

# Assessment of agreement between cervical vertebrae skeletal and dental age estimation with chronological age in an Indonesian population

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The authors declare that they have no conflict of interest.

## KEYWORDS

Cervical vertebrae,  
Dental, age estimation,  
Panoramic,  
Cephalometric

J Forensic Odontostomatol  
2020. Dec;(38): 3-16:24  
ISSN :2219-6749

## ABSTRACT

Age estimation significantly contributes to forensic medicine and law enforcement in Indonesia. However, lateral cephalometric radiographs of cervical vertebrae have not been used to estimate age in the Indonesian population. This study developed a formula to estimate the skeletal age of cervical vertebrae using multiple linear regression analyses, estimating the dental age and evaluating the agreement between cervical vertebrae skeletal-chronological, dental-chronological, and cervical vertebrae skeletal-dental ages. Several anatomical parameters were measured to obtain cervical vertebrae ratios from 100 lateral cephalometric radiographs, and followed by the calculation of dental tooth crown index data from 100 panoramic radiographs of subjects 9–18 years old. The Bland-Altman plot of cervical vertebrae skeletal and dental ages showed a mean difference of  $-0.094 \pm 1.52$  years, with upper and lower limits of 2.882 and  $-3.070$  years, respectively. The means of the cervical vertebrae skeletal, dental, and chronological ages were 13.97 (2.67), 14.06 (2.45), and 13.97 (2.97), respectively. The mean differences between cervical vertebrae skeletal-chronological and dental-chronological ages were 0.566 (2.26) and 4.005 (2.07), respectively. Furthermore, a validation trial (group 2,  $n = 10$ , three males and seven females) was conducted to test the accuracy of the cervical vertebrae skeletal age estimation formula using consecutive sampling. The age range was 9–11 years. Cervical vertebrae skeletal age showed a better agreement with chronological age than did dental age.

## INTRODUCTION

In forensic medicine, age is estimated for purposes of identification in natural disasters, criminal cases, and civil cases. Recently, several significant investigations have required age estimation. For example, the identification of tsunami victims in Aceh (2013), Palu (2018), and Banten (2018) required age estimation to distinguish between children and adults. Criminal cases of minor prostitution and the investigation of age fraud in various sports also require age confirmation, especially for those aged between nine and 18 years.

Indonesian minors and adults are classified on the basis of several laws. According to Indonesian law in the child justice system, someone less than 18 years of age is a child and cannot be convicted of a crime. The Indonesian Marriage Act stipulates that the minimum age of marriage for a female is 16 years and that for a male is 19 years. Thus, accurate age estimation in the 16 to 19 year old group is crucial in civil cases.<sup>1-6</sup>

Age can be determined from legal documents (chronological age) and by using biological indicators, such as body height, menarche, voice change, and skeletal and dental development.<sup>7-9</sup> Lamparski (1975) introduced the estimation of cervical vertebrae (CV) maturation by evaluating the shape of the inferior border of the first (atlas, C1) to the sixth (C6) CV using lateral cephalometric radiographs, but his method was deemed subjective and lacking in detail.<sup>10,11</sup> Mito and Sato (2002) measured the bodies of the third (C3) and fourth (C4) CV using lateral cephalometric radiographs in a Japanese population and developed a formula to estimate CV skeletal age.<sup>12</sup> To evaluate its accuracy, they calculated skeletal age using the Tanner-Whitehouse 2 (TW2) hand-wrist method and compared the CV skeletal and chronological ages.<sup>12,13</sup> The formula proved highly reliable in estimating CV skeletal age.<sup>14-16</sup>

Dental age estimation based on periapical, lateral cephalometric, and panoramic radiograph features can be highly accurate. According to Drusini, Ito was the first to estimate dental age by comparing the enamel and pulp chamber to dentine, but the correlation was weak.<sup>17</sup> Ikeda (1985) perfected the formula by including the variable of height to the crown and observed a strong correlation.<sup>18</sup> Nehemia et al. (2012) estimated dental age based on the radiographic pulp chamber size in the mandibular first premolar using the tooth coronal index (TCI) and analyzed histological features in the pulp chamber in an age range of 9-21 years in an Indonesian population. The results showed a strong correlation between the TCI and biological age ( $r^2 = 0.6407$ ).<sup>5,6,17</sup>

