold of 14 years.

Materials and Methods: Routinely taken lateral cephalograms from 408 subjects aged 10 to 18 years were evaluated retrospectively using the CVM stages described by Baccetti et al. Descriptive statistics, accuracy, sensitivity, specificity, positive and negative predictive values, and likelihood ratios were calculated for stages 2, 3, and 4 of CVM.

Results: Real age increased as the CVM stage gradually increased. The results of 2×2 contingency tables showed that CVM stage 4 produced an accuracy of 71% and 73%, a false positive rate of 7% and 18%, and a post-test probability of 59% and 68% for boys and girls, respectively.

Conclusion: Based on these findings, it can be concluded that the stages of CVM are of limited use for predicting the attainment of the legal age threshold of 14 years. Future studies should investigate whether combinations of skeletal and dental methods could achieve better accuracy and post-test probability. ***(Imaging Sci Dent 2020; 50: 209-16)***

KEY WORDS: Cervical Vertebrae; Growth and Development; Age Determination by Skeleton; Child; India

Introduction

Age estimation in living individuals who lack valid documentation is an important element of forensic practice. Age assessments in children should be conducted in a safe, child- and gender-sensitive manner with due respect for human dignity. It is also important to safeguard children by providing them with age-related services.[1] Registration of birth is a fundamental human right. However, in many countries, it is not always practiced, leading to a considerable number of unregistered births. According to the United Nations Children's Fund, by 2012, only 65% of children under 5 years of age were registered worldwide.[2] In South Asia and sub-Saharan Africa, 61% and 56% of births were unregistered, respectively.

No age is fully immune from medico-legal scrutiny and each age is of medico-legal importance. A few examples of age groups that have medico-legal importance in India are the age of majority (18 years), the age of criminal responsibility (16 years), and the legal ages to provide consent for sex, to consume alcohol, and for marriage. In India, hiring children below the 14 years of age for any kind of work is a cognizable offence. According to statistics from 2017, India is among the countries in Asia with

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the highest burdens of child labour, with approximately 33 million children employed in various forms.[3] Over the years, experts have shown increasing interest in this problem and investigated the reliability of various age assessment methods. Some researchers have stated that there is little or no difference in developmental parameters between populations, while others have stated that such differences do exist and should be considered seriously.[4]

In general, experts assess the level of craniofacial maturity in patients to identify the optimal time to initiate orthodontic/orthopaedic treatment planning for certain craniofacial skeletal imbalances.[5] Dental and skeletal parameters such as the hand and wrist bones, clavicle, pelvis, and cervical vertebrae have been analysed to assess age with reasonable accuracy. Reliable indicators are an increase in statural height, maturation of the hand and wrist bones, and changes in the morphology of cervical vertebrae. Greulich and Pyle presented atlas images of the left hand and wrist of children at different age intervals, which were then modified and adapted by Tanner and Whitehouse.[6] The main disadvantage of this method is that the complexity of landmark identification leads to inaccurate classification.[7] Similarly, using teeth, Demirjian and co-workers presented a series of formation stages and developed a reference dataset using dental maturation.[8] When tested, it was found to consistently overestimate the age of tested individuals from various populations.

Due to the drawbacks of using the hand and wrist bones (e.g., the complexity of landmark identification, the need for increased radiation exposure, and inconsistencies in age estimation), the cervical vertebral maturation (CVM) method has started to gain importance over conventional methods due to the direct visibility of these structures on lateral cephalograms and their established validity and reliability.[9] Changes in the size and shape of cervical vertebrae in growing individuals have received increasing interest in the past few decades. In 1972, Lamparski introduced the CVM index for growth assessment.[10] Later, Baccetti et al. simplified the CVM index, which included the second to fourth cervical vertebrae.[11] A few authors have investigated the ossification events of CVM as a biological indicator of skeletal maturity.[12-14] Others have studied the geometry of stages of the cervical vertebrae — that is, the relationship between the growth of the vertebrae and the age of children and young adolescents — for forensic purposes.[15-17] The growth of the cervical vertebrae follows a somatic pattern and they reach their final maturation at approximately 15-16 years, with the maximum cervical vertebral growth rate at between 10 and 12 years.[18]

