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Original Research Article

Identification and quantitative assessment of human dehydroepiandrosterone sulphate in saliva and its co-relation with skeletal age

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Abstract

Background and Objectives: Evaluation of the accurate skeletal maturity status is one of the most important tool in treatment planning in orthodontics. Over the decades, various methods have emerged for correct estimation of skeletal maturity. In the recent years, biomarkers has gained popularity as it is a non-invasive technique. DHEAS is one of the biomarker which has a role in triggering growth and proliferation of epiphyseal cartilage, augment bone density and also plays role in prompting pituitary and hypothalamus to start its action. Thus, this cross sectional study was undertaken to assess the salivary level of DHEAS and co-relate it with different skeletal age as determined by hand wrist radiograph using the Hagg and Taranger method and projecting it as a potential non-invasive tool for assessment of skeletal age.

Materials and Methods: Right hand wrist radiographs and unstimulated saliva samples were collected from routine 80 patients visiting to SDM College of Dental Sciences under their consent. Unstimulated saliva samples were collected by "passive drool" method. Unstimulated saliva samples were stored at -800℃ and were assayed by Human DHEAS ELISA kit. According to skeletal age determined by hand wrist radiograph, 80 subjects were divided into 5 skeletal groups classified by Hagg and Taranger method; S-0, S-1, MP-3, DP-3 and R-J. Data thus obtained were subjected to suitable statistical analysis.

Results: DHEAS level in saliva increased in accordance with the skeletal maturation with in females in general showing higher concentration than males. However, there was significant high concentration of DHEAS in males than females at R-J stage was observed which might be due to influence of both body weight of the patient on DHEAS level. Maximum increase in level of DHEAS was observed at S-1 stage, marking adrenarche and followed by gradual increase in level.

Interpretation and Conclusions: There is a positive correlation between DHEAS and skeletal maturation as there is significant increase in DHEAS level in saliva with advancing skeletal maturation. Thus, the study confirms that DHEAS has a role in skeletal maturation and can be used potentially more sensitive and reliable biomarker for assessing growth. However weight being the limitation of the study, further longitudinal studies involving weight as a variable should be carried out to further support this hypothesis.

Keyword-Biomarker, DHEAS, Skeletal maturity, Hand wrist radiograph, Non-invasive technique.

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1. Introduction

A comprehensive understanding of growth is the key for accurate treatment planning in orthodontics. Complex interactivity of genes, hormones, growth factors and environment leads to skeletal growth and maturation. Maturational status is best evaluated by different stages of physiologic maturity rather than chronologic age as the latter is not a reliable indicator.[1] Various methods have been reported to determine maturity, i.e. increase in body weight,[2] body height,[3] hand-wrist skeletal maturation stages,[4-6] dental development and eruption,[7,8] chronological age,[9] sexual maturation,[10] cervical vertebrae maturation,[11-13] frontal sinus[14] and recently biomarkers.[15]

As stated by WHO, biomarkers are "almost any measurements reflecting an interaction between a biological system and potential hazard, which may be chemical, physical or biological. The measured response may be functional and physiological, biochemical at the cellular level or molecular interaction.[16] Mechanism of bone remodeling involves interaction between biochemical mediators which

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are released in the circulation[17] and detection of such mediators would assist the orthodontist to predict the growth status of an individual. Initially biomarkers were detected in serum, but nowadays they are also being identified in saliva.

Saliva is the most abundant oral fluid with average individual salivation ranging from 0.3 to 0.7 ml of saliva per minute, i.e.1 to 1.5 liters per day. Saliva is the mirror of the body. Collection of saliva in comparison to serum and gingival crevicular fluid is easy as it is non-invasive, doesn't require highly trained personnel, safe to handle and procedure is economical. However, very few studies have been carried out to evaluate biomarkers in saliva because of its low sensitivity as a diagnostic fluid.[18,29]

At puberty, there exists a strong interlinkage between growth of craniofacial structures and somatic changes. Puberty is predominantly a neuroendocrinal event. Hypothalamus and pituitary are jointly called as gonadostat. Adrenal gland stimulates gonadostat to initiate its action. Adrenal gland secretes dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS), 3 years prior to puberty.

