TURKISH JOURNAL of ORTHODONTICS

DOI: 10.5152/TurkJOrthod.2016.0015

ORIGINAL ARTICLE

The Role of Blood Spot Dehydroepiandrosterone Sulfate Levels in Adjunct to Hand Wrist Radiographs as Skeletal Maturity Indicator

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ABSTRACT

Objective: The purpose of this study was to find out whether blood spot Dehydroepiandrosterone Sulfate (DHEAs) levels can be used as Skeletal Maturity Indicators (SMI) by correlating them to hand-wrist maturation stages.

Methods: The cross sectional study population consisted of 107 subjects (62 females and 45 males) 5-25 years old. Hand-wrist radiographs were obtained, and the subjects were divided into 5 groups based on the hand-wrist skeletal maturity stages. A blood spot sample was collected and immunoassayed for DHEAs. Analysis of variance was used to compare the mean DHEAs levels corresponding to the hand-wrist maturation stages. Pearson's correlations were performed to determine the DHEAs trends relating to the various hand-wrist maturation stages.

Results: DHEAs levels increased continuously from the prepubertal stage to the acceleration, high growth velocity/peak, deceleration, and postpubertal stages (r=0.17), but the total increase was not found to be statistically significant (p=0.08).

Conclusion: The blood spot DHEAs level cannot be used as an SMI in individuals. Nevertheless, it can be used in conjunction with hand wrist radiographs to predict and validate the skeletal maturation. The role of DHEAs in the termination of growth and residual growth may be a subject for further research.

Keywords: Dehydroepiandrosterone sulfate, growth, skeletal maturity indicator, hand-wrist radiography, dried blood spot

INTRODUCTION

Assessment of skeletal maturity is one of the most important diagnostic and treatment planning criteria in the field of orthodontics. It is critical to the modality and timing of treatment of various skeletal malocclusions. Clinical alteration of the craniofacial skeletal pattern with orthopedic devices and functional appliances may be best achieved when the rate of growth is highest and pubertal growth is not yet complete. Even though there are many indicators for skeletal maturation, including body height, peak height velocity, tooth mineralization, hand-wrist radiographs, and cervical vertebrae development, hormone measurements are central to all the biological maturity indicators. Many investigators have studied the cellular organization of and the local and systemic factors controlling longitudinal bone growth (1-10). Systemic factors include growth hormone (GH), insulin-like growth factor 1 (IGF-1), thyroid hormones, and sex steroids. An increase in gonadotropin secretion consequent to the maturation of the central nervous system pituitary system plays a critical role in the initiation of puberty (11,12).

The growth spurt that occurs at the time of puberty is due in part to the anabolic effect of androgens. The adrenal cortex secretes significant levels of the androgenic hormone dehydroepiandrosterone (DHEA) and its sulfated derivative dehydroepiandrosterone sulfate (DHEAs). The concentration of DHEAs in the circulation increases until the early twenties and then falls to very low values in old age (13). DHEAs have been found to stimulate the growth and proliferation of epiphyseal cartilage and potentiate the action of growth hormones (13). As DHEAs is found to progressively increase during puberty and to enhance bone deposition because of its androgenic action, it could be related to skeletal maturation.

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Received: 1 July 2016
Accepted: 20 August 2016

Dehydroepiandrosterone levels have been explored to assess growth on the basis of chronological age during orthodontic treatment (14). A recent study using an invasive venipucture technique showed that DHEAs is associated with growth and plays a direct role in skeletal maturation (15). If DHEAs can be used as reliable Skeletal Maturity Indicators (SMI), the use of the hand-wrist method can be done away with, which will reduce the amount of radiation a patient has to be exposed to for diagnostic purposes. Thus, the purpose of this study was to find out whether DHEAs levels can be used as SMI by correlating them to hand-wrist maturation stages utilizing the blood spot technique (which is less invasive than the previously used venipuncture method).

METHODS

The cross sectional study population consisted of 107 subjects (62 females and 45 males) in the age group of 5-25 years visiting the Department of Orthodontics and Dentofacial Orthopedics and Department of Pedodontics, Government Dental College & Research Institute, Bangalore, India. Subjects were selected randomly from patients who had not yet begun orthodontic treatment and who had no systemic illness, growth abnormalities, bleeding disorders or obesity. Patients who regularly smoked or consumed alcohol were also excluded from the study. Ethical clearance for the study was obtained from the ethical committee of the institution. The study procedure was explained to the patients, and written informed consent was obtained from those who voluntarily agreed to participate in this study.

