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The Relationship Between Salivary Dehydroepiandrosterone Sulphate (DHEAS) Levels and Skeletal Maturation Parameters Before and During Pubertal Growth Spurt in Children

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Abstract

Objectives: This study aimed to assess the skeletal maturation by using salivary DHEAS levels and its correlation with existing skeletal maturity parameters represented by cervical vertebrae and MP3 region radiographs in adolescents in pre-pubertal and pubertal age groups.

Methods: In this study, 80 children in the age range of 8 - 14 years were divided into two equal groups based on their chronological age as group I (pre-pubertal group) and group II (pubertal group). Pre-existing lateral cephalograms and medial phalanx of third finger (MP3) radiographs of their left hands were assessed. The levels of the DHEAS of each individual were analysed by ELISA kit. ANOVA with post-hoc Tukey's test and student t-test were used for statistical analysis. P-value < 0.05 was considered significant.

Results: The mean level of DHEAS values shown in the present study was 4.36 +/- 0.32 ng/mL in group I and 5.73+/-0.39 ng/mL in group II. This study showed that in group I, more subjects were in stage 1 of cervical vertebral maturation than stage 2 and there were significant differences between the two stages (P-value = 0.011); also, in group II, more subjects were in stage 3 than 4 and there were significant differences between the two stages (P-value = 0.017). This study revealed the highest salivary DHEAS levels to be in the F stage of pre-pubertal MP3 development in addition to the H stage of MP3 development in pubertal children. This study noted that there were significant differences for salivary DHEAS levels between males and females not only in pre-pubertal (P-value = 0.031), but also in pubertal stages (P-value < 0.001).

Conclusions: Salivary DHEAS, like cervical vertebra and MP3 radiographs, can be used for growth assessment in young adolescents.

Keywords: Saliva, Biomarkers, Salivary DHEAS, Cervical Vertebrae Maturation

1. Background

Biological variability can be regarded as one of the most important laws of nature. Growth and development play a major role in the orthodontic diagnosis and treatment planning. The outcome of treatment in a growing patient is more complex to predict than a non-growing patient. Analysing radiographs has broadly been utilized in order to evaluate the skeletal maturation stage, as well as to speculate the time of pubertal growth, growth rate and the amount of growth remaining. Various tools have been used for growth assessment, such as hand wrist radiographs and cervical vertebrae (1).

Biochemical markers render new possibilities in the determination of skeletal maturation. Recent literature has put much emphasis on biochemical methods for the detection of skeletal maturity. They are found to be conservative, non-invasive, and non-radiographic and represent agents that are directly involved in bone growth and remodeling. Further, salivary hormonal assessment usually involves several sample collections in a short time. This fact suggests that saliva collection is a great option to attain this goal (2).

Saliva as a diagnostic tool has been found to be in competition with blood for its diagnostic potential. Measurement of salivary hormone levels reflects significant correlation with the serum hormone levels. Due to its potential advantages, salivary diagnosis provides a useful and dependable diagnostic alternative to more invasive, time-consuming and complicated approaches. Also, steroid lev-

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els in the saliva are reflective of the circulating levels of free steroid as opposed to total circulating levels, that are perplexed by the high affinity binding proteins present in the circulation (3). Alkaline phosphates in the serum and gingival crevicular fluid (GCF), proteins in GCF, serum Insulin-like growth factor-1, serum parathyroid hormone related peptide (PTHrP), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) (2) have been used to explore their role in the assessment of maturation in an individual.

2. Objectives

The main goal of this study was to evaluate skeletal maturation by using salivary DHEAS levels and their correlation with existing skeletal maturity indicators represented by cervical vertebrae and medial phalanx of third finger (MP3) radiograph of left hand in pre-pubertal and pubertal adolescents.

3. Methods

The study population comprised of 80 children who visited our institution. They were divided into 2 equal groups:

Group I: Girls aged 8 - 10 and boys aged 10 - 12 years were included in the pre-pubertal age group.

Group II: Girls aged 10 - 12 and boys aged 12 - 14 years were included in the pubertal age group.

Age in all groups represented the chronological age of children. Children with hormonal disturbances such as cretinism, hyperparathyroidism or premature puberty which would affect their growth as well as the children with a history of trauma to their face or hand were in included in the study.

