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Is there any relationship between pubertal growth spurt and dental or bone age estimation methods?

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ABSTRACT

The aim of this study was to compare the accuracy of the Willems method, Nolla method, Cameriere dental age estimation method (CDM), Greulich-Pyle Atlas(GPA) method, and Cameriere bone age estimation method(CBM) for age estimation in healthy children aged between ten and 14.9 years, according to the pubertal growth stage(PGS). The accuracy was determined as the difference between estimated age(EA) and chronological age(CA) was assessed by analysing orthopantomograms and hand-wrist radiographs. At the onset of the PGS, the Willems method showed the closest value to the CA for both sexes(girls = 0.17 ± 0.78, boys = 0.006 ± 0.96 years). At the peak, the Willems method showed the closest value to the CA for girls(0.005 ± 1.09 years), and the GPA method for boys(0.05 ± 0.68 years). At the end of the PGS, the Willems method showed the closest value to the CA for girls (0.09 ± 1.23 years), and the CBM for boys(0.24 ± 0.74 years). It was observed that all methods concluded with the closest result to the CA at the onset period of the PGS. Our study demonstrates that sex and PGS are effective in over- or under- estimations that are obtained according to the CA.

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Bone age estimation; dental age estimation; hand-wrist bones; puberty; tooth development

Introduction

Age estimation is one of the principal issues in various disciplines. In the area of forensic sciences, age estimation assists with solving legal or civil problems, namely adoption, determining criminal, legal liability, child abuse for minors, and identification for victims. Within clinical dentistry and medical disciplines, estimation of biological age aids diagnosis and treatment planning especially in terms of the success of the treatment[1,2].

Presently, histological, radiological, physical, and chemical methods can be used for age estimation[3–5]. In the radiological method, which is the most common method of age estimation, the age is estimated using the developmental stages of the bones or teeth[3,6,9]. Especially hand-wrist bones and teeth are selected because their radiographic images are obtained using a low radiation level compared to other structures, and a significant amount of bone or tooth structure information can be obtained using a simple radiography[10].

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Although radiographic images of various bones in the body are used in the assessment of bone age (BA), the most common method is the evaluation of hand-wrist radiographs due to their reliability and easy accessibility[11]. Among others[12–14] the Greulich-Pyle Atlas (GPA)[3] is one of the most used methods for age estimation because it is quick and relatively easy to learn and to perform[15]. The method involves matching the hand-wrist radiograph of the person to be age estimated to the closest one from a collection of photographs representing a series of skeletal maturation.

The formation of teeth is widely used to predict age and assess maturity[8,9,16,18]. In the literature, dental age (DA) estimation methods are considered to be more reliable than a skeletal analysis, as it is assumed that teeth are less affected by environmental and racial factors[1]. Many methods have been developed for DA estimation, and the most widely used are Nolla[8], Haavikko[17], Demirjian[18], and Willems methods[9]. In these methods, the age is calculated according to the tables developed by the researchers using the developmental stages of the teeth or calcification degrees to estimate the DA for individuals who have not reached skeletal maturity. However, in the methods developed by Cameriere et al.[6,7], unlike other methods, hand-wrist bones and teeth are not compared with the developmental stages that have been defined by the researchers; based on the measurements of these structures on radiographs. So they are metric rather than morphological. This may mean that they are not dependent on the experience and may be preferable to qualitative assessments of morphology.

Puberty is a maturational, hormonal, and developmental process that is dependent on biological factors[19]. The normal pubertal development process is characterized by major physical changes, such as sexual maturation, changes in body composition, and rapid skeletal growth[20]. The age for the onset of puberty changes according to sex, generation, population, and environment and varies greatly from person to person[21].

There is a strong correlation between puberty and skeletal development[21,22]. A radiological evaluation of the hand-wrist bones is generally used to determine the pubertal status in dental practices[23,24]. However, the relationship between puberty and dental development has not been clearly determined. Although some studies state that there is a weak association between them[21,25], there are also studies claiming the opposite[26,27]. Similarly, in addition to the studies that indicate that dental development can be used to determine pubertal status[28,29], some studies show that this method is unreliable[30,31].

Ye et al.[27] reported that individuals who were aged over ten years showed greater deviations in the DA assessment, which could be related to the onset of puberty. Similarly, other studies reported that there were differences in the age assessments during the pubertal growth period[32–35], which suggests that the pubertal spurt status may need to be considered in the age estimation.

This study aims to evaluate five different age estimation methods (Willems method, Nolla method, Cameriere dental age estimation method [CDM], GPA method, and Cameriere bone age estimation method [CBM]) in healthy children aged between ten and 14.9 years, according to the pubertal growth stages in a sample of Turkish children.

Materials and methods

The study was ethically approved with the decision (dated 26.12.2018 and numbered 920) of Clinical Research Ethics Committee of Medical Faculty of Akdeniz University.

Determination of patient groups

This retrospective study includes 400 healthy children aged between ten and 14.9 who applied to the Akdeniz University Faculty of Dentistry between 01.01.2013 and 03.12.2018 and who had orthopantomograms (OPGs) and hand-wrist radiographs taken for treatment.

