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# The Cameriere, Haavikko, Demirjian, and Willems methods for the assessment of dental age in Croatian children

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## Abstract

**Aim** This study aimed to evaluate the accuracy and precision of the Cameriere European formula, Demirjian, Haavikko, and Willems methods for estimating dental age in a sample of children with permanent dentition in Croatia.

**Material and methods** The study consisted of a sample of 1576 panoramic radiographs; a sub-sample of 84 OPGs, in which all first seven mandibular teeth were matured, was excluded from the study. A final sample of 1492 (704 males and 788 females) aged 6.0 to 13.9 years was evaluated. Seven mandibular teeth from the left side of the mandible were analyzed, and dental age (DA) was determined by the Cameriere European formula, Demirjian method from 1976, Haavikko, and Willems methods and compared to chronological age (CA). In addition, the mean age difference (DA-CA), the mean absolute error (MAE) between dental and chronological age, the percentage of the individuals of dental age within  $\pm 0.25$  to  $\pm 2$  years of chronological age, and intra-observer and inter-observer statistics were calculated.

**Results** The Cameriere European formula estimated the best dental age compared to the chronological age; the mean underestimation was  $-0.4$  years for both sexes, Haavikko underestimated by  $-0.17$  years, while Demirjian and Willems overestimated by 1.02 years and 0.48 years, respectively. The most significant difference showed the Demirjian method in 11-year-old and 12-year-old females. The MAE were 0.50 years, 1.01 years, 0.61 years, and 0.78 years in males and 0.51 years, 1.18 years, 0.61 years, and 0.70 years in females for the Cameriere European formula, Haavikko, Willems, and Demirjian methods, respectively. Furthermore, the Cameriere European formula showed the highest proportions of individuals with DA within  $\pm 0.5$  year difference of the CA, 61.5% in males and 59.6% in females. In addition, the Cameriere method showed the best intra-observer and inter-observer agreements.

**Conclusions** Although the Demirjian method was used previously in Croatian children for legal, medical, and clinical purposes, the Cameriere European formula, Haavikko, and Willems were more accurate in the tested sample. According to our findings, the Cameriere European formula showed the best accuracy and precision in dental age assessment in Croatian children following Haavikko, and we recommend it as the method of the first choice in forensic and clinical analyses.

**Keywords** Age assessment · Dental maturity · Forensic dentistry · Anthropology · Cameriere European formula · Haavikko · Demirjian and Goldstein · Willems

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## Introduction

Determining dental age is of great importance in clinical disciplines such as pedodontics and orthodontics, research on archeological skeletal specimens, and forensic analyses of living people and cadavers [1–11].

Various methods for determining age in children, include general physical examination, molecular biomarkers, DNA isolation, racemization of aspartic acid, and radiographic images of the hand, knee, and dental images, were recognized [12–19].

Different methods based on radiographic analysis of dental age have been proposed, focusing on the qualitative

aspect of tooth calcification or the shape of tooth development and apex width [12, 20–22]. For example, Haavikko [23] published median age values of twelve developmental stages of upper and lower permanent teeth based on an analysis of panoramic radiographs (OPGs) of children from Helsinki, Finland. Next, according to Demirjian, the method based on eight developmental stages of seven mandibular permanent teeth was set on French-Canadian children [24]. Demirjian's method showed contradictory results when applied to estimate age in different populations and therefore underwent numerous modifications and the creation of new tables [11]. The Demirjian method mostly overestimates the known chronological age [25–28]. Willems et al. [26] used Demirjian stages and adopted age estimation standards based on the Belgian sample of OPGs of the children. Previous research in determining dental age in Croatian samples were mainly based on the Demirjian and Willems methods and compared children in different parts of the world [26, 29, 30]. Cameriere et al. [20] presented a linear regression formula based on variables resulting from measurements of projections of open apices and heights of mandibular developing teeth and teeth with closed apex.

This study aimed to validate the accuracy and precision of the Cameriere European formula, Demirjian method from 1976 (Demirjian), Haavikko, and Willems methods on a sample of children in Croatia aged 6 to 13 years and to assess which of the four methods is most accurate for estimating the dental age of children in Croatia.

## Material and methods

Panoramic radiographs (OPGs) of individuals aged from 6.0 to 13.9 years from the database of the Department of Dental Anthropology, School of Dental Medicine, the University of Zagreb, taken from 2000 to 2020 were used in this study. All individuals live in the Zagreb metropolitan area, continental and coastal parts of the Republic of Croatia. Totally, 117 OPGs of low quality, medical history of systemic diseases, congenital anomalies, and hypodontia of permanent teeth, except for third molars, were excluded. The material consists of 1576 consecutive OPGs, and a sub-sample of 84 OPGs, in which all first seven mandibular teeth were matured, was excluded. A final sample of OPGs for analysis was 1492, with a comparable ratio of males (704) and females (788). There was a recording date for each OPG and the child's date of birth and sex. The chronological age was calculated by subtracting the date of birth given by the patient/caregiver from the date the OPG was taken. All OPGs were divided into seven age groups according to chronological age: 6–6.9; 7–7.9; 8–8.9; 9–9.9; 10–10.9; 11–11.9; and 12–12.9 years, Table 1.

All data were entered into an Excel spreadsheet. In addition, informed consent for using OPGs was obtained from

**Table 1** The cohort of panoramic radiographs of the Croatian children

Age group	Males	Females	Both
6.0–6.9	39	45	84
7.0–7.9	71	67	138
8.0–8.9	110	112	222
9.0–9.9	112	123	235
10.0–10.9	100	99	199
11.0–11.9	109	137	246
12.0–12.9	99(5)	133(14)	232(19)
13.0–13.9	64(21)	72(44)	136(65)
<b>Total</b>	<b>704(26)</b>	<b>788(58)</b>	<b>1492(84)</b>

Note: The values within parentheses represent the number of panoramic radiographs where completed mineralization of all first seven teeth of the left side of the mandible

parents and guardians. The Ethics Committee of the School of the Dental Medicine University of Zagreb approved this research, decision number: 05-PA-30-XVIII-6/2020.

The second author, IG, analyzed the sample of OPGs. Measurements and stages estimation were performed on OPGs on seven permanent mandibular teeth on the left side. Standard methods for age estimation according to Cameriere et al. [31], Demirjian and Goldstein [11], and Willems [26] were applied to this study.

