European Journal of Orthodontics 34 (2012) 447–451
doi:10.1093/ejo/cjr041
Advance Access Publication 20 April 2011

Assessment of serum dehydroepiandrosterone sulphate in subjects during the pre-pubertal, pubertal, and adult stages of skeletal maturation

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SUMMARY The aim of this cross-sectional study was to evaluate serum levels of the hormone, dehydroepiandrosterone sulphate (DHEAS), during the pre-pubertal, pubertal, and adult stages of skeletal maturation based on the methods of Björk and Grave and Brown of assessing hand–wrist radiographs. The levels of the DHEAS of each individual were measured using quantitative enzyme-linked immunosorbent assay and correlated with the corresponding stages in their hand–wrist radiograph. This study was performed on 60 subjects (30 females and 30 males) aged from 7 to 30 years.

Analysis of variance followed by a Tukey honestly significant difference test showed that the serum levels of the DHEAS were statistically significant at ($P < 0.01$) in all three groups. The serum levels were significant ($P < 0.05$) when each of the three groups were individually compared with the other two groups. The mean DHEAS levels were 0.43 ± 0.28, 2.17 ± 0.92, and 4.60 ± 1.34 μg/ml in the pre-pubertal, pubertal, and adult groups, respectively. There was a gradual increase in the hormonal level with progressing skeletal age. The adult group showed the highest DHEAS level and the pre-pubertal group the lowest.

Serum levels of DHEAS showed a constant increase from pre-puberty to adulthood, and at the same level of skeletal maturation, both females and males had similar hormone levels. This finding highlights the fact that the hormone DHEAS plays a significant role and can be a valuable tool in assessing skeletal maturation.

Introduction

Appropriate timing of the interception of a skeletal malocclusion is a key to success in dentofacial orthopaedics. Growth assessment is carried out using various skeletal maturity assessment tools such as hand–wrist and cervical vertebrae radiographs (Bambha and Natta, 1963; Björk and Helm, 1967; Mitani, 1977; Moore *et al.*, 1990). Skeletal maturation accelerates during puberty. There is a strong correlation between craniofacial growth and somatic changes in puberty (skeletal maturation; Nanda, 1955). Puberty is primarily a neuroendocrinal event, with the pituitary and hypothalamus playing major roles in its initiation. Both the pituitary and hypothalamus are together called 'gonadostat'. Puberty is marked by the pulsatile secretion of the gonadotropin-releasing hormone from the hypothalamus, which stimulates the pituitary to secrete gonadotropins (follicle stimulating and luteinizing hormones). Gonadotropins stimulate the gonads to secrete the sex steroids testosterone and oestrogen, which in turn affects somatic changes or skeletal maturation in puberty (Carlson and Ribbens, 1986).

For the gonadostat to initiate its action, it requires stimulation from the adrenal gland, which secretes a significant amount of a steroid, dehydroepiandrosterone (DHEA), and its sulphated conjugate dehydroepiandrosterone sulphate (DHEAS; Auchus and Rainey 2004). They are present in circulation approximately 3 years prior to puberty. This period is termed as adrenarche (Sizonenko and Paunier 1975; Carlson and Ribbens 1986; Remer *et al.*, 2004) and the steroids DHEA and DHEAS are responsible for the stimulation of the gonadostat to take effect. Both DHEA and DHEAS have similar actions. They are found to stimulate growth and proliferation of epiphyseal cartilage and potentiate the action of growth hormone (GH). The phenotypic result of adrenarche is pubarche or the development of secondary sexual characteristics such as axillary and pubic hair that occurs in both girls and boys at approximately 11 years of age. Premature and exaggerated adrenarche can be indicative of future onset of adult diseases, thus increasing clinical relevance.

