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Menarchal status as an indicator of mandibular second molar maturation in female adolescents

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Title: Menarchal Status as an Indicator of Mandibular Second Molar Maturation in Female Adolescents

Running Title: Menarchal Status and Dental Maturation

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ABSTRACT

Background: Menarchal status is a key pubertal milestone in females, yet its relationship with dental maturation has not been adequately explored using multivariate approaches. This study aimed to evaluate the association between menarchal status and mandibular second molar calcification stages in female adolescents, and to determine whether menarchal status is associated with dental maturation after controlling for chronological age and vertical facial pattern.

Methods: This retrospective cross-sectional study included 180 Turkish female patients aged 9–16 years, equally distributed into three groups: pre-menarche (n=60), early post-menarche (0–1 year, n=60), and late post-menarche (>1 year, n=60). Mandibular second molar calcification was assessed using the Demirjian index (stages D–H). Chronological age across groups was compared using the Kruskal–Wallis test with Dunn post-hoc comparisons. Demirjian stages were analysed using the Kruskal–Wallis test with post-hoc comparisons, Spearman correlation, and ordinal logistic regression analysis controlling for vertical facial pattern (α=0.05).

Results: Demirjian stage distribution differed significantly across menarchal groups (H=110.49, p<0.001), with median stages progressing from E to G to H. Menarchal status showed a strong positive correlation with dental maturation (ρ=0.785, p<0.001). In multivariate analysis, menarchal status was significantly associated with Demirjian stage (OR=26.82, 95% CI: 13.66–52.68, p<0.001); however, the magnitude of this effect partly reflects the equal-group design, and model fit was comparable to that of chronological age (AIC: 377.3 vs. 379.6).

Conclusions: Menarchal status is strongly associated with mandibular second molar maturation, with model fit comparable to chronological age. These findings suggest that menarchal history may serve as a clinically accessible, non-invasive biological marker to complement dental maturation assessment in orthodontic treatment planning.

Keywords: Menarche; Dental calcification; Demirjian index; Mandibular second molar; Orthodontic diagnosis; Biological maturation

Background

Dental maturation is widely used as a reliable indicator of biological development in growing individuals, particularly in orthodontic diagnosis and treatment planning. Among available methods, the system proposed by Demirjian et al. remains one of the most widely accepted approaches for assessing dental age based on calcification stages of mandibular teeth [1]. Compared with skeletal maturation indicators, dental development is considered less affected by environmental factors and exhibits relatively lower variability [2].

Pubertal development is a complex biological process influenced by genetic, hormonal, and environmental factors. In females, menarche represents a key milestone of sexual maturation and typically occurs after the peak pubertal growth spurt [3,4]. Previous studies have demonstrated that the timing of menarche is closely associated with skeletal maturation and overall somatic

development [5,6]. More recent evidence continues to support this relationship, indicating that menarche is closely linked to skeletal and somatic growth patterns [7,8], and that dental maturation remains a robust, environmentally stable indicator of biological development in contemporary adolescent populations [9].

Although skeletal maturation methods such as the cervical vertebral maturation (CVM) method are widely used in orthodontics, they require radiographic evaluation and may be subject to observer variability [10,11]. Therefore, alternative indicators that are non-invasive, easily obtainable, and biologically meaningful are of increasing clinical interest.

The relationship between dental maturation and pubertal development has been investigated in several studies, with varying results. While some authors reported significant correlations between dental calcification stages and skeletal maturity [12,13], others found weak or inconsistent associations with sexual maturation indicators [14]. Recent evidence suggests that the strength of this relationship may depend on the specific tooth evaluated, with the mandibular second molar showing stronger associations with skeletal maturity compared with other teeth [12,15]. Furthermore, dental calcification stages of the second molar have been shown to coincide closely with the peak pubertal growth phase with high diagnostic accuracy in females [15]. For these reasons, the mandibular second molar was selected as the index tooth in the present study.

Vertical facial pattern reflects the direction of craniofacial growth and has been associated with differences in the magnitude and timing of skeletal development; individuals with differing vertical patterns may therefore differ in maturational tempo. It was included in the present analysis as a potential confounder, in order to test whether any association between menarchal status and dental maturation is independent of facial morphology.

In addition, hormonal changes during puberty may directly or indirectly influence dental development. The presence of sex hormone receptors in dental tissues supports the biological plausibility of such interactions [16,17].