To the best of our knowledge, there is no evidence in the scientific literature regarding the use of CVM stages to predict the attainment of legal age thresholds. Keeping in mind the easy visibility of cervical vertebrae on lateral cephalograms, the routine use of lateral cephalograms for orthodontic patients, the relatively low radiation exposure for patients, and the elimination of the need for additional radiographs (hand-wrist films), the authors of the present study formulated a hypothesis with the primary aim of testing the validity of CVM stages in predicting the legal age threshold of 14 years using lateral cephalograms in a sample of south Indian children.

Materials and Methods

Sample

The sample of this study included 408 lateral cephalometric radiographs of children (181 boys and 227 girls) aged between 10 and 18 years, which were collected retrospectively from the archives of the Department of Oral Medicine and Radiology and from private dental clinics. Table 1 shows the age and sex distribution of the overall sample. All the radiographs were taken for the purpose of orthodontic treatment. Ethical approval was obtained from the institutional ethical committee (PMVIDS&RC/IEC/OMFP/PR/0261-18). Parents and guardians of the children were provided a detailed explanation and informed consent was obtained regarding the use of personal data for research.

The inclusion criteria for the study were 1) high-quality cephalograms that allowed good visualization, 2) the absence of craniofacial abnormalities, 3) radiographs of children aged between 10 and 18 years, 4) clearly visualized bones that were not affected by systemic and bone metabolic diseases, and 5) inclusion of the first 4 cervical

Table 1. Age and sex distribution of the total sample

Age groups	Boys	Girls	Total
10-10.9	26	27	53
11-11.9	22	31	53
12-12.9	31	34	65
13-13.9	49	48	97
14-14.9	33	42	75
15-15.9	10	21	31
16-16.9	8	12	20
17-17.9	2	12	14
Total	181	227	408

vertebrae with clear inferior borders. The exclusion criteria were 1) cephalograms of patients with an unknown date of birth and 2) patients with congenital anomalies of the second, third, or fourth cervical vertebrae such as fusion of vertebrae or the presence of a secondary ossicle.

Evaluation of cervical vertebrae maturation

The maturation of cervical vertebrae was evaluated using the method developed by Baccetti et al.[19] In this method, 2 sets of variables were examined in the cervical vertebrae C2, C3, and C4: 1) the presence or absence of concavity along the inferior border and 2) differences in the morphological shapes of the vertebral bodies (trapezoidal, rectangular horizontal, square, and rectangular vertical). Figure 1 and Table 2 show schematic illustrations with typical radiographs and a detailed description of the stage classifications.

The analysis was carried out independently by a single examiner, a forensic odontologist with 6 years of experience in evaluating radiographic images and in age estimation analysis, with the exception of a subset used to investigate inter-observer variability, as described below. The chronological age of each subject was calculated as the difference between the date of birth and the date of exposure of the radiograph, and the obtained age was converted into decimal form. All the radiographs were provided with numerical codes so that the examiners did not have any external information regarding the age of the subjects. The data were then entered into a Microsoft Excel spreadsheet and subjected to statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). The significance threshold was set at $P<0.05$. Descriptive statistics were performed for all CVM stages. Inter-examiner variability was evaluated to test the feasibility and reliability of the skeletal method. Radiographs of 30 subjects were selected and evaluated by a second examiner. The number of matches, indicating agreement between the 2 examiners, was counted. After an interval of 1 month, 40 radiographs were randomly selected and evaluated to study intra-ex-