The exclusion criteria were as follows:

(1) Patients with systemic, developmental, or endocrine disorders.
(2) Patients with a history of previous orthodontic treatments.
(3) Patients with dental anomalies, tooth extractions, or congenital tooth deficiencies in the left seven mandibular teeth (except the third molar).
(4) Patients with deep caries, restoration, apical lesion, canal treatment, or any pathologies in the left seven mandibular teeth or jawbone.
(5) Patients with any major traumas or operations on the face and hand-wrist.
(6) OPGs and hand-wrist radiographs of insufficient quality (distorted, projection error, or artefact).
(7) Subjects who had not reached the onset of PGS were also excluded.

After the radiographs that met the appropriate criteria for the study had been selected, the children's birth dates were recorded, and their chronological ages (CA) were calculated. Study groups were formed in accordance with the CA and sex factors. Accordingly, five groups were formed for children between the ages of ten and 14.9, and each group consisted of an equal gender ratio (40 girls, 40 boys). Groups were formed by assigning the first 40 eligible participants from the pre-selection. The DA estimation was carried out using three different methods (Willems method, Nolla method, and CDM) using the OPGs that were taken from the patients. The BA estimation was carried out using two different methods (GPA and CBM) using hand-wrist radiographs, and the skeletal maturation was assessed according to the method described by Grave and Brown[22].

To avoid any prejudices during the assessment of the teeth and hand-wrist bones, the children's radiographs were recorded numerically, and the calculations were carried out at different times by the same researcher without knowing the CA of the patients.

CA calculation

The children's CAs were calculated by subtracting their date of birth from the date of the panoramic or hand-wrist radiographs after they had both been converted to the decimal age.

DA estimation

The Willems, Nolla, and CDM radiographic methods on the left permanent mandibular teeth (except the third molar) were used for the dental age estimation. The Willems method, which is based on a revised Demirjian method using adopted scores, was

developed by Willems et al.[9]. In the method, the mineralization stages of the left seven permanent mandibular teeth were examined in the OPG and matched with the A-H mineralization stages defined by Demirjian et al. The scores obtained as a result of the matching were scored according to the tables which were created regarding the sex by Willems et al. and age estimation was made by adding these scores.

In the Nolla method, calcification degrees of the teeth were evaluated and assigned a stage of between 1 and 10 determined by Nolla. If a tooth was between stages a suitable fraction (0.2–0.5 or 0.7) was added as recommended by Nolla. The sum of scores was compared to the average sum for each sex and DA was calculated[8].

DA was also estimated by the CDM was explained in detail by Cameriere et al. [6]. Briefly in this method, root development and apex opening of the seven left permanent mandibular teeth were evaluated. For teeth with one root, the distance (Ai, i = 1, . . .,5) between the inner sides of the open apex was measured, for teeth with two roots (Ai, i = 6, 7), the sum of the distances between the inner sides of the two open apices was evaluated. Radiographic tooth length was measured (Li, i =1, . . ., 7). All measurements were made by ImageJ (1.52a, National Institutes of Health, USA), using JPG format radiographs. The xi = Ai/Li (i =1, . . ., 7) value was calculated to eliminate accountable differences in magnification and angulation during radiographic imaging and the DA was calculated with the following European linear regression formula[36].

(the sum of normalized open apices (s), number of teeth with complete root development (N_0), (g) is a variable equal to 1 for boys and 0 for girls, (x5 = A5/L5) A, is the radiographic distance between the inner sides of the open apex, L is the radiographic tooth length, The '5' in the formula is the A/L ratio of the second premolar)

BA estimation

The GPA and CBM on the left hand-wrist radiographs were used for BA estimation. In GPA[3] the left hand-wrist radiographs of each patient were matched with the radiographic images found separately for girls and boys in the atlas, the most appropriate image was selected and the age of that image was accepted as the bone age. Since the bone age corresponding to each image is given as month and year in GPA, the parts after the comma of the values found as a result of the evaluation are converted to decimal values by mathematical calculation for compatibility.

BA was also estimated by the CBM was explained in detail by Cameriere et al.[7]. While calculating; The carpal area (Ca) and the epiphyses of the ulna and radius were identified and defined by the polygonal lasso instrument of ImageJ. The areas of the eight carpal bones were selected, the pixel areas were calculated, and added together to yield the global values of bone areas (Bo). If two bones are overlapped, the common area was calculated only once. BA was calculated according to the following formula:

Age = −3.253 ± 0.719 g ± 20.610 Bo/Ca

(carpal area (Ca), bone area (Bo), (g) is a variable equal to 1 for boys and 0 for girls)

Table 1. Description of the ossification events [22].

Stage	Abbreviation	Ossification events
Onset	1. PP2 =	Proximal phalanx of second finger; epiphysis is as wide as its diaphysis
	2. MP3 =	Middle phalanx of third finger; epiphysis is as wide as its diaphysis
	3. H-1	Hooking of hamate-Stage 1
	4. Pisi	Appearance of pisiform
	5. R =	Radius; epiphysis is as wide as its diaphysis
Peak	6. S	Appearance of ulnar sesamoid at metacarpophalangeal joint of first fmger
	7. H-2	Hooking of hamate-Stage 2
	8. MP3cap	Middle phalanx of third finger; epiphysis caps its diaphysis
	9. PP1cap	Proximal phalanx of first finger; epiphysis caps its diaphysis
	10. Rcap	Radius; epiphysis caps its diaphysis
End	11. DP3u	Distal phalanx of third finger; complete epiphyseal union
	12. PP3u	Proximal phalanx of third finger; complete epiphyseal union
	13. MP3u	Middle phalanx of third finger; complete epiphyseal union
	14. Ru	Radius; complete epiphyseal union

Pubertal growth stage estimation

Skeletal maturation was assessed according to the method described by Grave and Brown[22] using hand-wrist radiographs for each patient. This method includes 14 stages of bone ossification, which represent the following three growth periods: the onset, the peak, and the end of PGS. These stages and ossification events are shown in Table 1.