Growth hormone's action is increased by DHEA and DHEAS. Both DHEA and DHEAS accelerate growth and multiplication of epiphyseal cartilage. However, DHEA exhibits less valid results than DHEAS as the latter has a greater half- life, slower clearance and strongly bound to albumin. DHEAS being charged molecule, is actively transported through salivary membranes via organic anion transport polypeptides.[15]

A cross-sectional study was conducted to evaluate serum levels of dehydroepiandrosterone sulphate (DHEAS), during the pre-pubertal, pubertal and adult stages of skeletal maturation and there was positive co- relation.[15] Another study was done to find the possible relationship between bone density and serum levels of dehydroepiandrosterone sulphate (DHEAS) and a significant positive relationship was established between DHEAS and BMD.[19] An investigation was carried out to find relationship between BMD and serum DHEA-S in both men and women and a positive co-relation was obtained between serum DHEA-S level and BMD, especially with BMD in the femur.[20,21-28]

Thus, objective of the present study is to identify and estimate level of DHEAS in whole unstimulated saliva at different skeletal ages as determined by the hand-wrist radiograph and also to correlate the salivary DHEAS level at different stages of skeletal development as assessed by Hagg and Taranger method and projecting salivary DHEAS axis in saliva as a reliable and potentially non-invasive tool for assessment of skeletal age. The study aims to assess the co relation between the salivary levels of DHEAS and different stages of skeletal maturation as assessed by hand wrist radiograph using Hagg and Taranger method (**Figure 1**).

2. Materials and Methods

80 healthy subjects from North Karnataka ethnical background, aged from 6 to 19 years with equal sex distribution were selected from routine patients (**Table 1**). Approval for the study was procured from the Ethical board and Committee; IRB No. 2017/P/OR/47.

2.1. Inclusion criteria

Healthy subjects (both males and females) in the group of 6-19 years of age.

2.2. Exclusion criteria

1. Subjects suffering from systemic disease which affects growth such as disorders related to thyroid, parathyroid and growth hormone, vitamin D metabolism, renal impairment, diabetes mellitus, etc.
2. Subjects under medication that could affect bone metabolism during the previous six months, for example: vitamin preparations, calcium supplements etc.
3. Subjects with history of xerostomia.
4. Subjects undergoing fixed orthodontic or functional orthopaedic treatment.

2.3. Sample size calculation

F tests - ANOVA: Fixed effects, omnibus, one-way

Analysis:A priori: Compute required sample size	
Input: Effect size f	0.41
α err prob	0.05
Power (1-β err prob)	0.80
Number of groups	5

Output: Noncentrality parameter λ =	13.448000
Critical F	2.493696
Numerator df	4
Denominator df	75
Total sample size	80
Actual power	0.824678

A power analysis was established by G*power, version 3.0.1(Franz Faul universitat, Kiel, Germany). A sample size of 80 subjects (16 in each group) would yield 80% power to detect significant differences, with effect size of 0.41 and significance level at 0.05.

Consent form was procured from each and every subject who participated in the study.

Saliva samples were collected from 9.00 am to 11.00 am in the morning for all patients. Patients were instructed to rinse mouth with deionized water prior to sample collection to assure the mouth is free from food debris etc. Using passive drool method 1ml of unstimulated saliva was collected and immediately stored at -80^{0}C. Unstimulated

whole saliva was collected as it is predominant for major part of the day and reflects both the oral cavity and entire body's physiologic status. Subject's right hand wrist radiograph was obtained on the same day. The hand wrist region has various small bones which show a predictable and scheduled pattern of appearance, ossification and union from birth to maturity. Thus, this region is one of the most suited to study growth.[21] Radius, ulna, selected metacarpals and phalanges, carpal and sesamoid bones were traced on matte acetate paper by 1 examiner and analyzed by 2 examiners after an hour to avoid any bias in grouping the participants to the skeletal groups. The investigator was blinded about each patient's age, pubertal status and salivary DHEAS levels. The hand wrist radiographs were retraced by the same investigator at a different point in time for estimation of method error. 80 subjects were divided into 5 subgroups of different skeletal stages as given by Hagg and Taranger[9] where each group consisted of 16 subjects. Enzyme-linked immunosorbent assay (ELISA) kits (KinesisDx, USA) was used to measure the level of DHEAS and results were expressed as nanograms/millilitre.

