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Research Article

Discrepancies and associations among chronological age, dental age, and cervical vertebral maturation in growing orthodontic patients: A cross-sectional study in a Spanish orthodontic clinical sample

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ABSTRACT

Background: Biological maturation varies substantially among children of the same chronological age, which may affect growth-based orthodontic decisions.
Methods: In this cross-sectional study, 120 patients aged 8 to 16 years were evaluated using routine orthodontic diagnostic records. Chronological age was compared with dental age estimated using the Nolla and Demirjian methods, and skeletal maturation was assessed using cervical vertebral maturation stages (CVMS). Associations with sex, BMI, BMI-for-age z-score, and menarcheal status in females were examined.
Results: Both dental methods systematically overestimated chronological age. Mean discrepancies were +0.59 years for the Nolla method (95% CI: 0.39–0.79; Cohen's dz = 0.52) and +1.09 years for the Demirjian method (95% CI: 0.87–1.31; Cohen's dz = 0.89) (both $P < 0.001$). Female participants exhibited a higher proportion of pubertal and postpubertal CVMS than males (Cramer's V = 0.41; $P < 0.001$), although sex differences in dental age discrepancies were small (Cohen's d ≤ 0.14). BMI and BMI-for-age z-scores were positively correlated with dental age discrepancies ($\rho = 0.27$–0.31) and CVMS stage ($\rho = 0.35$–0.36) (all $P \leq 0.003$), indicating small-to-moderate associations. Menarcheal status was not significantly associated with CVMS ($P = 0.727$) or dental age discrepancies ($P > 0.05$).
Conclusions: Chronological age alone provides an incomplete estimate of biological maturation. Dental age estimation and CVM assessment offer complementary but nonequivalent information, and their interpretation should consider sex and basic anthropometric context.

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1. Introduction

Accurate assessment of growth and maturation is a cornerstone of orthodontic diagnosis and treatment planning in growing patients. The timing of orthodontic and orthopaedic interventions is highly dependent on an individual's biological development rather

Abbreviation: BMI, Body mass index; BMI-for-age z-score, Body mass index–for-age z-score; CVM, Cervical vertebral maturation; CVMS, Cervical vertebral maturation stage(s); ICC, Intraclass correlation coefficient; SD, Standard deviation; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

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than on chronological age alone, as growth-related responses to treatment may vary substantially between patients of the same age [1,2].

Chronological age, although easily obtainable and widely used in clinical practice, does not reliably reflect the individual variability of somatic and craniofacial growth [3]. Numerous studies have demonstrated that children and adolescents of identical chronological age may present markedly different stages of dental and skeletal maturation, which may lead to suboptimal treatment timing if chronological age is used as the sole diagnostic reference [4,5].

Dental age estimation has therefore been proposed as a useful biological indicator, as dental development follows a relatively stable and genetically regulated pattern and is less influenced by environmental factors than other growth markers [6]. Radiographic methods based on tooth mineralisation, those described by Nolla and Demirjian, are among the most widely used techniques in orthodontic and forensic settings due to their simplicity, reproducibility and clinical feasibility [7–9]. However, the accuracy of these methods may vary depending on sex, ethnicity and population-specific growth patterns, with a tendency to either overestimate or underestimate dental age when applied outside the populations in which they were originally developed [10–12].

In parallel, skeletal maturation assessment provides essential information regarding pubertal growth status and remaining growth potential. Among the available techniques, cervical vertebral maturation (CVM) analysis, as described by Baccetti and colleagues, has gained widespread acceptance in orthodontics because it allows skeletal maturity evaluation using routine lateral cephalograms, avoiding additional radiation exposure [13,14]. The CVM method has been shown to correlate with mandibular growth velocity and is therefore relevant for treatment planning in patients undergoing growth modification therapies [15].

Sex-related differences further complicate age assessment, as females generally exhibit earlier dental and skeletal maturation than males, particularly during prepubertal and pubertal stages [16,17]. These differences underline the importance of analysing biological age indicators separately by sex to avoid diagnostic bias and inappropriate treatment timing.

Despite extensive research on dental and skeletal age estimation, discrepancies between chronological, dental, and cervical vertebral age remain inconsistently reported across different populations. In European and Mediterranean cohorts, available data are limited and heterogeneous, particularly regarding studies that simultaneously assess dental age using more than one radiographic method together with skeletal maturation within the same clinical sample [18–20].

In European and Iberian populations, most validation studies have focused on the accuracy of individual dental age methods against chronological age, often in forensic or population-calibration contexts, or within comparative validation frameworks, consistently reporting method-specific bias (e.g., overestimation with Demirjian) [21,22].

Although some European studies have examined the relationship between dental maturation and cervical vertebral maturation, they have typically relied on a single dental method and have only recently begun to incorporate simple clinical variables that may influence maturation patterns, such as anthropometric indicators or pubertal milestones [23].

Consequently, there remains a clinically relevant gap in describing—within a single routine orthodontic sample—how two widely used dental age methods (Nolla and Demirjian) differ in their discrepancy patterns relative to chronological age, and how these discrepancies relate to cervical vertebral maturation and basic clinical maturation proxies.

Therefore, this cross-sectional observational study aimed (i) to quantify the discrepancy between chronological age and dental age estimated using the Nolla and Demirjian methods, and (ii) to examine the associations between dental age estimates, CVMS, and selected clinical variables (sex, BMI/BMI-for-age z-score, and menarcheal status in females) in a Spanish orthodontic clinical sample. This study did not seek to determine diagnostic accuracy or interchangeability between indicators; rather, it sought to describe agreement/discrepancy patterns and their clinical correlates to support interpretation in routine orthodontic assessment.

