# Reliability of Serum Dehydroepiandrosterone Sulphate (DHEAs) as an Indicator of Skeletal Maturation – A Comparative Study

*A Dissertation Submitted*

*in partial fulfillment of the requirements*

*for the degree of*

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**BRANCH – V**

**ORTHODONTICS**


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# Certificate

This is to certify that **Dr.S.Bhadrinath**, Post Graduate student (2005 – 2008) in the Department of Orthodontics, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003, has done this dissertation titled **"Reliability of Serum Dehydroepiandrosterone sulphate as an Indicator of Skeletal Maturation- a Comparative Study "** under our direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr.M.G.R. Medical University, Chennai - 600032** for **M.D.S.** (Branch–V) **Orthodontics** (Part II) degree examination.

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# *Introduction*

# ***Introduction***

Dentofacial orthopedics has been an integral part of orthodontic practice for more than a century. Growth modification, an important orthodontic treatment procedure, encompasses the use of functional and/or orthopedic appliances. Timing is the most important factor that determines the outcome of growth modification treatment. Treatment at the appropriate time will yield best results. The use of these appliances at the correct time can modify skeletal growth. By beginning growth modification at patient's optimal maturational stage, the most favorable response can be anticipated.

The issue of optimal timing for dentofacial orthopedics is linked intimately to the identification of periods of accelerated growth [spurts] that can contribute significantly to the correction of skeletal imbalances in patients. There is a strong correlation between craniofacial growth and the somatic changes in puberty. A thorough clinician has to look beyond the chronological age to identify growth spurts. Chronological age is not a reliable indicator for assessment of skeletal maturation. In fact, the biological indicators of skeletal maturity refer mainly to the somatic changes in puberty.

Puberty is a period of development during which mammals typically acquire their reproductive capability. Somatic structural alterations of body generally occur in synchrony with the physiological changes associated with μpuberty and thus they can be measured clinically when one wants to monitor the sexual maturation of the individual.

Clinical orthodontic considerations associated with pubertal onset generally is concerned with somatic changes in skeletal system; this is particularly true for oro- facial musculoskeletal frame work. Nanda[19] (1986) demonstrated that the development of musculoskeletal system has comparable growth rate parameters to that of the somatic skeletal growth and that acceleration and deceleration in mandibular and maxillary growth rates can be correlated nearly with somatic skeletal growth.

Clinical alteration of craniofacial skeletal pattern with orthopedic devices and functional appliances may be best achieved when the rate of growth of the face is highest and prior to completion of pubertal growth rate acceleration. Even though there are many indicators for skeletal maturation which include body height, peak height velocity, tooth mineralization, hand wrist radiographs and cervical vertebrae but hormones adorn the center stage of all the biological maturity indicators.

Pubertal maturation is initiated and sustained by developments in central nervous system, that results in increased secretion of gonadotropin releasing hormone (GnRH), gonadotropins ( FSH and LH) , sex steroids, growth hormone (GH) and stomatomedin (Smc)(Gruber 1976)[19]

During early infancy, plasma concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) are greater than during childhood due to CNS restraint mechanisms (Reiter and Grumbach1982[19], Kletch et al 1983[19]). The onset of pubertal growth acceleration is marked by pulsatile secretion of GnRh by hypothalamus particularly during sleep (Jackacki et al 1982[19]). This secretion occurs after the maturation of hypothalamus pituitary complex called as the gonadostat which is related to blood levels of adrenal sex steroids. The adrenal cortex secretes significant levels of androgenic hormones dehydroepiandrosterone (DHEA) and its sulfated derivative dehydroepiandrosterone sulfate (DHEAs). These androgens appear to be transformed into estrogen in peripheral fatty tissues and stimulate the gonadostat (Parker and Mehesh 1977[19]). This phenomenon is termed as ***adrenarche.*** It occurs two years before the pubertal growth spurt.

DHEA serves as a precursor of testosterone and estrogen synthesis. Serum DHEA levels are relatively high in fetus and neonate, low during

childhood and increases during puberty. Serum DHEA levels progressively decline after the third decade of life.

DHEAS is the sulfated derivative of DHEA and it is bound to albumin. DHEAs is significantly involved in the androgenesis. Blood levels of DHEAs during various periods of life time parallel that of DHEA and it is 100-1000 times that of DHEA. DHEAs is found to stimulate the growth and proliferation of epiphyseal cartilage and potentiate the action of growth hormone. DHEAs is also found to enhance bone deposition.