Reproducibility

In our study, the radiographic images of randomly selected 20 female and 20 male patients were re-evaluated by the same researcher with each method used in the study one month after the first radiographic evaluation in order to evaluate the integrity and reliability of the researcher. The weighted Cohen's Kappa coefficient was used to determine the reproducibility of the statistics.

Statistical analysis

Statistical analyses were performed using the SPSS (IBM SPSS Statistics 18.0; IBM Co., Armonk, NY, USA) software package. The mean difference between estimated age (EA) and CA was calculated for each sex and PGS, and the total sample. A positive result indicated an overestimation, and a negative result indicated an underestimation of age. To better understand the difference between EA and CA without being affected by negative and positive values, the standard error of estimation (SEE) was calculated. Box-plot graphs are used to present the mean EA-CA of each sex and the total sample (Figures 1–3). In addition to the descriptive statistical methods, a paired t-test was used to compare the EA and CA. Non-parametric Wilcoxon signed-rank test was also applied to assess the significances of the difference between EA and DA because the sample size was small and had non-normal distribution in some PGS groups. For all tests, a p value less than 0.05 was considered statistically significant.

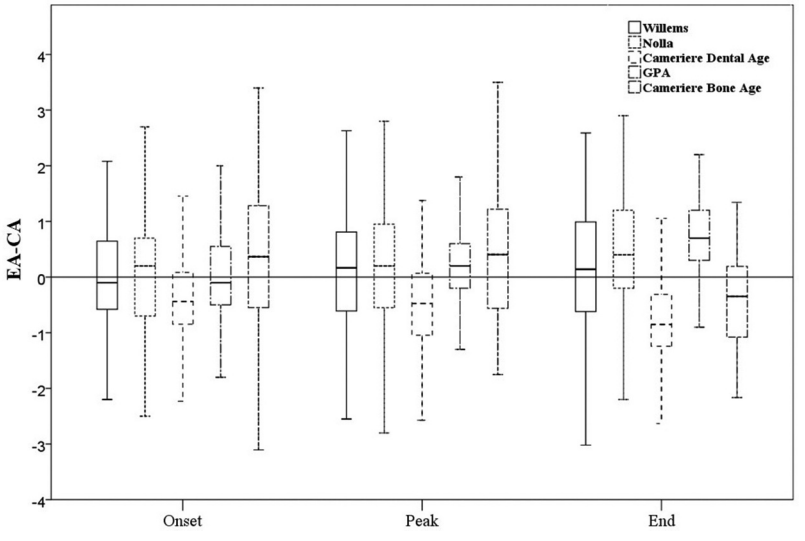

Figure 1. Box plot of the mean differences between estimated age (EA) and chronological age (CA) by all methods according to pubertal growth stages.

Results

A total of 400 children (200 boys, 200 girls) between the ages of ten and 14.9 (mean CA: 12.45 ± 1.41) were included in this study. When they were grouped according to their sex, the mean CA of the boys was 12.45 ± 1.43, and the mean CA of the girls was 12.44 ± 1.39. There was no statistically significant difference in the CA distribution between the girls and boys ($p = 0.921$). The results of Cohen Kappa scores were found to be higher than 0.95 for all methods.

Upon examining all individuals, the Willems method showed the closest value according to the CA (0.09 ± 1.07). It was observed that there was a statistically significant difference according to the CA in all methods except the Willems method ($p < 0.05$). When all the individuals were evaluated according to sex, the closest value for the girls was obtained by the Willems method (0.02 ± 1.12), followed by the Nolla method (0.06 ± 1.26) and CBM (0.13 ± 1.20). For the boys, the closest result was obtained by the CBM (0.14 ± 1.25), and a statistically significant difference was observed in all other methods ($p < 0.05$) (Table 2).

The distribution of all individuals in the ten to 14.9 age group, according to their pubertal growth stages, is shown in Table 3. Although most of the girls were at the end of the PGS, most of the boys were at the onset period of PGS. According to the pubertal growth stages, the mean CAs of the girls and boys were calculated as 10.80 ± 0.69 and 11.49 ± 0.99 at the onset period of PGS, 11.67 ± 0.92 and 13.53 ± 0.78 at the peak period of the PGS, and 13.53 ± 0.92 and 14.40 ± 0.32 at the end of the PGS, respectively.