CV lateral cephalometric radiographs have not been used to estimate age in the Indonesian population. We developed a formula to estimate CV skeletal age using multiple linear regression analyses of C3 and C4 bodies. Dental age was estimated by analyzing the height of the dental pulp and crown using the TCI. The extent of the mean difference between these two methods was analyzed, after which we measured the reliability of both methods in estimating biological age compared to chronological age.

All the parameters were measured twice (days 1 and 15) by one rater. To measure the intra-rater agreement, we randomly selected 15 lateral cephalometric and 15 panoramic radiographs from group 1 (100 lateral cephalometric and 100

## MATERIAL AND METHODS

### *Study subjects*

The study was approved by the Research Ethics Committee of the Faculty of Dentistry Universitas Indonesia. Medical records were used to select samples by purposive sampling. The inclusion criteria were age 9-18 years, healthy growth and development of the cervical bones and teeth, and high quality digital lateral cephalometric and panoramic radiographs to analyze C3, C4, and the mandibular first premolar. The exclusion criteria comprised CV trauma (cervical dislocations or fracture) and abnormal mandibular first premolars (attrition, caries, tooth loss, periapical lesions, post-root canal treatment, artificial crowns, fillings, and orthodontic appliances).<sup>5,6,17</sup> We obtained panoramic and lateral cephalometric radiographs from patients undergoing orthodontic and paedodontic treatment. We estimated the sample size and selected 100 people ( $n = 10$ /group; five males and five females) aged 9-18 years. They were traced using Digimizer image analysis software version 5.3.5.

### *Radiography*

Digital lateral cephalometric and panoramic radiographs were obtained from the medical records of the Dental Hospital of Universitas Trisakti, Jakarta, Indonesia. We used the lateral cephalometric radiographs to develop the CV skeletal age estimation formula based on Mito and Sato's (2002) method of measuring the parameters of anterior height (AH), posterior height (PH), antero-posterior height (AP), and body height (H) from C3 and C4 and then determining their ratios (AH/AP, H/AP, PH/AP, AH/H, H/PH, AH/PH) (Fig. 1).<sup>12</sup> We used panoramic radiographs to estimate dental age using the TCI, which calculates the coronal pulp cavity height (CPCH) and coronal height (CH) of the mandibular first premolar (Fig. 2). We selected the right or left region from the panoramic radiographs depending on which more clearly showed the pulp space as previously described.<sup>5</sup>