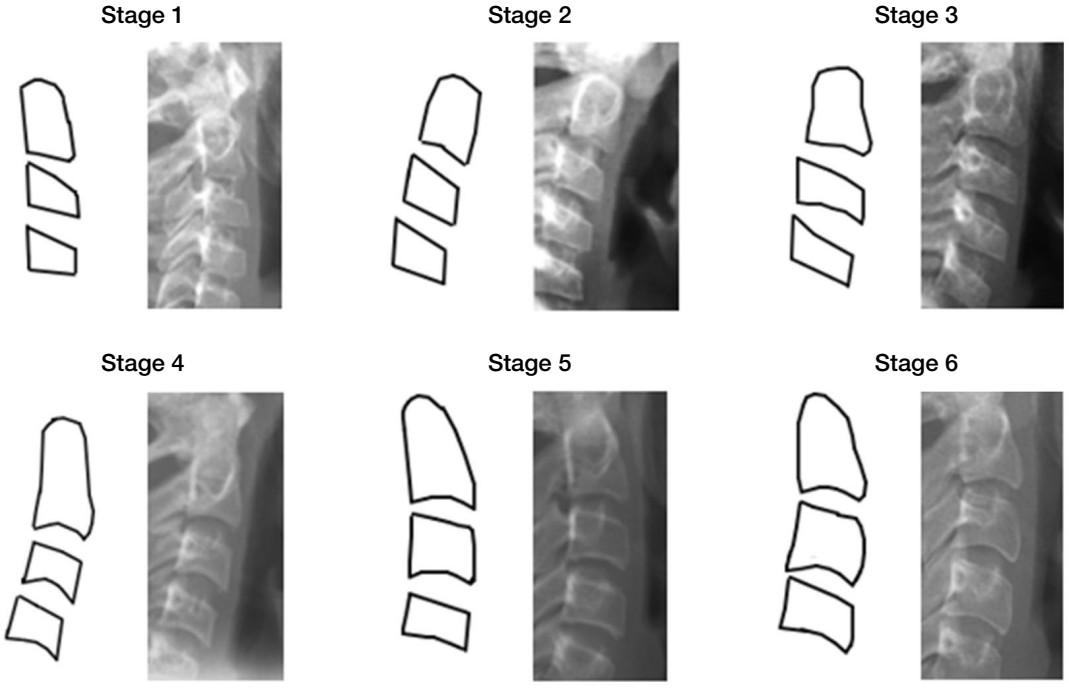

Fig. 1. Schematic representation of cervical vertebral maturation stages according to the newly modified method by Baccetti et al. (2005).[19] In stage 1, the lower borders of all 3 vertebrae (C2-C4) are flat. The bodies of both C3 and C4 are trapezoid. In stage 2, a concavity is present at the lower border of C2, and the bodies of both C3 and C4 are still trapezoid in shape. In stage 3, concavities at the lower borders of both C2 and C3 are present. The bodies of C3 and C4 may be either trapezoid or rectangular horizontal in shape. In stage 4, concavities at the lower borders of C2, C3, and C4 are present. The bodies of both C3 and C4 are rectangular horizontal in shape. In stage 5, the concavities at the lower borders of C2, C3, and C4 are still present. At least one of the bodies of C3 and C4 is square. In stage 6, the concavities at the lower borders of C2, C3, and C4 are still evident. At least 1 of the bodies of C3 and C4 is rectangular vertical in shape. If not rectangular vertical, the body of the other cervical vertebra is square.