For the Cameriere method, open apices ( $A_i$ ,  $i = 1, \dots, 7$ ) and heights ( $L_i$ ,  $i = 1, \dots, 7$ ) were measured, normalized scores of open apices ( $X_i = A_i/L_i$ ) were calculated, and the number of teeth with closed apices ( $N_0$ ) was estimated for the Cameriere method. The European formula was used for age calculation:

$$\text{Age} = 8.387 + 0.282_g - 1.692X_5 + 0.835N_0 - 0.116S - 0.139SN_0 \quad (1)$$

where the  $g$  variable in the formula was taken as 0 for females and 1 for males, and  $S$  in the normalized scores with open root apices ( $S = X_1 + \dots + X_7$ ) [31]. The Demirjian and Willems methods used the same scoring system of eight stages of mineralization (A–H). For the Demirjian method, the system for dental maturity based on seven teeth from 1976 was used [11]. Briefly, a maturity self-weighted score for Demirjian dental stages using seven mandibular teeth was used to calculate each individual's total maturity score, and dental age was calculated from the 50th dental maturity percentile of total maturity scores from Demirjian and Goldstein [11]. The Willems method was created on the adopted scoring system based on the Belgian sample of OPGs, uses the same Demirjian stages, and dental age was calculated from new age scores expressed in years [26]. The dental age for the Haavikko method was calculated as the mean age of age medians of estimated tooth stages of the first seven mandibular teeth from the left side. The final stage of apex

closure or  $A_c$  was excluded for age calculation to avoid the bias of matured teeth for age calculation [23]. Haavikko estimated the median age and 10% to 90% age range of mineralization stages from the OPTs of Finnish children from Helsinki. The results of dental age were presented separately for each sex.

The randomized sample of 100 OPGs was examined by the IG and the fifth author (FM) for estimating intra-observer and inter-observer agreements 2 weeks after the analysis of the sample.

**Statistical analysis**

Statistical analysis was done using the statistical software package MedCalc® Statistical Software version 20.027 (MedCalc Software Ltd., Ostend, Belgium; <https://www.medcalc.org>; 2022). The precision or reliability of methods was calculated by intraclass correlation coefficient (ICC) for the continuous variables and by the mean Cohen kappa (kappa) for the dental stages [32]. A value of  $ICC < 0.5$  may be interpreted as a poor agreement,  $ICC \geq 0.50$  and  $< 0.75$  as moderate agreement,  $ICC \geq 0.75$  and  $< 0.90$  as good agreement, and  $kappa \geq 0.90$  as excellent agreement [33]. A value of  $kappa < 0.4$  may be interpreted as a poor agreement,  $kappa \geq 0.40$  and  $< 0.60$  as moderate agreement,  $kappa \geq 0.60$  and  $< 0.80$  as good agreement, and  $kappa \geq 0.80$  as very good agreement [32]. The continuous variables were presented as mean and standard deviation (SD). A difference between dental age (DA) and chronological age (CA) or (DA-CA) was assessed with paired samples *t*-test for males and females and additionally across different age groups. Additionally, the values of DA-CA were presented as 95% confidence interval (95% CI), minimal, median, maximal, first, and third quartiles. Positive values of DA-CA indicate overestimation and negative underestimation of dental age to chronological age. The method’s accuracy can be determined by the difference between dental and chronological age, regardless of whether overestimating or underestimating, by the mean absolute error (MAE). The MAE can be presented as follows:

$$MAE = \frac{1}{n} \sum_{i=1}^n |DA_i - CA_i|, \tag{2}$$

where “*n*” is the number of individuals in the tested sample.

A one-way repeated measures ANOVA was conducted to compare mean DA-CA and MAE among methods in males and females. In addition, a Fisher LSD post hoc test was used to compare means of DA-CA and MAE between pairs of each method [34].

The accuracy of the methods can also be presented by the proportion of individuals with dental age within a specific age range. Therefore, the proportions of individuals with dental age within  $\pm 0.25, \pm 0.5, \pm 1$ , and  $\pm 2$  years of chronological age were calculated. A  $p < 0.05$  was considered statistically significant.

**Results**

The intra-observer and inter-observer agreements showed that the mean ICC for the Cameriere normalized scores,  $X_i$ , were 0.91 and 0.89, which are excellent and very good. Furthermore, the mean kappa for the intra-observer and inter-observer agreements for Haavikko stages were good, 0.78 and 0.77, and for Demirjian stages, 0.81 or very good and 0.78 or good, respectively, Table 2.

The Cameriere European formula has shown the best age prediction or accuracy. Cameriere European formula showed the slightest difference between dental and chronological age, underestimated by  $-0.05$  years in males and  $-0.04$  years in females and comparatively better accuracy than the Haavikko method, which underestimated by  $-0.04$  years in males and  $-0.30$  years in females, Table 3. Conversely, the Demirjian method showed the most significant difference, overestimating the chronological age by 0.94 years in males and 1.09 years in females. The Willems method, with an overestimation by 0.61 years in males and 0.37 years in females, was significantly more accurate than Demirjian ( $p < 0.001$ ), Table 3. The most significant underestimation of DA-CA was in 13-year-old males ( $-0.50$  years) for the Cameriere European formula and 13-year-old females for the Haavikko method ( $-1.25$  years), while 12-year-old males (1.17 years) and females (1.32 years) overestimated DA-CA the most, Tables 4 and 5.

The mean absolute error between dental and chronological age or MAE was the smallest for the Cameriere European

**Table 2** Intra-observer and inter-observer agreement of the Cameriere normalized scores ( $X_i, i = 1, \dots, 7$ ) by interclass coefficients (ICC) and Demirjian and Haavikko stages by Cohen kappa (kappa) on 100 randomly selected panoramic radiographs of the Croatian children

Tooth	31	32	33	34	35	36	37	Mean
Intra-observer								
Cameriere $X_i$ (ICC)	0.82	0.84	0.92	0.94	0.95	0.98	0.94	0.91
Haavikko (kappa)	0.77	0.72	0.75	0.82	0.79	0.81	0.79	0.78
Demirjian (kappa)	0.84	0.75	0.82	0.79	0.80	0.76	0.88	0.81
Inter-observer								
Cameriere $X_i$ (ICC)	0.80	0.88	0.96	0.85	0.92	0.88	0.96	0.89
Haavikko (kappa)	0.75	0.81	0.75	0.85	0.81	0.82	0.86	0.77
Demirjian (kappa)	0.80	0.69	0.80	0.82	0.77	0.74	0.86	0.78

**Table 3** A paired samples *t*-test for comparison of chronological age (CA) and dental age (DA) (years) estimated and calculated using Cameriere European formula, Demirjian, Haavikko, and Willems methods on Croatian sample of panoramic radiographs of 704 males and 788 females