2. Material and methods

2.1. Study design

This study was designed as a cross-sectional observational investigation. The reporting of the study was conducted in strict accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for cross-sectional studies, with the aim of ensuring transparency, completeness, and methodological rigor in the presentation of the design, conduct, analysis, and interpretation of the findings [24].

2.2. Setting and study population

The study was conducted at the Dental Clinics of the Universidad Católica de Valencia San Vicente Mártir (UCV), Spain, using data obtained from routine orthodontic diagnostic records. Radiographic and clinical information was retrospectively analysed from patients attending the Orthodontics Department as part of standard clinical care. Accordingly, the study sample corresponds to an orthodontic clinical population, recruited exclusively from individuals attending the Orthodontics Department for diagnostic assessment or treatment.

The source population consisted of children and adolescents, presenting mixed or permanent dentition and having both a panoramic radiograph (orthopantomograph) and a lateral cephalometric radiograph of sufficient diagnostic quality available for evaluation. In addition to radiographic data, routinely recorded clinical information, including anthropometric measurements (weight and height) and pubertal status in female patients (menarcheal status), was extracted from the clinical records when available.

A consecutive sampling approach was applied. All clinical records of patients attending the Orthodontics Department between September 2024 and June 2025 were screened against the eligibility criteria described in section 2.3, and every eligible record within this period was included in the analysis until the predefined target sample size was reached. No preselection, stratification or matching by sex was performed; the approximately balanced numerical distribution between male and female participants in the final sample therefore reflects the chronological order of consecutive presentation rather than an intentional sex-matching procedure.”

2.3. Eligibility criteria

Participants were included in the study if they were between 8 and 16 years of age at the time of radiographic examination, were of either sex, presented mixed or permanent dentition, and had both a panoramic radiograph and a lateral cephalometric radiograph of adequate diagnostic quality available for analysis. In addition, complete clinical records including anthropometric data (weight and height) were required for inclusion.

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Participants were excluded if they presented only primary dentition, craniofacial syndromes or skeletal malformations potentially affecting growth and development, systemic diseases or conditions known to influence dental or skeletal maturation, previous or ongoing orthodontic or orthopaedic treatment, or dental agenesis in the third mandibular quadrant, excluding third molars. Radiographs with insufficient diagnostic quality, including motion artefacts, inadequate contrast, or incomplete visualisation of the anatomical structures required for dental or skeletal staging, were also excluded from the analysis.

2.4. Ethical approval and consent

The study protocol was reviewed and approved by the appropriate institutional Ethics Committee (UCV/2024-2025/127). The study was conducted in accordance with the principles of the Declaration of Helsinki and in compliance with current Spanish legislation on biomedical research and personal data protection (Law 14/2007 and Organic Law 3/2018).

This study was based on the retrospective analysis of clinical and radiographic data obtained during routine orthodontic care. Written informed consent for the use of clinical, anthropometric, and radiographic data for educational and research purposes had been obtained from all participants and/or their legal guardians at the time of the initial orthodontic assessment. All data were fully anonymised prior to analysis, and no additional diagnostic or therapeutic procedures were performed specifically for the purposes of this study.

2.5. Variables and outcomes

The variables analysed in this study were derived from clinical records and radiographic assessments. Chronological age was calculated in years as the difference between the participant's date of birth and the date of radiographic acquisition. Sex was recorded as male or female according to the clinical record.

Biological maturation was evaluated using dental and skeletal indicators. Dental age was estimated using the Nolla and Demirjian methods, and skeletal maturation was assessed using CVMS.

Somatic growth was characterised using anthropometric variables, including body weight, height, and body mass index (BMI). In addition, pubertal development in female participants was assessed using menarcheal status.

The primary outcomes of the study were dental age estimated by the Nolla and Demirjian methods and skeletal maturation stage assessed by the CVM method. Secondary outcomes included discrepancies between chronological age and dental age, calculated for each method as the difference between estimated dental age and chronological age (Δ = dental age−chronological age), to quantify the magnitude and direction of disagreement between chronological and biological maturation indicators.

2.6. Anthropometric assessment

Anthropometric data, including body weight and height, were obtained from routine clinical records as part of the standard orthodontic assessment. Measurements had been recorded at the initial clinical visit, corresponding to the same period in which the diagnostic radiographs were obtained. Body weight was registered in kilograms and height in centimetres using calibrated clinical equipment available at the dental clinics.

For each participant, BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m^2). When required for age- and sex-adjusted analyses, BMI values were further standardised using age- and sex-specific reference data to obtain BMI-for-age z-scores, allowing comparison across the wide age range included in the study.

Anthropometric data were analysed as continuous variables and were considered potential explanatory factors for interindividual variability in dental and skeletal maturation.

2.7. Pubertal status assessment

Pubertal development was clinically recorded only in female participants using menarcheal status (yes/no) at the time of radiographic assessment; when available, age at menarche was also extracted for exploratory description. No equivalent clinical pubertal milestone (e.g., Tanner staging or voice change) was routinely documented for male participants in the clinical records; therefore, skeletal maturation in both sexes was primarily operationalised using CVMS as the radiographic proxy of pubertal timing.

2.8. Dental age assessment

Dental age was assessed on panoramic radiographs using two widely accepted radiographic methods based on tooth mineralisation: the Nolla method and the Demirjian method. Both methods were applied following their original descriptions and using sex-specific reference standards. The Nolla and Demirjian methods were selected to allow methodological comparability with previous studies conducted in Spanish and Iberian orthodontic populations [18,21,22].