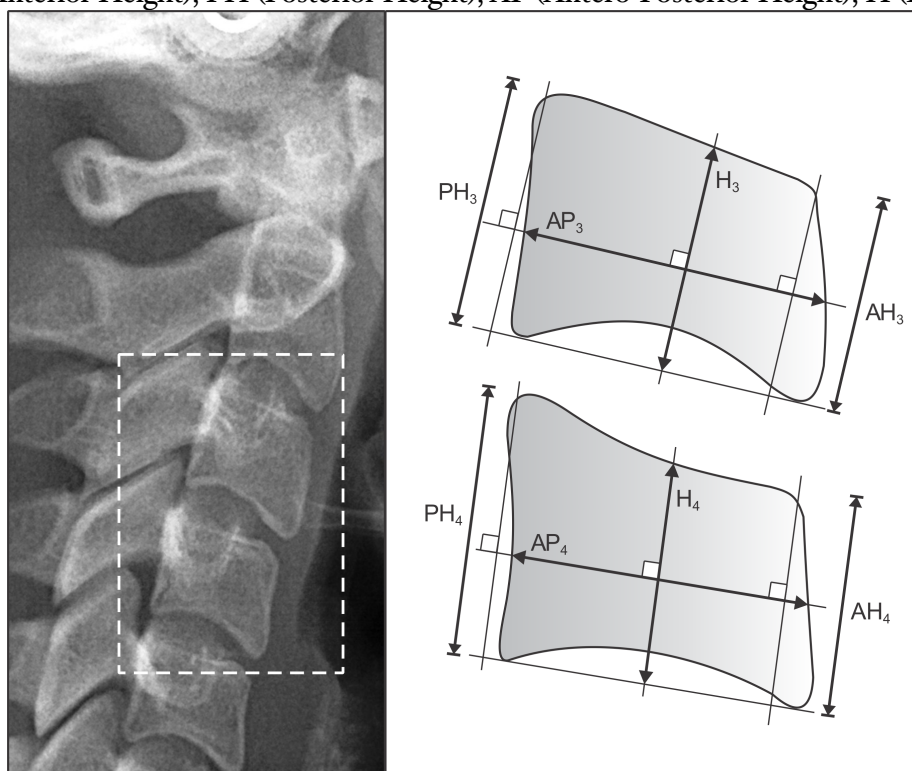
### *Statistical analysis*

panoramic radiographs) using Research Randomizer<sup>19</sup> and analyzed them using the intraclass correlation coefficient (ICC) (an ICC >0.9 indicated excellent agreement). Multiple linear regression analysis was used to devise the

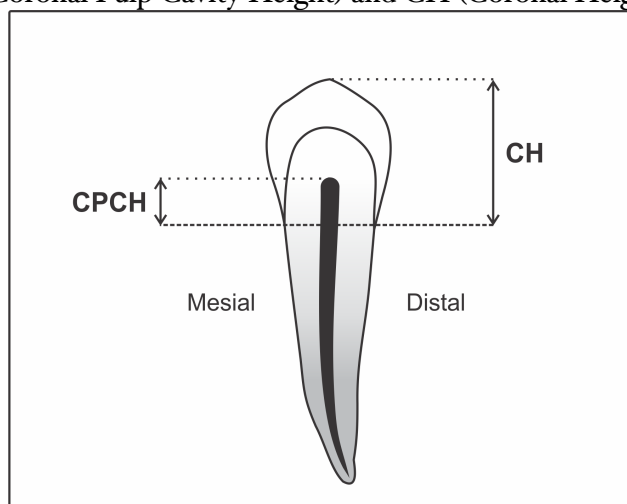
CV skeletal age estimation formula. Dental age was estimated using  $TCI = (CPCH \times 100)/CH$  and Nehemia et al.'s formula (2012), which is  $29.16 + (-0.4)TCI$ , with  $r^2 = 0.6407$  and  $r = 0.8$ .<sup>5,6</sup> The Bland-Altman plot was used to evaluate the agreement between CV skeletal-chronological, dental-chronological, and CV skeletal-dental ages. One-way analysis of variance (ANOVA) was then used to measure the difference between CV skeletal, dental, and chronological ages.  $P < 0.05$

was considered statistically significant. To test the accuracy of the CV skeletal age estimation formula, we performed a trial on group 2 (10 subjects, three males and seven females, with 10 lateral cephalometric and 10 panoramic radiographs) using consecutive sampling and the same eligibility criteria. We compared the age estimation results to chronological age using unpaired *t*-test analysis.  $P < 0.05$  was considered statistically significant.<sup>8</sup>

**Figure 1.** The third and fourth cervical vertebrae bodies from cephalometric radiographs. The parameters were AH (Anterior Height), PH (Posterior Height), AP (Antero-Posterior Height), H (Body Height)



**Figure 2.** TCI (Tooth Coronal Index) from the lower first premolar. The parameters were CPCH (Coronal Pulp Cavity Height) and CH (Coronal Height)