A written consent formed was signed by the patients as well as from their parents upon explanation of the entire procedure. Ethical clearance for this study was obtained from the Institutional Review Committee (IRC).

Radiographic examination of each subject included:

1) Pre-existing lateral cephalogram (Figure 1);

2) Medial phalanx of third finger (MP3) radiograph of left hand (Figure 2).

The lateral cephalogram of each subject was then interpreted following the Hassel and Farman's (4) classifications (Table 1). All the lateral cephalograms obtained were assessed for skeletal maturation by estimating the concavity of the lower border of the cervical vertebrae. MP3 radiographs were evaluated using the method suggested by Hagg and Taranger (5) later modified by adding E 3/4 stages by Liete et al. (Table 2) (6). The MP3 radiographs were taken by placing the cone of the X-ray machine directed towards the middle phalanx, perpendicular to the film (Figure 3). The assessment, analysis and measurements of all the records were carried out by a single examiner using 0.003-inch lacquered polyester matte acetate tracing paper and a 0.3 mm lead pencil using a radiographic viewer.

Estimation of the concentration of salivary DHEAS was done using a standard FDA approved ELISA based DHEA-S Estimation KIT (Immuno-Biological Laboratories, IBL-America-8201 Central Ave. NE, Suite P, Minneapolis, Minnesota, USA) (Figure 4). Salivary samples were collected from all subjects using polypropylene tubes every morning between 10am to 12am and were transferred to the laboratory within 2 to 3 hours of collection in an appropriate storage media.

The principle of the test was centered on a solid phase enzyme-linked immunosorbent assay (ELISA), as described in the principle of competitive binding. An inverse relation is found between the amount of bound peroxidase conjugate and the concentration of DHEA in the sample. The colour intensity obtained is negatively associated with the amount of DHEA in the sample after adding the substrate solution.

ANOVA with post-hoc Tukey's test and student t-test were used for statistical analysis. P-value < 0.05 was considered significant.

4. Results

This study showed that in group I, more subjects were in stage 1 of cervical vertebrae maturation than stage 2 and there were significant differences between the two stages (P-value = 0.011); also, in Group II, more subjects were in stage 3 than 4 and there were significant differences between the two stages (P-value = 0.017) (Table 3).

MP3 stages and salivary DHEAS levels of the subjects in pre-pubertal and pubertal age groups were assessed using ANOVA with post-hoc Tukey's test as shown in Table 4. The table shows that the highest salivary DHEAS levels were in F stage of pre-pubertal MP3 development, as well as in H stage of MP3 development in pubertal children.

Salivary DHEAS levels in pre-pubertal and pubertal age groups were compared using student t-test as shown in Table 5, which was revealed to be statistically meaningful ($P < 0.001$).

According to Table 6, there were substantial differences for salivary DHEAS levels between males and females not only in pre-pubertal (P-value = 0.031), but also in pubertal stage (P-value < 0.001).