Dental maturation according to the Nolla method was evaluated by classifying tooth development into ten stages (0–10), ranging from absence of calcification to complete apical closure [7]. Permanent teeth in the third mandibular quadrant, excluding third molars, were assessed individually, and each tooth was assigned a developmental stage based on morphological criteria. The overall dental maturity score was calculated according to Nolla's original methodology and subsequently converted into dental age (years) using the corresponding sex-specific reference tables. The Nolla method has been extensively applied in paediatric and orthodontic populations and has demonstrated acceptable validity, although population- and sex-related differences in dental maturation have been reported, particularly when applied outside the original reference population [7,9,10,18].

In parallel, dental age was estimated using the Demirjian method, which is based on the assessment of mineralisation stages of the seven permanent mandibular teeth on the left side, excluding third molars [8]. Each tooth was classified into one of eight calcification stages (A–H), and maturity scores were assigned and summed to obtain a global dental maturity score. Dental age in years was then derived using the original sex-specific conversion standards.

2.9. Skeletal maturation assessment (cervical vertebral maturation)

Skeletal maturation was assessed on lateral cephalometric radiographs using the CVM method as described by Baccetti et al. [13]. This method evaluates morphological changes in the second (C2), third (C3), and fourth (C4) cervical vertebrae and classifies skeletal maturation into six consecutive stages (CVMS 1–CVMS 6), corresponding to prepubertal, pubertal, and postpubertal phases of growth.

CVM staging was performed according to the original morphological criteria proposed by Baccetti et al., considering vertebral

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body shape and the presence or absence of concavities on the inferior borders of C2, C3, and C4 [13].

2.10. Sample size estimation

An a priori sample size estimation was conducted for the primary within-subject comparison between chronological age and estimated dental age (paired design). Previous cross-sectional studies comparing chronological age with dental and/or skeletal maturation indicators have reported mean discrepancies of approximately 0.8 to 1.5 years, with standard deviations ranging from 1.0 to 1.8 years [1–3]. Based on these data, a moderate standardised effect size was assumed for the paired difference (Cohen's dz = 0.6).

The required sample size was calculated using G*Power software (version 3.1, Universität Düsseldorf, Germany) for a two-tailed paired-samples t-test with an alpha level of 0.05 and a statistical power of 80%. Under these assumptions, the minimum sample size required was 45 participants.

2.11. Examiner assessment and reliability

All radiographic assessments, including dental age estimation using the Nolla and Demirjian methods and skeletal maturation staging using the CVM method, were performed by a single examiner with formal training in orthodontic radiographic diagnostics.

Prior to the main evaluation, the examiner underwent a calibration phase using a set of training radiographs not included in the final sample, in order to standardise scoring criteria in accordance with the original method descriptions and to ensure consistent application of the staging systems. Calibration focused on the identification of tooth mineralisation stages and the morphological characteristics of the cervical vertebrae, as defined in the original publications.

To assess intraobserver reliability, a randomly selected subset comprising 20% of the total sample was re-evaluated after a two-week washout period, with the examiner blinded to the initial assessments. Intraobserver agreement for ordinal categorical variables, including tooth development stages and CVMS classification, was evaluated using weighted Cohen's kappa coefficients, with corresponding 95% confidence intervals. For continuous variables derived from conversion tables, such as dental age expressed in years, reproducibility was assessed using the intraclass correlation coefficient (ICC) based on a two-way mixed-effects model with absolute agreement, also reported with 95% confidence intervals. Interobserver reliability was not assessed, as all evaluations were performed by a single calibrated examiner.

Kappa and ICC values were interpreted according to conventional thresholds for agreement and reliability [25]. These procedures were implemented to ensure methodological robustness and to minimise the risk of classification bias.

2.12. Radiographic acquisition, software and image handling

Radiographic examinations were obtained as part of routine orthodontic diagnostic procedures and were not performed specifically for the purposes of this study. Panoramic radiographs (orthopantomographs) and lateral cephalometric radiographs were acquired following standard clinical protocols routinely applied in the academic orthodontic practice in which the study was conducted.

All radiographs were obtained with the patient in natural head position and using manufacturer-recommended positioning procedures. Digital radiographic images were stored in the institutional imaging system and subsequently retrieved for analysis. Image evaluation was performed using dedicated dental imaging software routinely employed in clinical practice (Planmeca Romexis, version 6.4; Planmeca Oy, Helsinki, Finland).

Radiographs were assessed under standardised viewing conditions, including consistent monitor use and controlled ambient lighting. Image magnification and contrast adjustments were permitted when necessary to optimise visualisation of anatomical structures, without altering the interpretation of morphological features relevant to dental or skeletal staging.

2.13. Statistical analysis

Data were entered into Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and analysed using IBM SPSS Statistics (v.30, 2024, IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables. Continuous variables were summarised as mean ± standard deviation or median and interquartile range, depending on data distribution, while categorical variables were expressed as frequencies and percentages. Normality was assessed using visual inspection (histograms and Q–Q plots) and the Shapiro–Wilk test. A two-tailed significance level of $\alpha = 0.05$ was adopted.

Primary analyses focused on the comparison between chronological age and dental age estimates obtained using the Nolla and Demirjian methods. These comparisons were performed using paired-samples t-tests or the Wilcoxon signed-rank test, as appropriate. Effect sizes were reported as Cohen's d (paired or independent, as appropriate) for parametric comparisons and as rank-biserial correlation for nonparametric tests, together with 95% confidence intervals. Sex-based differences in age discrepancies were analysed using independent-samples t-tests or the Mann–Whitney U test.