Despite these findings, the relationship between menarchal status and dental maturation has not been fully elucidated. In particular, to our knowledge, no previous study has employed multivariate regression approaches to assess whether menarchal status is associated with dental calcification independently of chronological age and vertical facial pattern. Therefore, the aim of the present study was to evaluate the association between menarchal status and mandibular second molar maturation in female adolescents using ordinal logistic regression analysis, while controlling for chronological age and vertical facial pattern.

Methods

Study Design and Ethical Approval

This retrospective cross-sectional study included 180 female patients aged 9–16 years who presented to the Department of Orthodontics. The sample was equally divided into three groups based on menarchal status: pre-menarche (n=60), early post-menarche (0–1 year, n=60), and late post-menarche (>1 year, n=60). Patient records from January 2020 to December 2025 were systematically reviewed to identify eligible participants. As this was a retrospective study based on existing records, no a priori sample-size calculation was feasible. A post-hoc sensitivity analysis (G*Power 3.1.9.7) confirmed that the achieved sample (total N=180; 60 per group) provided 80% power at $\alpha=0.05$ to detect a small-to-medium effect (Cohen's $f=0.23$) in a three-group comparison; the effects observed in the present study were considerably larger, indicating that the sample was adequately powered for the primary comparisons. Ethical approval for this study was obtained from the Non-Interventional Clinical and Observational Research Ethics Committee of Istanbul

Kent University Faculty of Dentistry (Approval No: 2026/2-1, dated 28.01.2026), and all procedures were conducted in accordance with the Declaration of Helsinki.

Inclusion criteria were: (1) availability of high-quality panoramic radiographs, (2) known menarchal history obtained from patient records, and (3) absence of systemic diseases affecting growth and development. Exclusion criteria included previous orthodontic treatment, craniofacial anomalies, missing mandibular second molars, and poor radiographic quality.

This study was reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Assessment of Dental Maturation

Dental maturation was evaluated using the Demirjian method, based on calcification stages (D–H) of the left mandibular second molar observed on panoramic radiographs. It should be emphasized that no dental-age estimation was performed: the seven-tooth Demirjian dental-age scoring system was not used. Instead, only the individual-tooth staging criteria of Demirjian were applied, and the ordinal calcification stage of the single index tooth was used directly as the maturation outcome. When the left second molar was unavailable, the right side was used (n=3); given the high intra-individual symmetry of contralateral tooth development, this substitution is unlikely to have affected staging. All radiographs were assessed independently by two calibrated orthodontists in a calibrated digital environment (Nemotec, version 23.0.0.0; Nemotec Dental Systems, Madrid, Spain), displayed on a diagnostic monitor rather than a negatoscope, under standardized viewing conditions with on-screen magnification permitted. Inter-examiner reliability was excellent (Cohen's κ=0.89, 95% CI: 0.84–0.94). Intra-examiner reliability was assessed by re-evaluating 30 randomly selected radiographs after a minimum two-week interval (κ=0.92 and κ=0.91 for each examiner, respectively). In cases of disagreement (n=8, 4.4%), consensus was reached through

joint re-evaluation. Both examiners were blinded to the participants' menarchal status and group allocation throughout the radiographic assessment.

Assessment of Menarchal Status and Covariates

Menarchal status was obtained from contemporaneously recorded institutional clinical data. As a university hospital, the date of menarche is routinely documented for every patient during the initial clinical anamnesis and stored in the patient record. Menarchal status was therefore extracted from these prospectively recorded data rather than obtained retrospectively by patient recall, and patients without a documented menarche date were excluded from the study. Based on this information, patients were categorized into three groups: pre-menarche, early post-menarche (0–1 year), and late post-menarche (>1 year). Chronological age was recorded in years at the time of radiographic examination. Vertical facial pattern was determined using lateral cephalometric analysis on a single cephalogram and therefore represents a cross-sectional morphological classification rather than a longitudinally assessed growth trajectory. All lateral cephalometric radiographs were obtained under standardized conditions using the same imaging system (Castellini X-Radius Trio; Cefla S.C., Bologna, Italy), and the manufacturer's magnification factor was accounted for during analysis; cephalometric measurements were performed digitally by two operators in the calibrated software described above. Two angular measurements were used: the SN-GoGn angle and the Frankfort-Mandibular Angle (FMA). Based on the widely accepted Steiner and Tweed normative values [18,19], patients were classified as hypodivergent (SN-GoGn <27° and FMA <22°), normodivergent (SN-GoGn 27–37° and FMA 22–28°), or hyperdivergent (SN-GoGn >37° and FMA >28°). Final assignment required concordance between the two parameters; patients in whom SN-GoGn and FMA yielded discordant classifications were excluded from the study.