Descriptive statistics results according to the pubertal growth stages are summarized in Table 4. The closest value to the CA was obtained by the Willems method for girls and boys at the onset period of PGS (girls = 0.17 ± 0.78, boys = 0.006 ± 0.96 years). While the results obtained for the girls were statistically different for the BA estimation methods, a statistically significant difference was found in all other DA estimation methods, apart

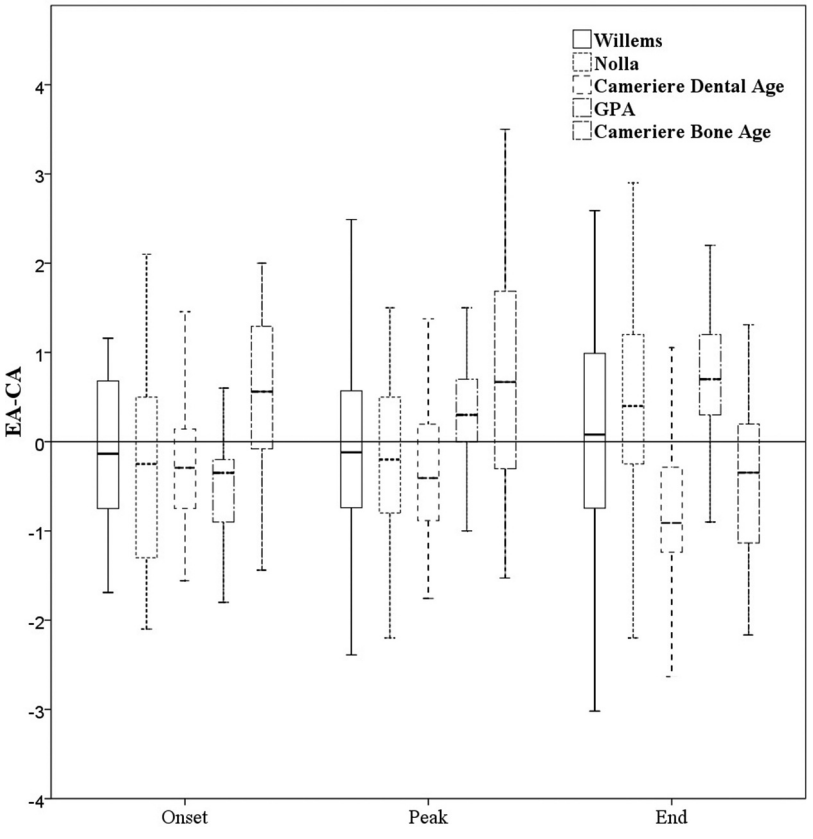

Figure 2. Girls – Box plot of the mean differences between estimated age (EA) and chronological age (CA) by all methods according to pubertal growth stages.

from the Willems method, for the boys ($p < 0.05$). Regarding the girls, only the CBM overestimated the age, while all other methods underestimated it. In the boys, all methods, except the CDM, overestimated the age.

At the peak, in the girls, the closest mean age difference was 0.005 ± 1.09 for the Willems method and −0.13 ± 1.10 for the Nolla method. Additionally, there was a statistically significant difference for all methods except these two methods ($p < 0.05$). In the boys, the closest mean age differences were obtained by the GPA method (0.05 ± 0.68) and CBM (0.07 ± 0.96), and there were statistically significant differences for three DA estimation methods compared to the BA estimation methods ($p < 0.05$). The Willems method, GPA method, and CBM overestimated the age in both girls and boys, whereas the Nolla method overestimated the age for boys and underestimated it for girls, and the CDM underestimated age for both sex.

At the end of the PGS, the closest value to the CA for girls was obtained using the Willems method (0.09 ± 1.23), and it was determined that the other methods differed statistically ($p < 0.05$). For the boys, the closest value to the CA was obtained by the CBM (0.24 ± 0.74), followed by the Willems (0.43 ± 1.11) and Nolla methods (0.48 ± 1.06). Moreover, there was a statistically significant difference

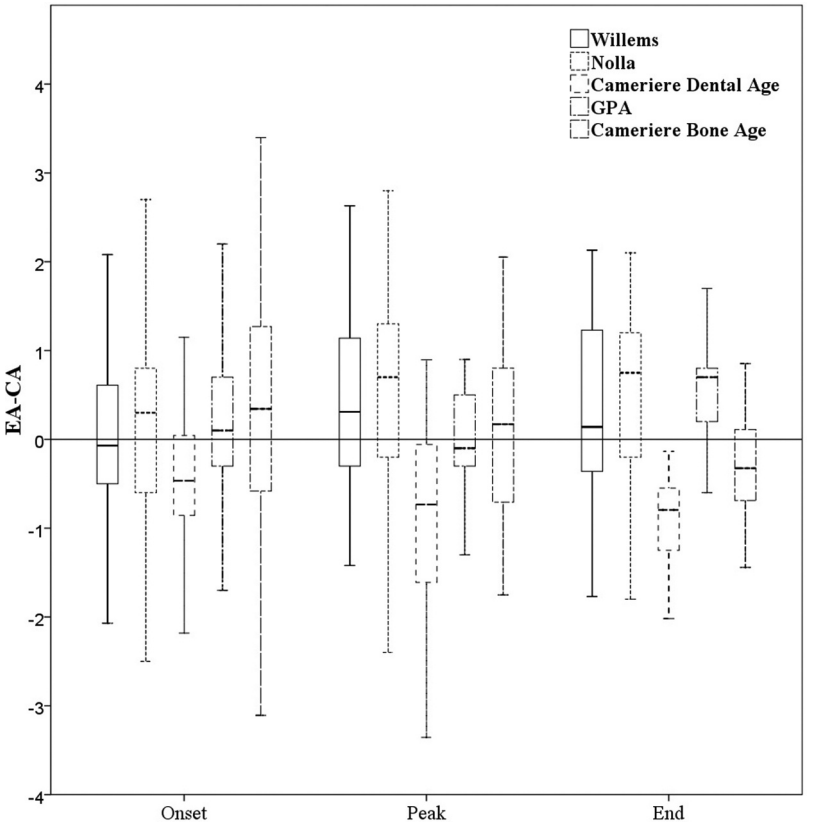

Figure 3. Boys – Box plot of the mean differences between estimated age (EA) and chronological age (CA) by all methods according to pubertal growth stages.