Table 2. Detailed description of the stages of cervical vertebrae

Stages	Description
CS1	The lower borders of all 3 vertebrae (C2-C4) are flat. The bodies of both C3 and C4 are trapezoid in shape (the superior border of the vertebral body is tapered from posterior to anterior).
CS2	A concavity is present at the lower border of C2 (in 4 of 5 cases, with the remaining subjects still showing cervical stage 1). The bodies of both C3 and C4 are still trapezoid in shape.
CS3	Concavities at the lower borders of both C2 and C3 are present. The bodies of C3 and C4 may be either trapezoid or rectangular horizontal in shape.
CS4	Concavities at the lower borders of C2, C3, and C4 are now present. The bodies of both C3 and C4 are rectangular horizontal in shape.
CS5	The concavities at the lower borders of C2, C3, and C4 are still present. At least one of the bodies of C3 and C4 is square. If not square, the body of the other cervical vertebra still is rectangular horizontal.
CS6	The concavities at the lower borders of C2, C3, and C4 are still evident. At least one of the bodies of C3 and C4 is rectangular vertical in shape. If not rectangular vertical, the body of the other cervical vertebra is square.

Table 3. Output of the contingency table

	Description
True positive	Subjects whose CVM is below stage 2 and are younger than 14 years of age
False positive	Subjects whose CVM is below stage 2, but are older than 14 years of age
True negative	Subjects whose CVM is above stage 2 and are older than 14 years
False negative	Subjects whose CVM is above stage 2, but are younger than 14 years

CVM: cervical vertebral maturation

aminer variability. The paired t-test was performed to evaluate intra- and- inter-examiner variability.

To test the performance of CVM stages (2, 3, and 4), the results were summarized as 2 × 2 contingency tables for each sex separately, as presented in Table 3. The accuracy, sensitivity, and specificity of all the stages were evaluated, as well as the positive predictive values (PPV) and negative predictive values (NPV). The PPV determines how many subjects were correctly classified as positive, while the NPV indicates how many subjects were correctly classified as negative. The likelihood ratio of a positive test (LR+) and a negative test (LR−) for different stages was also considered. Values of LR+ above 10 and LR− values below 0.1 are considered to provide strong evidence for most assessment situations.

According to Bayes' theorem, post-test probability may be expressed as[20]

$$p = \frac{Se \times p_0}{Se \times p_0 + (1 - Sp) \times (1 - p_0)}$$

where p is the post-test probability and p_0 is the probability that the subject in question is 14 years old or older, given that he or she is between 10 and 18 years of age, which represents the target population. The probability p_0 was calculated as the proportion of subjects between 14 and 18 years of age residing in Andhra Pradesh and Telangana according to demographic data from the 2011 census (http://www.censusindia.gov.in/2011census/C-series/C-13.html), and the number of those between 10 and 18 years was obtained from the same data source. This proportion was determined to be 49% for boys and 48% for girls.

Results

The paired t-test showed no statistically significant difference for inter-examiner ($P = 0.321$) or intra-examiner ($P = 0.547$) evaluations.

There were 181 (44.4%) males and 227 (55.6%) females in the tested sample. The mean age ± standard deviations of males and females were 13.11 ± 1.7 and 13.45 ± 1.9 years, respectively. Real age gradually increased as CVM stage increased in both sexes (Fig. 2). A summary of descriptive statistics (i.e., the number of individuals, mean, minimum and maximum ages, standard deviation, and percentile data for both sexes) is presented in Table 4. The earliest appearance of stage 1 occurred at 10 years in both sexes, stage 2 first appeared at 10 years in boys and

Table 4. Summary data of chronological age for each of the cervical vertebral maturation (CVM) stages for boys and girls, respectively

	CVM	Number	Mean age	Min	25th	50th	75th	Max
Boys (n=181)	Stage 1	44	11.8±1.2	10.0	10.7	11.6	13.1	14.6
	Stage 2	40	12.3±1.5	10.0	10.9	12.4	13.3	16.2
	Stage 3	32	13.6±1.6	10.2	12.3	13.7	14.6	17.7
	Stage 4	45	14.1±1.1	11.3	13.3	14.2	14.6	16.7
	Stage 5	15	14.3±1.3	11.9	13.3	13.7	15.6	16.7
	Stage 6	5	14.6±1.3	12.5	13.5	14.7	15.7	16.4
Girls (n=227)	Stage 1	30	11.3±1.1	10.0	10.3	10.8	12.2	13.7
	Stage 2	27	11.9±1.2	10.2	10.8	11.8	13.1	14.8
	Stage 3	30	13.3±1.9	10.2	11.8	13.0	14.9	17.7
	Stage 4	61	13.7±1.3	11.0	12.8	13.5	14.5	17.1
	Stage 5	65	14.5±1.5	11.2	13.5	14.5	15.6	17.7
	Stage 6	14	14.8±1.8	12.2	13.2	15.6	17.1	17.9