As dehydroepiandrosterone is found to progressively increase during puberty and enhance bone deposition due to its androgenic action, it could be an indicator for assessing skeletal maturation. Hence in this study serum dehydrepiandrosterone sulphate levels of normal individuals was measured and compared with their respective stages of skeletal maturation, which is assessed by hand wrist radiographs to find out the relationship between serum levels of the hormone and the level of skeletal maturity.

# Aims & Objectives

## ***Aims and objectives***

### ***Aim:***

To find out whether the hormone dehydroepiandrosterone sulphate can be used as an indicator of skeletal maturation.

### ***Objectives:***

1. To measure the serum levels of dehydroepiandrosterone suphate in individuals categorized as prepubertal, pubertal and adult based on skeletal maturation assessed by of hand-wrist radiographs using Bjork (1972), Grave and Brown method (1976), in Chennai population.
2. To compare the levels of DHEAs of the individuals with their respective stages of skeletal maturation.
3. To compare the value of DHEAs of males and females in each of the three groups.

# *Review of Literature*

## ***Chronological age***

***Bjork A and Helm S*** **(1967)**[11] from the longitudinal study on Danish children and that appearance of adductor sessamoid is a preferable indicator as compared to chronological age or dental development.

***Fishman L.S***. **(1979)**[24] in his longitudinal study on sixty boy and 68 girls investigated the correlation between chronological age and skeletal age. He stated that no matter how simple or complex the method of growth prediction the establishment of skeletal not chronological age would lend itself to more accurate thereby clinically beneficial results and thereby concluding that skeletal age is more reliable.

***Fishman L.S.***[25] in his later (1982) longitudinal and cross sectional study on more than one thousand four hundred children established a system of scoring for skeletal malocclusion assessment. He concluded that (SMA) provide a more valid basis than chronological age for grouping of individuals.

***Smith R.J.***[84] (1980) in a review article stated that chronological age is a poor indicator of the stage of adolescent development and accurate measure will be biological age.

***Hagg U. and Taranger J.*** **(1982)**[36] in a longitudinal perspecetive interdisciplinary study of growth and development of two hundred and twelve Swedish children from birth to adulthood, reported that because of wide individual variation in timing of pubertal growth spurt, chronological age cannot be used for evaluation of pubertal growth.

***Fishman L. S. (1987)***[26] conducted cross sectional study on a sample of 4000 data records including 2225 hand wrist radiographs of females and 1775 males. He concluded that healthy children of any age do not demonstrate any chronological specificity regarding particular stage of maturation. Skeletal maturational indicator provides a more reliable mean of evaluating individual maturational levels.

Contrary to the above reports ***Engstrom C. and Engstrom H. and Sagne. S*****(1983)**[23] who investigated the development of the lower third molar angle in relation to chronological age and skeletal age among two hundred and twenty one individuals reported a correlation between lower third molar development and skeletal maturation and chronological age. Their finding was in agreement with earlier investigations of Demish and Watsman(1956) Lanstersteen (1961), Tanner (1971) and Taranger (1976).

## ***Dental age***

***Bambha J.K and Natta P.V.*** **(1959)**[5] in a longitudinal study on sixty children, (28 boys and 32 girls) followed at the child research council concluded that there is no association between time of tooth eruption and skeletal maturation.

***Nanda R.S.*** **(1960)**[63] analyzed the longitudinal records of dentition at the child research council at Denver during the past 25 years. The data of 34 children of each sex is studied with the objective to determine the correlation between dental and physical maturation. His findings suggested no correlation between timing of dental maturation and puberty. Referring to his previous studies in 1955 and 1956 found that the pattern of the human face and body height were found to be similar and closely related, though the level of correlation between the dentition and face is low.

***Bjork and Helm. S.*** **(1967)**[11] conducted a longitudinal study in facial growth among Danish children involving 32 boys and twenty boys. The following data were recorded

I. Maximum pubertal growth and body height(H)
II. Ossification of ulnar sesamoid (US).

III. Menarche.

IV. Dental stages DS4: all canines and premolars fully erupted.

DSM2: all second molars fully erupted.