Table 2. The mean differences between the estimated ages (EA) and chronological ages(CA) for boys and girls and in total according to the methods (years).

			CA	EA	EA-CA	p
Methods	Sex	N	Mean ± SD	Mean ± SD	Mean ± SD	
Willems	Girls	200	12.44 ± 1.39	12.47 ± 1.76	0.02 ± 1.12	0.737
	Boys	200	12.45 ± 1.43	12.62 ± 1.75	0.16 ± 1.00	0.022
	Total	400	12.45 ± 1.41	12.54 ± 1.75	0.09 ± 1.07	0.074
Nolla	Girls	200	12.44 ± 1.39	12.51 ± 2.01	0.06 ± 1.26	0.460
	Boys	200	12.45 ± 1.43	12.82 ± 1.84	0.36 ± 1.16	0.0001
	Total	400	12.45 ± 1.41	12.66 ± 1.93	0.21 ± 1.22	0.001
CDM	Girls	200	12.44 ± 1.39	11.87 ± 1.16	−0.57 ± 0.85	0.0001
	Boys	200	12.45 ± 1.43	11.80 ± 1.31	−0.65 ± 0.94	0.0001
	Total	400	12.45 ± 1.41	11.83 ± 1.23	−0.61 ± 0.90	0.0001
GPA	Girls	200	12.44 ± 1.39	12.85 ± 1.69	0.41 ± 0.76	0.0001
	Boys	200	12.45 ± 1.43	12.61 ± 1.35	0.15 ± 0.75	0.005
	Total	400	12.45 ± 1.41	12.73 ± 1.53	0.28 ± 0.77	0.0001
CBM	Girls	200	12.44 ± 1.39	12.57 ± 0.98	0.13 ± 1.20	0.116
	Boys	200	12.45 ± 1.43	12.60 ± 1.54	0.14 ± 1.25	0.099
	Total	400	12.45 ± 1.41	12.59 ± 1.29	0.14 ± 1.23	0.022

CDM: Cameriere dental age estimation method GPA: Greulich-Pyle Atlas CBM: Cameriere bone age estimation method

Table 3. Distribution of the girls and boys grouped by skeletal maturation.

	Girls			Boys		
Age group (years)	Onset	Peak	End	Onset	Peak	End
10–10.9	19	20	1	40	0	0
11–11.9	5	31	4	38	2	0
12–12.9	2	20	18	27	13	0
13–13.9	0	7	33	6	32	2
14–14.9	0	1	39	2	22	16
Total	26	79	95	113	69	18

in the other two age estimation methods ($p < 0.05$). For both sex, although the Willems, Nolla, and GPA methods overestimated the age compared to the CA, the CDM and CBM underestimated it.

Discussion

Age estimation is of great significance in forensic and medical sciences, and the number of studies for age estimation has been increasing over time. However, the current methods are generally developed according to different populations, and it is difficult to clearly state which method provides the most accurate result, as bone and tooth development varies according to social and individual factors. Upon analysis, similar results may occur between societies, and different results can be seen in different regions of the same country[27,33,37,39].

The accuracy of an age assessment method is influenced by the following three factors: the quality and applicability of the reference standards of the method; the correct interpretation of the radiographic image; and individual variability in biological development[39]. Therefore, individual variability should be considered an important factor in age assessment. Additionally, individuals with different syndromes or pathologies may have deviations in the age assessment. Ozerovic[40], after evaluating the relationship between the CA and DA, concluded that cerebral palsy affects tooth and skeletal development. In another study, it was reported that although there was no significant difference between the CA and DA in boys with cerebral palsy or down syndrome, the DA was significantly delayed in girls compared to the CA[41]. However, it was found that dental variations affect the DA[42,43]. Moreover, dental development may be affected by problems related to the pubertal period when growth and development accelerate. One study reported that dental maturation was delayed in boys with a constitutional delay of growth and puberty[26]. However, Lee et al.[44] stated that early maturation was observed in mandibular teeth in girls with central precocious puberty.

Puberty is a developmental process that is characterized by major physical changes, such as sexual maturation, changes in body composition, and rapid skeletal growth, and is controlled by complex neuro-hormonal mechanisms throughout[20,45]. The effect of hormones on dental maturity or jaw growth is controversial[46]. Cantu et al.[47] reported that although the BA was delayed in the radiographs of patients with a growth hormone deficiency, there was no delay in the DA. In contrast, Kjellberg et al.[48] reported that patients with a growth hormone deficiency showed delayed dental eruption. Moreover, several studies found a relationship between the hormones secreted by the pituitary

Table 4. The mean differences between the estimated age (EA) and chronological ages (CA) according to pubertal growth stages.