3. Results

Significant differences among five sub-groups with all respective parameters were tested using one way ANOVA test. Tukeys multiple posthoc procedure was used to find the significant differences between five sub-groups with all respective parameters after one way ANOVA. Statistical significance was tested at $p < 0.05$.

The mean chronological at peak pubertal growth; regardless of gender is 12years of age (**Table 1**). There is a positive correlation between salivary levels of DHEAS and all skeletal maturity groups with a p value of 0.0001 by one way ANOVA test. Pair wise comparisons of skeletal ages by Tukeys multiple posthoc procedures shows significant rise in DHEAS between the groups (**Table 2**). Apart from skeletal age group MP-3 and R-J, there weren't any significant differences in the mean hormone level between male and female (**Table 3**).

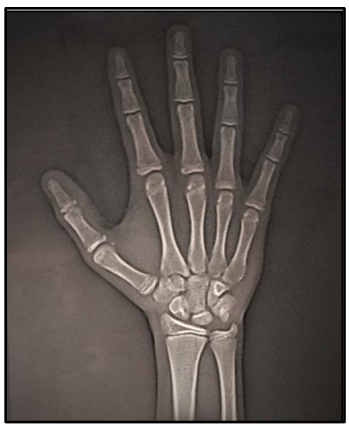

Figure 1: Right hand wrist radiograph

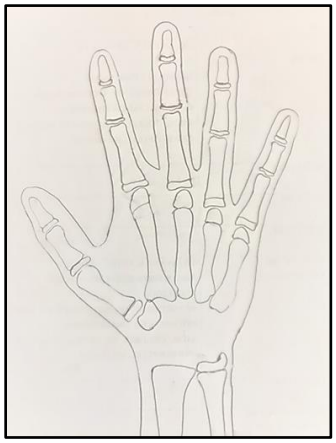

Figure 2: Tracing of radiograph

Table 1: Comparison of skeletal ages with mean chronological ages by one way ANOVA

Skeletal ages	Mean	SD	SE
S-0	6.38	1.20	0.30
S-1	11.50	0.73	0.18
MP3-G	11.13	1.75	0.44
DP3-1	15.44	1.67	0.42
R-J	18.19	1.05	0.26
F-value	182.3634		
P-value	0.0001*		
Pair wise comparisons of skeletal ages by Tukeys multiple posthoc procedures			
S-0 vs S-1			p=0.0001*
S-0 vs MP3-G			p=0.0001*
S-0 vs DP3-1			p=0.0001*
S-0 vs R-J			p=0.0001*
S-1 vs MP3-G			p=0.9317
S-1 vs DP3-1			p=0.0001*
S-1 vs R-J			p=0.0001*
MP3-G vs DP3-1			p=0.0001*
MP3-G vs R-J			p=0.0001*
DP3-1 vs R-J			p=0.0001*

*p<0.05

Table 2: Comparison of skeletal ages with DHEAS scores by one way ANOVA

Skeletal ages	Mean	SD	SE
S-0	0.19	0.07	0.02
S-1	0.58	0.18	0.05
MP3-G	0.74	0.17	0.04
DP3-1	0.92	0.16	0.04
R-J	0.94	0.17	0.04
F-value	62.2844		
P-value	0.0001*		
Pair wise comparisons of skeletal ages by Tukeys multiple posthoc procedures			
S-0 vs S-1	p=0.0001*		
S-0 vs MP3-G	p=0.0001*		
S-0 vs DP3-1	p=0.0001*		

S-0 vs R-J	p=0.0001*
S-1 vs MP3-G	p=0.0500*
S-1 vs DP3-1	p=0.0001*
S-1 vs R-J	p=0.0001*
MP3-G vs DP3-1	p=0.0126*
MP3-G vs R-J	p=0.0038*
DP3-1 vs R-J	p=0.9948

Table 3: Comparison of male and females with DHEAS scores in each skeletal age by independent t test