DHEAS has also been found to enhance bone deposition (Christian *et al.*, 1997; Remer *et al.*, 2003; Bonofiglio *et al.*, 2004; Auchus and Rainey, 2004; Adachi and Takayanagi, 2006), to increase bone mineral density, and to maintain the cancellous and cortical bone mass by way of protective action in osteoblasts (Bing *et al.*, 1988; Bonofiglio *et al.*, 2004; Remer *et al.*, 2004; Adachi and Takayanagi, 2006). Christian *et al.* (1997) stated that DHEA and DHEAS have actions similar to dehydrotestosterone on human osteoblast

cell metabolism. They exert their mitogenic influence on osteoblasts through androgen receptor-mediated mechanisms and stimulate the action of alkaline phosphatase activity through tumour growth factor β expression.

DHEAS is the immediate metabolite of DHEA and the concentration of DHEAS is 100- to 1000-fold greater than DHEA. Unlike DHEA, DHEAS does not show any diurnal variation. Hence, measurement of the serum levels of DHEAS is more reliable and convenient when compared with measurement of DHEA.

As hormones play a primary role in the initiation of puberty, it would be more appropriate to measure their serum levels (the cause) rather than study the effects of hormones or puberty. This study aimed to determine the relationship between serum levels of DHEAS and skeletal maturation assessed using hand–wrist radiographs.

Subjects and methods

Sixty individuals (30 males, 30 females) between 7 and 30 years of age (mean 22.95 years) participated in the cross-sectional study. All individuals had a Class I occlusion. Subjects with any systemic disease or under any medication were excluded from the study. Informed consent was obtained from the selected individuals and/or their parents. The study was approved by the institutional ethical committee of Madras Medical College, Chennai.

The individuals were divided into three groups: 20 pre-pubertal (Figure 1A), pubertal (Figure 1B), and adult (Figure 1C) based on the skeletal maturation stage assessed using the method of Björk (1972) and Grave and Brown (1976). Each group contained 10 males and 10 females. Hand–wrist radiographs were taken with 60 mA X-ray machine with 40 kV and 12–16 mAs speed using extra-oral radiographic film (Kodak T-mat Blue; Kodak Limited, Hemel Hempstead, Hertfordshire, UK). The radiographs were taken by placing the cassette with its long axis parallel to the long axis of the hand. The subjects were seated on an adjustable stool with their left forearm resting on the table and the palm of the hand facing downward with the lower end of the radius and ulna also included on the film. The radiographs were evaluated in a dark room on a cephalometric table with posterior illumination and traced on an acetate paper by the same author (BS). Evaluation of hand–wrist radiographs was carried out using the method of Björk (1972) and Grave and Brown (1976).

Estimation of the concentration of serum DHEAS was undertaken using quantitative enzyme-linked immunoassay (ELISA kit, Dia Metra, Milano, CE Italy). Approximately 2.5 ml of venous blood was collected from each patient and centrifuged for serum separation. The samples were stored in Ependorf tubes at −20°C.

The standard curve (Figure 2) for hormone concentration was plotted in the *X*-axis and absorbance on the Y-axis using the set of six standards provided in the ELISA kit, by a semi-automated ELISA reader. When the absorbance values of the samples had been obtained, the corresponding DHEAS concentrations were calculated from the standard curve on the *X*-axis. The measured serum DHEAS values for each group were calculated. Analysis of variance was used to compare the mean hormone value of the three groups followed by a Tukey honestly significant difference (HSD) test to assess the level of significance for multilevel comparison. A Student's *t*-test was used to compare the difference between the males and females in each group.

Results

The distribution of the hormone levels in the pre-pubertal, pubertal, and adult groups is shown in Figures 3A, 3B, and 3C. The mean DHEAS values in each group were 0.43 (pre-pubertal), 2.17 (pubertal), and 4.60 μg/ml (adult). The standard deviation for each of the groups was 0.28, 0.92, and 1.34, respectively. There was a significant difference ($P < 0.001$) in the means of hormone levels between the groups (Table 1).