		Onset						Peak						End					
			Mean ± SD						Mean ± SD						Mean ± SD				
Method	Sex	N	CA	EA	EA-CA	SEE	p	N	CA	EA	EA-CA	SEE	p	N	CA	EA	EA-CA	SEE	p
Willems	Girls	26	10.80 ± 0.69	10.62 ± 1.02	−0.17 ± 0.78	0.82	0.262	79	11.67 ± 0.92	11.68 ± 1.36	0.005 ± 1.09	1.09	0.963	95	13.53 ± 0.92	13.63 ± 1.40	0.09 ± 1.23	1.24	0.431
	Boys	113	11.49 ± 0.99	11.49 ± 1.17	0.006 ± 0.96	0.97	0.943	69	13.53 ± 0.78	13.89 ± 1.16	0.35 ± 1.02	1.1	0.005	18	14.40 ± 0.32	14.83 ± 1.12	0.43 ± 1.11	1.23	0.117
	Total	139	11.36 ± 0.98	11.33 ± 1.19	−0.02 ± 0.93	0.94	0.727	148	12.54 ± 1.26	12.71 ± 1.68	0.16 ± 1.07	1.09	0.058	113	13.66 ± 0.91	13.82 ± 1.43	0.15 ± 1.21	1.23	0.183
Nolla	Girls	26	10.80 ± 0.69	10.42 ± 1.33	−0.37 ± 1.00	1.1	0.067	79	11.67 ± 0.92	11.54 ± 1.42	−0.13 ± 1.10	1.12	0.290	95	13.53 ± 0.92	13.88 ± 1.60	0.35 ± 1.38	1.43	0.015
	Boys	113	11.49 ± 0.99	11.70 ± 1.35	0.21 ± 1.14	1.17	0.045	69	13.53 ± 0.78	14.10 ± 1.35	0.56 ± 1.20	1.34	0.0001	18	14.40 ± 0.32	14.88 ± 1.02	0.48 ± 1.06	1.21	0.068
	Total	139	11.36 ± 0.98	11.46 ± 1.43	0.10 ± 1.13	1.15	0.271	148	12.54 ± 1.26	12.73 ± 1.88	0.19 ± 1.20	1.22	0.053	113	13.66 ± 0.91	14.04 ± 1.56	0.37 ± 1.33	1.39	0.003
CDM	Girls	26	10.80 ± 0.69	10.57 ± 0.76	−0.22 ± 0.73	0.77	0.131	79	11.67 ± 0.92	11.34 ± 0.91	−0.33 ± 0.76	0.84	0.0001	95	13.53 ± 0.92	12.65 ± 0.82	−0.87 ± 0.87	1.24	0.0001
	Boys	113	11.49 ± 0.99	11.01 ± 0.86	−0.47 ± 0.87	1	0.0001	69	13.53 ± 0.78	12.71 ± 1.00	−0.82 ± 0.96	1.29	0.0001	18	14.40 ± 0.32	13.30 ± 1.13	−1.09 ± 1.09	1.61	0.001
	Total	139	11.36 ± 0.98	10.93 ± 0.86	−0.42 ± 0.85	0.96	0.0001	148	12.54 ± 1.26	11.98 ± 1.17	−0.56 ± 0.89	1.06	0.0001	113	13.66 ± 0.91	12.76 ± 0.91	−0.90 ± 0.91	1.29	0.0001
GPA	Girls	26	10.80 ± 0.69	10.20 ± 0.79	−0.59 ± 0.70	0.95	0.0001	79	11.67 ± 0.92	12.00 ± 0.87	0.32 ± 0.59	0.69	0.0001	95	13.53 ± 0.92	14.29 ± 0.78	0.76 ± 0.63	1	0.0001
	Boys	113	11.49 ± 0.99	11.62 ± 0.77	0.13 ± 0.78	0.8	0.065	69	13.53 ± 0.78	13.58 ± 0.43	0.05 ± 0.68	0.69	0.542	18	14.40 ± 0.32	15.02 ± 0.58	0.62 ± 0.61	0.92	0.0001
	Total	139	11.36 ± 0.98	11.36 ± 0.95	0.0007 ± 0.82	0.83	0.992	148	12.54 ± 1.26	12.74 ± 1.05	0.19 ± 0.65	0.69	0.0001	113	13.66 ± 0.91	14.41 ± 0.80	0.74 ± 0.63	0.98	0.0001
CBM	Girls	26	10.80 ± 0.69	11.28 ± 1.10	0.48 ± 1.03	1.17	0.025	79	11.67 ± 0.92	12.38 ± 0.81	0.70 ± 1.24	1.44	0.0001	95	13.53 ± 0.92	13.09 ± 0.63	−0.43 ± 0.94	1.04	0.0001
	Boys	113	11.49 ± 0.99	11.74 ± 1.42	0.25 ± 1.45	1.49	0.068	69	13.53 ± 0.78	13.61 ± 0.82	0.07 ± 0.96	0.98	0.505	18	14.40 ± 0.32	14.15 ± 0.59	−0.24 ± 0.74	0.81	0.187
	Total	139	11.36 ± 0.98	11.65 ± 1.37	0.29 ± 1.38	1.42	0.013	148	12.54 ± 1.26	12.95 ± 1.02	0.41 ± 1.16	1.24	0.0001	113	13.66 ± 0.91	13.26 ± 0.74	−0.40 ± 0.91	1	0.0001