**RESULTS**

Table 1 shows the characteristics of group 1 by chronological age and sex. The mean ICC was 0.998 (95% confidence interval [CI] = 0.995–0.999) for CV skeletal parameters and 0.975 (95% CI = 0.925–0.992) for dental parameters. These values show excellent agreement between the two measurements on days 1 and 15. According to chronological age, the C3 and C4 measurements increased with increasing age (Fig. 3) whereas the mean TCI of the mandibular

first premolar decreased (Fig. 4). The mean CV parameters increased in the age range of 9–18 years. AH, H, and PH in both C3 and C4 increased whereas AP4 did not change. A Pearson correlation test (Table 2) revealed the correlation of all the CV parameters with chronological age: AH3/AP3 ( $r = 0.829$ ) and AH4/AP4 ( $r = 0.834$ ) were strongly correlated whereas H3/AP3, PH3/AP3, PH3/AP3, H3/PH3, H4/AP4, PH4/AP4, AH4/H4, and H4/PH4 were moderately correlated ( $0.3 < r < 0.7$ ).

**Table 1.** Characteristics of group 1 according to chronological age and sex.

Age	Male (n = 50)		Female (n = 50)		Total (n = 100)	
	Mean	SD	Mean	SD	Mean	SD
9 (n = 10)	9.40	0.26	9.33	0.13	9.37	0.20
10 (n = 10)	10.33	0.28	10.32	0.22	10.33	0.23
11 (n = 10)	11.45	0.40	11.50	0.36	11.48	0.36
12 (n = 10)	12.20	0.14	12.37	0.25	12.28	0.21
13 (n = 10)	13.33	0.39	13.65	0.19	13.49	0.33
14 (n = 10)	14.56	0.25	14.52	0.29	14.54	0.26
15 (n = 10)	15.32	0.07	15.65	0.17	15.48	0.21
16 (n = 10)	16.42	0.35	16.57	0.17	16.49	0.27
17 (n = 10)	17.85	0.09	17.38	0.17	17.62	0.28
18 (n = 10)	18.58	0.22	18.60	0.22	18.59	0.21
Total (n = 100)	13.95	3.01	13.99	2.98	13.97	2.98

