ORIGINAL RESEARCH

Cortisol: A Biomarker in Assessing Skeletal Maturation during Circumpubertal Development

Jangam Sowmya[1], Y. N. Sasidhar[2], N.Satish Varma[3], R. Preetam[4], K. Gangadhara Prasad[5]

1-PG student, Dept of Orthodontics and Dentofacial Orthopedics, GITAM Dental College and Hospital, Vishakhapatnam, Andra Pradesh. 2,3-Professor, Dept of Orthodontics and Dentofacial Orthopedics, GITAM Dental College and Hospital, Vishakhapatnam, Andra Pradesh. 4-Senior Lecturer, Dept of Orthodontics and Dentofacial Orthopedics, GITAM Dental College and Hospital, Vishakhapatnam, Andra Pradesh. 5-Prof and Head, Dept of Orthodontics and Dentofacial Orthopedics, GITAM Dental College and Hospital, Vishakhapatnam, Andra Pradesh.

Correspondence to:
Dr. Jangam Sowmya, PG student, Dept of Orthodontics, GITAM Dental College and Hospital, Vishakhapatnam, Andra Pradesh.
Contact Us: www.ijohmr.com

ABSTRACT

Objective: To assess the Salivary Cortisol levels at different pubertal status and to correlate with cervical vertebrae maturation stages to use Salivary Cortisol as a biological indicator for skeletal maturation. **Materials and Methods:** The study was conducted on 27 girls who were categorized into 3 groups based on their Cervical vertebrae maturation. Mean Salivary Cortisol was estimated using Electrochemiluminescence(ECL) Immunoassay. **Results:** Mean salivary Cortisol levels were higher in the post pubertal group compared to the prepubertal and pubertal groups. Pearson correlation showed a positive correlation between Pre pubertal and Postpubertal groups. **Conclusion:**Peak Salivary Cortisol levels correlated with the pubertal Group. Hence, Salivary Cortisol can be used to predict peak growth velocity.

KEYWORDS:Cortisol, Biomarker, Circumpubertal Development

INTRODUCTION

In clinical orthodontics, an understanding of growth events plays an important role. Estimating the amount of growth and the maturational status will be of help for the orthodontist in taking clinical decisions depending upon the pubertal status of a child, the Orthodontist will be able to formulate ideal treatment goals, treatment planning and eventual outcome of the orthodontic treatment. Some of the mixed dentition treatment philosophies like orthodontic treatment, functional appliance philosophy, extraction and nonextraction treatment planning will always be based on the maturational/Pubertal status of the child. Hence, predicting both the time and amount of active growth of craniofacial complex would be useful to the orthodontist's selection of the appropriate appliance for the child.[1]

The pubertal status of the patient and subsequent evaluation of growth spurt during the preadolescence and adolescence is important.[2] There is a strong correlation between craniofacial growth and somatic changes in puberty as stated by Nanda. The radiographic methods for assessing skeletal maturity are hand wrist radiographs[3], cervical vertebrae maturation[4], dental eruption; dental calcification stages and frontal sinus enlargement. These methods are highly subjective techniques involving radiation exposure. In the recent past, a lot of emphases has been given on biochemical methods for detection of skeletal maturity. Some of them are Serum Alkaline phosphates (Himes et al 1993)[5], Alkaline phosphates in gingival crevicular fluid (Perinetti et al 2012)[6], Serum Insulin-like growth factor-1 (Gupta et al 2012)[7], DHEAS (Premkumar 2012)[8] and Serum PTHrP (Hussain et al 2013)[9] have been used to explore their role in assessing the skeletal maturation of an individual.