Tukey HSD test showed that each group was significantly different from the other two groups (Table 1). The 95 per cent confident interval for mean for the hormone levels in

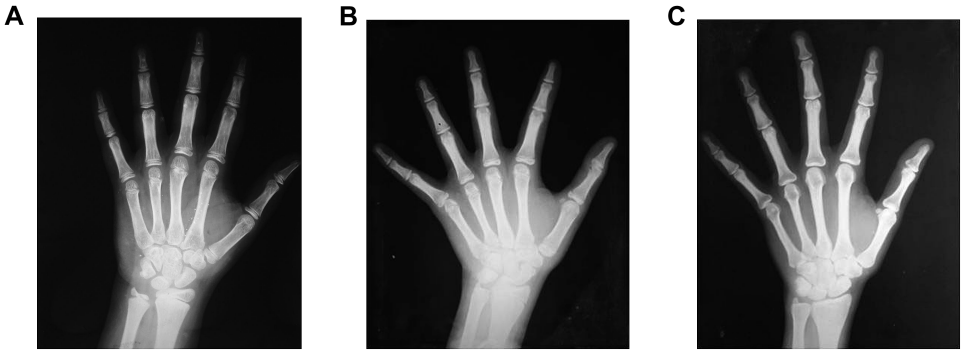

Figure 1 Hand–wrist radiograph of a pre-pubertal (A), pubertal (B), and adult (C) subject based on the methods of assessment of Björk and Grave and Brown.

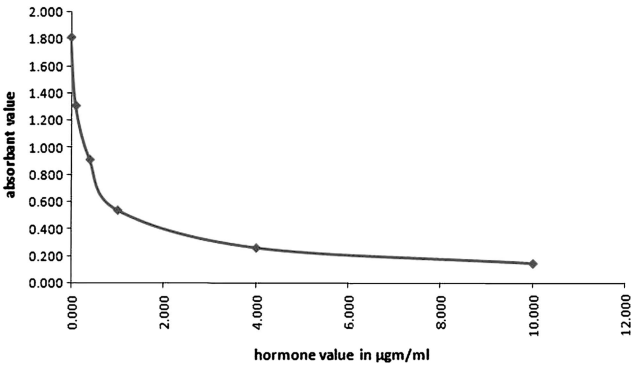

Figure 2 Standard curve obtained for the hormone, dehydroepiandrosterone sulphate, using enzyme-linked immunosorbent assay. The hormone levels are marked on *X*-axis and the absorbant values in Y-axis.

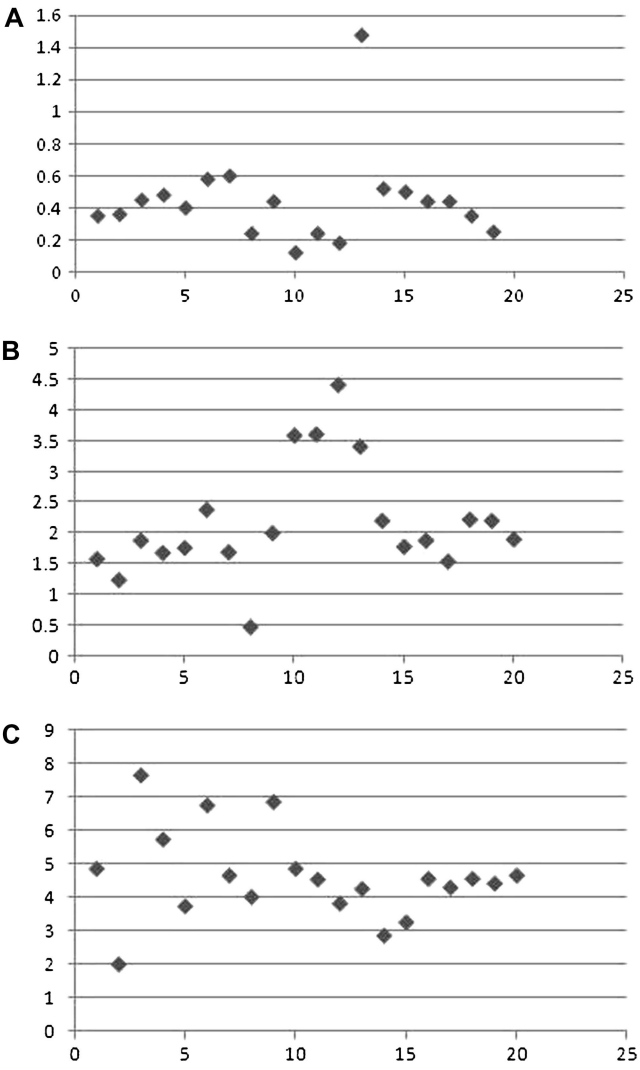

Figure 3 Scatter diagrams depicting the distribution of dehydroepiandrosterone sulphate values in (A) the pre-pubertal, (B) pubertal, and (C) adult groups.

