

Salivary Cortisol Levels Across Cervical Vertebral Maturation Stages In Girls Aged 8–18 Years

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Article type	ABSTRACT
<p>Research Paper</p>	<p>Introduction: Accurate assessment of skeletal maturation is important for determining the timing of growth-modification orthodontic treatment. Cervical vertebral maturation (CVM) assessed by lateral cephalography is commonly used to estimate skeletal maturity, but it requires radiographic imaging. We investigated whether salivary cortisol concentrations vary across CVM stages and whether salivary cortisol could serve as a non-invasive complementary marker of skeletal maturation in adolescent girls.</p> <p>Materials & Methods: In this observational study, 60 female patients aged 8–18 years who presented for orthodontic assessment were enrolled. Lateral cephalograms were used to classify participants according to CVM stage into four groups (CS1–CS2, CS3, CS4, and CS5–CS6). Unstimulated morning saliva samples were collected, and salivary cortisol concentrations were measured using an electrochemiluminescence assay. Statistical analysis included one-way ANOVA with post-hoc testing and independent samples t-tests. $P < 0.05$ was considered statistically significant.</p> <p>Results: Salivary cortisol concentrations increased progressively across CVM stages ($p < 0.001$), with the highest concentrations observed in participants classified as CS5–CS6. Post-hoc comparisons showed significant differences between CS1–CS2 and the later maturation stages, as well as between CS3 and CS5–CS6. Salivary cortisol concentrations were also higher in post-menarcheal than in pre-menarcheal participants ($p < 0.001$).</p> <p>Conclusion: Given the strong correlation between salivary cortisol levels and CVM stages, salivary cortisol may serve as a non-invasive marker for assessing skeletal maturity in adolescent patients, particularly when cephalometric evaluation is not a priority.</p> <p>Keywords: Cephalometry, Hydrocortisone, Saliva, Orthodontics, Sexual Maturation, Menstruation.</p>
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Introduction

Accurate assessment of skeletal maturation is important for determining the timing of growth-modification treatment in orthodontics. Skeletal maturity is commonly estimated using radiographic methods, including Cervical Vertebral Maturation (CVM) assessed on lateral cephalograms, hand–wrist radiography, and dental developmental indicators. The CVM method has been widely adopted in orthodontic practice because it can be assessed from lateral cephalograms obtained routinely for orthodontic diagnosis. However, concerns have been raised regarding its reproducibility and reliability, including interobserver variability and methodological limitations^[1].

These limitations have prompted interest in non-invasive biomarkers that might complement conventional radiographic approaches. Pubertal growth is accompanied by measurable changes in endocrine and metabolic pathways, providing a potential biological basis for the use of salivary biomarkers to assess skeletal maturation. A scoping review of non-invasive biomarkers reported that several salivary biomarkers show associations with radiographic measures of skeletal maturity, suggesting that biochemical measures might provide quantitative information on maturational status^[2].

Among these biomarkers, insulin-like growth factor 1 (IGF-1) and alkaline phosphatase (ALP) have shown potential. Studies combining salivary biomarkers with chronological age have also reported promising performance in predicting CVM stages, although these findings require further validation^[3].

Cortisol is another biomarker of interest because of its role in stress physiology and its effects on bone metabolism. Nevertheless, its relationship with skeletal maturation remains less well established than that of biomarkers such as IGF-1 and ALP. Previous research has reported differences in salivary cortisol concentrations across CVM stages, suggesting a possible association between cortisol and skeletal maturation, whereas systematic-review evidence indicates that the clinical utility and reliability of cortisol remain uncertain^[4]. Moreover, findings from previous studies^[5, 6] have largely focused on specific populations, and evidence regarding the relationship between salivary cortisol and skeletal maturation in girls is limited. Pubertal development in girls is also accompanied by substantial endocrine changes, with menarche representing an important clinical marker of pubertal progression. Whether salivary cortisol concentrations differ according to CVM stage and menarcheal status therefore remains unclear^[7].

Clarifying this relationship could help determine whether salivary cortisol has value as a non-invasive complementary measure of skeletal maturation, particularly when interpreted alongside established clinical and radiographic assessments. We therefore aimed to investigate the association between salivary cortisol concentrations and CVM stage in girls aged 8–18 years and to compare cortisol concentrations between pre-menarcheal and post-menarcheal participants.

Materials & Methods

This cross-sectional analytical study included 60 girls aged 8–18 years who were referred for orthodontic assessment and required lateral cephalometric radiography. Participants were

recruited from the Department of Orthodontics, School of Dentistry, Babol University of Medical Sciences. The study protocol was approved by the institutional ethics committee (IR.MUBABOL.REC.1399.315), and written informed consent was obtained from all participants and, where applicable, their parents or legal guardians before enrolment.