Statistical Analysis

All statistical analyses were performed using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Demirjian stages were treated as an ordinal scale and numerically coded (D=1, E=2, F=3, G=4, H=5) for analysis; group medians and interquartile ranges are accordingly reported as the corresponding stage letters. Differences in Demirjian stages among menarchal groups were evaluated using the Kruskal–Wallis test, followed by post-hoc pairwise comparisons with Bonferroni correction, with statistical significance set at $p<0.05$. Because chronological age departed from normality (Shapiro –Wilk $p<0.05$), differences in age across groups were also compared using the Kruskal–Wallis test with Dunn post-hoc comparisons (Bonferroni), and the distribution of vertical facial pattern across groups was compared using the chi-square test. The association between variables was assessed using Spearman correlation analysis. To evaluate whether menarchal status is associated with dental maturation independently of age, separate ordinal logistic regression models were constructed with Demirjian stage (D–H) as the dependent variable. Given the high collinearity between menarchal status and chronological age ($\rho=0.831$; variance inflation factor >10), covariates were entered into two independent models: Model 1 (chronological age + vertical facial pattern) and Model 2 (menarchal status + vertical facial pattern). A combined Model 3 was additionally constructed as a sensitivity analysis and should be interpreted with caution because of this collinearity. Odds ratios (OR) with 95% confidence intervals (CI) were reported, and model fit was compared using the Akaike Information Criterion (AIC). Statistical significance was set at $p<0.05$. The proportional odds assumption was tested using the test of parallel lines and was not violated.

Results

Sample Characteristics

A total of 180 female patients were included, with 60 patients in each menarchal group. Inter-examiner reliability for Demirjian staging was excellent (κ=0.89, 95% CI: 0.84–0.94), and intra-examiner reliability was κ=0.92 and κ=0.91. Chronological age differed significantly across groups [median (IQR): pre-menarche 10.5 (10.0–11.0), early post-menarche 12.0 (11.8–13.0), late post-menarche 15.0 (14.0–15.0); Kruskal–Wallis H=123.77, p<0.001], with all three pairwise Dunn comparisons significant (p<0.001). Partial age overlap was observed between adjacent groups (Table 1). The distribution of vertical facial patterns did not differ significantly among groups (χ^2=3.22, p=0.521).

Table 1. Descriptive characteristics of the study sample (n = 180)

Characteristic	Pre-menarche (n=60)	Early Post-menarche (0–1 yr, n=60)	Late Post-menarche (>1 yr, n=60)	p-value
Chronological age (years)				
Median (IQR)	10.5 (10.0–11.0)	12.0 (11.8–13.0)	15.0 (14.0–15.0)	**p < 0.001**
Range (min–max)	9–13	11–14	11–16	
Vertical Facial Pattern, n (%)				
Low angle (hypodivergent)	13 (21.7)	20 (33.3)	21 (35.0)	p = 0.521
Normal angle (normodivergent)	28 (46.7)	22 (36.7)	22 (36.7)	
High angle (hyperdivergent)	19 (31.7)	18 (30.0)	17 (28.3)	

Values are presented as median (interquartile range) for age and as number (percentage) for categorical variables. Age was compared using the Kruskal–Wallis test (H = 123.77) with Dunn post-hoc comparisons (Bonferroni correction); all pairwise comparisons were significant (p < 0.001). Vertical facial pattern was compared using the chi-square test (χ^2 = 3.22, p = 0.521). Cut-off values: hypodivergent (SN-GoGn < 27° and FMA < 22°), normodivergent (SN-GoGn 27–37° and FMA 22–28°), hyperdivergent (SN-GoGn > 37° and FMA > 28°).

Distribution of Demirjian Stages

The distribution of mandibular second molar calcification stages showed a clear shift toward advanced stages with increasing menarchal status (Figure 1; Table 2). Early stages were more

frequent in the pre-menarche group, whereas advanced stages predominated in post-menarche groups.