Method	Sex	N	CA ± SD	DA ± SD	DA-CA	95% CI of DA-CA		Min	Q1	Median	Q3	Max	MAE ± SD	<i>t</i> ( <i>df</i> )	<i>p</i>	
						Lower	Upper									
Cameriere	Males	704	10.22 ± 2.00	10.18 ± 1.89	-0.05 ± 0.67	0.03	-0.10	0.01	-2.47	-0.44	-0.02	0.37	2.10	0.50 ± 0.44	-1.85(703)	0.065
	Females	788	10.36 ± 1.99	10.33 ± 1.84	-0.04 ± 0.66	0.02	-0.08	0.01	-2.52	-0.45	-0.03	0.38	2.29	0.51 ± 0.42	-1.55(787)	0.123
	Males	704	10.22 ± 2.00	11.16 ± 2.20	0.94 ± 0.77	0.03	0.88	1.00	-1.36	0.43	0.91	1.41	3.38	1.01 ± 0.67	32.28(703)	< 0.001
	Females	788	10.36 ± 1.99	10.19 ± 2.23	-0.04 ± 0.77	0.03	-0.09	0.02	-2.31	-0.54	-0.09	0.48	2.53	0.61 ± 0.47	-1.25(703)	0.212
Demirjian	Males	704	10.22 ± 2.00	10.83 ± 2.13	0.61 ± 0.76	0.03	0.55	0.67	-1.76	0.12	0.62	1.04	3.52	0.78 ± 0.58	21.38(703)	< 0.001
	Females	788	10.36 ± 1.99	11.45 ± 2.27	1.09 ± 0.84	0.03	1.03	1.15	-1.12	0.52	1.06	1.57	3.68	1.18 ± 0.88	36.46(787)	< 0.001
	Males	704	10.22 ± 2.00	10.07 ± 1.92	-0.30 ± 0.70	0.02	-0.34	-0.25	-2.11	-0.74	-0.29	0.17	2.25	0.61 ± 0.45	-11.93(787)	< 0.001
	Females	788	10.36 ± 1.99	10.73 ± 2.16	0.37 ± 0.80	0.03	0.31	0.42	-1.87	-0.18	0.35	0.90	2.98	0.70 ± 0.54	12.75(787)	< 0.001

Note: DA-CA, mean difference between dental and chronological age; SD, standard deviation; SEM, standard error of the mean; CI, confidence interval; Min, minimal DA-CA; Q1, 1st quartile of DA-CA; Median, median DA-CA; Q3, 3rd quartile of DA-CA; Max, maximal DA-CA; MAE, mean absolute error between dental and chronological age; *df*, degrees of freedom

formula, 0.50 years in males and 0.51 years in females, following the Haavikko, 0.61 years for both sexes. The MAE for Willems method was 0.78 years and 0.70 years, while the Demirjian method was the least accurate, 1.01 years and 1.18 years in males and females, respectively, Table 3. The values of MAE ranged from 0.35 years for 10-year-old males for the Cameriere method to 1.50 years for 11-year-old females for the Demirjian method, Tables 4 and 5.

Tables 4 and 5 show mean differences of DA-CA and MAE across different age groups for males and females. Figures 1 and 2 show the relationship between age groups and dental and chronological age differences. A one-way repeated measures ANOVA was conducted to compare the mean DA-CA among methods, and there was a significant main effect of methods on the mean DA-CA in males, Wilks' lambda = 0.165,  $F(3, 701) = 1185.8$  ( $p < 0.001$ ), and in females, Wilks' lambda = 0.122,  $F(3, 785) = 1875.4$ ,  $p < 0.001$ . A Fisher LSD post hoc test showed only no statistically significant difference in DA-CA between Cameriere European formula and Haavikko methods in males ( $p = 0.647$ ), while differences between other pairs of each method were statistically significant ( $p < 0.001$ ).

A one-way repeated measures ANOVA was conducted to compare the mean MAE among methods, and there was a significant main effect of methods on the mean DA-CA in males, Wilks' lambda = 0.619,  $F(3, 701) = 143.8$  ( $p < 0.001$ ), and in females, Wilks' lambda = 0.538,  $F(3, 785) = 223.6$ ,  $p < 0.001$ . In addition, a Fisher LSD post hoc test showed a statistically significant difference in mean MAE between pairs of each method in both sexes ( $p < 0.001$ ).

The Cameriere European formula showed the highest proportions of individuals with DA within ± 0.5 year difference of the CA, 61.5% in males and 59.6% in females, following Haavikko with 48.3% in males and 48.7% in females, Willems with 37.2% and 43.5% while the Demirjian method was the least accurate with 25.1% in males and 22.6% in females, Table 6. In addition, the proportions of the individuals with dental age within ± 0.25, ± 0.5 years, ± 1 year, and ± 2 years across different age groups are also presented in Table 6.

## Discussion

In this study, we compared four different methods for age assessment in developing permanent teeth to recommend the most reliable method for children in Croatia.

Two observers tested the precision, and the results indicate that all three approaches using developmental stages by Demirjian and Haavikko and measuring projections of open apices and height by Cameriere are reliable. Furthermore, our findings indicate that the Cameriere European formula is the most accurate considering DA-CA, MAE, and proportions of individuals with estimated dental age

**Table 4** A paired samples *t*-test for comparison of chronological age (CA) and dental age (DA) (years) across different age groups estimated and calculated using Cameriere European formula, Demirjian, Haavikko, and Willems methods on Croatian sample of panoramic radiographs of 704 males