One of the Hormones which plays a significant role in the growth is Glucocorticoid[10].It was observed that chronic administration of Glucocorticoid in vivo inhibits GH secretion and in turn hampers the growth (Malerba M et al 2005)[11].Cortisol is the principal Glucocorticoid, which is produced and secreted by the adrenal cortex and is regulated by hypothalamic pituitary- adrenal (HPA) axis. Cortisol circulates in the plasma mostly in bound form. It binds to globulin or albumin. Trace amounts of Cortisol are found in saliva due to its properties of low molecular weight and lipophilic nature. Clemens Kirschbaum[12] has described in detail the passive diffusion property of Cortisol. The present study was conducted to evaluate the level of Cortisol in saliva and its role as a biomarker in assessing skeletal maturation during circumpubertal development growth.

MATERIALS AND METHODS

The study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, GITAM Dental College and Hospital, Vishakhapatnam, Andhra Pradesh, India. A total of 27 female patients of age 9 -16 years were selected and they were categorized into 3 groups based on their maturational status according to the CVM stages given by Mc Namara, Franchii, and Baccetti (2005) (Table 1):

How to cite this article:
Sowmya J, Sasidhar YN, Varma NS, Preetam R, Prasad KG. Cortisol: A Biomarker in Assessing Skeletal Maturation during Circumpubertal Development. Int J Oral Health Med Res 2016;3(1):10-12.

Sample	Sample Size	Cervical Vertebrae Stage
Group I	9	Cs1-Cs2
GROUP II	9	Cs3-Cs4
GROUP III	9	Cs5-Cs6

Table 1: Sample Groups

Individuals who were in need of Orthodontic therapy were included in the study. Individuals with systemic illness, growth abnormalities, who were under medication, were excluded from the sample.

Collection of Saliva: Saliva was collected by using passive drool method into sterile containers and Stored at 5°C in a vaccine box (Spensors vaccine carrier-model no:1181-SFR-001 Series). To standardize the time of collection, all the samples were collected at 7'Oclock in the morning at a local biochemical lab.

Analysis of salivary Cortisol: In the laboratory the salivary samples were transferred into 15 ml Centrifuge tubes and subjected to centrifugation at the rate of 3000rpm in the R-8C Laboratory Centrifuge REMI. After the centrifugation, with the help of Eppendorf pipette supernatant solution was drawn from the centrifuge tubes and they were transferred into 2ml Eppendorf tubes The caps were sealed with parafilm. Later on, the estimation of salivary Cortisol was done using Electrochemi-luminescence (ECL) immunoassay using Roche Cobas E411 Immunoanalyserby CENTRAL LAB, Bengaluru, Karnataka.

Statistical analysis: Continuous data was represented as the Mean and Standard deviation. Difference between the groups was analyzed using Analysis of Variance (ANOVA). If P value obtained in ANOVA is less than 0.05, Post Hoc Bonferroni analysis was done to detect the test difference between the groups. The Pearson correlation test was performed to study the correlation of the Salivary Cortisol levels and the group sample.

RESULTS

Mean and Standard deviation values for Group – I were found to be 0.28±0.05 microgram per deciliter, Group – II are 0.50 ±0.11 microgram per deciliter, Group - III are 0.61 ±0.13 microgram per deciliter.

Groups	Mean	Std.Deviation
Group I	0.2878	0.05869
Group II	0.5011	0.11297
Group III	0.6156	0.13078
Total	0.4681	0.17149

Table 2: Mean and Standard Deviation

One way ANOVA showed that mean Salivary Cortisol levels between the groups were statistically significant as the p-value was less than 0.05

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.498	2	.249	22.433	.000*
Within Groups	.266	24	.011		
Total	.765	26			

Table 3: ANOVA, Salivary Cortisol level (*. The mean difference is significant at the 0.05 level)

Post Hoc Bonferroni analysis showed that there was a statistical significant value when Group I was compared to either of Group II or Group III, but there was a statistical insignificant difference between the Salivary Cortisol levels for Group II and Group III

(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
gr1	gr2	-.21333*	.04967	.001	-.3412	-.0855
	gr3	-.32778*	.04967	.000	-.4556	-.1999
gr2	gr2	.21333*	.04967	.001	.0855	.3412
	gr3	-.11444	.04967	.091	-.2423	.0134
gr3	gr2	.32778*	.04967	.000	.1999	.4556
	gr3	.11444	.04967	.091	-.0134	.2423

Table 4: Multiple Comparisons, Dependent Variable: Salivary Cortisol (*. The mean difference is significant at the 0.05 level)

Pearson Linear Correlation of Mean Salivary Cortisol values between the Group I and Group III showed statistical significant value.