The sample size was calculated using a predefined sample-size formula based on the expected difference in salivary cortisol concentrations across cervical vertebral maturation (CVM) stages.

$$n = \frac{\lambda_{g,\alpha,1-\beta}}{\Delta} \quad \Delta = \frac{1}{\sigma^2} \sum_{i=1}^k (\mu_i - \bar{\mu})^2 \quad \bar{\mu} = \frac{1}{k} \sum_{j=1}^k \mu_j \quad \alpha = 0.05, \beta = 0.20, \sigma^2 = 0.17$$

Participants were eligible if they were in good general health and had no history of systemic disease, psychiatric disorders, developmental abnormalities, congenital or hereditary disorders, or previous facial or maxillofacial trauma. Participants were excluded if they were taking psychiatric or other long-term medications, or if they had experienced substantial stress or anxiety within 24 h before saliva collection.

Cephalometric Assessment and Grouping

Standard lateral cephalograms were obtained as part of the orthodontic assessment. Skeletal maturation was assessed using the cervical vertebral maturation (CVM) method, and participants were classified into four groups according to CVM stage: CS1–CS2 (n=17), CS3 (n=14), CS4 (n=14), and CS5–CS6 (n=15).

Saliva Sample Collection

On the same day as the radiographic examination, unstimulated whole saliva samples were collected before 10:00 a.m. using the passive drooling method^[7]. A total volume of 1.5–2.0 mL was collected in sterile tubes. Samples were centrifuged at 2500 rpm for 15 minutes on the day of collection to remove cellular debris. The supernatant was transferred into 1.5 mL microtubes and stored at –20°C until analysis.

Measurement of Salivary Cortisol

Before testing, samples were thawed at room temperature. Salivary cortisol concentration was measured using an ELISA-based electrochemiluminescence assay. All samples were analyzed using the same diagnostic kit and protocol to ensure consistency. Cortisol levels were determined using the Cobas e 411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) with the Elecsys Cortisol II assay (REF 06687733, LOT 41946601; Roche, USA), following the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA). Mean salivary cortisol levels among the four CVM groups (CS1–CS2, CS3, CS4, CS5–CS6) were compared using one-way analysis of variance (ANOVA). Post-hoc pairwise comparisons were performed using the Scheffé test. An independent samples t-test was used

to compare cortisol levels between participants with and without menstrual cycles. A p-value < 0.05 was considered statistically significant.

Results

Table 1 shows the mean salivary cortisol levels across the four CVM groups. A significant difference in mean cortisol concentration was observed among the groups ($P < 0.001$).

Table 1. Mean salivary cortisol level (nmol/L) in the four study groups

Group	N	Mean	Std. Deviation	P-value*
CS1–CS2	17	3.318	1.216	< 0.001
CS3	14	5.403	1.6	
CS4	14	7.835	2.598	
CS5–CS6	15	9.415	3.687	

*ANOVA

Given the significant overall difference, post-hoc pairwise comparisons were performed using the Scheffé test (Table 2). The analysis demonstrated significantly higher salivary cortisol levels in:

- Group CS4 and group CS5–CS6 compared with group CS1–CS2 ($P < 0.001$), and
- Group CS5–CS6 compared with group CS3 ($P < 0.001$).

No other pairwise comparisons reached statistical significance.

Table 2. Comparison of salivary cortisol levels (nmol/L)

Group	Mean Difference	P-value*	95% Confidence Interval	
			Lower	Upper
CS1–CS2	CS3	0.097	-4.4220	.2528
	CS4	< 0.001	-6.8542	-2.1794
	CS5–CS6	< 0.001	-8.3914	-3.8028
CS3	CS4	0.052	-4.8800	.0157
	CS5–CS6	< 0.001	-6.4192	-1.6057
CS4	CS5–CS6	0.314	-3.9871	.8264

*Scheffé post-hoc pairwise

An independent-samples t-test comparing salivary cortisol levels between girls with and without menstruation demonstrated that post-menarchal participants had significantly higher cortisol levels than pre-menarchal girls (8.064 ± 3.205 nmol/L vs. 4.701 ± 2.679 , respectively; $P < 0.001$).

Receiver operating characteristic (ROC) curve analysis was conducted to determine a cut-off cortisol level predictive of menstrual onset. The area under the curve (AUC) was 0.851 (95% CI: 0.741–0.961), indicating strong discriminatory performance ($P < 0.001$). A threshold value of 5.51 nmol/L yielded a sensitivity of 0.90 and a specificity of 0.80 (Figure 1).