Age groups	N	Method	CA ±SD	DA ±SD	DA-CA		95% CI of DA-CA		Min	Q1	Median	Q3	Max	MAE ±SD	<i>t</i> ( <i>df</i> )	<i>p</i>
					Mean ±SD	SEM	Lower	Upper								
6.0–6.9	39	Cameriere	6.60 ±0.31	6.96 ±0.70	0.36 ±0.60	0.10	0.17	0.56	-0.59	-0.02	0.36	0.66	2.10	0.52 ±0.47	3.78(38)	<0.001
		Demirjian		7.48 ±0.69	0.88 ±0.55	0.09	0.70	1.06	0.03	0.51	0.83	0.83	1.16	0.88 ±0.55	10.06(38)	<0.001
		Haavikko		6.77 ±0.67	0.17 ±0.59	0.17 ±0.59	0.09	-0.02	0.36	-0.71	-0.24	0.06	0.52	2.30	0.43 ±0.44	1.82(38)
7.0–7.9	71	Willems		7.27 ±0.85	0.67 ±0.69	0.11	0.44	0.89	-0.35	0.16	0.65	1.13	2.43	0.76 ±0.58	6.02(38)	<0.001
		Cameriere	7.52 ±0.31	7.69 ±7.66	0.18 ±0.62	0.07	0.03	0.32	-1.54	-0.10	0.19	0.50	1.68	0.48 ±0.43	2.41(70)	0.019
		Demirjian		8.32 ±0.71	0.80 ±0.65	0.80 ±0.65	0.08	0.65	0.96	-1.07	0.48	0.79	1.26	2.85	0.88 ±0.54	10.32(70)
8.0–8.9	110	Haavikko		7.44 ±0.55	-0.07 ±0.49	0.06	-0.19	0.04	0.04	-0.33	-0.16	0.12	1.33	0.38 ±0.33	-1.23(70)	0.223
		Willems		8.25 ±0.66	0.73 ±0.62	0.07	0.59	0.88	-1.14	0.46	0.76	1.13	2.58	0.85 ±0.46	9.94(70)	<0.001
		Cameriere	8.55 ±0.30	8.62 ±0.72	0.07 ±0.66	0.06	-0.05	0.20	-1.19	-0.41	0.06	0.47	2.10	0.52 ±0.41	1.18(109)	0.239
9.0–9.9	112	Demirjian		9.38 ±0.75	0.83 ±0.70	0.07	0.70	0.97	-0.26	0.29	0.70	1.25	3.38	0.85 ±0.68	12.54(109)	<0.001
		Haavikko		8.13 ±0.62	-0.42 ±0.61	0.06	-0.53	-0.30	-1.66	-0.89	-0.52	-0.01	1.74	0.61 ±0.42	-7.21(109)	<0.001
		Willems		9.11 ±0.59	0.56 ±0.55	0.05	0.45	0.66	-0.43	0.12	0.55	0.87	2.82	0.61 ±0.50	10.57(109)	<0.001
10.0–10.9	100	Cameriere	9.51 ±0.32	9.64 ±0.73	0.13 ±0.67	0.06	0.01	0.26	-2.22	-0.27	0.12	0.63	1.65	0.53 ±0.44	2.07(111)	0.041
		Demirjian		10.64 ±0.89	0.95 ±0.81	0.08	0.80	1.11	-1.36	0.43	0.97	1.49	3.28	1.05 ±0.69	12.40(111)	<0.001
		Haavikko		9.10 ±0.68	-0.40 ±0.63	0.06	-0.52	-0.29	-2.31	-0.82	-0.39	-0.39	0.05	0.96	0.60 ±0.45	-6.78(111)
11.0–11.9	109	Willems		10.07 ±0.82	0.57 ±0.76	0.07	0.42	0.71	-1.03	0.10	0.48	0.96	3.52	0.71 ±0.63	7.84(111)	<0.001
		Cameriere	10.51 ±0.28	10.45 ±0.45	-0.06 ±0.44	0.04	-0.15	0.03	-1.11	-0.35	-0.07	0.25	1.34	0.35 ±0.27	-1.40(99)	0.166
		Demirjian		11.44 ±0.75	0.93 ±0.73	0.07	0.79	1.08	-0.92	0.56	0.95	1.30	2.78	1.01 ±0.62	12.72(99)	<0.001
12.0–12.9	99	Haavikko		10.62 ±0.94	0.11 ±0.90	0.09	-0.07	0.29	-1.85	-0.51	-0.12	0.75	2.53	0.74 ±0.53	1.18(99)	0.239
		Cameriere	11.51 ±0.30	11.32 ±0.73	-0.19 ±0.68	0.07	0.30	0.61	-1.22	-0.06	0.34	0.93	2.67	0.68 ±0.59	5.86(99)	<0.001
		Demirjian		12.55 ±0.97	1.04 ±0.90	0.09	0.87	1.21	-0.68	0.36	0.93	1.58	3.01	1.10 ±0.83	-2.87(108)	0.005
13.0–13.9	64	Haavikko		11.91 ±0.95	0.40 ±0.92	0.09	0.23	0.58	-1.88	-0.12	0.52	1.17	2.21	0.84 ±0.54	4.58(108)	<0.001
		Willems		12.17 ±1.03	0.66 ±0.97	0.09	0.48	0.85	-1.52	-0.04	0.66	1.25	2.55	0.94 ±0.70	7.14(108)	<0.001
		Cameriere	12.48 ±0.30	12.24 ±0.74	-0.24 ±0.71	0.07	-0.38	-0.10	-2.08	-0.56	-0.10	0.21	1.27	0.54 ±0.52	-3.31(98)	0.001
13.0–13.9	64	Demirjian		13.65 ±0.83	1.17 ±0.79	0.08	1.01	1.33	-1.20	0.71	1.16	1.77	2.77	1.24 ±0.67	14.72(98)	<0.001
		Haavikko		12.82 ±0.57	0.33 ±0.60	0.06	0.21	0.46	-2.15	0.04	0.42	0.74	1.22	0.57 ±0.38	5.51(98)	<0.001
		Willems		13.31 ±0.81	0.83 ±0.77	0.08	0.67	0.98	-1.76	0.30	0.92	1.38	2.30	0.98 ±0.57	10.64(98)	<0.001
13.0–13.9	64	Cameriere	13.43 ±0.28	12.92 ±0.58	-0.50 ±0.60	0.08	-0.66	-0.35	-2.47	-0.86	-0.41	-0.06	0.33	0.55 ±0.56	-6.68(63)	<0.001
		Demirjian		14.19 ±0.77	0.76 ±0.77	0.10	0.57	0.96	-1.07	0.20	0.94	1.41	1.80	0.95 ±0.53	7.90(63)	<0.001
		Haavikko		13.06 ±0.43	-0.36 ±0.44	0.05	-0.47	-0.26	-1.92	-0.60	-0.31	-0.07	0.54	0.43 ±0.37	-6.68(63)	<0.001
		Willems		13.84 ±0.65	0.42 ±0.66	0.08	0.25	0.56	-1.21	-0.11	0.55	0.95	1.34	0.68 ±0.38	5.03(63)	<0.001

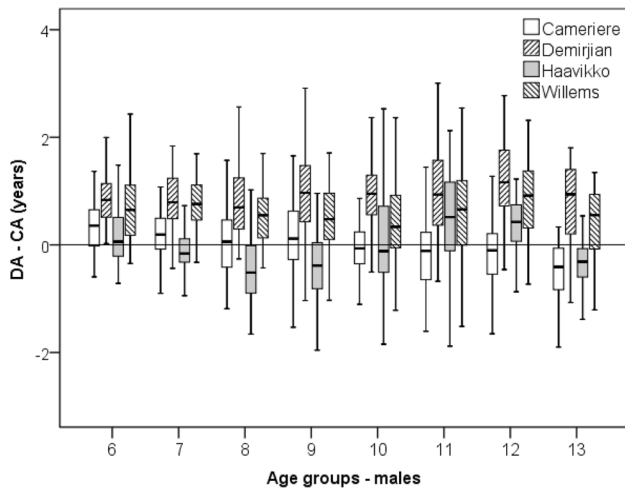
Note: DA-CA, mean difference between dental and chronological age; SD, standard deviation; SEM, standard error of the mean; CI, confidence interval; Min, minimal DA-CA; Q1, 1st quartile of DA-CA; Median, median DA-CA; Q3, 3rd quartile of DA-CA; Max, maximal DA-CA; MAE, mean absolute error between dental and chronological age; *df*, degrees of freedom