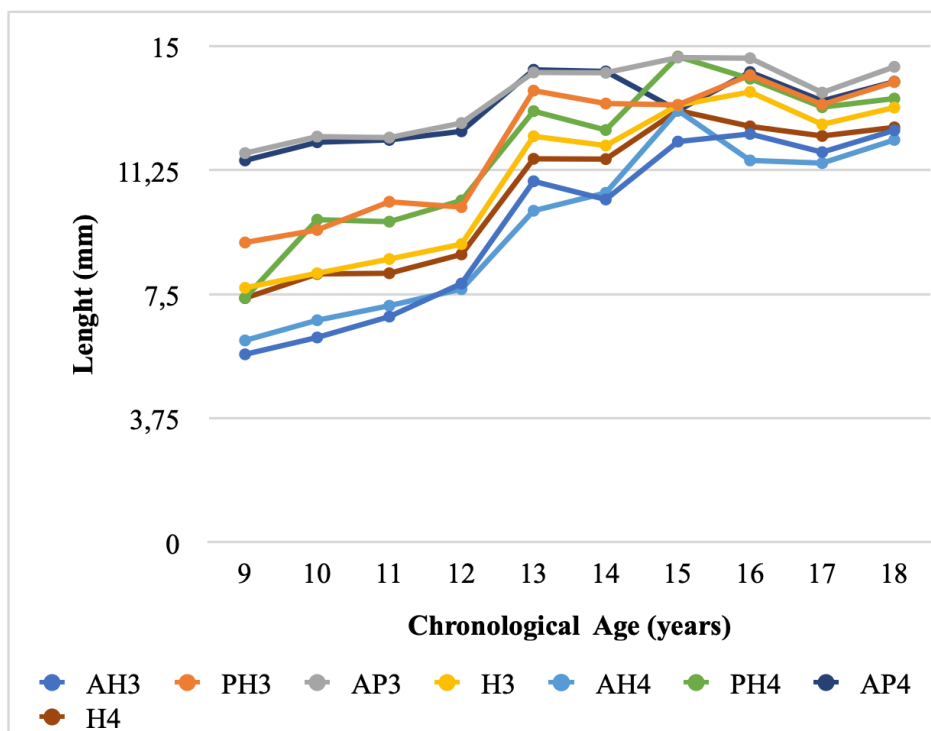
SD: standard deviation

**Table 2.** Correlation test between CV parameters and chronological age.

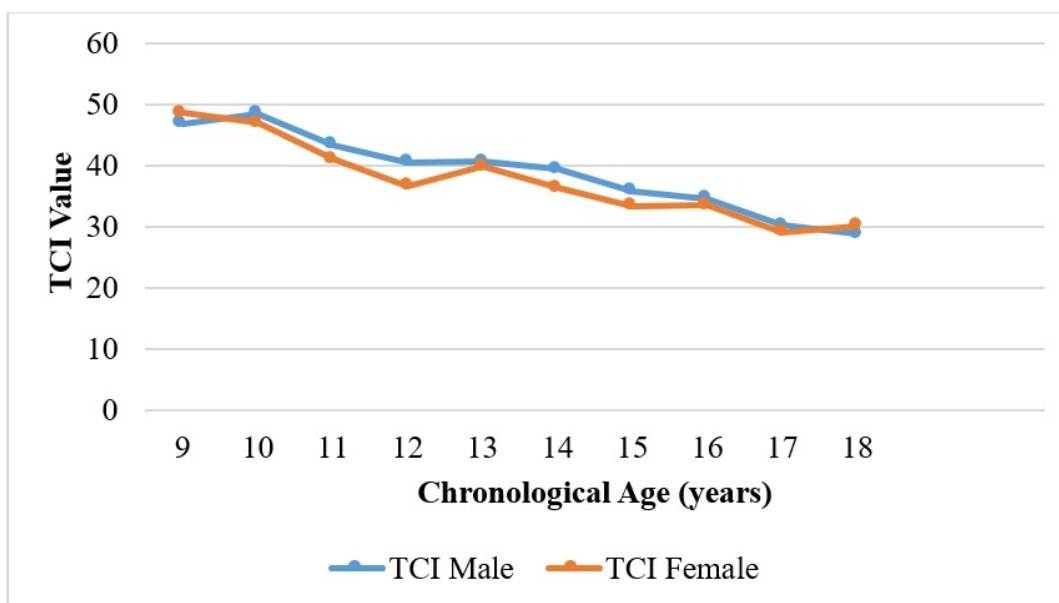
Ratio	AH/AP		H/AP		PH/AP		AH/H		H/PH		AH/PH		
	C3	C4	C3	C4	C3	C4	C3	C4	C3	C4	C3	C4	
Age	<i>r</i>	0.829	0.834	0.695	0.696	0.598	0.549	0.740	0.531	0.492	0.544	0.757	0.740
	<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	<i>n</i>	100	100	100	100	100	100	100	100	100	100	100	100

CV: cervical vertebrae; AH: anterior height; AP: antero-posterior height; H: body height; PH: posterior height

**Figure 3.** Mean value of parameter C<sub>3</sub> and C<sub>4</sub> from the CV body by chronological age group



**Figure 4.** Mean value of TCI lower first premolar by chronological age group



The adjusted  $r^2$  of the CV age estimation formula was 0.789, with a Durbin-Watson value of 1.192 and a one-way ANOVA of  $p < 0.001$ . The average residual value was 0. The CV skeletal age estimation formula was as follows:

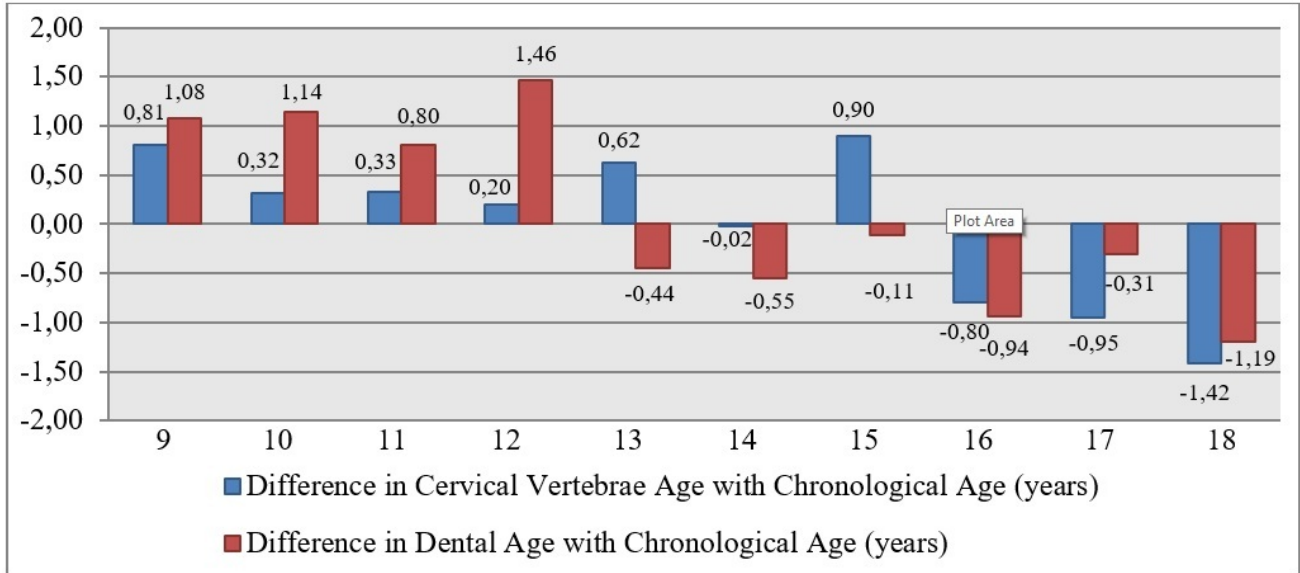
$$y = 15.827 + 14.19*(AH_3AP_3) + 15.604*(AH_4AP_4) - 7.432*(PH_3AP_3) - 7.407*(PH_4AP_4) - 10.823*(H_3PH_3) + \text{residual value}$$

Figure 5 shows the reliability analysis between CV skeletal and dental age estimation and chronological age. CV skeletal age was overestimated at 9–12 years and underestimated at 16–18 years. The most accurate CV skeletal age estimation was at 14 years, and the least accurate was at 18 years. Dental age estimation had the smallest mean difference at 15 years and the largest mean difference at 12 years.