Salivary Cortisol		Salivary Cortisol	Groups
	Pearson Correlation	1	.795
	Sig. (2-tailed)		.000
	Sum of Square and cross products	.765	2.950
	Covariance	.029	.113
	N	27	27
Groups	Pearson Correlation	.795	1
	Sig (2-tailed)	.000	
	Sum of Square and Cross- product	2.950	18.000
	Covariance	.113	.692
	N	27	27

Table 5: Correlations (Statistically significant *p value <0.05)

DISCUSSION

This study was attempted to establish a relationship between different pubertal stages and the mean Salivary Cortisol levels during the circumpubertal growth spurt. This method would enable the Orthodontist to use Salivary Cortisol as a biomarker to assess patient's skeletal maturity.

One of the hormones which influence growth is the Growth Hormone. An in-vitro study conducted by D. Swolin-Eide. It was shown that Cortisol has the capability to modulate the Growth hormone action at the cellular level, by GH-receptor expression in hOB-like cells.[13] In an another in-vitro study done by Senaris RM et al[14] it was shown that Cortisol boosts GH synthesis and release in vitro by activating on GH gene transcription and upregulating GH-releasing hormone (GHRH) receptor expression. In an invivo study conducted by Martinelli CE Jr and Moreira AC[15] where they assessed the relationship between the Growth Hormone and Cortisol secretion in children. Their results showed that there was a positive relationship. Hence, we conclude by these studies that Growth hormone and Glucocorticoid hormones are interrelated.

In the present study, Cortisol hormone was analyzed to establish a relationship between the pubertal status of the individual and the hormone levels. In the recent years, it

was proved that the salivary Cortisol is directly correlated with plasma free Cortisol and this plasma free Cortisol is the biologically active form of the hormone.[16]

The results of the present study showed that the salivary Cortisol levels are less in the Prepubertal group (Group I) when compared with the Pubertal (Group II) and Post pubertal groups (Group III). The results of the present study are in accordance with the study conducted by Linder B.L et al[18], Gandia A et al[19], Sarah L. Tsai [20], Kiess W[21]. There was a sudden increase in the salivary Cortisol level in the pubertal group. The probable reason for the sudden increase may be due to the increase in the growth hormone release in the pubertal spurt and the high activity of the adrenal glands. The Salivary Cortisol levels in the Post pubertal group increased gradually in comparison to other two groups.

CONCLUSION

The individual skeletal maturation stages identification is crucial to the success of functional and orthopedic treatment. Radiographic methods are used conventionally for the identification of pubertal growth status of the individual. Their main drawback was the radiation exposure. Hence, to overcome this noninvasive procedure was used in the study. The results of the study show that the Salivary Cortisol levels are increased in the pubertal group. The validity of using salivary Cortisol as a biomarker for assessing the pubertal status need to be confirmed by longitudinal studies with larger sample size.