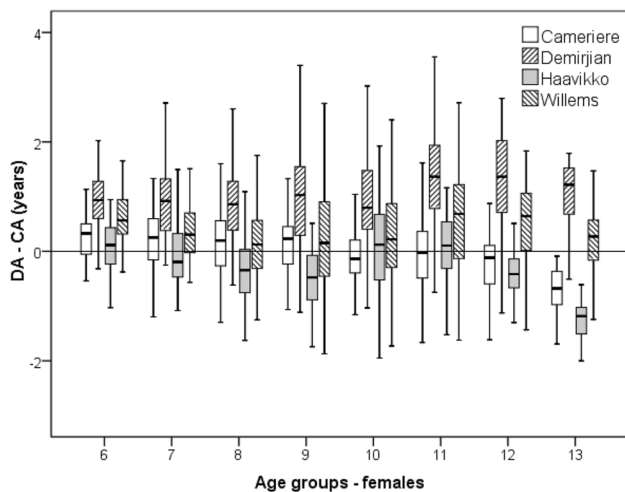
**Table 5** A paired samples *t*-test for comparison of chronological age (CA) and dental age (DA) (years) across different age groups estimated and calculated using Cameriere European formula, Demirjian, Haavikko, and Willems methods on Croatian sample of panoramic radiographs of 788 females

Age groups	<i>N</i>	Method	CA ±SD	DA ±SD	DA-CA	95% CI of DA-CA				Q1	Median	Q3	Max	MAE ±SD	<i>t</i> ( <i>df</i> )	<i>p</i>
					Mean ±SD	Lower	Upper	Min	Max							
6.0–6.9	45	Cameriere	6.61 ± 0.27	6.84 ± 0.58	0.23 ± 0.52	0.25	0.58	-1.38	-0.07	0.33	0.51	1.34	0.46 ± 0.33	2.98(44)	0.005	
		Demirjian		7.47 ± 0.56	0.86 ± 0.54	0.70	1.02	-0.47	0.59	0.93	1.29	2.02	0.91 ± 0.45	10.76(44)	< 0.001	
		Haavikko		6.70 ± 0.47	0.09 ± 0.47	-0.05	0.24	-1.03	-0.23	0.11	0.44	0.94	0.39 ± 0.26	1.35(44)	0.185	
		Willems		7.18 ± 0.49	0.57 ± 0.48	0.43	0.72	-0.82	0.24	0.57	0.95	1.65	0.64 ± 0.38	7.95(44)	< 0.001	
7.0–7.9	67	Cameriere	7.60 ± 0.29	7.88 ± 0.75	0.28 ± 0.67	0.12	0.44	-1.20	-0.17	0.25	0.62	2.29	0.54 ± 0.49	3.44(66)	0.001	
		Demirjian		8.54 ± 0.79	0.95 ± 0.71	0.78	1.12	-0.25	0.35	0.92	1.34	2.88	0.96 ± 0.69	10.97(66)	< 0.001	
		Haavikko		7.53 ± 0.63	-0.06 ± 0.60	-0.21	0.09	-1.08	-0.47	-0.19	0.33	1.49	0.50 ± 0.34	-0.83(66)	0.410	
		Willems		7.97 ± 0.60	0.38 ± 0.56	0.24	0.51	-0.57	-0.03	0.30	0.74	2.30	0.50 ± 0.44	5.54(66)	< 0.001	
8.0–8.9	112	Cameriere	8.53 ± 0.29	8.70 ± 0.62	0.17 ± 0.57	0.07	0.28	-1.30	-0.28	0.20	0.56	1.60	0.48 ± 0.34	3.21(111)	0.002	
		Demirjian		9.41 ± 0.71	0.88 ± 0.65	0.76	1.01	-0.62	0.38	0.86	1.28	2.60	0.92 ± 0.60	14.32(111)	< 0.001	
		Haavikko		8.18 ± 0.59	-0.35 ± 0.57	-0.45	-0.24	-1.63	-0.76	-0.34	0.04	1.09	0.54 ± 0.39	-6.44(111)	< 0.001	
		Willems		8.70 ± 0.68	0.17 ± 0.63	0.05	0.29	-1.25	-0.32	0.13	0.57	1.75	0.52 ± 0.39	2.85(111)	0.005	
9.0–9.9	123	Cameriere	9.47 ± 0.29	9.65 ± 0.66	0.17 ± 0.64	0.06	0.29	-1.71	-0.25	0.23	0.47	1.82	0.52 ± 0.41	3.04(122)	0.003	
		Demirjian		10.45 ± 1.34	0.98 ± 1.33	0.75	1.22	-1.11	0.29	1.03	1.55	3.65	1.21 ± 1.12	8.22(122)	< 0.001	
		Haavikko		8.97 ± 0.63	-0.50 ± 0.61	-0.61	-0.40	-2.11	-0.89	-0.48	-0.07	2.25	0.61 ± 0.50	-9.21(122)	< 0.001	
		Willems		9.75 ± 0.94	0.28 ± 0.91	0.12	0.45	-1.87	-0.46	0.15	0.93	2.98	0.74 ± 0.59	3.46(122)	0.001	
10.0–10.9	99	Cameriere	10.52 ± 0.28	10.42 ± 0.62	-0.10 ± 0.58	-0.22	0.01	-2.42	-0.40	-0.14	0.21	1.67	0.43 ± 0.39	-1.77(98)	0.079	
		Demirjian		11.44 ± 0.92	0.92 ± 0.86	0.74	1.09	-1.03	0.40	0.80	1.49	3.02	1.03 ± 0.72	10.58(98)	< 0.001	
		Haavikko		10.61 ± 0.86	0.08 ± 0.80	-0.08	0.25	-1.95	-0.52	0.12	0.70	1.92	0.66 ± 0.46	1.05(98)	0.296	
		Willems		10.78 ± 0.98	0.26 ± 0.91	0.08	0.44	-1.73	-0.31	0.22	0.89	2.40	0.74 ± 0.58	2.82(98)	0.006	
11.0–11.9	137	Cameriere	11.48 ± 0.28	11.46 ± 0.72	-0.02 ± 0.70	-0.14	0.10	-1.66	-0.49	-0.03	0.37	1.88	0.54 ± 0.44	-0.30(136)	0.767	
		Demirjian		12.76 ± 1.49	1.28 ± 1.44	1.04	1.53	-0.75	0.76	1.36	1.94	3.68	1.50 ± 1.20	10.45(136)	< 0.001	
		Haavikko		11.54 ± 0.64	0.07 ± 0.63	-0.04	0.17	-1.69	-0.33	0.11	0.54	1.16	0.52 ± 0.36	1.23(136)	0.219	
		Willems		11.48 ± 0.28	0.60 ± 0.95	0.44	0.76	-1.62	-0.14	0.69	1.22	2.72	0.91 ± 0.65	7.40(136)	< 0.001	
12.0–12.9	133	Cameriere	12.45 ± 0.29	12.21 ± 0.57	-0.25 ± 0.57	-0.35	-0.15	-1.91	-0.59	-0.12	0.11	0.87	0.45 ± 0.42	-5.08(132)	< 0.001	
		Demirjian		13.77 ± 0.88	1.32 ± 0.85	1.17	1.47	-1.12	0.70	1.36	2.04	2.79	1.38 ± 0.76	17.84(132)	< 0.001	
		Haavikko		12.00 ± 0.38	-0.45 ± 0.45	-0.53	-0.37	-2.04	-0.67	-0.41	-0.14	0.51	0.49 ± 0.41	-11.67(132)	< 0.001	
		Willems		12.96 ± 0.80	0.50 ± 0.79	0.37	0.64	-1.84	0.01	0.64	1.08	1.83	0.79 ± 0.50	7.36(132)	< 0.001	
13.0–13.9	72	Cameriere	13.45 ± 0.27	12.71 ± 0.41	-0.74 ± 0.48	-0.85	-0.62	-2.52	-0.97	-0.68	-0.36	-0.09	0.74 ± 0.48	-13.03(71)	< 0.001	
		Demirjian		14.44 ± 0.65	1.00 ± 0.68	0.84	1.16	-0.78	0.58	1.22	1.52	1.79	1.10 ± 0.49	12.46(71)	< 0.001	
		Haavikko		12.19 ± 0.15	-1.25 ± 0.30	-1.32	-1.18	-2.00	-1.51	-1.18	-1.02	-1.02	1.25 ± 0.30	-35.11(71)	< 0.001	
		Willems		13.57 ± 0.59	0.12 ± 0.62	-0.02	0.27	-1.68	-0.20	0.27	0.57	1.47	0.51 ± 0.37	1.67(71)	0.098	

