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The Correlation Between Salivary Cortisol Levels And Cervical Vertebral Maturation Stages

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ABSTRACT

Introduction: Dentofacial abnormalities in growing children are a common reason for orthodontic referral. Studies have suggested the use of biomarkers like cortisol to predict puberty stages. The aim of the present study was to investigate the correlation between salivary cortisol levels and Cervical Vertebral Maturation Stages (CVMSs) on lateral cephalometry.

Materials and Methods: This cross-sectional study was conducted on 60 male patients aged 8 to 18 years. After preparing unstimulated saliva in the morning and determining the level and amount of cortisol in saliva by laboratory method, the level of cortisol in saliva was compared with the maturity of cervical vertebrae in lateral cephalometric view. Individuals were divided into four groups (CS-1, CS-2); (CS-3); (CS-4); and (CS-5, CS-6) based on the CVMS. The collected data were entered into SPSS 18 and analyzed. A value of $P<0.05$ was considered statistically significant.

Results: The results showed that the mean salivary cortisol level in the first, second, third, and fourth groups was 1.37±1.34, 2.29±2.01, 2.62±1.86, and 4.51±2.34, respectively. The mean salivary cortisol level of the fourth group was significantly higher than that of the first and second groups ($P<0.001$ and $P=0.029$, respectively) but compared with the third group, it was not statistically significant.

Conclusion: Salivary cortisol level increases with the development of the CVMS from CS-3. Therefore, salivary cortisol levels can be used as an indicator to determine the growth peak.

Keywords: Hydrocortisone, Puberty, Cephalometry, Dental Radiography, Cervical Vertebrae

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Introduction

Dentofacial abnormalities in growing children are a common reason for referring patients for orthodontic treatment. These patients may require functional or orthopedic treatments during growth or orthognathic surgical treatment after growth is complete. [1] Skeletal maturity is a key factor in

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orthodontic treatment planning. [2] When growth modification treatment is performed during peak pubertal growth, the treatment duration is shorter and the results are more successful. [3, 4] Various decisions in the treatment plan, such as tooth extraction or not, treatment with functional appliances, orthopedic corrections, jaw expansion, and orthognathic surgery, depend on the patient's growth status. [3, 5]

Various indicators have been introduced to estimate the stage of skeletal maturity. Some of these indicators include height, weight, chronological age, sexual maturity, frontal sinus, biological age or physiological age, wrist maturity, cervical vertebrae, tooth eruption, stages of dental calcification, and more recently biomarkers. [6] The cervical vertebral maturation (CVM) method is used to evaluate growth stages. Some researchers propose to predict maturation stages by biomarkers. Because lateral cephalometry alone may not be reliable in suspicious cases, the use of biomarkers along with imaging increases the accuracy of determining pubertal timing. [2, 7, 8] Biochemical methods are noninvasive and do not require X-rays. Biomarkers can be measured from various biological fluids such as blood, urine, gingival crevicular fluid (GCF), and saliva. [9]

Collecting saliva is far less invasive than collecting other body fluids such as GCF, serum, and urine. [10, 11] Therefore, in addition to the CVM method to validate results, the use of alternative tests such as salivary cortisol levels has been suggested to evaluate skeletal maturity. [3, 12] Puberty is essentially a hormonal phenomenon. Biological changes that occur during puberty include changes in nervous factors or hormones. The entire endocrine system changes during puberty, and growth, thyroid, and adrenal hormones are also involved in puberty. [4, 13]

Glucocorticoids are one of the hormones that play an important role in growth. Cortisol is the main glucocorticoid produced and secreted by the adrenal cortex and is regulated by the hypothalamus, pituitary, and adrenal axis. [3] Severe depression, the use of steroid medications, stressors such as exams, emotional pressure, pain and anxiety, physical and emotional trauma, adrenal disorders, decreased pituitary activity and other cases lead to a decrease in cortisol levels. [10, 14]

Cortisol can be measured in serum and saliva. Measurement of cortisol level in saliva can replace measurement of cortisol level in serum because measurement of cortisol level in saliva is a sign of the free and biologically active part of the hormone and is also a simple, noninvasive, and stress-free method. [15] Recently, studies have been done on cortisol as a biomarker of skeletal maturity, and the results of the studies have shown an increase in cortisol levels during puberty, but most of these studies have been conducted on serum. [3, 12]

Since in orthodontics the target population for determining maturity status is children and adolescents, the use of a minimally invasive method such as salivary cortisol testing may be useful in obtaining maximum patient cooperation. The aim of the present study was to investigate the relationship between the salivary hormone cortisol and the assessment of the maturity status of an individual's skeleton based on the cervical vertebrae using lateral cephalometry.