Associations between chronological age, dental age estimates, CVMS (treated as an ordinal variable), and anthropometric variables were explored using Spearman rank correlation coefficients or Pearson correlation, as appropriate according to measurement level and distribution. Agreement between chronological age and estimated dental age was further evaluated using Bland–Altman analysis and Lin's concordance correlation coefficient, given the clinical relevance of agreement beyond simple correlation.

Given the cross-sectional design and the strong coupling between age, sex, and maturation stage distributions, the primary analytical strategy focused on (i) within-subject comparisons between chronological age and dental age estimates, and (ii) bivariate association analyses (correlation and agreement metrics) to characterise relationships among maturation indicators. Multivariable modelling was considered as an exploratory approach; however, to avoid overfitting and unstable estimates across uneven CVMS strata by sex, results are presented primarily as effect sizes with confidence intervals and as agreement/association measures rather than as fully adjusted predictive models. Where clinically relevant, sex-stratified analyses were used to reduce confounding by sexual dimorphism in maturation timing.

3. Results

3.1. Sample characteristics

The final analytical sample comprised 120 growing patients, with an approximately equal numerical distribution by sex; however, skeletal maturation stages were unevenly distributed between males and females, with females showing a higher proportion of pubertal and postpubertal stages. The mean chronological age was 12.3 ± 2.1 years, with comparable age distributions in boys and girls. Anthropometric characteristics showed a wide range consistent with the age span of the sample, with a mean BMI of

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Table 1
Baseline characteristics of the study sample.

Variable	Total sample (n = 120)	Boys (n = 60)	Girls (n = 60)
Age (years), mean ± SD	12.13 ± 1.94	12.06 ± 2.00	12.19 ± 1.89
Weight (kg), mean ± SD	45.67 ± 11.81	47.34 ± 12.35	44.01 ± 11.09
Height (cm), mean ± SD	152.67 ± 12.82	153.84 ± 15.14	151.49 ± 9.97
BMI (kg/m²), mean ± SD	19.88 ± 5.64	20.41 ± 5.97	19.34 ± 5.28
BMI-for-age z-score, mean ± SD	0.00 ± 1.00	0.10 ± 1.06	−0.10 ± 0.94
BMI category (WHO), n (%)			
Normal weight (−2 ≤ z ≤ +1)	100 (83.3%)	47 (78.3%)	53 (88.3%)
Overweight (+1 < z ≤ +2)	16 (13.3%)	11 (18.3%)	5 (8.3%)
Obesity (z > +2)	4 (3.3%)	2 (3.3%)	2 (3.3%)
Underweight (z < −2)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Menarche status, n (%)	-	-	28 (46.7%)

Baseline demographic, anthropometric and pubertal characteristics of the study population are presented for the total sample and stratified by sex. Continuous variables are expressed as mean ± standard deviation. BMI was calculated as weight (kg) divided by height squared (m²), and BMI-for-age z-scores were derived using age- and sex-specific reference standards. BMI categories were defined according to WHO BMI-for-age cut-offs (underweight: z < −2; normal weight: −2 ≤ z ≤ +1; overweight: +1 < z ≤ +2; obesity: z > +2). Menarcheal status is reported only for female participants and expressed as number and percentage.

19.9 ± 5.6 kg/m² and a mean BMI-for-age z-score of 0.00 ± 1.00, indicating a distribution closely centred around the international reference mean. According to WHO BMI-for-age cut-offs, 100 participants (83.3%) were classified as normal weight, 16 (13.3%) as overweight (z > +1 to +2), and 4 (3.3%) as obese (z > +2); no participant was classified as underweight (z < −2). Overweight and obesity cases were comparably distributed between sexes (boys: 11 overweight, 2 obese; girls: 5 overweight, 2 obese). Among female participants, menarche had occurred in 14 of 60 girls (23.3%) at the time of data collection. Detailed baseline characteristics are summarised in Table 1.

3.2. Intraobserver reliability

Prior to the main analyses, intraobserver reliability was assessed to ensure measurement consistency. intraobserver reliability was high across all radiographic assessments. Weighted Cohen's kappa coefficients indicated substantial to almost perfect agreement for ordinal variables, with values of 0.86 (95% CI: 0.79–0.92) for the Nolla method, 0.88 (95% CI: 0.81–0.93) for the Demirjian method, and 0.83 (95% CI: 0.75–0.90) for CVMS classification.

For continuous variables derived from dental age conversion, intraclass correlation coefficients (ICC) demonstrated excellent reproducibility, with values of 0.94 (95% CI: 0.90–0.97) for the Nolla method and 0.96 (95% CI: 0.92–0.98) for the Demirjian method.

These findings support the consistency of the examiner's measurements and the reliability of the radiographic assessment procedures.

3.3. Chronological age, dental age and skeletal maturation

Mean dental age estimates exceeded chronological age for both methods. Dental age assessed using the Nolla method was, on average, approximately 0.6 years higher than chronological age, whereas estimates derived from the Demirjian method showed a larger difference of approximately 1.1 years. Across both methods, girls presented higher mean dental age values than boys (Table 2). Between-sex differences in CVMS distribution were statistically significant and of moderate magnitude (Cramer's V = 0.41; $P < 0.001$).

Skeletal maturation spanned all cervical vertebral maturation stages. When grouped into clinically relevant phases, 41% of participants were classified as prepubertal (CVMS: 1–2), 35% as pubertal (CVMS: 3–4), and 24% as postpubertal (CVMS: 5–6). Girls showed a higher proportion of pubertal and postpubertal stages compared with boys, as illustrated in Fig. 1 and detailed in Table 2.