each group were pre-pubertal: 0.2993–0.5627 µg/ml; pubertal: 1.7388–2.60002 µg/ml and adult: 3.9732–5.2268 µg/ml.

Table 1 Analysis of variance to test the significance in serum dehydroepiandrosterone sulphate among the pre-pubertal, pubertal, and adult groups.

Groups	Hormone value		*P* value
	Mean	SD	
Pre-pubertal	0.43*	0.28	
Pubertal	2.17*	0.92	**
Adult	4.60*	1.34	

*Tukey honestly significant difference indicates significant at the 5 per cent level when an individual group was compared with the other two groups.
**$P < 0.001$.

Table 2 Comparison of the hormone dehydroepiandrosterone sulphate levels between male and female subjects in the pre-pubertal, pubertal, and adult groups.

Groups	Gender	Mean ± SD	*P* value
Pre-pubertal	Male	0.48 ± 0.38	0.489
	Female	0.39 ± 0.14	
Pubertal	Male	2.17 ± 1.21	0.998
	Female	2.17 ± 0.56	
Adult	Male	4.93 ± 1.81	0.276
	Female	4.27 ± 0.53	

The mean DHEAS was pre-pubertal group: male 0.48 ± 0.38 µg/ml; female 0.39 ± 0.14 µg/ml, pubertal group: male 2.17 ± 1.21 µg/ml; female 2.17 ± 0.56 µg/ml, and adult group: male 4.93 ± 1.81 µg/ml; female 4.27 ± 0.53 µg/ml. There was no significant difference in hormone levels between males and females in any group at a particular stage of skeletal maturation at the 5 per cent level (Table 2).

Discussion

Time, the fourth dimension in orthodontics, is important when planning growth modification therapy. Successful growth modification is related to the amount of remaining growth and accurate prediction of growth spurts. The adolescent growth spurt is the most important for orthodontic treatment because the physical changes at adolescence significantly affect the face and dentition (Nanda, 1955; Hunter, 1966).

Earlier methods of assessing growth included physical stature, peak height velocity, and growth charts. All these methods assessed growth in relation to chronological age. These were later followed by using skeletal maturation as an indicator of physical development and maturation, which led to the radiographic method of determining skeletal maturation (Ranke, 1896; Todd, 1930). Greulich and Pyle (1959) compiled a radiographic atlas of skeletal development of the hand and wrist, which were developed on the basis of skeletal age as opposed to chronological age (Carlson and Ribbens, 1986).

Timing of tooth eruption was related to skeletal maturation by Engstrom *et al.* (1983) who reported a correlation between lower third molar development, skeletal maturation, and chronological age. Nanda (1960), Lewis (1991), and Demirjian *et al.* (1985) found less association between the time of tooth eruption and skeletal maturation. Conversely, Coutinho *et al.* (1993) stated that canine calcification can be used as a tool for assessing skeletal maturation, in agreement with the findings of Chertkow and Fatti (1979).

Hellman (1927), Björk and Helm (1967), Houston *et al.* (1979), Singer (1980), Fishman (1982), Hägg and Taranger (1982), Leite and O'Rielly (1987), Pileski *et al.* (1973), and Abdel-Khader (1998) associated the ossification events of the hand–wrist bones with the pubertal growth spurt. Although other bones such as the carpals, the femur, the elbow joint, the shoulder joint, and the skull can be used to assess skeletal maturation, the hand–wrist proved to be a more effective method because of numerous centres of ossification which undergo changes at different times and rates.