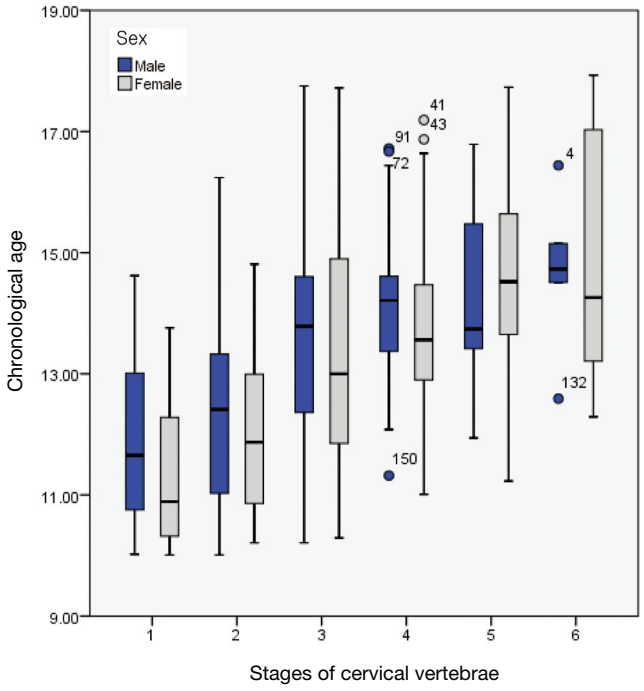

Fig. 2. Box plot shows the relationship between chronological age and stages of cervical vertebrae in boys and girls.

10.2 years in girls, stage 3 was first recorded at 10.2 years in both sexes, stage 4 was first documented at 11.3 years in boys and 11 years in girls, stage 5 at 11.9 years in boys and 11.2 years in girls, and stage 6 at 12.5 years in boys and 12.2 years in girls, respectively. As shown in Table 4, mature stages of CVM (stages 4, 5, and 6) were observed earlier in girls than in boys.

Table 5 shows the results of the criterion validity of CVM stages 2, 3, and 4 for evaluating the number of true positives, true negatives, false positives, and false negatives for each stage. The overall performance measures for CVM stages 2, 3, and 4 for our sample are presented in Table 6 for both sexes separately. Among the tested CVM stages, stage 4 showed the best accuracy (0.71 and 0.73 in boys and girls, respectively). Stage 4 had a sensitivity and specificity of 0.73 and 0.50 in boys and 0.77 and 0.67 in girls, respectively.

The estimated post-test probability for CVM stage 2 (the probability that a subject who was classified as stage 2 of CVM or below was under 14 years of age) was 62% in boys and 64% in girls. For CVM stage 3, the probability of a subject being under 14 years of age was 64% for boys and 63% for girls. For CVM stage 4, the probability for boys was 59% and for girls it was 68%.