3.4. Discrepancies between chronological age and dental age

Positive discrepancies between dental age and chronological age were observed for both estimation methods, indicating systematic overestimation. The mean difference was approximately +0.6 years for the Nolla method and approximately +1.1 years for the Demirjian method. These within-subject differences were statistically significant and were accompanied by moderate effect sizes, with corresponding 95% confidence intervals reported to facilitate interpretation of clinical magnitude (Table 3).

Sex-stratified analyses revealed larger discrepancies in girls than in boys for both methods, consistent with the earlier maturation timing observed in females. Effect sizes for sex-based comparisons were calculated and are presented alongside *P*-values to provide an estimate of practical relevance beyond statistical significance.

From a categorical perspective, overestimation of dental age relative to chronological age was more frequent than underestimation, while a smaller proportion of participants demonstrated no discrepancy.

Agreement analysis further contextualised these findings. The Bland–Altman plot (Fig. 2) illustrates the distribution of individual differences between chronological age and Demirjian dental age across the age range, highlighting both the mean bias and the limits of agreement. The mean bias was +1.09 years, with limits of agreement ranging from −1.30 to +3.48 years, reflecting substantial variability at the individual level.

For completeness, an equivalent agreement analysis for the Nolla method showed a smaller mean bias of +0.59 years with 95% limits of agreement of −1.62 to +2.81 years, consistent with the lower systematic overestimation observed for this method. The Bland–Altman plot is shown only for the Demirjian method, as it represented the case with the larger and more clinically relevant systematic bias.

Although correlation analyses indicated a strong association between indicators, the agreement analysis revealed a consistent pattern of systematic overestimation, particularly for the Demirjian method. The width of the limits of agreement indicates considerable dispersion of individual differences across the sample.

3.5. Associations with anthropometric and pubertal variables

Anthropometric variables demonstrated measurable but modest associations with maturation indicators. BMI and BMI-for-age z-scores were positively correlated with both dental age discrepancies and cervical vertebral maturation stage (CVMS), with correlation coefficients within the small-to-moderate range (Table 4).

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Table 2
Chronological age, dental age and skeletal maturation by sex (with effect sizes).

Variable	Boys (n ≈ 60) Mean ± SD	Girls (n ≈ 60) Mean ± SD	Mean difference (G−B)	Cohen's d	95% CI
Chronological age (years)	12.37 ± 2.27	12.58 ± 2.11	+0.21	0.10	−0.59 to 1.01
Dental age—Nolla (years)	12.88 ± 2.13	13.25 ± 1.94	+0.37	0.18	−0.39 to 1.13
Dental age—Demirjian (years)	13.43 ± 2.27	13.70 ± 1.93	+0.27	0.13	−0.53 to 1.07
Skeletal maturation (CVMS grouped)					
VMS Phase	Boys n (%)	Girls n (%)	χ^2	Cramer's V	*P*-value
Prepubertal (1–2)	35 (58.3%)	14 (23.3%)			
Pubertal (3–4)	18 (30.0%)	24 (40.0%)	19.8	0.41	<0.001
Postpubertal (5–6)	7 (11.7%)	22 (36.7%)			

Chronological age, dental age estimated using the Nolla and Demirjian methods, and skeletal maturation assessed by CVMS are presented for the total sample and stratified by sex. Continuous variables are expressed as mean ± standard deviation. Between-sex comparisons for continuous variables include mean differences (girls − boys), Cohen's d effect sizes, and 95% confidence intervals. Skeletal maturation stages were grouped into prepubertal (CVMS 1–2), pubertal (CVMS 3–4), and postpubertal (CVMS 5–6) phases for descriptive purposes. Between-sex differences in CVMS distribution were analysed using the chi-square test, with Cramer's V reported as a measure of effect size.

Table 3
Discrepancies between chronological age and dental age estimates (with size effects).

Variable	Total Mean ± SD	95% CI	Cohen's dz	Boys Mean ± SD	Girls Mean ± SD	Mean diff (G−B)	Cohen's d
Δ Nolla (years)	+0.59 ± 1.13	0.39 to 0.79	0.52	+0.51 ± 1.19	+0.67 ± 1.07	+0.16	0.14
Δ Demirjian (years)	+1.09 ± 1.22	0.87 to 1.31	0.89	+1.06 ± 1.27	+1.12 ± 1.18	+0.06	0.05

Categorical discrepancies

Category	Boys n (%)	Girls n (%)	χ^2	Cramer's V
Dental age > chronological age	34 (56.7%)	41 (68.3%)		
Dental age < chronological age	18 (30.0%)	14 (23.2%)	2.1	0.13
No discrepancy	8 (13.3%)	5 (8.3%)		

Differences (Δ) were calculated as dental age minus chronological age, with positive values indicating overestimation. Within-subject discrepancies were analysed using paired comparisons, and Cohen's dz with 95% confidence intervals are reported to quantify effect magnitude. Between-sex comparisons include mean differences (girls − boys) and Cohen's d. Categorical differences were assessed using chi-square tests, with Cramer's V reported as a measure of effect size.