Note: DA-CA, mean difference between dental and chronological age; SD, standard deviation; SEM, standard error of the mean; CI, confidence interval; Min, minimal DA-CA; Q1, 1st quartile of DA-CA; Median, median DA-CA; Q3, 3rd quartile of DA-CA; Max, maximal DA-CA; MAE, mean absolute error between dental and chronological age; *df*, degrees of freedom



**Fig. 1** The relationship between age groups in the Croatian sample of males and the difference between dental and chronological age (DA-CA) according to the Cameriere, Demirjian, Haavikko, and Willems methods



**Fig. 2** The relationship between age groups in the Croatian sample of females and the difference between dental and chronological age (DA-CA) according to the Cameriere, Demirjian, Haavikko, and Willems methods

within  $\pm 0.5$  years. Precisely, we found that the accuracy of the Cameriere European formula in estimating dental age had no statistically significant difference from chronological age; the mean underestimations of DA-CA were in males by  $-0.05$  years,  $p=0.065$ , and females by  $-0.04$  years,  $p=0.123$ . The Haavikko method was less accurate than Cameriere European formula, with a mean underestimation in males by  $-0.04$  years,  $p=0.212$ , and in females by  $-0.30$  years,  $p < 0.001$ , following the Willems method, which overestimated dental age by  $0.61$  years in males and by  $0.37$  years in females. Finally, our study showed the

most significant mean overestimating dental age using the Demirjian method, by  $0.94$  years in males and  $1.09$  years in females. The difference between dental and chronological age varies among age groups for each method in our study. For example, the Cameriere European formula showed an overestimation of dental age for 6-year-old ( $0.36$  years in males and  $0.23$  years in females) and decreasing trend of overestimation from the youngest and underestimation for 10-year-old in both sexes. Finally, older age groups showed a trend of underestimating dental age, while in 13-year-old, the underestimation was the greatest.

For the Haavikko method, a significant underestimation of dental age was evident in 8- and 9-year-old and especially in the children of the oldest age, in 13-year-old females, by  $-1.25$  years. On the other hand, Demirjian's method overestimated the chronological age across all age groups, from  $0.76$  years in 13-year-old males to  $1.32$  years in 12-year-old females. However, the results of the Willems method were significantly better than Demirjian; dental age was overestimated, from  $0.12$  years in 13-year-old females to  $0.83$  years in 12-year-old males. Comparing the results of the Demirjian and Willems methods, it is evident that Demirjian more than doubled the overestimation of dental age, and in some age groups, significantly more. Therefore, in the case of the need to use Demirjian's stages, the method of estimating the dental age in Croatian children is Willems.

The results of mean MAE follow the sequence from the best to the least accurate method of mean DA-CA; from MAE of  $0.50$  years in males and  $0.51$  years in females for the Cameriere European formula to MAE of  $1.01$  years in males and  $1.18$  years in females for Demirjian method, which is a difference of 6 months.

Studies that compare individual methods for estimating dental age mostly give only a partial answer, whether they show the average results of DA-CA, MAE, or the percentage of individuals in which the dental age is within a deviation of half or one year [28, 35–41]. This study comparing different methods for age estimation in Croatian children aligns with some previous studies. A study assessed the Cameriere method's accuracy vs the Demirjian and Willems methods on the sample of 756 OPGs taken from Caucasian Italian, Spanish, and Croatian children aged 5 to 15 [35]. The results showed that the Cameriere method slightly underestimated the chronological age; the median underestimation was  $-0.036$  years in males and  $-0.081$  years in females. The Willems method overestimated dental age in males by median age of  $0.247$  years and underestimated it in females by the median age of  $-0.073$  years. The Demirjian method overestimated the median age by  $0.61$  years in males and  $0.75$  years in females [35]. The mean values of MAE were from  $0.48$  years in females for the Cameriere method to  $1.13$  years in females for the Demirjian method [35]. Sezer