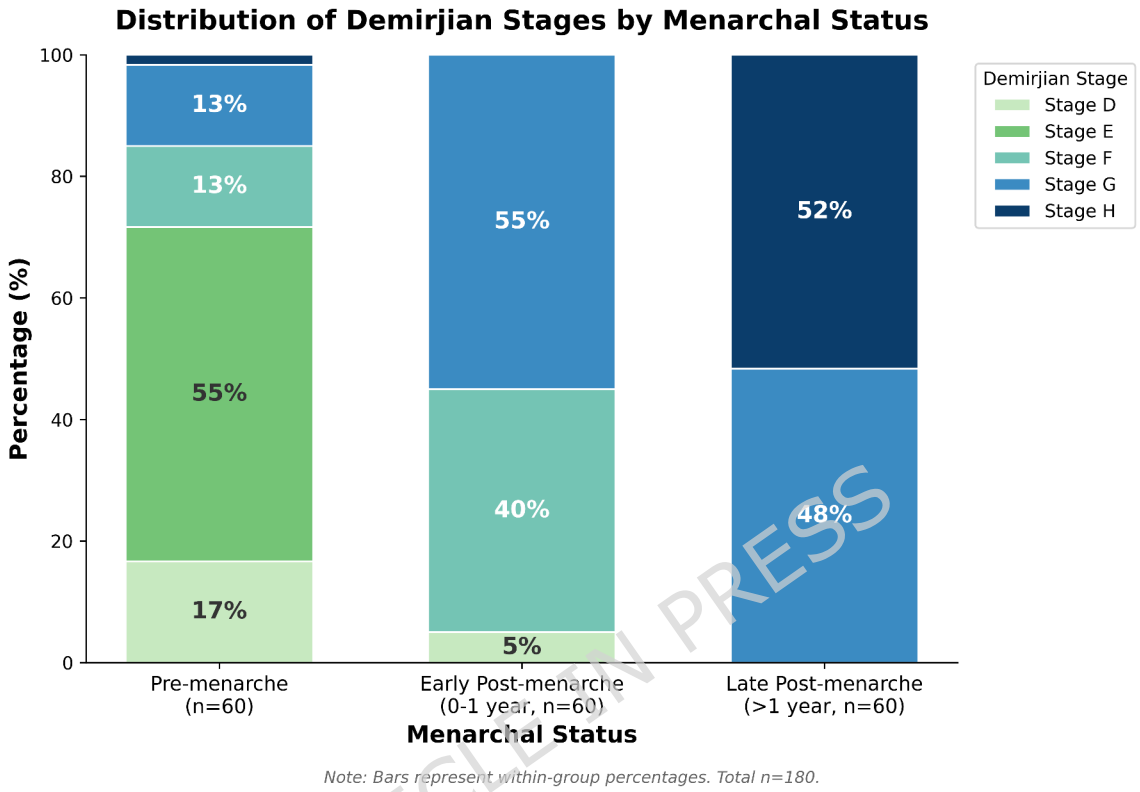


Figure 1. Distribution of mandibular second molar Demirjian calcification stages (D–H) according to menarchal status. Stacked bars represent within-group percentages for each stage (n = 60 per group; total n = 180). The colour gradient from light to dark indicates ordinal stage advancement (D → H).