Materials & Methods

The present study was approved by the Ethics Committee of Babol University of Medical Sciences (IR.MUBABOL.HRI.REC.1400.109). In this cross-sectional study, sixty 8-18-year-old male patients who needed orthodontic treatment and lateral cephalometric radiography were referred to the Orthodontic Department of the Faculty of Dentistry in Babol. All participants were selected with full

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knowledge of the study conditions. They or their parents (for those under 18) signed a written consent form.

All boys were apparently healthy. Exclusion criteria were patients with systemic diseases, long-term medication use, congenital and hereditary diseases, smoking, developmental disorders, trauma to the jaw or face, periodontal disease, depression, anxiety within the past 24 hours, and a history of neuropsychiatric medication use.

The head was in the Natural Head Position (NHP) during radiographs. Lateral cephalometric radiographs taken (70BD- NewTom Go, Imola, Italy) to determine Cervical Vertebral Growth Stages (CVMSs) were evaluated separately by two orthodontists. If two experts disagreed on the CVMS, both experts agreed with each other after consultation.

The valuation of the CVMS was based on the classification of Baccetii et al. (16). In this way, patients were divided into four groups: CS-1, CS-2; CS-3; CS-4; and CS-5, CS-6 (Figure 1).

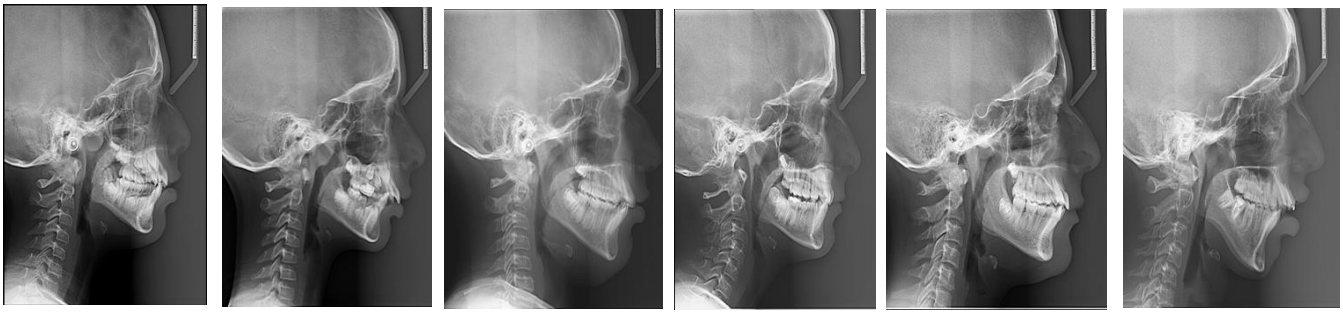

Figure 1. An example of lateral cephalometric radiographs examined in the present study at each of the six maturation stages of cervical vertebrae (A: CS-1, B: CS-2, C: CS-3, D: CS-4, E: CS-5, F: CS-6)

Sampling

On the same day that the lateral cephalometric radiograph was taken, saliva samples were collected from the patients under almost the same conditions and at the same time of day (the patient fasted between 8 and 10 am). First, the patients' mouths were rinsed with physiological serum and then their unstimulated saliva was collected in an amount of 2-5 ml using the spitting method. On the same day, the tubes were centrifuged at a speed of 3000 rpm for 15 minutes and the clear liquid above the tube was separated and frozen. Then, they were kept in the freezer at -20 degrees Celsius until the day of the experiment.