Skeletal age	Gender	Mean	SD	SE	t-value	P-value
S-0	Male	0.18	0.06	0.02	-0.3355	0.7422
	Female	0.20	0.09	0.03		
S-1	Male	0.61	0.14	0.05	0.4821	0.6372
	Female	0.56	0.22	0.08		
MP3-G	Male	0.61	0.10	0.04	-5.4129	0.0001*
	Female	0.87	0.10	0.03		
DP3-1	Male	0.89	0.23	0.08	-0.8411	0.4145
	Female	0.96	0.04	0.01		
R-J	Male	1.05	0.13	0.05	3.0817	0.0081*
	Female	0.84	0.15	0.05		

4. Discussion

Accurate evaluation of the developmental stage forms an integral part of both diagnosis and treatment planning for orthodontic patients. Numerous methods have been developed with time to accurately assess the maturation status starting from indicators like height[3] and weight.[2] Both the methods evaluated growth with respect to chronologic age. However, chronologic age is not a reliable indicator for maturational status as there lies a wide individual variation in timing of pubertal growth spurt with respect to chronologic age.[22] This led to the use of skeletal maturation (hand-wrist maturity, cervical vertebrae, dental development and eruption)[4,8,11,13] in lieu of indicator of physical development and maturation status where radiographic method is used to assess the same. Recently introduced, biomarkers have been more of interest in the growth studies.

The neuroendocrine system controls puberty. During adrenarche, which is 3 years before puberty, dehydroepiandrosterone (DHEA) and its sulphated conjugate dehydroepiandrosterone sulphate (DHEAS) are secreted from adrenal gland which stimulates gonadostat (pituitary and hypothalamus together) to initiate puberty. A study conducted by Srinivasan et al[15] Believed DHEAS has a relation which is directly proportional to the skeletal maturation.

Initially biomarkers were detected in serum, but recently they are also being identified in saliva. Saliva is more convenient as it doesn't require trained personnel for sample collection, adequate quantities can be easily obtained for analysis, is non-invasive, safe to handle, and procedure is economical.

The hand wrist radiographs obtained in this study were from a North Karnataka population group. These were in agreement with the fact that the peak growth, irrespective of gender was observed at a mean age of 12 years and the phase of deceleration was noted to begin at an age of 15 years.

In the cross sectional study conducted, there was statistically significant progressive rise in DHEAS level for both male and female from S-0 to S-1 which supports the fact that biochemically adrenarche is marked by elevation of DHEAS. From S-1 to DP-3 there was a statistically significant gradual rise in salivary concentration of DHEAS. However, the salivary concentration of DHEAS did not show any statistically significant increase from DP-3 to R-J. This insignificant rise is in accordance with the result obtained by the study conducted by Shashikalakumari et al[23] and Anusuya et al.[24] DHEA and DHEAS levels are high in fetal life, decrease after birth, and show a marked pubertal increase to a maximal level during young adulthood; after young adulthood, levels of DHEA decline and are between 10% and 20% of young adult levels by age 70.[25] In the present study there was rise in DHEAS level with skeletal age, attaining its highest value following complete fusion of both the epiphysis and diaphysis of the radius[29]. Apart from S-1 and R-J skeletal age, females had maintained higher values of DHEAS level as compared to boys in all other skeletal age group, reflecting the fact that females matures earlier than males. In the R-J stage, male had higher salivary concentration of DHEAS. This could be due to obese male in that group, which was not taken into account in the present study. Study conducted by Camila et al concluded that obesity is positively associated with dehydroepiandrosterone sulfate.[26]

4.1. Clinical implications

1. To assess growth of the patient, so that proper treatment modalities like growth modification, orthognathic surgery can be planned depending upon the growth remaining.
2. Use of biomarker for assessing growth will prevent the patient from undue radiation exposure.

5. Conclusion

As, DHEAS level in whole unstimulated saliva was identified and estimated at different skeletal ages as determined by the hand-wrist radiograph it can be concluded that there is a positive correlation between DHEAS and skeletal maturation as there is significant increase in DHEAS level in saliva with advancing skeletal maturation and projecting salivary DHEAS axis in saliva as a reliable and potentially non-invasive tool for assessment of skeletal age.

Thus, the results obtained from the study confirms that DHEAS has a role in skeletal maturation and can be used as a potentially sensitive and reliable biomarker for assessing growth.

6. Source of Funding

None.

7. Conflict of Interest

None.

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