These two well defined stages in dental maturation were from that introduced by ***Bjork, Krebs and Solow*** (1964). Ages at maximum pubertal growth, ossification of sesamoid, and menarche showed ranges of variation of 3-4 years while those for the two dental ages were nearly twice for 5-6 years. The results showed that in most of the girls menarche occurred on an average 17±2.5 months after the peak height velocity. Thus they concluded that dental development was less strongly associated with chronological age than the growth maximum, ossification and menarche and dental development based on tooth eruption was of little value as criteria for puberty.

***Demirjian, Goldstein and Tanner*** **(1973)**[20] describe a new method for estimating dental maturity or dental age by reference to radiological appearance of seven teeth on left quadrant of mandible. Each tooth is given a point value according to the stage of development, rather than the change in size. Nine stages O, A to H were defined for each tooth mineralization stage. The sum of the individual points on all teeth gave the dental maturity directly.

***Gupta D.S and Chawla T.N.*** **(1973)**[35] examined 522 school going 268 males and 254 females Lucknow, UP India to find out the average shape and size of the wrist bones at various stages of dental development. Six groups were formulated on the basis of presence of dentition. Milli-square graph paper was used for recording areas of individual carpal bone. The shape of each bone was determined by the method of superimposition. They concluded that size of carpal bone increase with the eruption of teeth from all deciduous dentition to the level of eruption of permanent second molars. The average shape of the various wrist bones at different stages of dental development was also represented. The increase in size of the carpal bones show a homogenous enlargement which gets slightly accentuated close to puberty i.e. around dentition group IV in males and group III in females. This represents a prepubertal growth spurt which represents a general body growth.

***Cehrtkow .S and Fatti .P.*** **(1979)**[15] investigated the relationships between the stages of mineralization of different teeth the early radiographic evidence of calcification of ulnar sesamoid of the first metacarpophalalngeal joint among South African Caucasoid boys and girls. One hundred and forty individuals, ninety three girls and forty seven boys were studied that calcification if adductor sesamoid was closely related to the root

mineralization of mandibular canine prior to apical closure. No significant sex difference was noted in state of maturation. He reported that completion of root formation of mandibular canine prior to apical closure could be used as a maturity indicator for circumpubertal growth spurt with a similar degree of confidence as calcification of adductor sesamoid of thumb.

Subsequently in **1980** ***Chertkow.S***[16] conducted a similar study in a mixed sample of 197 black and white South African boys and girls. Results showed that completion of root formation of mandibular canine prior to apical closure was closely related to other maturational indicators namely degree of calcification of hook of hamate, calcification of adductor sesamoid of thumb and state of development of the epiphysis of the middle phalanx of the third finger among white children. However these relations could not be verified in black children as the apical closure has already occurred. Marked racial variation was noticed on tooth mineralization, state of tooth maturity and other parameters of development between black and white children. Tooth development was accelerated in black. He confirmed his earlier finding that completion of root formation of mandibular canine prior to apical closure may be used as a maturity indicator of pubertal growth among children of Caucasoid origin and caution should be exercised in the application of this finding to other racial group.

***Hagg and Taranger* (1985)**[37] investigated the pubertal growth spurt dental and skeletal and pubertal development in perspective longitudinal study of 212 randomly selected Swedish children. Tooth eruptions were recorded annually by direct inspection. A tooth was considered to be erupted if any part of the crown was visible in the oral cavity. Dental development was measured by dental eruption stages (DES). The association between the attainment of dental emergence states and pubertal growth events was statistically was statistically significant only in girls which was however weak. It was concluded that dental development was not useful as an indicator of pubertal growth spurt.

***Egstrom. C, Egstrom H. and Sagne .S.***(1983) did a statistical evaluation to find a positive correlation between third molar development and skeletal maturation. Five developmental stages of lower third molar were determined from orthopantomograph. Strong correlations were found between lower third molar development, skeletal maturation and chronological age.

***Hagg U. and Taranger* J.(1985)**[36] did a longitudinal study on Swedish children from birth to 18 years to test the validity of dental age based on tooth counts of the deciduous and permanent tooth. They determined the age of deciduous teeth and first 29 permanent teeth and established tooth emergence curve. It was reported that there was a wide variation in dental

development in relation to chronological age. At about 8.5 year chronological age in girls the dental age ranged from 7.1 year to 10.7 year.

***Demirjian A, et al*** **(1985)**[21]evaluated the inter relationships among the following five measures of physiological maturity for 50 French Canadian girls.