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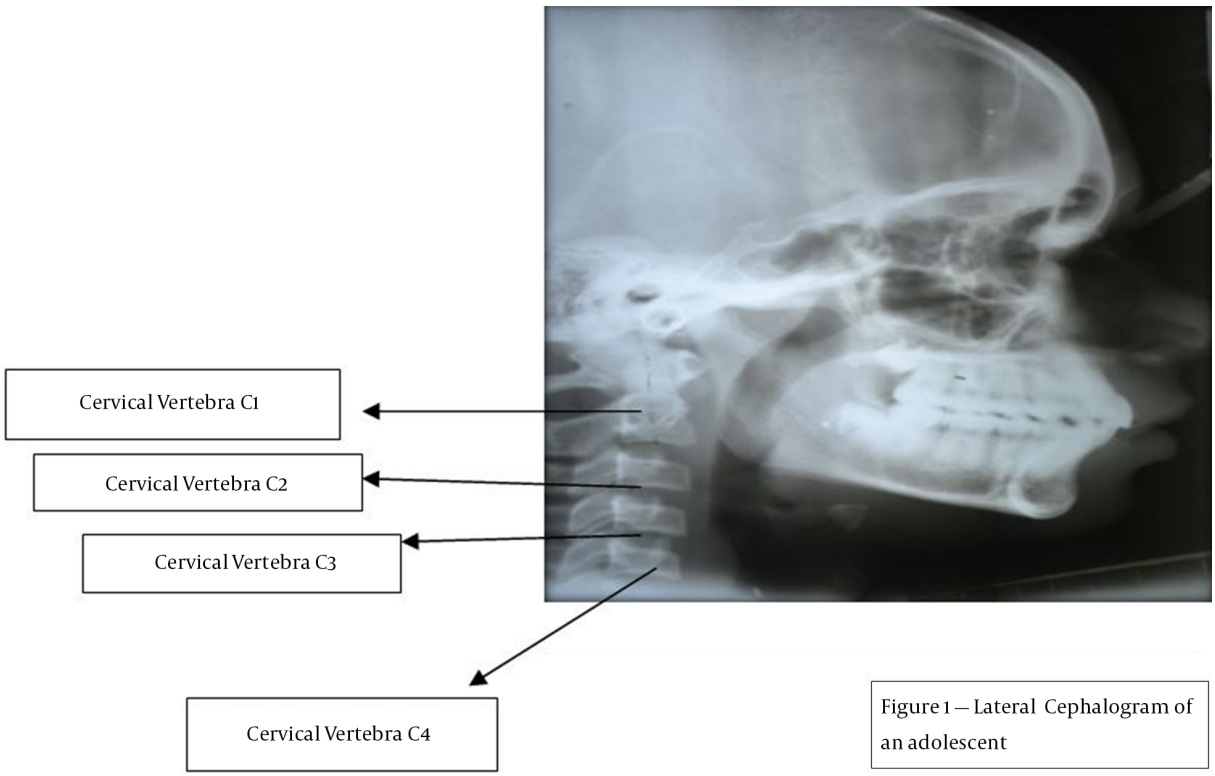


Figure 1. Lateral Cephalogram of an adolescent

Table 1. Developmental Stages of Cervical Vertebrae (Hassel and Farman Method)

Stage	Description	Percentage of Growth Remaining
CVMI 1 (initiation)	The lower borders of C2, C3, and C4 are flat in this stage. The vertebrae have wedge- like shapes, and the upper vertebral borders are tapered from posterior to anterior.	80% - 100% of adolescent growth is left.
CVMI 2 (acceleration)	Concavities develop on the lower borders of C2 and C3. The inferior border of C4 is flat. The bodies of C3 and C4 are almost shaped rectangularly.	65% - 85% of adolescent growth is left.
CVMI 3 (transition)	Clear concavities are observed in the lower borders of C2 and C3. A concavity is starting to form in the lower border of C4. The bodies of C3 and C4 are rectangular in shape.	25% - 65% of adolescent growth is left.
CVMI 4 (deceleration)	Clear concavities are observed in the lower borders of C2, C3, and C4. The vertebral bodies of C3 and C4 are getting squarer in shape	10% - 25% of adolescent growth is left.
CVMI 5 (maturation)	More accentuated concavities are observed in the lower borders of C2, C3, and C4. The bodies of C3 and C4 are square or almost squarely shaped.	5% - 10% of adolescent growth is left.
CVMI 6 (completion)	Deep concavities are observed in the lower borders of C2, C3, and C4. The bodies of C3 and C4 are square or have greater vertical dimension than horizontal dimension.	Little or no adolescent growth is left.

5. Discussion

Biomarkers have gained tremendous popularity in recent years because of the non-invasive nature of saliva based diagnostic tests. Biomarkers have been used as a method of assessment of skeletal maturation in children and adolescents (2, 7). Various biomarkers have been used for skeletal age assessment, among which DHEA and DHEAS are found to be correlated with growing in the pubertal growth spurt and might play a major part in skeletal maturation (2). DHEA is an adrenal precursor of steroid biosynthesis, which shows neurosteroid action on central nervous system (8). It is also reported to have anti-obesity, anti-atherosclerosis, anti-diabetes and anti-osteoporosis effects. As osteoblasts express aromatase, androgen is converted to estrogen and DHEA may act protectively against osteoporosis through its metabolites (9, 10).