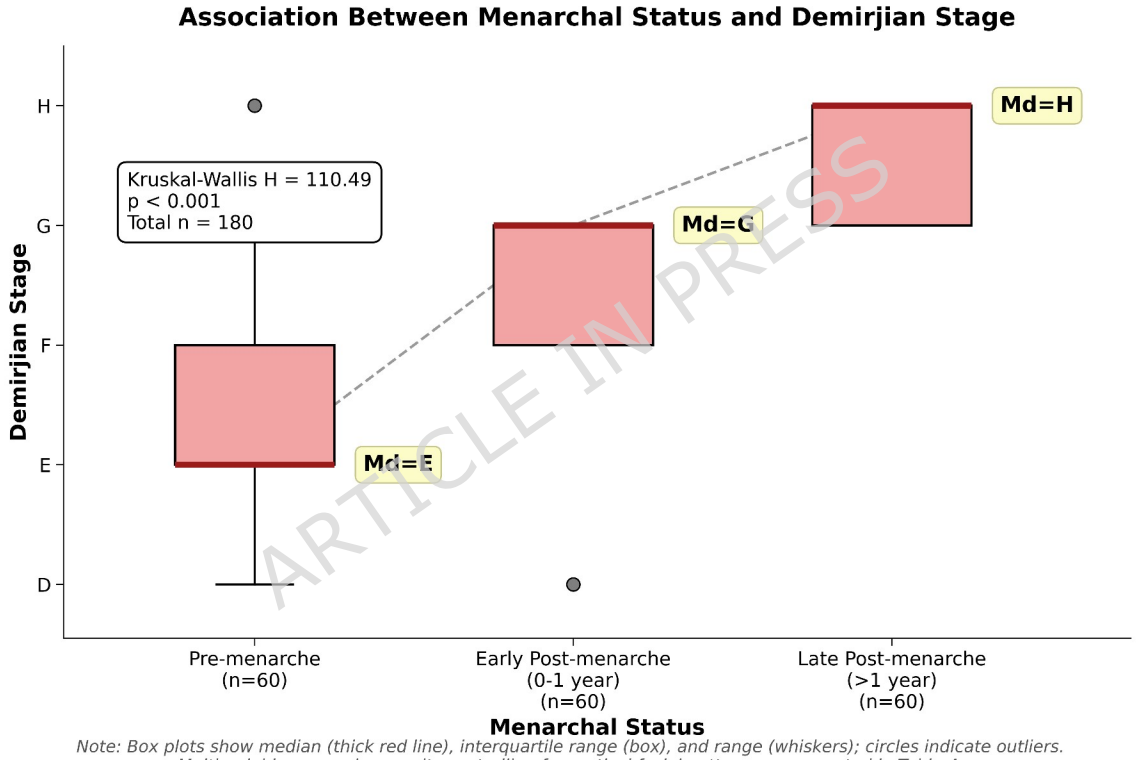
Table 2. Distribution of Demirjian stages according to menarchal status (n = 180)

Demirjian Stage	Pre-menarche (n=60)	Early Post-menarche (n=60)	Late Post-menarche (n=60)	p-value
Stage D	10 (16.7)	3 (5.0)	0 (0)	
Stage E	33 (55.0)	0 (0.0)	0 (0)	
Stage F	8 (13.3)	24 (40.0)	0 (0)	
Stage G	8 (13.3)	33 (55.0)	29 (48.3)	
Stage H	1 (1.7)	0 (0.0)	31 (51.7)	
Summary statistics				
Median (IQR)	**E (E–F)**	**G (F–G)**	**H (G–H)**	
Kruskal–Wallis H	**H = 110.49**			**p < 0.001**
Pre vs Early post-menarche				**p < 0.001**
Pre vs Late post-menarche				**p < 0.001**
Early vs Late post-menarche				**p < 0.001**

Data are presented as number (percentage). Group comparisons used the Kruskal–Wallis test with Dunn post-hoc test (Bonferroni correction). Demirjian stages were treated as an ordinal scale and numerically coded (D = 1 to H = 5); group medians and IQRs are therefore expressed as the corresponding stage letters. IQR = interquartile range.

Group Comparisons

Significant differences in Demirjian stages were observed among the three menarchal groups (Kruskal–Wallis H=110.49, p<0.001). Median stages increased sequentially from E (pre-menarche) to G (early post-menarche) to H (late post-menarche) (Figure 2). Post-hoc comparisons confirmed significant differences between all groups (p<0.001) (Table 2).



Note: Box plots show median (thick red line), interquartile range (box), and range (whiskers); circles indicate outliers. Multivariable regression results controlling for vertical facial pattern are presented in Table 4.

Figure 2. Box plots of Demirjian stage by menarchal status. The thick line denotes the median, the box the interquartile range, and the whiskers the range; circles indicate outliers. Group differences were significant (Kruskal–Wallis H = 110.49, p < 0.001; n = 60 per group). The dashed line connects group medians (stages E, G, and H for the pre-, early post-, and late post-menarche groups, respectively).

Correlation Analysis

Menarchal status showed a strong positive correlation with Demirjian stage (ρ=0.785, p<0.001). Chronological age was also strongly correlated with both menarchal status (ρ=0.831, p<0.001) and

Demirjian stage (ρ=0.777, p<0.001). Vertical facial pattern showed no significant correlation with any study variable (all p>0.05) (Figure 3, Table 3).