CDM: Cameriere dental age estimation method, GPA: Greulich-Pyle Atlas, CBM: Cameriere bone age estimation method, SD: Standard deviation, SEE: Standard error of the estimate (SEE)

gland and the growth of the jaw or the development of the dentition[49,50]. Embryologically, the pituitary gland is an important structure for the migration of neural crest cells in oral formation[51]. The anterior pituitary gland has the same mesenchymal origin as the oral neural crest cells, and the posterior pituitary gland has the same mesenchymal origin as the maxillofacial region[52]. Additionally, deformities belonging to the sella turcica, which form a bony seat for the pituitary gland, are associated with dental developmental disorders[53,54]. Moreover, many studies found deviations in the DA during the pubertal growth period, which is closely related to pituitary gland hormones[27,32,35]. Akkaya et al.[32] reported that the deviations in age differences detected in the pubertal period in both girls and boys increased, and they concluded that it may be related to the fluctuations in the pubertal growth period. Similarly, as a result of their study, in which they calculated the DA using different methods, Ye et al.[27] reported that there was a tendency to overestimate the age of older individuals (> ten years), and the overestimation was larger for boys than for girls, which could be due to the differences between boys' and girls' pubertal growth processes. However, there are no studies on the relationship between pubertal growth periods and age estimation methods in children with normal pubertal development.

Although several methods have been developed for age estimation, radiographic methods are more frequently preferred due to their ease of application, non-invasiveness, and reproducibility. In addition, more reliable results are obtained since hard tissues that are less influenced by many factors such as nutritional and environmental effects are evaluated[55]. Generally, these methods are based on comparing radiographic images with the schemes that are created by the researchers according to the development stages of the teeth or bones. On the other hand, in Cameriere age estimation methods, which are relatively newer, age is estimated by carrying out measurements on radiographs. These methods can be considered to be more quantitative as they do not base on a visual assessment read from published charts as in other radiographic methods. In this study, the validity of the Willems, Nolla, and GPA methods, CDM, and CBM were evaluated in 400 Turkish children between the ages of ten and 14.9.

We could not find any studies in which the DA and BA were directly evaluated according to the pubertal growth periods, and in studies that mentioned pubertal growth stages, the pubertal classification was estimated according to age. Therefore, there are no studies in the literature that we can directly compare the results of our study to. In addition, it would be more appropriate to evaluate boys and girls separately due to the prevalence of sexual dimorphism, which is commonly reported in dental and skeletal development. Since the selection of radiographs in our study was made according to chronological age, the rates between boys and girls, especially during the onset and end period of PGS, were significantly different, which is one of the limitations of our study.

Several previous studies that used the Willems method have been reported to overestimate the age compared to the CA (Table 5) [56,58]. Our study and other studies that were conducted in Turkey obtained similar results. On the other hand, the overestimation values that were obtained from our study using the Willems method were less than other Turkish population studies' overestimations for both sexes (Table 5) [32,59,62]. Upon evaluating the method according to the PGS, the method only underestimated the age at the onset period of the PGS in girls. The closest value to the CA was obtained at the peak period for the girls and the onset period of the PGS for the boys.

Table 5. Previous studies that show mean differences between estimated age (EA) and chronological ages (CA), comparing methods in different populations for different age groups (Positive results show overestimation, negative results show underestimation).

Authors, year of publication	Population	Number of subjects	CA (years)	Method	Sex	Mean differences between the EA and CA (EA-CA)
Loder et al.[66], 1993	ABD	841 (389 F, 452 M)	0–18.5	GPA	Girls	0.31
					Boys	0.08
Maber et al.[16], 2006	Bangladeshi and British Caucasian	946 (455 F, 491 M)	3.00 −16.99	Nolla	Girls	−1.18
					Boys	−0.87
					Total	−1.02
				Willems	Girls	−0.20
					Boys	−0.05
					Total	−0.12
Buken et al.[67], 2007	Turkish	492 (241 F-251 M)	11–18 (F) 11–19(M)	GPA	Girls	0.54
					Boys	0.13
El-Bakary et al.[56], 2010	Egyptian	286 (152 F, 134 M)	5–16	Willems	Girls	0,14
					Boys	0,29
				CDM	Girls	−0.26
					Boys	−0.49
Miloglu et al.[63], 2011	Caucasian Turkish	719 (409 F, 310 M)	7–18	Nolla	Girls	−0.5
					Boys	−0.3
					Total	−0.3
Galić et al.[57],2011	Bosnian-Herzegovian	1089 (591 F, 498 M)	6–13	Willems	Girls	0.25
					Boys	0.42
				CDM	Girls	0.10
					Boys	−0.02
Kırzıoglu et al.[34], 2012	Turkish	425 (213 F, 212 M)	7–13	Nolla	Girls	−0.57
					Boys	−0.53
					Total	−0.54
Nur et al.[64], 2012	Turkısh	673 (331 F, 342 M)	5–15.9	Nolla	Girls	−0.57
					Boys	−0.50
					Total	−0.54
Cameriere et al.[65], 2012	Italian	288 (136 F, 152 M)	5–15	CDM	Girls	−0.150
					Boys	−0.073
				CBM	Girls	0.135
					Boys	0,052
Akkaya et al.[32], 2015	Turkish	799 (412 F, 387 M)	2.2–15.99	Willems	Girls	0.157
					Boys	0.076
Mohammed et al.[39], 2015	Indıan	660 (330 F, 330 M)	6–16	Nolla	Girls	0.63
					Boys	0.31
					Total	0.47
				Willems	Girls	−0,11
					Boys	−0.69
					Total	−0.4

(*Continued*)

Table 5. (Continued).