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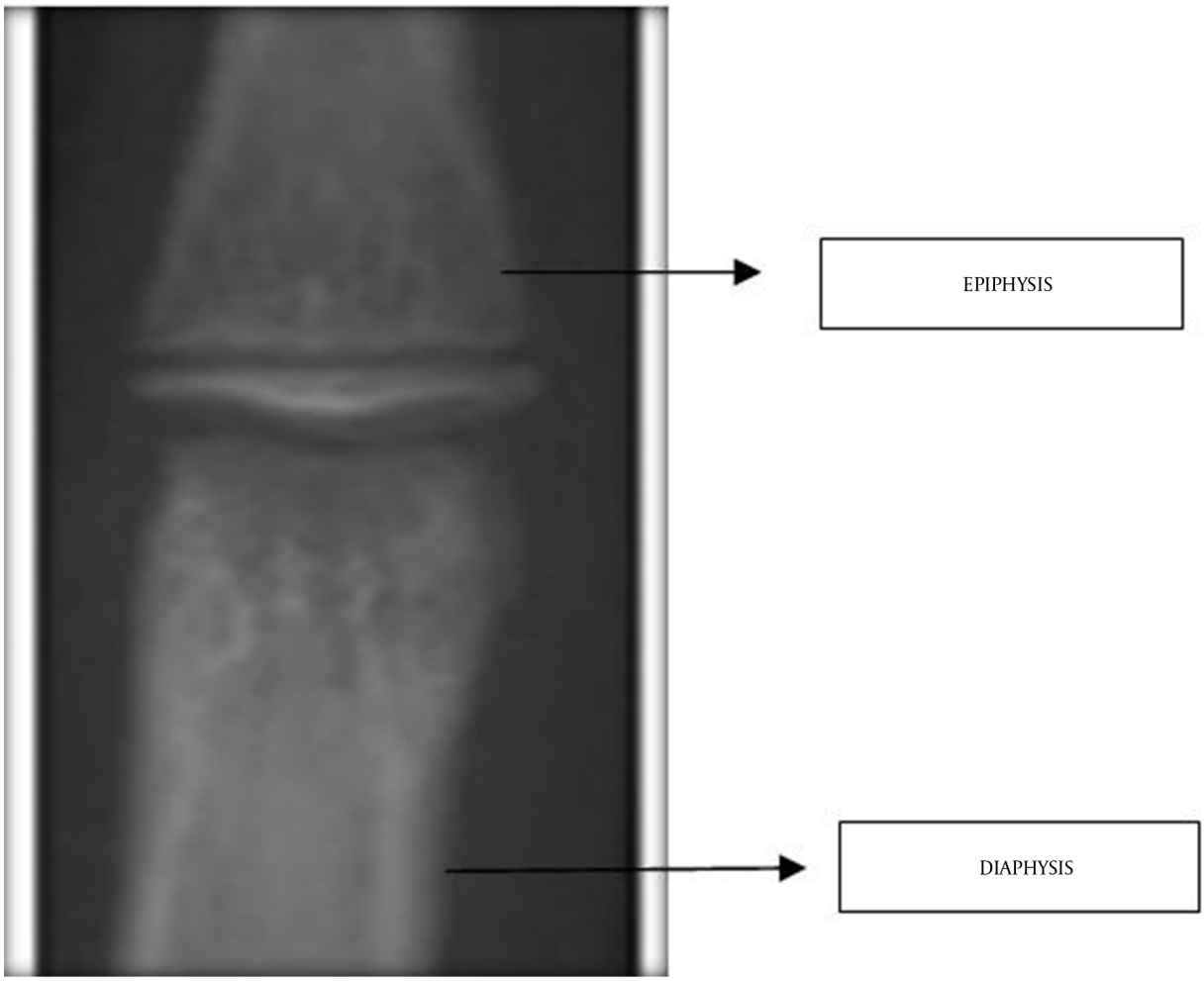


Figure 2 — IOPA radiograph showing
Middle phalanx of an adolescent

Figure 2. IOPA radiograph showing Middle phalanx of an adolescent

In children, as they progress towards maturation, decreased levels of 3 beta hydroxy steroid dehydrogenase in the adrenal reticularis contribute to the increased production of DHEA observed during adrenarche (11). DHEA has a positive effect on bone metabolism. It promotes growth by proliferation of osteoblasts and mitogen-activated protein kinase signalling pathways which are necessary for the expression of osteoblast-specific genes. It has been reported that DHEA inhibits osteoblast apoptosis and inhibits osteoclast maturation, thereby promoting bone growth and maturation (12). DHEAS, the sulphate form of DHEA, is an important health biomarker, which is considered as the marker of Adrenarche (8, 13). Hence the present study was undertaken with an objective to assess salivary DHEAS levels in children and young adolescents.