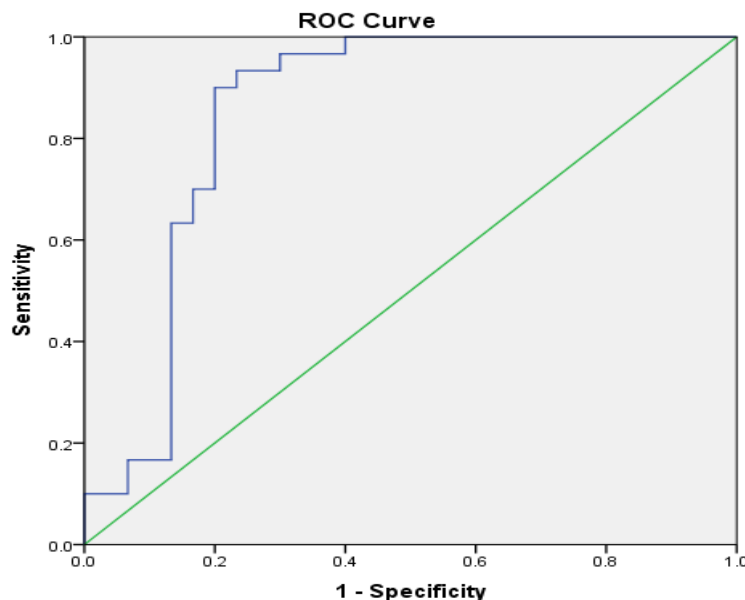


Figure 1. ROC curve for determining cortisol cut-off associated with menstrual onset.

Discussion

In this study, we observed a progressive increase in salivary cortisol levels across advancing CVM stages, with the highest levels in the later stages (CS5–CS6). This pattern suggests that cortisol may reflect biological changes associated with skeletal maturation, potentially mirroring the hormonal environment of puberty.

Although there is limited literature on cortisol in this exact context, our findings align with the broader concept that salivary hormonal profiles are linked to skeletal maturity. For example, the cross-sectional cohort study by Gandhi et al. observed associations between salivary cortisol and CVM staging^[8]. This finding was consistent with the results of Sownaya et al., who examined the relationship between salivary cortisol levels and CVM stage in 27 girls^[9]. Gavate et al. found a progressive increase in salivary cortisol concentration as skeletal maturation progressed from CVM stages 1 and 2 to CVM stages 3 and 4, with the highest values at CVM stages 5 and 6 in both males and females^[10]. Together, these results support the idea that salivary hormones could serve as noninvasive indicators of maturation.

We found that post-menarche females had significantly higher cortisol levels than pre-menarche participants, which is consistent with previous studies on this topic^[11, 12]. Moreover, Salehi Delaver et al. reported a relationship between CVM and salivary cortisol levels in boys^[7]. Therefore, it can be acknowledged that regardless of gender, salivary cortisol levels are associated with CVM.

During puberty, salivary and serum cortisol levels are influenced by various factors, including endocrine changes related to menstruation^[13]. Cortisol levels in bodily fluids rise in response to stress caused by menstrual changes^[14].

This aligns with the understanding that menstrual cyclicity and reproductive hormones interact with HPA axis activity. During and after menarche, fluctuations in estrogen, progesterone, and other hormones can modulate cortisol secretion, potentially explaining the higher levels in post-menarche individuals. Clinically, these findings suggest that salivary cortisol measurement could complement radiographic skeletal maturity assessment. Unlike cephalograms or hand–wrist films, saliva collection is noninvasive, repeatable, and free of radiation exposure, making it well studied for repeated monitoring.

There are still important limitations. First, the study design does not allow causal or temporal inferences; longitudinal studies are needed to map the trajectory of cortisol relative to skeletal and pubertal maturation. Second, we studied only female adolescents because HPA dynamics differ by gender; further research should include male participants. Third, cortisol is inherently sensitive to stress, sleep, circadian rhythm, and other confounders. Although we standardized sample collection (morning, unstressed), unmeasured variables may have influenced cortisol levels.

Finally, compared with more established biomarkers such as IGF-1 or ALP, cortisol's predictive power is likely more modest or complementary. Noninvasive biochemical indicators should augment, rather than replace, radiographic methods. Future research should explore multimarker models (e.g., combining cortisol with IGF-1, ALP, or other salivary proteins) to improve the accuracy and utility of skeletal maturity assessment in orthodontics.

Conclusion

Given the strong correlation between salivary cortisol levels and CVM stages, salivary cortisol may serve as a non-invasive marker for assessing skeletal maturity in adolescent patients, particularly when cephalometric evaluation is not a priority.

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Conflict of interest

There is no conflict of interest to declare.

Author's Contribution

Valiollah Arash, Mehdi Pouramir and Sedigheh Sheikhzadeh developed the original idea and protocol. Saba Mahmoudzadeh and Valiollah Arash conducted the literature and data collection. Saba Mahmoudzadeh, Mehdi Pouramir and Valiollah Arash summarized the

data, drafted the manuscript, and edited the article. Saba Mahmoudzadeh analyzed the data. The study was supervised by Mehdi Pouramir and Valiollah Arash.

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