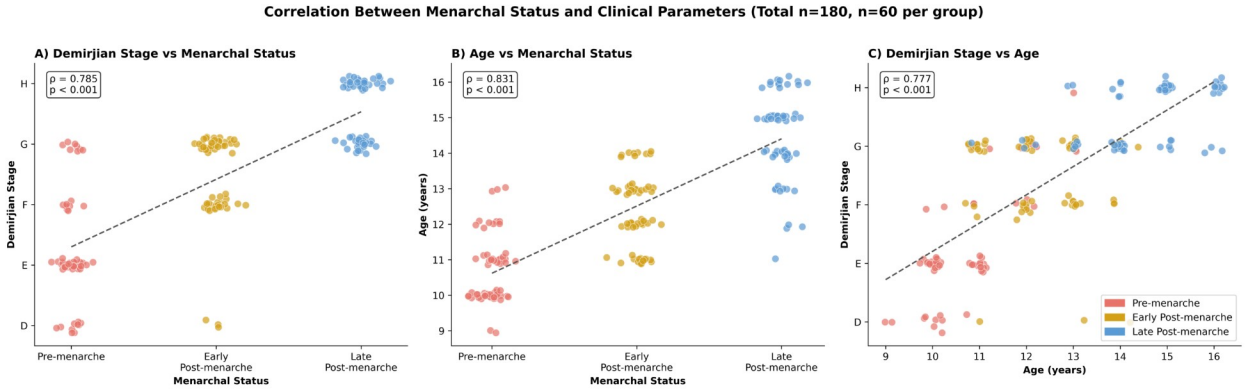


Figure 3. Scatter plots of the relationships between study variables (total n = 180; n = 60 per group). (A) Menarchal status versus Demirjian stage (ρ = 0.785, p < 0.001); (B) menarchal status versus chronological age (ρ = 0.831, p < 0.001); (C) chronological age versus Demirjian stage (ρ = 0.777, p < 0.001). Data points are jittered for visibility and coloured by menarchal group; dashed lines indicate linear trends. Spearman coefficients (ρ) are shown in each panel.

Table 3. Spearman correlation coefficients between study variables (n = 180)

Variable Pair	ρ	p-value
Menarchal status – Demirjian stage	**0.785**	**p < 0.001**
Chronological age – Demirjian stage	**0.777**	**p < 0.001**
Menarchal status – Chronological age	**0.831**	**p < 0.001**
Vertical facial pattern – Demirjian stage	−0.031	0.679
Vertical facial pattern – Chronological age	0.015	0.840
Vertical facial pattern – Menarchal status	−0.088	0.241

ρ = Spearman rank correlation coefficient. Menarchal status was treated as an ordinal variable (pre-menarche, early post-menarche, late post-menarche). A p-value < 0.05 was considered statistically significant.

Ordinal Logistic Regression

In Model 2, menarchal status was significantly associated with Demirjian stage after controlling for vertical facial pattern (OR=26.82, 95% CI: 13.66–52.68, p<0.001). In Model 1, chronological age was similarly significant (OR=3.86, 95% CI: 2.97–5.01, p<0.001). Vertical facial pattern was not significant in either model (p>0.05) (Table 4). Model 2 (menarchal status) and Model 1 (chronological age) showed comparable fit (AIC: 377.3 vs. 379.6; ΔAIC=2.3), indicating that menarchal status provides model fit comparable to, rather than greater than, that of chronological age for dental maturation stage.

Table 4. Ordinal logistic regression models for Demirjian stage (n = 180)

Variable	OR	95% CI	p-value
Model 1: Age + Vertical Facial Pattern			
Age (years)	**3.86**	**2.97 – 5.01**	**p < 0.001**
Vertical: High (ref. Normal)	1.03	0.50 – 2.11	0.943
Vertical: Low (ref. Normal)	1.43	0.72 – 2.83	0.311
Model 2 (Primary): Menarchal Status + Vertical Facial Pattern			
Menarchal Status	**26.82**	**13.66 – 52.68**	**p < 0.001**
Vertical: High (ref. Normal)	0.75	0.37 – 1.52	0.425
Vertical: Low (ref. Normal)	0.52	0.26 – 1.03	0.062
Model 3 (Sensitivity): Menarchal Status + Age + Vertical Facial Pattern			
Menarchal Status	7.25	3.20 – 16.41	< 0.001
Age (years)	2.22	1.60 – 3.10	< 0.001
Vertical: High (ref. Normal)	0.86	0.42 – 1.79	0.694
Vertical: Low (ref. Normal)	0.83	0.40 – 1.72	0.620

Odds ratios (OR) and 95% confidence intervals (CI) from ordinal logistic regression. Menarchal status was treated as an ordinal variable (pre-menarche, early post-menarche, late post-menarche). The normal vertical facial pattern was the reference category. Model 3 should be interpreted with caution due to collinearity between age and menarchal status.