Puberty is hugely influenced by the neuro-endocrine system. The neuroendocrine part in the start of puberty begins with the maturation of hypothalamus pituitary complex shown by a major increase in the secretion of DHEA and its conjugate DHEAS (9).

The measurement of steroid levels in saliva can be a useful and practical diagnostic tool in the assessment of skeletal changes in relation to pubertal growth and maturation (14). Therefore, in the present study, salivary assessment was undertaken, as saliva offers many distinctive advantages. The most important benefit of saliva is that the sampling is not stressful and can be easily repeated, whereas blood collection might be tough or unpleasant.

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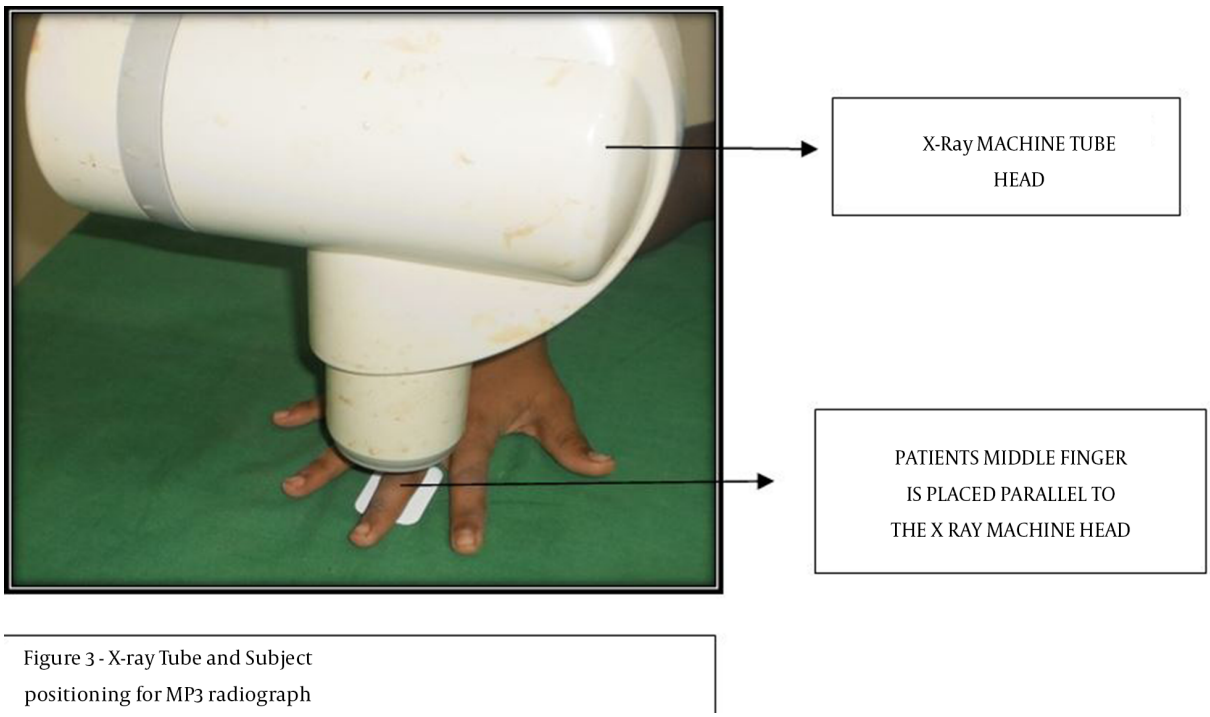


Figure 3. X-ray Tube and Subject positioning for MP3 radiograph

Table 2. The Developmental Stages of MP3 Region Were Categorized as Follows Based on the Method by Hagg and Taranger

Stage	Features
MP3- E3/4	The epiphysis approached 3/4 of the width of the diaphysis (Leite et al. (6)).
MP3-F	The epiphysis is as wide as the metaphysis; this denotes the onset of the pubertal growth spurt.
MP3-FG	The epiphysis is the same width as the metaphysis and there is an obvious medial and/or lateral border of the epiphysis shaping a line of demarcation at right angles to the distal border.
MP3-G	The sides of the epiphysis are thickened and cap its metaphysis forming a sharp edge distally on one or both sides.
MP3-H	Fusion of the epiphysis and metaphysis has begun. This is the deceleration period of the pubertal growth spurt.
MP3-I	Fusion of the epiphysis and metaphysis is complete. This marks the end of the pubertal growth spurt.