**Table 6** Dental age within  $\pm 0.25$ ,  $\pm 0.5$ ,  $\pm 1$ , and  $\pm 2$  years of chronological age

Age	Method	Sex	<i>N</i>	$\pm 0.25$ year	$\pm 0.5$ year	$\pm 1$ year	$\pm 2$ years
6	Cameriere	Males	39	30.8%	56.4%	92.3%	97.4%
	Demirjian			10.3%	20.5%	61.5%	94.9%
	Haavikko			38.5%	64.1%	94.9%	97.4%
	Willems			17.9%	43.6%	64.1%	94.9%
	Cameriere	Females	45	28.9%	68.9%	93.3%	100%
	Demirjian			4.4%	17.8%	57.8%	97.8%
	Haavikko			35.6%	68.9%	97.8%	100%
	Willems			20.0%	37.8%	82.2%	100%
7	Cameriere	Males	71	40.8%	64.8%	88.7%	100%
	Demirjian			11.3%	22.5%	66.2%	97.2%
	Haavikko			49.3%	73.2%	94.44%	100%
	Willems			8.5%	21.1%	69.0%	98.6%
	Cameriere	Females	67	29.9%	55.2%	86.6%	95.5%
	Demirjian			16.4%	32.8%	55.2%	92.5%
	Haavikko			23.9%	59.7%	91.0%	100%
	Willems			22.0%	62.7%	89.6%	98.5%
8	Cameriere	Males	110	31.8%	56.4%	88.2%	99.1%
	Demirjian			22.7%	36.4%	66.4%	92.7%
	Haavikko			23.6%	41.8%	80.0%	100%
	Willems			27.3%	46.4%	80.9%	98.2%
	Cameriere	Females	112	27.7%	58.0%	91.1%	100%
	Demirjian			12.5%	50.7%	56.2%	92.9%
	Haavikko			24.1%	50.1%	86.6%	100%
	Willems			27.7%	58.0%	88.4%	100%
9	Cameriere	Males	112	29.5%	53.6%	89.3%	99.1%
	Demirjian			13.4%	22.3%	49.1%	92.0%
	Haavikko			25.9%	50.0%	83.9%	99.1%
	Willems			19.6%	44.6%	77.7%	96.4%
	Cameriere	Females	123	28.5%	63.4%	87.0%	100%
	Demirjian			17.1%	26.8%	48.0%	86.9%
	Haavikko			29.3%	52.8%	78.9%	98.4%
	Willems			26.8%	38.2%	74.8%	95.9%
10	Cameriere	Males	100	46.0%	79.0%	97.0%	100%
	Demirjian			9.0%	18.0%	56.0%	89.0%
	Haavikko			17.0%	40.0%	73.0%	96.0%
	Willems			33.0%	46.0%	74.0%	97.0%
	Cameriere	Females	99	41.4%	67.7%	91.9%	99.0%
	Demirjian			11.1%	25.3%	57.6%	87.9%
	Haavikko			20.2%	41.4%	78.8%	100%
	Willems			26.3%	43.4%	72.7%	96.0%
11	Cameriere	Males	109	31.2%	53.2%	81.7%	100.0%
	Demirjian			12.8%	32.1%	53.2%	83.5%
	Haavikko			19.3%	31.3%	63.3%	99.9%
	Willems			14.7%	32.1%	70.0%	89.0%
	Cameriere	Females	137	31.4%	59.1%	84.7%	100%
	Demirjian			7.3%	16.1%	34.3%	77.4%
	Haavikko			27.7%	54.0%	89.8%	100%
	Willems			17.5%	32.8%	62.8%	91.2%

**Table 6** (continued)

Age	Method	Sex	<i>N</i>	±0.25 year	±0.5 year	±1 year	±2 years	
12	Cameriere	Males	99	39.4%	65.7%	80.8%	98.0%	
				Demirjian	6.0%	16.2%	33.3%	86.9%
				Haavikko	20.2%	45.5%	85.9%	99.0%
				Willems	12.1%	25.2%	54.5%	94.9%
	Cameriere	Females	133	47.4%	60.6%	85.4%	100%	
				Demirjian	8.3%	15.0%	33.1%	73.7%
				Haavikko	36.8%	55.5%	88.7%	99.2%
				Willems	20.3%	34.6%	66.2%	100%
13	Cameriere	Males	64	40.6%	64.0%	81.2%	96.9%	
				Demirjian	10.9%	26.6%	51.6%	100%
				Haavikko	40.6%	64.1%	89.1%	100%
				Willems	18.7%	35.9%	75.0%	100%
	Cameriere	Females	72	12.5%	40.3%	79.2%	97.2%	
				Demirjian	6.9%	19.4%	36.1%	100%
				Haavikko	0%	0%	22.2%	100%
				Willems	25.0%	52.8%	90.3%	100%
6.0–13.9	Cameriere	Males	704	36.1%	61.5%	87.2%	98.9%	
				Demirjian	12.5%	25.1%	54.3%	91.2%
				Haavikko	27.6%	48.3%	80.1%	99.1%
				Willems	19.6%	37.2%	70.9%	96.0%
	Cameriere	Females	788	32.2%	59.6%	87.6%	99.2%	
				Demirjian	10.8%	22.6%	45.7%	87.7%
				Haavikko	25.6%	48.7%	80.5%	99.6%
				Willems	24.1%	43.5%	75.6%	97.3%

Abbreviation: *N*, Number of individuals; %, the percentage of individuals

et al. [39] compared the accuracy of the adopted Haavikko method on four teeth and the Cameriere European formula in 980 Turkish children aged 6 to 15 years from North-western Turkey. The Haavikko method underestimated dental age by  $-0.3$  years and  $-0.7$  years and Cameriere European formula by  $-0.15$  years and  $-0.07$  years in males and females, respectively [39]. The results of MAE of 0.36 years and 0.30 years favor the Haavikko method to 0.44 years and 0.48 years in males and females, respectively, as estimated by the Cameriere European formula [39]. Galić et al. [40] compared the accuracy of the European Cameriere formula, adopted Haavikko using four teeth, and Willems method in age estimation in 1089 Bosnian and Herzegovinian children 6–13 years of age. The Cameriere European formula underestimated dental age by  $-0.02$  years in males and overestimated by 0.10 years in females, while the adopted Haavikko method underestimated by  $-0.09$  years in males and  $-0.23$  years in females. The Willems method overestimated dental age by 0.42 years in males and 0.25 years in females [40]. The mean values of MAE for the Cameriere European formula, Haavikko, and Willems methods were 0.55 years,