Measurement of cortisol level

On the day of the test, the samples were first brought to room temperature along with the ELISA kit (which was in the refrigerator at 2-8 degrees Celsius). The samples were centrifuged at a speed of 3000 rpm for 15 minutes according to the instructions of the manufacturer of the ELISA kit, and the liquid at the top of the tube was separated. The method and kit used were the same for all samples. In this experiment, the Enzyme immunoassay (EIA) kit from ZELL BIO, Germany with the number ZB-S11003M-H9648 was used. Finally, the samples were entered into the ELISA reader and the result was read at a wavelength of 450 nm according to the manufacturer's instructions.

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Statistical analysis

The resulting data were entered into SPSS 18 (IBM, Armonk, New York) and analyzed using the ANOVA with Tukey's post hoc test. A value of P<0.05 was considered statistically significant.

Results

In the present study, sixty 8-18-year-old male patients with a mean age of 12.36±2.27 years were examined. The frequency of CVMSs and the mean salivary cortisol level in each group are shown in Table 1.

Table 1. Frequency of individuals by cervical vertebral growth stage and comparison of their salivary cortisol levels

Cervical vertebral maturation stage	Frequency	Percentage	Mean cortisol level in the group (nanograms/ml)	Standard deviation	95% confidence interval for mean cortisol level		*P-value
					Lower limit	Upper limit	
CS-1,CS-2	18	30	1.37	1.34	0.7	2.04	
CS-3	13	21.666	2.29	2.01	1.07	3. 5	**0.001
CS-4	19	31.666	2.62	1.86	1. 72	3. 51	
CS-5,CS-6	10	16.666	4.51	2.34	2.84	6. 18	

* ANOVA test
** Statistically significant

The mean salivary cortisol level of the patients indicated a statistically significant difference in the boys with different CVMSs (P<0.001). The lowest concentration of salivary cortisol was found in the CS-1, CS-2 stages and the highest concentration in the CS-5, CS-6 stages, and as the CVMS developed, the salivary cortisol level increased (Diagram 1).

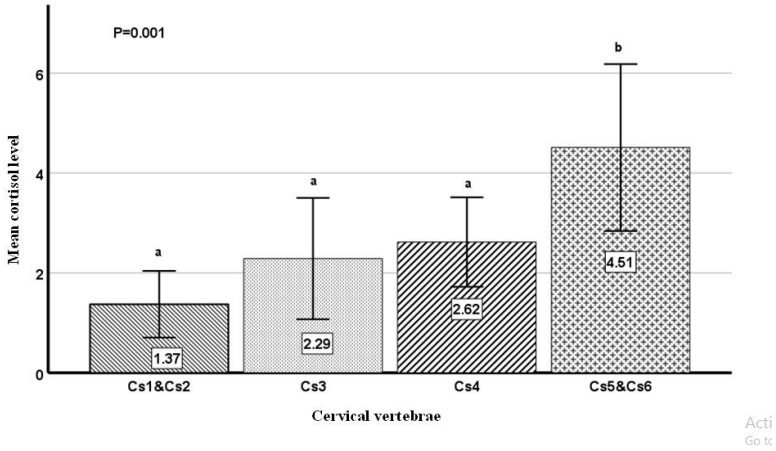

Diagram 1. Salivary cortisol levels of subjects according to cervical vertebral maturation stage

Pairwise comparison of CVMS in terms of salivary cortisol levels revealed a statistically significant difference between CS-1,CS-2 and CS-5,CS-6 as well as CS-3 and CS-5, CS-6 (P<0.001 and P=0.029, respectively). Although the difference between CS-4 and CS-5, CS-6 was not statistically significant. (Table 2).