This method is appropriate for paediatric and psychobiological studies (3). The method of saliva collection used in the present study was the spitting method, where the patient directly expectorates passive drools of saliva into the polypropylene tube. This method is believed to be a superior method used for saliva collection apart from draining method (15).

The timing of collection of saliva for DHEAS evaluation was often a crucial factor in most of the previous studies. A study by Whetzel and Klein (16) had stated that early morning collection of passive drool of saliva contains increased DHEAS levels, when compared to the samples in the rest of the day, hence in the present study, early morning samples of saliva were collected for evaluation of optimal DHEAS levels.

The outcomes of the current study clearly show that there was a continuing increase in salivary DHEAS level as skeletal maturation advanced. Salivary DHEAS levels reached a near maximum amount following the utter fusion of the epiphysis and diaphysis of the radius in both age groups, which was found to be statistically significant. Within each group, there were substantial variations in the mean hormone values among males and females, demonstrating gender dissimilarity in the hormone values in a specific stage of skeletal maturation. It was also observed that females had significantly higher salivary DHEAS levels than males in both age groups. Studies performed by several authors are in accordance with the finding of the present study, which displayed an initial rise in DHEAS levels in females at the age of 7.5 years (7, 17, 18). However, contradicting results were found, which showed no significant gender difference when total samples were considered. This finding acknowledges the results of previous

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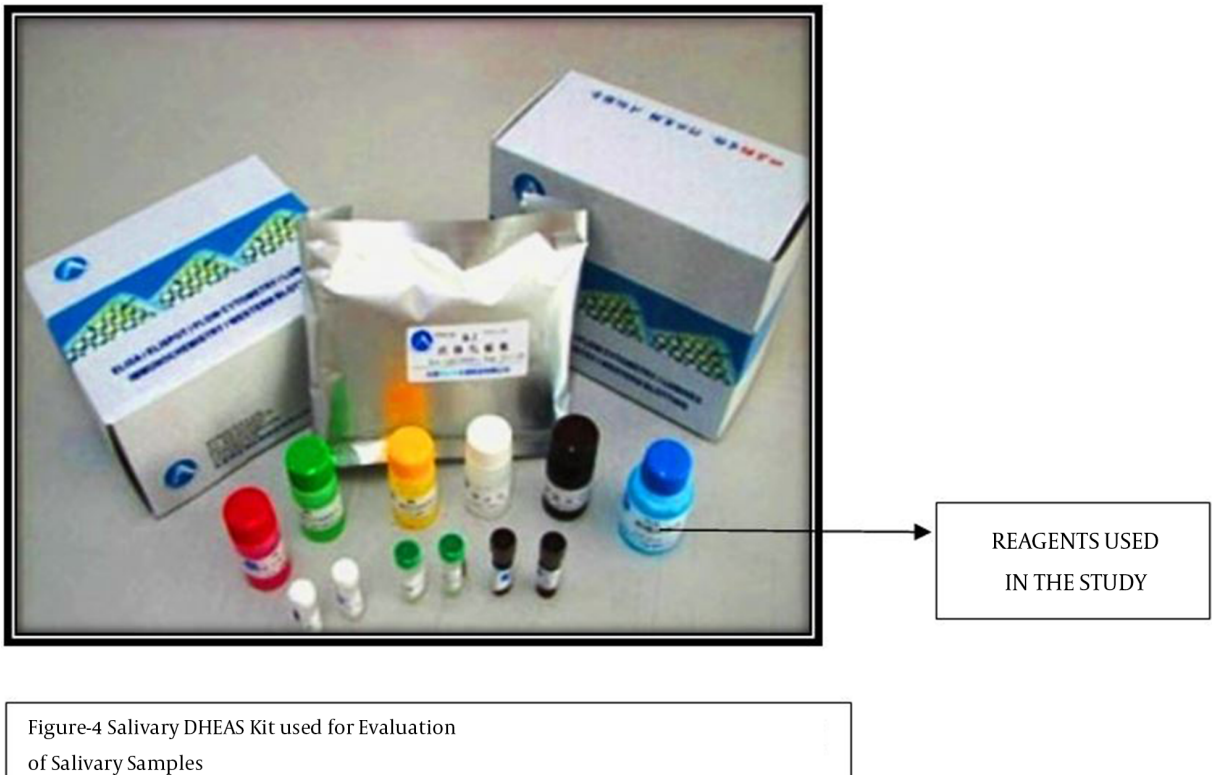