REFERENCES

1. Moore RN, Moyer BA, DuBois LM. Skeletal maturation and craniofacial growth. Am J Orthod Dentofacial Orthop 1990;98:33-40.
2. Hassel B, Farman AG. Skeletal maturation evaluation using cervical vertebrae. Am J Orthod Dentofacial Orthop 1995;107:58-66.
3. Fishman LS. Chronological versus skeletal age, an evaluation of craniofacial growth. Angle Orthod 1979;49:181-9.
4. Baccetti T, Franchi L, McNamara JA. The cervical vertebral maturation (CVM) method for the assessment of optimal treatment timing in dentofacialorthopedics. SeminOrthod. 2005; 11:119–29.
5. Himes JH, Huang Z, Haas JD, Rivera R, Pineda O. Serum alkaline phosphatase activity and skeletal maturation in Guatemalan adolescents. Ann Hum Biol 1993;20:39-46.
6. Perinetti G, Baccetti T, Contardo L, Di Lenarda R. Gingival crevicular fluid protein content and alkaline phosphatase activity inrelation to pubertal growth phase. Angle Orthod 2012;82:1047-52.
7. Gupta S, Jain S, Gupta P, Deoskar A. Determining skeletal maturation using insulin-like growth factor I (IGF-I) test. Prog Orthod 2012;13:288-95.
8. Srinivasan B, Premkumar S. Assessment of serum dehydroepiandrosterone sulphate in subjects during the pre- pubertal, pubertal, and adult stages of skeletal maturation. Eur J Orthod 2012;34:447-51.
9. Hussain MZ, Talapaneni AK, Prasad M, Krishnan R. Serum PTHrP level as a biomarker in assessing skeletal maturation during circumpubertal development. Am J Orthod Dentofacial Orthop 2013;143:515-21.
10. B. C. J. Van der eerden, m. Karperien, and j. M. Wit. Systemic and Local Regulation of the Growth Plate. Endocrine Reviews, December 2003, 24(6):782–801
11. Malerba M, Bossoni S, Radaeli A, Mori E, Bonadonna S, Giustina A, Tantucci C. Growth hormone response to growth hormone-releasing hormone is reduced in adult asthmatic patients receiving long-term inhaled corticosteroid treatment. Chest. 2005 Feb;127(2):515-21
12. Clemens kirschbaum and Dirk H. Hellhammer. Salivary Cortisol. Encyclopedia of Stress. 3; 379-383.
13. Ramirez-yenaz GO, Young WG, Daley TJ, Waters WJ. Influence of growth hormone on the mandibular condyle of rats. Arch Oral Biol 2004:49:585-90.
14. D Swolin-Eide, A Nilsson1 and C Ohlsson. Cortisol increases growth hormone-receptor expression in human osteoblast-like cells. Journal of Endocrinology (1998) 156, 99–105
15. Senaris RM, Lago F, Coya R, Pineda J, Dieguez C. Regulation of hypothalamic somatostatin, growth hormone-releasing hormone, and growth hormone receptor messenger ribonucleic acid by glucocorticoids. Endocrinology 1996 ; 137(12):5236-41.
16. Martinelli CE Jr and Moreira AC. Relation between growth hormone and cortisol spontaneous secretion in children. Clin Endocrinol (Oxf) 1994 Jul;41(1):117-2.
17. Aardal-Eriksson E, Karlberg BE, Holm AC. Salivary cortisol--an alternative to serum cortisol determinations in dynamic function tests. Clin Chem Lab Med 1998 Apr;36(4):215-22.
18. Linder B.L., Esteban N.V., Yergey A.L., Winterer J.C., Loriaux D.L., Cassorla F. (1990) Cortisol production rate in childhood and adolescence. J Pediatr 117: 892–896.
19. Gandia A, Bolufer P, Rodriguez A, Antonio P. Salivary cortisol increases with age in children. Journal of Clinical Chemistry and Clinical Biochemistry.1990;28:655.
20. Sarah L. Tsai, Kelly J. Seiler, Jill Jacobson. Morning Cortisol Levels Affected by Sex and Pubertal Status in Children and Young Adults. J Clin Res Pediatr Endocrinol 2013;5(2):85-89.
21. Kiess W, Meidert A, Dressendörfer RA, Schriever K, Kessler U, König A, Schwarz HP, Strasburger CJ. Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage, and weight. Pediatr Res 1995 Apr;37(4 Pt 1):502-6.

Source of Support: Nil
Conflict of Interest: Nil