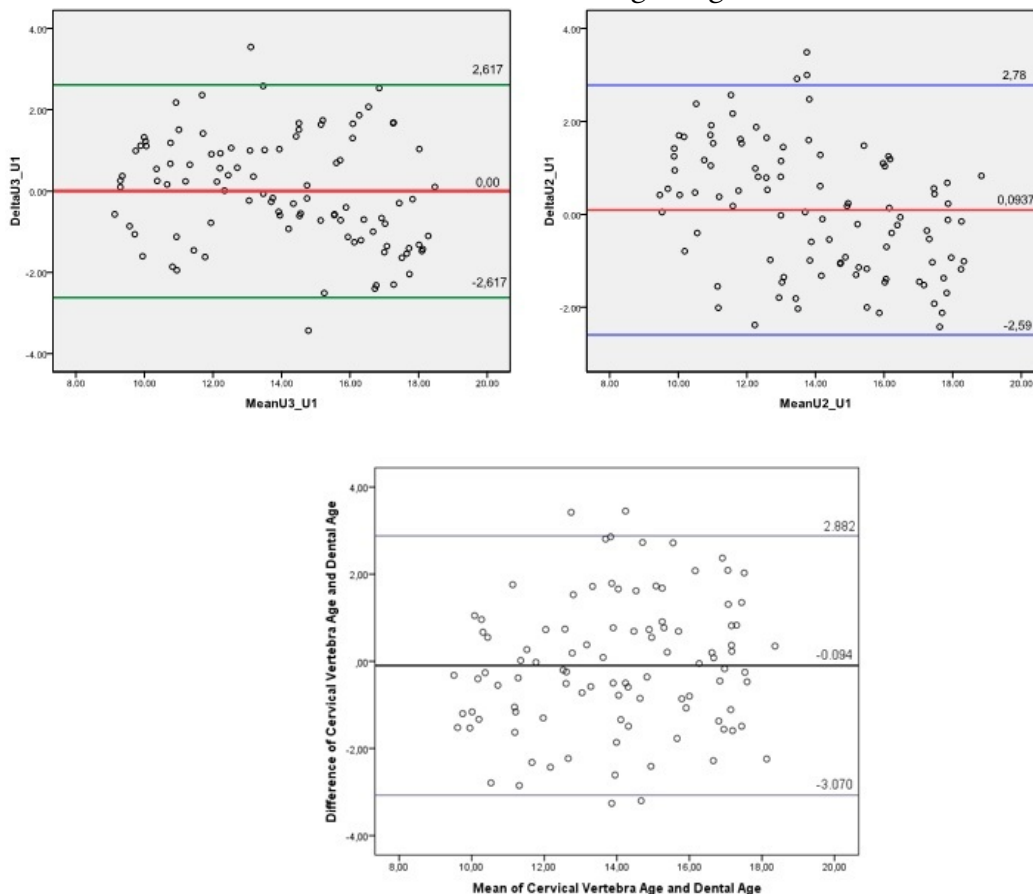
The Bland-Altman plot showed that the mean difference between CV skeletal age and chronological age and between dental age and chronological age was  $0.0000 \pm 1.34$  years and  $0.0937 \pm 1.37$  years, respectively (Fig. 6). The upper and lower limits of CV skeletal age were 2.63 and -2.63 years, respectively, while those of dental age

were 2.78 and -2.59 years, respectively. The Bland-Altman plot between CV skeletal age and dental age showed a mean difference of  $-0.094 \pm 1.52$  years, with upper and lower limits of 2.882 and -3.070 years, respectively. Therefore, compared to dental age estimation, CV skeletal age estimation conformed better to chronological age.

**Figure 5.** Difference in CV skeletal age and dental age estimation with chronological age per age group (n = 100)



**Figure 6.** The agreement Bland Altman plot between CV skeletal age (left) and dental age (right) estimation with chronological age



We found no statistically significant differences ( $p > 0.05$ ) between CV skeletal, dental, and chronological ages using one-way ANOVA (Table 3), which is consistent with previous studies.<sup>12,14,16</sup>

**Table 3.** One-way ANOVA for CV skeletal, dental, and chronological ages.

Age	Mean (SD)	P-value
CV skeletal age	13.97 (2.67)	0.961
Dental age	14.06 (2.45)	
Chronological age	13.97 (2.97)	

ANOVA: analysis of variance; CV: cervical vertebrae; SD: standard deviation

**Table 4.** Characteristics of group 2 according to chronological age and sex.

Age	Male (n = 3)		Female (n = 7)		Total (n = 10)	
	Mean	SD	Mean	SD	Mean	SD
9 (n = 6)	9.58	-	9.45	0.25	9.47	0.23
10 (n = 3)	10.33	-	10.38	0.18	10.36	0.12
11 (n = 1)	11.17	-	-	-	11.17	-
Total (n = 10)	10.36	0.80	9.72	0.50	9.7	0.64

SD: standard deviation

**Table 5.** Unpaired *t*-test analysis of the mean difference between CV skeletal and chronological ages and between dental and chronological ages (group 2).