Authors, year of publication	Population	Number of subjects	CA (years)	Method	Sex	Mean differences between the EA and CA (EA-CA)
Gülsahi et al.[38], 2015	Turkısh	573 (275 F, 298 M)	8–15	CDM	Girls	−0.24
					Boys	−0.47
					Total	−0.35
Kumaresan et al.[58], 2016	Malaysian	426(247 F, 179 M)	5–15	Nolla	Girls	0.57
					Boys	0.50
					Total	0.54
				Willems	Girls	0.53
					Boys	0.55
					Total	0.54
				CDM	Girls	−0.39
					Boys	−0.44
					Total	−0.41
Onat Altan et al.[59], 2016	Turkish	756 (378 F, 378 M)	5–14.99	Willems	Girls	0.26
					Boys	0.45
Özveren et al.[60], 2018	Turkısh	766 (386 F, 380 M)	6–14.99	Willems	Girls	0.17
					Boys	0.40
Apaydın et al.[33], 2018	Turkısh	330 (165 F, 165 B)	5–15.90	Willems	Girls	−0.062
					Boys	−0.056
					Total	−0.06
				CDM	Girls	−0.550
					Boys	−0.603
					Total	−0.58
Özveren et al.[61], 2019	Turkısh	636 (317 F, 319 M)	6–15	Willems	Girls	0.30
					Boys	0.52
				CDM	Girls	−0.37
					Boys	−0.18
Koç et al.[62], 2021	Turkısh	919 (459 F, 460 M)	6–14	Nolla	Girls	−0.57
					Boys	−0.41
					Total	−0.49
				Willems	Girls	0.34
					Boys	0.29
					Total	0.31

CDM: Cameriere dental age estimation method GPA: Greulich-Pyle Atlas CBM: Cameriere bone age estimation method

The age estimation values that were obtained using the Nolla method in some societies other than Turkey and Turkey were lower compared to the CA (Table 5) [16,34,62,64]. Similar to the studies conducted by Mohammed et al.[39] in India and Kumerasan et al.[58] in Malaysia, our study showed an overestimation of the DA for both genders. In our study, the closest results to the CA were obtained compared to previous studies that were conducted in Turkey, this proximity was more pronounced in girls. Although the method overestimated the age for boys in all three growth periods, for girls, it only overestimated the age at the end of the PGS.

The CDM showed conflicting results when it was applied for age estimation in different populations (Table 5) [33,38,56,58,61,65]. Most studies on the Turkish population reported an underestimation of the DA[33,38,61]. Similarly, in our study, the CDM underestimated the DA for both sexes, and the underestimation values were higher than other Turkish population studies' underestimations for both sexes. The method underestimated the DA in all three growth periods and the closest value to the CA was obtained at the onset period of the PGS for both sexes. While this result is similar to the results of other DA estimation methods for boys, it differs for girls.

Previous studies that used the GPA method have been reported to overestimate the age compared to the CA (Table 5) [66,67], as in studies that used the Willems method. It can be said that the results obtained using the GPA method are similar to the results obtained using the Willems method. In our study, the method only underestimated the age at the onset period of the PGS in girls. The closest value to the CA was obtained at the peak period for the girls, but at the peak period of the PGS for the boys, unlike the Willems method.

The literature research showed that only one study used a similar formula in the CBM[65]. Although Cameriere et al. reported an underestimation of the BA in both sexes in their study, which was conducted in Italy, our study showed overestimation values. The method overestimated the age at the onset and peak period of the PGS for both girls and boys, whereas it underestimated the age at the end of the PGS. Unlike the other methods, the closest value to the CA was obtained at the end period of the PGS for the girls.

At the peak and end periods of PGS, the DA estimation methods showed closer results to the CA in girls, and the BA estimation methods showed closer results in boys. Although the effect of puberty on tooth development is unclear, there are many studies on its effect on skeletal development. However, when we evaluated the methods that were used to calculate the BA in our study, we found similar differences to the DA, which could be due to the ethnic origins for which the GPA was created or the formulas by which the Cameriere methods were determined.

The results of the studies were largely different, despite some similarities. The differences in the methodology and analytical approaches that were used, as well as environmental factors, such as socioeconomic status, dietary habits, and lifestyle of the participants, could have contributed to these variable results[32,68]. These factors can also affect both dental formation and pubertal development. It has been reported that puberty onset/duration was affected by socioeconomic status[69] and children of high socioeconomic status showed an advancement in maturation of teeth[70]. However, we believe that one of the most significant factors in our study is the age range selection. Compared to other studies, our study was conducted on a population of a relatively narrower age range.

Conclusion

In summary, the Willems method showed the closest value to the CA for girls in all pubertal stages. On the other hand, the method that gave the closest result to the CA according to pubertal stages showed differences in boys. It was observed that all methods concluded with the closest value to the CA at the onset period of the PGS. Our study demonstrates that sex and PGS are effective in over- or under- estimations that are obtained according to the CA. Therefore, further studies are needed to verify the regional background of the sample, sex, and chronological age distribution, and statistical procedure, as well as whether the stages of pubertal growth represent the main factors that control accuracy and reliability in age assessments.

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