Hand-wrist radiographs were obtained as part of the standard treatment. A 100rnA X-ray machine with 40-45 KV, 6 mAs was used to take the hand-wrist radiographs using 8×10 inches extra oral films (Indu extra oral X-Ray films, India).

Patient positioning for the hand-wrist radiograph:
The film in the cassette was placed on the table with its long axis parallel to the long axis of the hand. Subjects were seated on an adjustable stool with his/her left forearm resting on the table with the hand placed on the table and the palm of the hand downward and fingers straight. The hand was placed on the film to include the lower end of the radius and ulna. The center of the ray was perpendicular to the center of the film. The distance between the hand and the X-ray source was fixed at variable distances, which can be adjusted by the machine, depending on the age of the subject. Radiographs were evaluated in a dark room on a cephalometric table with posterior illumination and traced on acetate tracing paper. Evaluation of the hand-wrist radiographs was done using Fishman's method (16). The subjects were divided into 5 groups based on the hand-wrist skeletal maturity stages.

The hand-wrist radiographs were staged using the 11-stage SMI technique described by Fishman (16). Based on Fishman's mandibular growth-increment data, the 11 stages were regrouped into 5 stages. The first stage, including SMIs 1 through 3, was considered prepubertal; the second stage, which included SMIs 4 and 5, was accelerating toward peak growth; the third stage, SMIs 6 through 8, included the peak and was called high growth velocity; the fourth stage included SMIs 9 and 10 and was decelerating from the peak growth; and the fifth stage, SMI 11, was postpubertal, and we made the assumption that the growth was complete.

Blood samples were collected from these subjects with dried blood spot kits donated by ZRT Laboratory (ZRT Laboratory; Beaverton, USA). After recording the name, date, and time on the card, it was taped to the table edge with its flap open. The subject's middle finger was pricked with a sterile lancet. After wiping away the first drop of blood, the finger was positioned over the circle, and the blood was milked from the palm to the tip of the finger. As the blood drop formed and was ready to fall, it was touched to the center of the circle. This was continued until all the circles were filled. The blood spot card was left open to dry for a minimum of 30 minutes before the flap was closed. The samples were stored in sealed plastic bags in a freezer at −70 degree Celsius for no more than 4 months and sent to ZRT Laboratory where the samples were immunoassayed for DHEAs.

Analysis of variance (ANOVA) was used to compare the mean DHEAs levels corresponding to the hand-wrist maturation stages. Pearson's correlations were performed to determine the DHEAs trends relating to the various hand-wrist maturation stages.

RESULTS

The mean DHEAs levels and the 95% confidence intervals for each skeletal stage are shown in Table 1, which gives the descriptive statistics for each stage and shows the one-way ANOVA for the overall group comparisons. DHEAs levels increased continu-

Table 1. Descriptive DHEAs statistics for each skeletal stage and P value of comparisons (one way ANOVA)

Skeletal Stage	No. of subjects	Mean DHEAs (µg/dL)	Standard Deviation	Minimum	Maximum	F*	p
Prepuberty	21	75.95	38.74	6	147	1.123	0.35
Acceleration	23	80.04	31.74	29	190		
Peak	25	95.20	56.95	19	262		
Deceleration	21	98.90	60.79	28	262		
Postpuberty	17	102.24	47.86	28	262		

DHEA: dehydroepiandrosterone; ANOVA: analysis of variance
*Analysis of variance test value

ously from the prepubertal stage (75.95 µg/dL) to acceleration (80.04 µg/dL), high growth velocity/peak (95.20 µg/dL), deceleration (98.90 µg/dL), and postpubertal (102.24 µg/dL) stages, but the total increase was not statistically significant at $p<0.05$. The Pearson linear correlation from the prepubertal to the postpubertal showed a correlation coefficient (r) of +0.169, which was insignificant (p=0.08). As the overall group comparison was not significant, intergroup comparisons were not done.

DISCUSSION

The current study assessed DHEAs levels using a blood spot technique among a population grouped into 5 stages based on hand-wrist radiographs. The data show that the DHEAS levels were low at the prepubertal hand-wrist stage, and they increased sharply between the acceleration stage and the high growth velocity stage to a mean of 95.20 µg/dL. Wide individual variations in the DHEAs levels were seen at each hand-wrist stage. A mild progressive increase from the high growth velocity stage to the postpubertal stage was also observed.