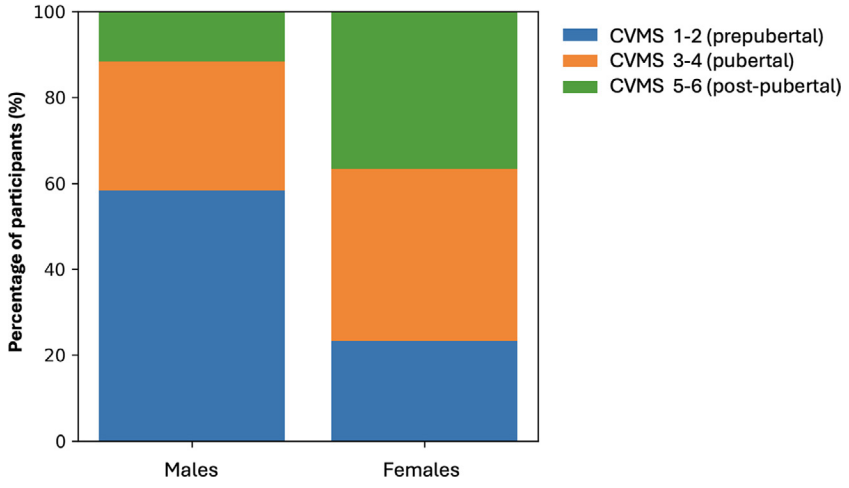


Fig. 1. Distribution of CVMS. Stacked bar chart showing the distribution of CVMS stratified by sex. CVMS were grouped into prepubertal (CVMS 1–2), pubertal (CVMS 3–4), and postpubertal (CVMS 5–6) phases to facilitate clinical interpretation. Female participants exhibited a higher proportion of pubertal and postpubertal maturation stages compared with males, indicating an overall advancement in skeletal maturation.

Although statistically significant, these associations suggest limited explanatory strength and should therefore be interpreted as indicative rather than determinative.

In female participants, menarcheal status (yes/no) was not significantly associated with skeletal maturation stage. The proportion of girls classified at CVMS ≥ 4 was comparable between those who had reached menarche and those who had not ($P = 0.727$). Likewise, no statistically significant differences were observed in dental age discrepancies between menarcheal groups for either the Nolla or Demirjian method (Table 4).

This pattern is biologically plausible. Menarche represents a relatively late pubertal milestone that typically occurs after peak height velocity; consequently, a proportion of girls who have not yet experienced menarche may nevertheless fall within advanced CVMS categories (particularly stages 3–5). In this cross-sectional sample, the age dispersion within the "no menarche" subgroup likely overlapped with the pubertal growth interval captured by CVM staging. The dichotomous recording of menarcheal status may therefore attenuate observable contrasts in maturation indicators when assessed at a single time point.

4. Discussion

4.1. Overview of the main findings

The present cross-sectional study examined the relationship between chronological age, dental age estimated using the Nolla and Demirjian methods, and skeletal maturation assessed by CVMS in

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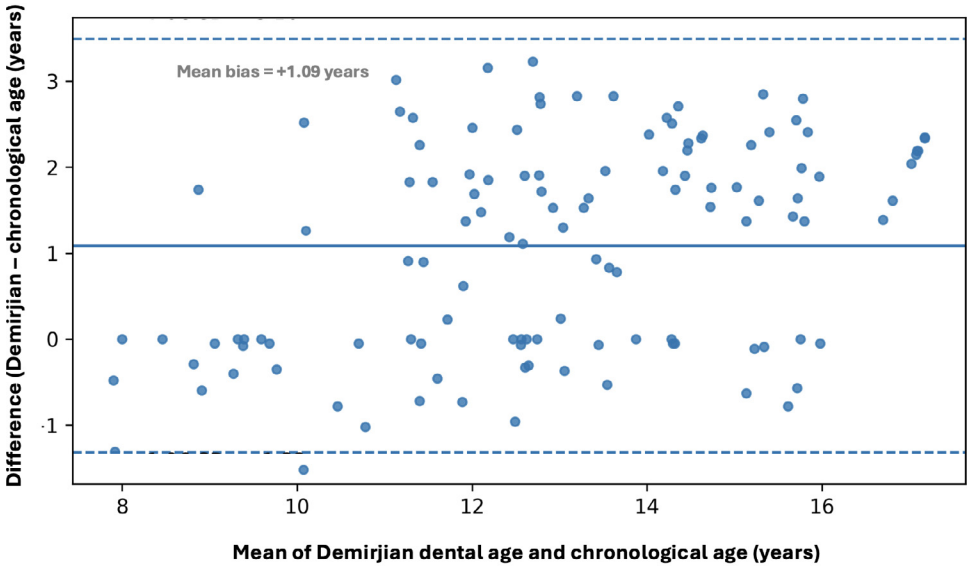


Fig. 2. Bland–Altman plot comparing Demirjian dental age and chronological age. Bland–Altman plot illustrating the agreement between dental age estimated using the Demirjian method and chronological age. The central horizontal line represents the mean difference (bias), and the upper and lower lines indicate the 95% limits of agreement (mean ± 1.96 SD). Positive values indicate overestimation of chronological age by the Demirjian method.

Table 4
Associations of anthropometric and pubertal variables with dental age discrepancies and skeletal maturation.