Discussion

The study group on forensic age diagnostics of the German Society of Legal Medicine recommended a combination of a physical examination, an X-ray of the hand, and a dental examination with a panoramic radiograph of the jaw region for age assessment. In individuals with complete hand skeletal maturation, an additional X-ray or computed tomography scan of the clavicles must be considered.[21] For many years, maturation of the cervical vertebrae (both in terms of size and shape) in growing individuals has received increasing interest in clinical practice for the assessment of skeletal maturation. Moreover, orthodontists are increasingly requesting information on patients' auxological stage from the development of cervical vertebrae on lateral X-rays, reducing unnecessary

Table 5. Criterion validity (chronological age > 14 years) according to cervical vertebral maturation (CVM) stages 2, 3, and 4 for boys and girls, number of individuals (%)

CVM	Sex	True positive	True negative	False positive	False negative
Stage 2	Boys	80 (62.5%)	49 (92.5%)	48 (37.5%)	4 (7.5%)
	Girls	55 (39.3%)	85 (97.7%)	85 (60.7%)	2 (2.3%)
Stage 3	Boys	98 (76.6%)	35 (66%)	30 (23.4%)	18 (34%)
	Girls	75 (53.6%)	75 (86.2%)	65 (46.4%)	12 (13.8%)
Stage 4	Boys	118 (92.2%)	10 (18.9%)	10 (7.8%)	43 (81.1%)
	Girls	114 (81.4%)	53 (60.9%)	26 (18.6%)	34 (39.1%)

Table 6. The quantities derived from 2-by-2 contingency tables (95% confidence interval) of stages of cervical vertebrae maturation (CVM) stages 2, 3 and 4 in south Indian boys and girls to discriminate between those who are 14 years of age and older or under 14 years of age

CVM	Quantities	Boys (95% CI)	Girls (95% CI)
Stage 2	Accuracy	0.71 (0.64-0.77)	0.61 (0.55-0.68)
	Sensitivity	0.95 (0.88-0.98)	0.96 (0.87-0.99)
	Specificity	0.50 (0.40-0.60)	0.50 (0.42-0.57)
	Positive predictive value	0.62 (0.57-0.67)	0.39 (0.35-0.43)
	Negative predictive value	0.92 (0.82-0.97)	0.97 (0.91-0.99)
	Positive likelihood ratio	1.92 (1.57-2.37)	1.93 (1.65-2.26)
	Negative likelihood ratio	0.09 (0.04-0.25)	0.07 (0.02-0.28)
	Bayes post-test probability	0.62 (0.58-0.67)	0.64 (0.60-0.68)
Stage 3	Accuracy	0.73 (0.66-0.79)	0.66 (0.59-0.72)
	Sensitivity	0.84 (0.76-0.90)	0.86 (0.77-0.92)
	Specificity	0.53 (0.41-0.66)	0.53 (0.44-0.62)
	Positive predictive value	0.76 (0.71-0.81)	0.53 (0.48-0.58)
	Negative predictive value	0.66 (0.54-0.75)	0.86 (0.78-0.91)
	Positive likelihood ratio	1.83 (1.39-2.41)	1.86 (1.53-2.26)
	Negative likelihood ratio	0.29 (0.18-0.47)	0.26 (0.15-0.45)
	Bayes post-test probability	0.64 (0.58-0.69)	0.63 (0.58-0.68)
Stage 4	Accuracy	0.71 (0.63-0.77)	0.73 (0.67-0.79)
	Sensitivity	0.73 (0.65-0.79)	0.77 (0.69-0.83)
	Specificity	0.50 (0.27-0.72)	0.67 (0.55-0.77)
	Positive predictive value	0.92 (0.88-0.94)	0.81 (0.75-0.85)
	Negative predictive value	0.18 (0.12-0.27)	0.61 (0.52-0.68)
	Positive likelihood ratio	1.47 (0.94-2.29)	2.34 (1.69-3.25)
	Negative likelihood ratio	0.53 (0.32-0.89)	0.34 (0.25-0.48)
	Bayes post-test probability	0.59 (0.52-0.64)	0.68 (0.62-0.74)

radiation exposure.[22]