0.62 years, and 0.67 years in males and 0.53 years, 0.59 years, and 0.69 years, respectively. These findings of MAE are close to ours using the same methods. Ambarkova et al. [28], in the study on children from the Former Yugoslav Republic of Macedonia, also showed a significant overestimation of dental age using the Demirjian and Willems methods, 0.86 years vs 0.52 years in males and 0.99 years vs 0.33 years in females, while MAE results were 1.03 years vs 0.80 years in males and 1.2 years vs 0.69 years in females, respectively. Marinković et al. [38] compared the Cameriere European formula and Willems method in the Serbian sample of 423 OPGs aged 5 to 15. In children between the ages of 7 and 13, there is no significant difference between the Cameriere European formula and the Willems method, but the European formula was somewhat more accurate, the mean values of DA-CA were  $-0.38$  years and  $-0.38$  years and 0.58 years and 0.63 years for males and females, respectively [38]. The proportions of individuals of DA within  $\pm 0.5$  years were 47.6% and 42.5% for the Cameriere European formula and 45.4% and 40.4% for the Willems method for males and females, respectively. In an Iranian study by

Javedinejad et al., on 287 males and 293 females, the Demirjian method overestimated dental age by 0.90 years in males and 0.85 years in females, the Cameriere method underestimated it by  $-0.27$  years in males and  $-0.11$  years in females, while Willems overestimated by 0.43 years in males and 0.31 years in females [42]. Wolf et al. [37] compared the Cameriere Italian formula and Demirjian method from 1973 on the German sample of 268 males and 211 females 6 to 14 years. They showed an overestimated DA-CA for the Demirjian method by 0.16 years in males and 0.18 years in females, while the Cameriere Italian formula was underestimated by  $-0.07$  years in males and overestimated by 0.08 years in females [37]. Wolf et al. [37] also showed an overestimation in younger ages and underestimation in 12-year-old males and 11-year-old females by the Cameriere Italian formula. These results are generally consistent with our findings; the results show a much more significant underestimation of dental age than in our study, especially in the older age groups [37]. A comparative study on the accuracy of dental age using the Cameriere European formula and three other methods showed that the values of dental age for Brazilians and Croats are relatively similar [43]. The Cameriere method underestimated dental age by  $-1.05$  years in Brazilians and  $-1.19$  years in Croats, while MAE values were 1.26 years in Croats and 1.38 in Brazilians. All four used methods were suitable for application [43].

A significant underestimation of dental age in the oldest individuals can be attributed to two things: first, the development of teeth is not linear to age and second, in older age groups, the reduced number of individuals who completed the development of the first seven mandibular teeth. Our study found 4.8% and 9.5% in 12-year-old and 24.7% and 37.9% in 13-year-old males and females, respectively, with closed apices of all first seven mandibular teeth. Although an individual with an open apex of the second molar and 14- or 15-year-old can be found, their number in the population is practically negligible, and thus, the possibility of age estimation error is very high and should be excluded from dental age assessment procedures [37]. It is especially evident in the Wolf et al. [37] study by using the Cameriere European formula, in which a dental age shift in the youngest individuals and a relatively small difference in 7–12-year-old males and 7–11-year-old females, but 13-year-old, it showed underestimated dental age, the most significant underestimation was in 14-year-old,  $-3.83$  years in males and  $-4.51$  years in females.

The decision about which method to apply for determining the age of a particular person in a particular case should be based on expert societies' recommendations and the examiner's experience. For example, the Forensic Age Diagnosis Study Group (AGFAD) proposed that tests be combined and performed independently: physical examination,

left arm X-ray, and dental development assessment, to increase the accuracy and precision of age assessment and improve estimation [44]. In addition, if skeletal development of the hand is complete, additional computed tomography of the clavicle is recommended [45].

In this research, we analyzed all teeth on one side of the lower jaw for the methods according to Demirjian, Willems, and Cameriere, and we applied the same approach to the analysis of the same teeth to the method according to Haavikko. The adopted Haavikko method from 1974 may not be helpful in forensic or age estimation procedures because it uses different sets of teeth for children under and 10 years of age and above [46]. However, our results of the Haavikko dental age were comparable to previous findings in different studies; the mean differences in age estimation and MAE were between Cameriere European formula and Willems. The applicability of the Haavikko method is especially useful if a deficiency of specific permanent teeth in children or archeological research with scanty remains [47].

The strength of this study is that the sample consisted of OPGs of children from different parts of Croatia, including continental and coastal regions. The limitations of this study were the lack of OPGs of very young individuals and the inability to determine the error range of the individual test for all used methods. In addition, the sample was limited to the age when deciduous teeth are exchanged with permanent ones, between 6 and 7 years. The method, according to Demirjian, allows the determination of the individual range of dental age from dental maturity percentiles, i.e., in addition to the 50th percentile, Demirjian and Goldstein presented the 3rd, 10th, 90th, and 97th dental maturity percentiles for each gender, while Haavikko published 10th and 90th percentile of ages for each developing permanent tooth in addition to the median dental age [11, 23]. According to Cameriere and Willems, the methods calculate precisely one value of the dental age without presenting the individual age range [26, 31].

When assessing which method is the best for determining the dental age in a sample of children's OPGs, the minimum preconditions are that the error in determining the age should not exceed  $\pm 1$  year of the error range or even up to  $\pm 0.5$  year of the error range [28]. The Demirjian method from 1976 does not meet any prerequisites; the other three methods satisfy this more comprehensive age range. However, only the results obtained by applying the Cameriere European formula can approach the values of  $\pm 0.5$  years of the age range.

There is an opportunity to increase the accuracy of specific methods for estimating dental age in children in a specific population. For example, for Demirjian and Goldstein, Croatian-specific self-weighted scores for dental stages and maturity percentiles can be created, or the Haavikko median ages with the age range of each stage of mineralization. For the Willems method, it is possible to create tables with corresponding age scores expressed directly in years or establish

a specific linear formula for the Cameriere method that analyzes teeth with an open apex [48, 49]. The adaptation of each method or standard should be checked on a test sample considering the sample's size and good cohort distribution [50].

## Conclusion

The results obtained in this study show that three of four methods are suitable for age estimation; Demirjian showed unacceptable accuracy for over a  $\pm 1$  year. On the other hand, the Haavikko method may show similar results to Cameriere's European formula in estimation variability. However, its error increases with age by underestimating chronological age, especially in 13-year-old females. Furthermore, Haavikko can be used in cases where some of the developing teeth in the lower jaw are missing, so the remaining three methods based on a complete set of seven teeth cannot be used. Therefore, the Cameriere and Haavikko methods are recommended for determining the dental age in Croatian children for the needs of forensic and clinical analyses because it shows the best accuracy and good to excellent precision.

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## Declarations

**Ethics approval** The Ethics Committee approved this research of the School of Dental Medicine, the University of Zagreb, at the 18th regular session held on June 4th, 2020, decision number: 05-PA-30-XVIII-6/2020. The study was carried out following the ethical standards of the Declaration of Helsinki (Finland).

**Consent to participate** Not applicable.

**Conflict of interest** The authors declare no competing interests.

**Research involving human participants and/or animals** Not applicable.

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