Predictor	Outcome	ρ (Spearman)	95% CI	*P*-value
BMI (kg/m²)	Δ Nolla	+0.27	0.09 to 0.43	0.003
BMI (kg/m²)	Δ Demirjian	+0.30	0.12 to 0.46	<0.001
BMI z-score	Δ Nolla	+0.28	0.10 to 0.44	0.002
BMI z-score	Δ Demirjian	+0.31	0.13 to 0.47	<0.001
BMI (kg/m²)	CVMS stage	+0.35	0.18 to 0.50	<0.001
BMI z-score	CVMS stage	+0.36	0.19 to 0.51	<0.001
Menarche (girls only)				
Outcome	Effect estimate	Effect size		*P*-value
CVMS ≥ 4	71.4% vs. 78.3%	φ = 0.05		0.727
Δ Nolla	+0.12 years	d = 0.10		0.692
Δ Demirjian	+0.06 years	d = 0.05		0.868

Associations were assessed using Spearman's rank correlation coefficient (ρ). Ninety-five percent confidence intervals were calculated using Fisher's z transformation. Menarcheal status (girls only) was analysed using between-group comparisons, with effect sizes reported as Cohen's d for continuous outcomes and phi (φ) for categorical outcomes. Statistical significance was set at $P < 0.05$.

a cohort of growing patients. The findings indicate that chronological age alone does not adequately reflect biological maturation, as dental and skeletal indicators showed systematic discrepancies relative to chronological age. Dental age tended to exceed chronological age, particularly when assessed using the Demirjian method, and sex-related differences were observed across maturation indicators. In addition, anthropometric variables and pubertal status provided complementary information, underscoring the multifactorial nature of growth and maturation during childhood and adolescence.

4.2. Discrepancies between chronological and dental age

The Demirjian method is one of the most widely used techniques for dental age estimation and has demonstrated good reproducibility; however, systematic over or underestimation has been reported when applied outside its original reference population, reinforcing the importance of population-specific evaluation [8–12].

In the present sample, both dental age estimation methods demonstrated positive discrepancies relative to chronological age, with a greater overestimation observed for the Demirjian method compared with the Nolla method. This pattern is consistent with previous studies reporting systematic overestimation when the Demirjian method is applied outside its original reference population [8,10,12]. Similar findings have been reported in European and Mediterranean populations, including Spanish cohorts, suggesting that population-specific growth patterns influence the accuracy of dental age estimation [18,21,22].

In contrast, the Nolla method showed smaller mean discrepancies, in line with earlier reports indicating that this method may provide closer estimates to chronological age in certain populations [9,11,18]. Nevertheless, substantial interindividual variability persisted, reinforcing the notion that dental age estimation should not be used in isolation for clinical decision-making [1,19].

In addition, agreement analysis further contextualised these findings. Although a consistent pattern of overestimation was observed, the relatively wide limits of agreement indicate considerable dispersion of individual differences. This reinforces that dental age estimation methods may be informative at the group level, but should be interpreted with caution when applied to individ-

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ual patients, particularly when clinical decisions depend on small differences in maturation timing.

4.3. Skeletal maturation and its relationship with dental age

The clinical relevance of CVM staging in growth-based orthodontic treatment planning is supported by previous evidence demonstrating significant correlations between CVMS and pubertal growth phases, as well as with mandibular growth velocity [14,15].

The association between skeletal maturation and dental age discrepancies observed in the present study supports earlier findings describing moderate correlations between dental and skeletal maturation indicators [3–5,15,26,27]. These findings are in line with previous European studies comparing dental age methods in Iberian populations, which have highlighted consistent relationships between dental development patterns and broader maturation indicators, although primarily within validation or comparative frameworks [22]. Comparable evidence from Portuguese and Middle Eastern cohorts has also demonstrated significant, though not interchangeable, relationships between CVM stages and dental development. Fernandes-Retto et al. reported wide distributions of CVM stages within mixed and early permanent dentitions, emphasizing that chronological age alone does not reliably discriminate prepubertal from pubertal phases [28]. Similarly, Mollabashi et al. observed strong correlations between CVM and Demirjian dental age in an orthodontic sample aged 8 to 16 years, while underscoring variability across maturation stages and sexes [29,30].

4.4. Sex-related differences in maturation patterns

Sex-related differences were evident across dental age estimates, skeletal maturation stages, and age discrepancies, with female participants exhibiting more advanced maturation than males of comparable chronological age. These findings are in agreement with extensive evidence indicating earlier dental and skeletal maturation in girls, particularly during prepubertal and pubertal periods [3,5,16,17].

Previous studies have shown that sexual dimorphism in maturation timing may influence the accuracy and interpretation of biological age indicators [1,2,16]. The present results reinforce the importance of stratifying analyses by sex and interpreting maturation indicators within a sex-specific framework to avoid diagnostic bias and suboptimal treatment timing.

4.5. Role of anthropometric and pubertal variables

Anthropometric variables, particularly BMI and BMI-for-age z-scores, were positively associated with both dental age discrepancies and skeletal maturation stage. Although the observed correlations were modest in magnitude, they are consistent with previous evidence indicating that somatic growth and body composition are linked to the tempo of biological maturation, with higher BMI values frequently associated with earlier or more advanced maturation stages [23,30]. Recent evidence in Spanish populations has also reported similar associations between BMI and dentoskeletal maturation using combined radiographic indicators, supporting the relevance of anthropometric context in interpreting maturation patterns, although with modest effect sizes [23]. In the present sample, these associations were statistically detectable but of limited explanatory strength, suggesting that anthropometric indicators may contribute to, but do not independently determine, maturation status.

Menarcheal status did not show a statistically significant association with skeletal maturation stage or dental age discrepancies in this cross-sectional analysis. This finding should be interpreted in context. Menarche is widely recognised as a late pubertal milestone that typically occurs after peak height velocity and therefore reflects a specific point within the broader maturation trajectory [30]. Because menarcheal status was recorded dichotomously (yes/no), its temporal resolution was limited, potentially obscuring gradations within the pubertal window. In addition, the relatively small number of participants who had reached menarche may have reduced the statistical power to detect potential differences between groups, which should be considered when interpreting the absence of significant associations.