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Table 2. Pairwise comparison of salivary cortisol levels of individuals by cervical vertebral maturation stage

Growth stage of the studied cervical vertebrae	Growth stage of the compared cervical vertebrae	Difference in mean cortisol level of two groups	95% confidence interval for the difference in mean cortisol level of two groups		*P-value
			Lower limit	Upper limit	
CS-1,CS-2	CS-3	-0.92	-2.69	0.86	0.527
	CS-4	-1.25	-2.85	0.36	0.181
	CS-5,CS-6	-3.14	-5.06	-1.21	**<0.001
CS-3	CS-4	-0.33	-2.09	1.43	0.96
	CS-5,CS-6	-2.22	-4.28	-0.17	**0.029
CS-4	CS-5,CS-6	-1.89	-3.8	0.01	0.052

* Tukey HSD post hoc test
** Statistically significant

Discussion

The results of the present study suggested that the mean salivary cortisol increased with the development of CVMS and reached its maximum at the CS-5, CS-6 stage. However, the difference in salivary cortisol levels was significantly higher only at the CS-5, CS-6 stage than at the CS-1, CS-2 and CS-3 stages. Of course, the difference in cortisol level in this stage compared with CS-4 was not significant. It seems that with the last CVMS, the salivary cortisol concentration increases dramatically, reaching almost three times the value in the first CVMS.

Due to the direct correlation between the salivary cortisol level and the CVMS, this finding can be used to determine the completion of the cervical vertebral growth, i.e., to confirm the end of the individual's growth period. However, because the difference in mean cortisol levels in the lower CVMSs is not significant, this index may not be able to distinguish the lower CVMSs from each other. However, this issue needs further investigation in future studies.

Sowmya et al., in a study on the salivary cortisol level and its relationship with the cervical vertebral maturation stage in girls, concluded that salivary cortisol levels reach their maximum during puberty. [3] The results of their study are consistent with the present study despite the difference between the sexes. In another study, Titman et al. reported that age and salivary cortisol levels are very strongly related to age. For every year that age increases, salivary cortisol levels increase by 11%. [17]

Although their study did not examine CVMSs and the present study focused on CVMSs rather than age, it was impossible to accurately compare the results of the two studies. However, from the point of view that people with a higher CVMS have a higher mean age, the results of their study can be considered in line with the present study.

Kiess et al. suggested that the highest level of salivary cortisol was found in children younger than one year and the lowest level was observed in 1-5-year-old children, and after five years, salivary cortisol levels also increased with age. [18] Moreover, the correlation of salivary cortisol level with pubertal stage was similar to that with age. First, it decreases from the first to the second stage and then increases until the fifth stage, with the difference that the highest salivary cortisol level is in the fifth developmental stage.

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Their study did not mention how to classify the stages of maturity. Moreover, in the current study, the CVMS was not investigated. In addition, in the present study, the minimum age of the participants was eight years, and there was no information on individuals younger than eight years. Despite these differences, the findings of both studies are consistent with the increase in salivary cortisol levels with increasing age. Despite an extensive database search, very few studies were found that examined the relationship between salivary cortisol levels and CVMS exclusively in boys. Even in the systematic review published by Khade et al., only one study was found that examined the relationship between salivary cortisol and CVMS. [5]

In general, the studies that have investigated salivary cortisol levels indicate a direct relationship with age, which is also confirmed in the present study. In this way, salivary cortisol level can be considered as an indicator of a person's growth rate and determination of the end CVMSs.

Very few studies have examined the relationship between salivary cortisol levels and CVMS exclusively in boys. Furthermore, there is no specific numerical value for normal salivary cortisol levels during developmental stages; this could be a topic for future research. Additionally, since parts of this study were conducted during the COVID-19 pandemic, we were unable to ensure that all of our sample groups were exactly the same in terms of numbers.

Conclusion

Salivary cortisol level increases with the development of CVMS. Hence, the lowest mean salivary cortisol level was observed in the CS-1, CS-2 stage and the highest in the CS-5, CS-6 stage. The difference in mean cortisol level between the different CVMSs was statistically significant. Therefore, salivary cortisol level can be used as an indicator to determine the end CVMSs.

Acknowledgments

This research project was approved by Babol University of Medical Sciences and conducted at the School of Dentistry. The authors would like to thank all those who sincerely cooperated in conducting this study.

Conflict of Interest

There is no conflict of interest to declare.