In the combined model including both age and menarchal status, both variables remained significant; however, interpretation was limited due to high collinearity between menarchal status and chronological age (ρ=0.831, VIF>10 for both variables). This combined model should be considered exploratory only.

Discussion

The present study demonstrated that menarchal status is strongly associated with mandibular second molar maturation in female adolescents. Notably, its model fit was comparable to that of chronological age, while offering a biologically relevant indicator of individual maturation. The principal contribution of this study is therefore not the existence of this association, which is biologically expected, but its quantification within a multivariable ordinal framework adjusted for vertical facial pattern, and the demonstration that a non-invasive, radiation-free, readily obtainable clinical history provides information regarding biological maturation that complements chronological age. Although the biological association itself is expected, previous studies have

largely relied on bivariate analyses, whereas the present study quantified this relationship using multivariable ordinal regression while accounting for chronological age and vertical facial pattern. To our knowledge, this is the first study to quantify the association between menarchal status and dental calcification stages using an ordinal regression framework with adjustment for vertical facial pattern.

Dental maturation has long been considered a reliable indicator of biological development, with the Demirjian method remaining one of the most widely used approaches [1,2]. However, its relationship with pubertal maturation markers has been inconsistently reported [14]. The present findings contribute to this field by demonstrating a clear association between menarchal status and dental calcification stages. The odds ratio of 26.82 indicates that, for each increase in menarchal status category, the likelihood of being in a more advanced Demirjian stage increases substantially. This large value should, however, be interpreted with caution. Its magnitude reflects the ordinal coding, in which each menarchal-status category spans a wide maturational range; the strong underlying biological coupling between pubertal and dental maturation; and the balanced extreme-group sampling design, which maximizes between-group separation and inflates the effect estimate relative to a sample reflecting the natural population distribution. The odds ratio should therefore be read as a strength-of-association index rather than a directly transferable clinical effect size.

Menarche represents a key event in female pubertal development and is closely related to hormonal and skeletal maturation processes [3–5]. The observed stepwise difference in Demirjian stages across menarchal groups in this study supports the biological relevance of menarchal status as a maturation indicator; because the design is cross-sectional, this pattern reflects association rather than a temporal or causal progression.

Previous studies have shown that mandibular second molar development correlates with skeletal maturation stages, particularly during the pubertal growth period [12,15]. Mostafavi et al. [15] reported high diagnostic accuracy (AUC: 0.84–0.92) for mandibular teeth in identifying the peak pubertal stage, with second molar stages G and H showing the highest coinciding probability. These stages correspond to those predominantly observed in our post-menarche groups, further supporting the biological coherence of the present results.

Systematic reviews have reported moderate to strong correlations between dental and skeletal maturation, although considerable heterogeneity exists across studies [20,21]. Bittencourt et al. [20] found that only four of ten included studies directly verified the association between dental development and the pubertal growth spurt, with significant heterogeneity (Q=51.00) likely reflecting variations in population characteristics, tooth selection, and assessment methods. More recently, Ferrillo et al. [21] concluded that the mandibular second molar provides the strongest correlation with skeletal maturity among all teeth evaluated by the Demirjian method. These findings collectively suggest that the strength of the dental–pubertal relationship depends critically on which teeth are evaluated. The stronger association observed in the present study may therefore be attributed to the specific focus on the mandibular second molar and the use of a homogeneous female adolescent sample.

Although chronological age showed strong correlations with dental maturation, it should be interpreted as a proxy rather than a direct biological indicator [6]. In contrast, menarchal status reflects underlying hormonal and physiological processes, providing a more individualized measure of maturation. Because the two groupings are strongly collinear (ρ=0.831), it is not possible to fully disentangle their independent contributions, and menarchal status is not claimed to outperform chronological age; rather, the comparable model fit indicates that it conveys

maturational information of similar magnitude while being biologically more interpretable. Two patients of the same chronological age may differ substantially in their pubertal development and, consequently, in their dental maturation stage—a distinction that menarchal status captures but chronological age does not, although this hypothesis requires confirmation in longitudinal studies. The biological plausibility of these findings is supported by evidence demonstrating the presence of estrogen and progesterone receptors in dental tissues and their role in regulating developmental processes [16,17]. Additionally, hormonal changes during puberty may influence bone metabolism, which could indirectly affect dental maturation [22]. Further evidence for this hormonal–dental link was provided by Tabakcilar et al. [23], who demonstrated that dental development was accelerated in children with precocious puberty and retarded in those with delayed puberty, indicating that alterations in pubertal timing are associated with the rate of tooth maturation.