Cervical vertebrae are also used for assessment of skeletal maturation (Lamparski, 1975; Hassel and Farman, 1995; Fernandez *et al.*, 1998; Franchi *et al.*, 2000; Baccetti *et al.*, 2000). The advantages of the cervical vertebrae include no additional radiation as they can be visualized on a lateral cephalometric radiograph. The disadvantages include difficulties in visualization of the subtle changes in the vertebrae, in visualization due to incorrect neck posture while taking the radiograph, and blocking out of cervical vertebrae due to the use of a thyroid collar (Leite and O'Rielly, 1987). The frontal sinus has also been used as an indicator of the pubertal growth spurt (Rossouw *et al.*, 1991; Ruf and Pancherz, 1996).

Puberty is under the control of the neuroendocrine system. Pubertal maturation is initiated by the central nervous system with increased secretion of gonadotropin-releasing hormone, gonadotropins (follicle-stimulating hormone and luteinizing hormones), sex steroids, GH, and somatomedin C. The neuroendocrine role in the onset of puberty starts with maturation of the hypothalamus pituitary complex, which is heralded by a significant increase in the secretion of DHEA and its conjugate DHEAS (Auchus and Rainey 2004). Serum levels of DHEAS are high in the neonate, after which there is a decrease, then a rapid increase in the serum level from 7 years of age in females and 8 years of age in males, with a gradual increase until it attains the adult value (Hopper and Yen, 1975; Sulcova *et al.*, 1984). The first peak in DHEAS concentration occurs between 6 and 8 years of age in both males and females and the second peak occurs at 11 years in females and 13 years in males (Sizonenko and Paunier, 1975; Rotteveel *et al.*, 1997; Kulik-Rechberger *et al.*, 2000).

Only one study has been conducted on the levels of DHEAS with regard to monitoring growth during orthodontic treatment (Ghafari *et al.*, 1995). The results of the present study clearly show that serum concentration of DHEAS for the pre-pubertal, pubertal, and adult groups was statistically significant. There was a progressive rise in serum concentration as skeletal maturation progressed, almost reaching a maximum value after the complete fusion of the epiphysis and diaphysis of the radius. The results also showed that low levels of DHEAS were present in the pre-pubertal group with increased values in the pubertal group and the highest levels in the adult group, showing a gradual increase as maturation progresses. Within each group, there was no significant difference in the mean hormone values between males and females, demonstrating that there was no gender difference in the hormone values at a particular stage of skeletal maturation. This finding is in contrast to the results of Ghafari *et al.* (1995) who found a significant gender difference in DHEAS level of the same age. The difference in DHEAS levels between males and females apparently reflects gender differences related to the earlier maturation of girls than boys. The difference in the findings could be due to the fact that in the present study, DHEAS levels were compared based on skeletal maturation and not on chronological age. Another finding of note was the individual variation in the DHEAS levels within the groups. In addition, some individuals in the pubertal group showed higher levels of DHEAS than those in the adult group. This could be attributed to a higher body mass index (l'Allemand *et al.*, 2002), which was not measured in the present study.

The evaluation of hormones can be repeated without the disadvantages of radiation exposure and can be charted to assess and predict skeletal maturation. The disadvantage of this procedure is the invasive venipuncture. However, non-invasive procedures such as evaluation of salivary DHEAS has proved to be unreliable (Read, 1993).

DHEA and DHEAS are considered as the markers of adrenarche. Their influence on bone growth and correlation with the stages of hand–wrist radiographs make them useful as a possible indicator of skeletal maturation to aid the assessment of growth status during adolescence.

Conclusions

The present study showed that serum levels of DHEAS in the pre-pubertal, pubertal, and adult groups were statistically significant. The findings also confirm that DHEAS is associated with growth during the pubertal growth spurt and possibly plays a direct role in skeletal maturation.

Acknowledgements

The authors acknowledge the help of Dr K S G A Nasser, and Professors W S Manjula and M C Sainath, from Tamil Nadu Government Dental College and Hospital, Chennai in completing the study.

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