Figure 4. Salivary DHEAS Kit used for evaluation of salivary samples

Table 3. Comparison of the CVM Stages with the Mean and Standard Deviation of Salivary DHEAS Levels

Group	CVM	Salivary DHEAS, ng/mL		P-Value
		Mean ± SD	Numbers	
Group I (pre-pubertal)	1	4.21 ± 0.30	16	0.011
	2	4.46 ± 0.30	24	
Group II (pubertal)	3	5.60 ± 0.45	22	0.017
	4	5.88 ± 0.24	18	

Table 4. Comparison of the MP3 Stages with the Mean and Standard Deviation of Salivary DHEAS Levels

Group	MP3	Salivary DHEAS, ng/mL		P-Value	Post-Hoc Test
		Mean ± SD	Numbers		
GROUP I (Pre-pubertal)	E3/4	3.98 ± 0.11	10	< 0.001	F, FG > E 3/4
	F	4.52 ± 0.26	14		
	FG	4.46 ± 0.26	16		
GROUP II (pubertal)	FG	5.40 ± 0.39	12	0.001	G, H > FG
	G	5.82 ± 0.34	16		
	H	5.92 ± 0.24	12		

studies9 that found no considerable gender variations in DHEAS levels in the same age.

In the group II of the present study, the SD values of DHEAS were highest for CVM3 stage (pre-pubertal stage)

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Table 5. Comparison of Saliva DHEAS Levels in Pre-Pubertal and Pubertal Age Groups

	Group		P-Value
	Pre-pubertal, Mean ± SD	Pubertal, Mean ± SD	
Saliva DHEAS, ng/mL	4.36 ± 0.32	5.73 ± 0.39	< 0.001

Table 6. Comparison of Salivary DHEAS Levels in Pre-Pubertal and Pubertal Age Groups with Respect to Gender[a]

	DHEAS, ng/mL	DHEAS, ng/mL	P-Value
Group I; Group II			
Pre-pubertal	4.26 ± 0.30	4.47 ± 0.31	0.031
Pubertal	5.48 ± 0.19	5.98 ± 0.38	< 0.001
Total sample	5.10 ± 0.90	4.99 ± 0.61	0.531

[a]Values are expressed as mean ± SD.

and lowest for CVM4 stage (pubertal stage), which is suggestive of an early orthodontic treatment for children in CVM3 stage. In both pre-pubertal and pubertal age groups, the CVMI and MP3 stages were more progressed in women compared to men. Close kind of sexual dimorphism concerning the maturational parameters is in accordance with older observations (19, 20). The findings also verify that DHEAS is related to growth in the pubertal growth spurt and might have a great role in skeletal maturation. This agrees with the study conducted by Ghafari et al. (7). The outcomes of the present study revealed that the maturation of the middle phalanx of third finger and cervical vertebrae elevate with increasing age. A significant correlation was observed among MP3 and CVM stages and salivary DHEAS levels in both groups, which indicates that the salivary DHEAS could instead be used for the evaluation of skeletal maturity.

5.1. Conclusions

This study showed that the pubertal group had significantly higher salivary DHEAS than the pre-pubertal group. The highest salivary DHEAS levels were shown in F stage of pre-pubertal MP3 development; also, in H stage of MP3 development in pubertal children. With respect to gender, it was also observed that females had significantly higher salivary DHEAS levels than males in both pre-pubertal and pubertal age groups, indicating the early age of maturational development for females.

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Footnotes

Authors' Contribution: TS did study concept and design, acquisition of data, and manuscript drafting. PS did analysis and interpretation of data and study supervision. NS did study supervision and critical revision of manuscript for intellectual content. CHSR did study supervision and manuscript editing. SOK did statistical analysis, administrative, and technical and material support. SH did drafting of manuscript.

Conflict of Interests: There is no conflict of interest.

Ethical Approval: Ethical clearance for this study was obtained from the Institutional Review Committee (IRC).

Funding/Support: There is no funding or support.

Informed Consent: A written informed assent was obtained from the patients and consent was obtained from their parents upon explanation of the entire procedure.

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