From a clinical perspective, menarchal history represents a simple, non-invasive, and readily available clinical parameter that can complement dental maturation assessment. Unlike radiographic methods such as the CVM method, it does not require additional radiation exposure and is not subject to inter-examiner variability in radiographic interpretation [10,11]. It is, however, intended as an adjunct and not as a substitute for established skeletal-maturity methods such as CVM, hand–wrist, or peak-height-velocity assessment, which the present study did not evaluate. Specifically, a post-menarchal patient presenting with Demirjian stage G or H in the mandibular second molar may be more likely to have limited remaining growth potential, which may contribute to the overall interpretation of developmental status during orthodontic assessment. Conversely, a pre-menarchal patient with early calcification stages (D or E) may retain greater growth potential. Integrating menarchal history with panoramic radiographic findings may

therefore support clinical decision-making without additional cost or radiation exposure, although it cannot replace direct skeletal-maturity assessment. Its specific added value is therefore not improved maturational estimation but a radiation-free means of flagging potential discordance between chronological age and biological maturation, which may prompt confirmatory skeletal assessment when clinically indicated.

This study has several limitations that should be acknowledged. Its cross-sectional design precludes causal inference regarding the temporal relationship between menarche and dental maturation, so the findings should be interpreted as associations rather than developmental sequences. Menarchal status was extracted from contemporaneously recorded institutional data rather than obtained by retrospective recall, which minimizes the recall bias documented in self-report studies [24]; nevertheless, the exclusion of patients without a documented menarche date may introduce selection bias, and residual misclassification cannot be entirely excluded, particularly for the early post-menarche group. The early post-menarche category (0–1 year) also spans a relatively broad biological interval, within which residual growth may differ considerably; finer stratification of time since menarche is recommended for future work. Furthermore, potential confounding factors such as body mass index, nutritional status, socioeconomic background, and endocrine influences were not evaluated, although they are known to influence pubertal timing [25], and may contribute to residual confounding. Because the groups were defined by menarchal status, chronological age differed substantially between them; although age-adjusted models were used, residual age confounding cannot be excluded. The equal allocation across menarchal groups strengthens internal comparisons but does not reflect the natural population distribution and contributes to the large effect estimate, reducing external validity. Vertical facial pattern was classified from a single cephalogram and does not represent a longitudinal growth assessment.

Finally, the study did not directly assess skeletal maturity (CVM, hand–wrist, or peak height velocity), which limits conclusions regarding growth potential and treatment timing. The study sample was drawn from a single university clinic in Turkey, which may limit generalizability to other ethnic populations. Because this study included only female adolescents, the findings cannot be generalized to male patients. In addition, the predominance of the terminal calcification stage (stage H) in the late post-menarche group may have introduced a ceiling effect that could partly account for the strength of the observed association. Future longitudinal studies with larger and more diverse samples are needed to validate these findings.

Conclusions

Menarchal status is strongly associated with mandibular second molar calcification stages in female adolescents, with model fit comparable to chronological age. It serves as a biologically relevant indicator of dental development in addition to chronological age and vertical facial pattern.

From a clinical perspective, menarchal history can be used as a simple and non-invasive adjunct to dental maturation assessment, with the potential to assist the clinical interpretation of developmental staging, although it does not replace direct skeletal-maturity assessment. Further longitudinal studies are required to validate these findings and to clarify the underlying biological mechanisms.

Declarations

Competing interests: The authors declare that they have no competing interests.

Funding: No external funding was received for this study.

Authors' Contributions: G.A. conceived and designed the study, supervised data collection and drafted the manuscript. E.K. and E.U. performed radiographic assessments and contributed to data

interpretation. S.B. contributed to manuscript review and editing and supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate: This study was approved by the Non-Interventional Clinical and Observational Research Ethics Committee of Istanbul Kent University Faculty of Dentistry (Approval No: 2026/2-1, dated 28.01.2026), and all procedures were performed in accordance with the Declaration of Helsinki. Owing to the retrospective design of the study, which used pre-existing, de-identified clinical records, the requirement for informed consent to participate was waived by the same committee.

Availability of Data and Materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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