Previous studies have shown that DHEAs levels show a marked increase just before puberty and increase consistently thereafter until the early twenties (16-21). The current study confirmed this finding, as the DHEAs values did show a marked elevation between the acceleration and high growth velocity stage. Nevertheless, there is a consistent increase in the levels in the subsequent stages that are not statistically significant. Srininvasan and Premkumar (15) reported a significant increase in the DHEAs levels from the prepubertal stage to the postpubertal stage. The current study showed a similar increase in the DHEAs levels from the prepubertal to the postpubertal stage, but this increase was not statistically significant. This could be attributed to the difference in the grouping of the subjects. Our study had five groups with minor skeletal age differences compared to the three groups considered in the previous study (15). This may also be due to the wide variation of the DHEAs values in the individual groups.

The blood spot method was used to collect blood in our study, whereas the venipuncture technique was used by Srininvasan and Premkumar (15). The blood spot method is a less invasive, less cumbersome technique and has a lower biohazard risk. The dried blood spot method was compared with whole blood in relation to the drug concentration-time profile, area under the curve, stability, precision, variability and data accuracy, and the results were similar (22,23). Worthman and Stallings documented that the blood spot DHEAs samples displayed good reliability, specificity, precision, accuracy, and convertibility of results to the plasma/serum equivalent concentrations (Pearson correlation coefficients value 0.99) (24).

Our study showed that the increased levels of DHEAs are statistically insignificant. So, DHEAs levels cannot be use as SMI in individuals, but they can be used in conjunction with hand-wrist radiographs to validate the predictability of skeletal maturation.

The current study did not consider male and female subjects separately as the DHEAs levels were similar in both groups, as reported previously (15). Additionally, there was no significant difference in the numbers of females and males in the current study.

An inherent disadvantage of hand-wrist radiographs is that the final stage of development does not necessarily indicate the completion of growth. Goto et al. (25) and Mitani et al. (26) have shown that mandibular growth continues even after radiographic skeletal maturity. It has been stated that besides being a factor for growth acceleration, androgens also play a role in the termination of growth (13). In a study conducted by Porcu et al. (27) on adolescent females, it was found that DHEAs levels were elevated in subjects with fused epiphyses (towards the end of growth). Our results also show an increase in the DHEAs levels in the postpubertal (towards the end of growth) group, which appears to suggest that DHEAs levels increase even after the completion of skeletal growth. This property of DHEAs can be explored in longitudinal studies to determine two important areas of growth termination, namely: what age do DHEAs levels start falling and can this point be considered as the cessation of growth for an individual; and is DHEAs via its androgenic properties responsible for residual growth?

To our knowledge, there are no pertinent data with regard to the role of DHEAs in residual growth and growth termination. We suggest that further research on the role of DHEAs as a determinant of the cessation of growth should be performed on a population group including subjects up to 35–40 years of age.

CONCLUSION

1. DHEAs levels increased progressively from prepubertal to postpubertal hand-wrist stages independent of the growth status. This increase was statistically insignificant. Thus, DHEAs levels cannot be used as Skeletal Maturity Indicators.
2. DHEA does have a role in the initiation of puberty, but it cannot be used as an SMI on an individual basis.
3. DHEAs levels can be used in conjunction with hand-wrist radiographs to predict and validate the skeletal maturation.
4. The role of DHEAs in the termination of growth and residual growth might be a subject for further research.

Ethics Committee Approval: Ethics committee approval for this study was received from the ethics committee of Government Dental College&Research Institute, Bangalore.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - S.V., A.R.; Design - S.V., A.R., A.S. ; Supervision - S.V.; Resource - A.R.; Materials - A.R., A.S.; Data Collection and/or Processing - S.V., A.R., A.S.; Analysis and/or Interpretation - A.R., A.S.; Literature Search - A.R., A.S.; Writing - S.V., A.R., A.S.; Critical Reviews - S.V., A.R., A.S.

Acknowledgements: The authors express their thanks to Dr. David Zava, Dr. Sanjay Kapur and ZRT Laboratory for providing blood spot kits, to Mr. Jagannatha PS, a Statistician for carrying all required statistics.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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