Author’s Contribution

Valiollah Arash and Mehdi Pouramir and Sedigheh Sheikhzadeh developed the original idea and protocol. Fatemeh Salehi Delavar and Valiollah Arash and Reza Ghorbanipour conducted the literature and data collection. Fatemeh Salehi Delavar and Reza Ghorbanipour and Mehdi Pouramir and Valiollah Arash summarized the data, drafted the manuscript, and edited the article. Hemmat Gholinia analyzed the data. The study was supervised by Mehdi Pouramir and Sedigheh Sheikhzadeh.

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References

1. Trakinienė G, Smailienė D, Kučiauskienė A. Evaluation of skeletal maturity using maxillary canine, mandibular second and third molar calcification stages. Eur J Orthod. 2016;38:398–403.
2. Alhazmi N, Trotman CA, Finkelman M, Hawley D, Zoukhri D, Papathanasiou E. Salivary alkaline phosphatase activity and chronological age as indicators for skeletal maturity. Angle Orthod. 2019;89:637–42.
3. Sowmya J, Sasidhar YN, Varma NS, Preetam R, Prasad KG. Cortisol: a biomarker in assessing skeletal maturation during circumpubertal development. Int J Oral Heal Med Res. 2016;3:10–12.
4. Gupta S, Deoskar A, Gupta P, Jain S. Serum insulin-like growth factor-1 levels in females and males in different cervical vertebral maturation stages. Dental Press J Orthod. 2015;20:68–75.
5. Khade DM, Bhad WA, Chavan SJ, Muley A, Shekokar S. Reliability of salivary biomarkers as skeletal maturity indicators: A systematic review. Int Orthod. 2023;21:100716.
6. Dhiman S, Maheshwari S, Verma SK. Assessment of maturity in orthodontics: A review. J Adv Clin Res Insights. 2015;2:100–3.
7. Perinetti G, Franchi L, Castaldo A, Contardo L. Gingival crevicular fluid protein content and alkaline phosphatase activity in relation to pubertal growth phase. Angle Orthod. 2012;82:1047–52.
8. Cericato GO, Bittencourt MA V, Paranhos LR. Validity of the assessment method of skeletal maturation by cervical vertebrae: a systematic review and meta-analysis. Dentomaxillofacial Radiol. 2015;44:20140270.
9. Irham F, Bahirrah S. The level of alkaline phosphatase in saliva as biomarker for pubertal growth phase. In: International Dental Conference of Sumatera Utara 2017 (IDCSU 2017). Atlantis Press; 2018. p. 102–5.
10. Ghalyani P, Tavangar A. Evaluation of anxiety and salivary cortisol level in patients with oral lichen planus. J Iran Dent Assoc. 2013;22:23–9.
11. Poorsoltan N, Mehrabi Y, Shadman Z, Akhoundan M, Rashidi A, Khoshniat Nikoo M. The association of dietary patterns with serum cortisol in type 2 diabetic patients. Iran J Diabetes Metab. 2015;14:27–36.
12. Tripathi T, Gupta P, Rai P. Biochemical markers as skeletal maturity indicators. Int J Orthod Rehabil. 2017;8:60.
13. Mendle J, Beltz AM, Carter R, Dorn LD. Understanding puberty and its measurement: ideas for research in a new generation. J Res Adolesc. 2019;29:82–95.
14. Wong DT. Text Book of Salivary Diagnostics. Ed First Publ. 2008;37–59.
15. Haratian M, Rajabian R, Ayatollahi H. Evaluation of the Level of Salivary and Serum Cortisol. J Mashhad Univ Med Sci [Internet]. 2008;51:13–18. Available from: https://mjms.mums.ac.ir/article_5524.html
16. Huang GJ, Graber LW, Vanarsdall RL, Vig KWL. Orthodontics: current principles and techniques. 7th ed. St. Louis: Elsevier Health Sciences; 2022.
17. Titman A, Price V, Hawcutt D, Chesters C, Ali M, Cacace G, et al. Salivary cortisol, cortisone and serum cortisol concentrations are related to age and body mass index in healthy children and young people. Clin Endocrinol (Oxf). 2020;93:572–8.
18. Kiess W, Meidert A, Dressendörfer RA, Schriever K, Kessler U, Köunig A, et al. Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight. Pediatr Res. 1995;37:502–6.

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