In 1972, Lamparski introduced a method to analyse CVM on lateral cephalometric radiographs.[10] Wong and colleagues evaluated the validity of the CVM method and concluded that it was a valid indicator of skeletal growth during the circumpubertal period.[23] Similar results were reported in another study by Al Khal and colleagues.[24] Alhadlaq and Al-Maflehi developed a statistical model based on the dimensions of C3 and C4, and concluded that skeletal maturation can be assessed using regression models of cervical bone.[9] Turkoz et al. measured the vertebral body heights of C3 and C4, derived a formula using regression analysis, and concluded that it could applied for age estimation in both legal and therapeutic settings.[25]

This present study compared the stages of CVM among different age groups. It is evident from the results that the mean chronological age increased as the cervical vertebrae matured in both boys and girls. No evidence was found of earlier skeletal maturation in either sex, similar to the findings of Montasser and colleagues.[26]

In boys, CVM stage 2 was more sensitive, with a sensitivity of 95% for being 14 years or younger, than specific, with a specificity of 50% for being 14 years or older. Its correct identification rate was 72% (129 out of 181 subjects). In girls, the sensitivity was 96% and the specificity was 50%, with a correct identification rate of 61%. When the discriminatory ability of stage 2 was tested, 48 boys who were below 14 years of age were identified as above 14 years (a 37.5% rate of false positives) and 4 subjects who were actually above 14 years were identified as below, corresponding to a false negative rate of 7.5%. In girls, the percentage of false positives was 61%, while that of false negatives was 2%. When stage 3 was tested in boys the percentage of false positives was 23% and that of false negatives was 34%, while the corresponding percentages in girls were 46% and 14%, respectively. For stage 4, in boys, the percentage of false positives was 7% and that of false negatives was 81%, while the corresponding proportions in girls were 18% and 39% respectively.

From the forensic point of view, it is important to have a small number of false positives, as in this context, it is a more serious error to consider a subject who is younger than 14 to be older than 14 than vice versa. Our results confirmed that, among of the 3 CVM stages that were reasonable possibilities, stage 4 produced the smallest proportion of false positives (i.e., 7% and 18% in boys and girls, respectively).

Owing to biological variability, any single method of age estimation is too imprecise in the forensic context and a combination of methods is needed to achieve satisfactory results. Evidence from the literature has reported that the combination of skeletal and dental age estimation methods provided higher accuracy than age assessments performed using a single method.[22,27] Improvements in the accuracy of age estimation and a reduced frequency of ethically unacceptable errors were observed when a combination of dental and skeletal methods was used.[28]

A possible limitation of the present study is the unpredictable growth of cervical vertebrae. At times, vertebrae do not develop notching at all or might develop notching very late, which makes staging difficult for examiners. In such instances, it is helpful to assess dental development as an adjunct to improve the accuracy of the outcome. Inconsistent changes in skeletal development during the growth period may result in higher inter- and intra-observer errors during staging. As with any subjective clinical evaluation, the reliability of CVM staging improves with experience. In addition, due to ethnic variations, the results obtained in this study may be limited to the population studied, and it is advised to test its validity in other populations of interest.

Future developments should consider using a larger sample size with an equal distribution of boys and girls in each age group, as well as assessing biological growth and age in children using the hand and wrist bones and second and third molar calcification in combination with CVM stages. As mineralization of the carpals lasts until the age of approximately 14 years, which is similar to the age when second molar mineralization is completed, the combination of all 3 parameters should be tested to reduce the number of false positives and to better discriminate children around 14 years of age.

This is the first study to verify the usefulness of CVM stages in determining whether children are older or younger than 14 years. Within the limitations of the study, our results showed that the stages of CVM are of limited use for predicting the attainment of the legal age threshold of 14 years. Of all the CVM stages, CVM stage 4 produced the smallest proportion of false positives, with a post-test probability of 59% for boys and 68% for girls, indicating that the individual in question has probably not reached 14 years of age if CVM has not reached stage 4. Future studies should consider a combination of methods (both skeletal and dental) to reduce the number of false positives and to achieve better estimated post-test probability.

Conflicts of Interest: None

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