Age	Mean (SD)	P-value
Skeletal-chronological age	0.366 (2.05)	0.002
Dental-chronological age	3.805 (2.26)	

CV: cervical vertebrae; SD: standard deviation

In the validation trial with group 2 (Table 4), the age range was 9–11 years, with a mean SD of 9.707 (0.64) years. Using the CV skeletal age estimation

formula for group 1, the CV skeletal age estimation for group 2 was 10.273 (1.59) years while the dental age estimation was 13.712 (2.32) years. Table 5 shows the mean difference between CV skeletal and chronological ages and between dental and chronological ages; the CV skeletal age result was more accurate ( $p = 0.002$ ).

**DISCUSSION**

Lateral cephalometric radiographs are often used to assess bone maturity level and determine skeletal age. However, CV skeletal age can be calculated objectively and reliably as in the TW2 hand-wrist method.<sup>11</sup> There is a significant correlation and no significant difference between hand-wrist skeletal age and the estimation of CV skeletal age from morphological CV parameters;<sup>13,15</sup> CV can replace the gold standard of hand-wrist bone for skeletal age estimation.<sup>10,13,16</sup> The CV body has a strong relationship with the development process in shape and size changes, is clearly visible, and can be measured using lateral cephalometric radiographs. C3 and C4 are measured because C1 and C2 do not show absolute changes and C5 and C6 are often invisible on lateral cephalometric radiographs.<sup>20–22</sup>

Teeth are often used to predict age with excellent accuracy.<sup>1</sup> The mandibular first premolar is highly accurate in age estimation, having the smallest standard of error among incisors, canines, and molars.<sup>1,5,6,23</sup> Nehemia et al. (2012) used panoramic radiographs for dental age estimation,<sup>5</sup> and Nurfitria et al. (2018) found no significant difference in age estimation using either periapical or panoramic radiographs.<sup>6</sup> Therefore, the TCI can be used with periapical or panoramic radiographs.<sup>5,6,17</sup>

In Figure 3, as previously reported, AP<sub>4</sub> remains constant, probably because of the AP dimensions of the vertebral body, which tend to develop in a superior-inferior manner with age.<sup>12,13,24</sup> Figure 4 illustrates the downward trend in TCI scores with increasing age group. This result indicates the decreased size of the pulp chamber with age, which can be caused by physiological factors such as the formation of secondary dentine, which starts after the crown and roots are fully formed and continues to develop as long as the tooth is viable. This secondary dentine deposit causes the pulp chamber to decrease in size and narrows the existing root canals. This narrowing tends to occur more quickly in the pulp horn region than

in other regions. Some researchers argue that changes in pulp chamber size can also occur due to pathological stimuli, including caries and traumatic occlusion.<sup>5,20</sup>

All the CV parameter ratios showed correlations with chronological age (Table 2). While the strength of the correlation is categorized as weak ( $0 < r < 0.3$ ), moderate ( $0.3 < r < 0.7$ ), or strong ( $r > 0.7$ ),<sup>25</sup> Mito, Caldas, and the present research found a diverse strength of correlation between the ratios. The correlation strength differs because of differences in the subject population and race.<sup>10,12</sup>

Age estimation using CV skeletal is more accurate than dental age estimation and can be used as a chronological age estimation tool. The CV skeletal age estimation formula from lateral cephalometric radiographs is limited to the 9–18 year age group of both sexes with no C3 or C4 abnormalities. Bones change with age; thus, the accuracy of the formula in other age groups needs to be tested further.

The limitation of this study is that the CV skeletal age estimation formula was validated

only by using consecutive sampling on 10 subjects aged 9–11 years. The testing of the formula in this age group does not provide a perfect analysis.

## CONCLUSIONS

Both CV skeletal and dental age estimation proved effective as tools for estimating chronological age, but CV skeletal age showed better agreement. We recommend estimating CV skeletal age in a larger sample differentiated according to sex for improved estimation.

## Acknowledgments

We thank the Dental Hospital of Universitas Trisakti, Jakarta, Indonesia for the digital radiograph samples and Denys Putra Alim, MD as the statistics mentor in this study.

## Financial support

This study was funded by Universitas Indonesia PITTA A Research Grant Year 2019 No. NKB-0416/UN2.R3.1/HKP 05.00/2019.

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