Accordingly, the distribution of advanced CVMS stages among some girls without menarche does not contradict biological expectations. Rather, it underscores that cervical vertebral maturation staging captures morphological changes across the pubertal continuum, whereas menarche represents a later clinical event within that continuum. From a clinical perspective, anthropometric and pubertal variables may complement radiographic indicators, particularly in longitudinal monitoring; however, the present cross-sectional findings do not support their use as standalone predictors of maturation status.

Finally, these associations should be interpreted in light of the potential for residual confounding by age and sex. Both factors are closely intertwined with biological maturation and are likely to influence not only anthropometric measures but also dental and skeletal development. Although we performed sex-stratified analyses where appropriate, the cross-sectional nature of the study and the overlap between age, sex, and maturation stage make it difficult to fully separate their individual contributions.

4.6. Clinical implications

From a clinical perspective, the present study reinforces that reliance on chronological age alone is insufficient for growth-based orthodontic diagnosis and treatment planning. Dental age estimation and CVM assessment each provide valuable but incomplete information when used independently. The systematic overestimation observed with the Demirjian method highlights the need for caution when interpreting absolute dental age values and supports the use of relative comparisons or population-adjusted references when available [2,10,12].

An integrated assessment combining dental age, skeletal maturation, and basic anthropometric and pubertal information may provide a more comprehensive understanding of individual growth status, particularly in patients approaching the pubertal growth spurt. These considerations are in line with previous European studies, which have emphasised the need for population-adjusted interpretation of dental age estimation methods rather than direct extrapolation across different populations [21,22].

4.7. Limitations and future directions

A particular strength of this study lies in its sample size, which substantially exceeded the minimum required by the a priori estimation. The inclusion of approximately 120 participants improved the precision of the estimates, allowed sex-stratified analyses, and ensured adequate representation across the full range of cervical vertebral maturation stages. Furthermore, because the analysis was based exclusively on existing clinical and radiographic records obtained during routine orthodontic assessment, no attrition related to follow-up occurred, and all eligible records meeting the predefined inclusion criteria within the study period were considered for analysis. The final sample size therefore reflects the number of

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complete records available for the required clinical, anthropometric, and radiographic variables.

Several limitations should be acknowledged. The cross-sectional design limits the ability to evaluate individual growth trajectories and precludes causal inference. The retrospective use of routinely collected clinical data may introduce variability in anthropometric measurements. In addition, pubertal status was clinically captured only in females (menarche yes/no), while no comparable clinical pubertal marker was available for males; consequently, sex-specific pubertal inferences rely mainly on CVMS distribution.

Moreover, the present sample was recruited exclusively from a single orthodontic clinical setting, and therefore the findings may not be directly extrapolated to the general paediatric population. Orthodontic samples may include a higher proportion of individuals with developmental or maturational variations, which could influence the magnitude of the discrepancies observed between chronological, dental, and skeletal age indicators. In addition, although the sample size exceeded the minimum required by the a priori estimation, it remained relatively modest for some sex-stratified comparisons and for the assessment of less frequent maturation categories. The results should therefore be confirmed in larger multicentre samples and, ideally, in nonorthodontic populations to establish their external validity. The use of CVM staging in the present study was supported by the fact that lateral cephalometric radiographs are routinely obtained as part of standard orthodontic diagnostic protocols, allowing skeletal maturation to be assessed without additional radiation exposure.

A further methodological consideration relates to the choice of dental age estimation methods. The Nolla and Demirjian methods were preferred because they are the techniques most extensively applied and validated in European and Iberian orthodontic populations, which favours comparability with previous regional literature. The Moorrees method, although internationally recognised, relies on a larger number of mineralisation stages per tooth and has been less frequently used in European orthodontic samples; its inclusion may be considered in future studies to allow direct cross-method comparisons.

Future longitudinal studies incorporating repeated assessments of dental, skeletal, anthropometric, and pubertal variables are warranted to better characterise maturation dynamics. The development of population-specific reference standards and the integration of advanced imaging and artificial intelligence-based approaches may further improve the accuracy of biological age assessment in orthodontics [19,20].

5. Conclusions

Within the limitations of this cross-sectional study, chronological age alone does not fully reflect biological maturation in growing orthodontic patients. Both dental age estimation and cervical vertebral maturation assessment demonstrated systematic discrepancies relative to chronological age and considerable interindividual variability across the studied age range. The Demirjian method showed a greater tendency toward overestimation compared with the Nolla method, while cervical vertebral maturation stages exhibited broad dispersion within similar chronological ages.

Sex-related differences were consistently observed, with female participants presenting more advanced dental and skeletal maturation than males of comparable chronological age. Anthropometric variables, particularly BMI and BMI-for-age z-scores, were statistically associated with maturation indicators; however, these relationships were modest in magnitude and should not be interpreted as independent determinants of biological age. Menarcheal status was not significantly associated with dental or skeletal maturation in this sample, likely reflecting both its late occurrence within the pubertal sequence and the cross-sectional design.

Taken together, these findings suggest that dental age and cervical vertebral maturation provide complementary—but not interchangeable—information regarding biological development. Chronological age should therefore be interpreted with caution when used as a proxy for maturation status. Further longitudinal and population-specific studies are warranted to clarify temporal relationships and to refine the clinical applicability of combined maturation indicators.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT author statement

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All authors contributed to the interpretation of the data, critically revised the manuscript for important intellectual content, and approved the final version for submission.

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