1) Menarche
2) Peak height velocity
3) 75% of skeletal maturity
4) appearance of adductor sesamoid
5) 90% of dental maturity.

They concluded that the age at which girls attend 90% of dental maturity is not significantly related to other maturity indicators. The mechanism controlling dental development is independent of somatic and or sexual maturity.

***Sierra*** **(1987)**[79] conducted study on 153 orthodontically treated Caucasian children ranging in age from 8-12 years to correlate the developmental stages by specific ossific centers by assessing hand wrist radiographs by Greulich- Pyle method and the calcification of upper and lower cuspids, bicuspids and second molars in the permanent dentition by the method given by Nolla (1980). In addition determination of overall skeletal age of hand

wrist was made by conventional Todd Inspection Method of skeletal age assessment. Results showed a strong correlation between skeletal maturation, highest correlation for the lower cuspids. It was determined that radiographic determination of skeletal maturation is of lesser clinical importance in children who falls with in normal developmental age particularly if the clinician can determine the calicification status of a teeth from a clear radiograph.

***Lewis A.B*** **(1991)**[56] conducted a study on 694 children (320 boys and 374 girls) seen in private orthodontic practice to analyze association between dental and skeletal maturity. Dental age was obtained by comparison with Bolton standards. Skeletal age was assessed using Greulich Pyle atlas. Results showed differences in dental and skeletal age as large as 36 months. The difference was less than 6 months in fewer 40% of the children. Only moderate associations were seen between these two measures.

***Mappes M. S. et al*** **(1992)**[57] did a comparative study in two groups of adolescent orthodontic patients one from the mid west and another from the mid south (USA) to confirm the clinical impression that permanent teeth of Southern children form and erupt and later significantly later stages. To compare the dental with that of skeletal maturation. Bone age was also assessed according to the method given by Fishman (1982). Results showed

that the mid south series achieved mineralization 1½ years after the average suggesting that regional differences are greater than previously suspected. Clinical consequences resolve on the use of conventional norm for tooth formation and predictive models of facial growth. In contrast analysis of rate of development of hand wrist (bone age) of the same subjects disclosed no difference. Thus comparing the individual development of dental and the osseous system.

***Rodney K et al*** **(1993)**[75] evaluated the tooth formation in children with idiopathic short stature before and after recombinant growth hormone (rHGH). Twenty nine short statured children ages 6-13 years were assigned in to two treatment groups. An experimental group n=18 which received rHGH and a control group which n=11 which was observed one year before commencing the rHGH. The tooth formation was initially delayed although the reduction in stature exceeded the initial delay in tooth formation. During the 2-year study rHGH therapy had a significant influence on the acceleration or gain in stature but did not have a significant influence on tooth formation. In conclusion during the 2- year study the recombinant growth hormone had a statistically significant influence on the increase in stature but not on tooth formation.

***Coutinho S, Buschang P.H, and Miranda F.*** **(1993)**[18] conducted a study to verify the relationship between the canine calcification and skeletal maturity. Hand wrist radiographs and dental panoramic radiographs of 200 boys and 215 girls were assessed. Most children having attained the canine stage G showed the presence of adductor sesamoid (81%) capping of the diaphysis of the third middle phalanx (77%) and capping of the fifth proximal phalanx (87%) suggested that growth reference data suggested that stage G occurred approximately 0.4 years and 1.3 years before peak height velocity for girls and boys respectively. It was concluded that canine calcification can serve as a useful tooth for evaluating children's skeletal maturation and by association skeletal maturity.

***Nykanen R. et al*** **(1998)**[65]studied dental age in 261 Norwegian children by using the maturation standards given by Demirjian and Goldstein (1976) to examine the applicability of these standards as a reference for overall dental maturity of Norwegian population. The sample comprised 128 boys and 133 girls included in Oslo growth material. The applied standards appeared to be adequate for studying dental age in groups of children from Norwegian population. However the estimation of chronological age in individual should be supplemented by other indicators of biological maturity.

***Krekmanova L, et al*** **(1999)**[49] investigated the 2- year follow up of longitudinal study examining the influence of the growth hormone on dental maturity in healthy short stature children. The children were divided into growth hormone deficient group and growth hormone non deficient group and the comparisons are made with healthy controls. The dental age of the growth hormone non substituted children was 10.2±2.6 years compared to their controls. Growth hormone substituted group show acceleration in dental maturation in contrast to controls, whereas in non substituted children the acceleration was less pronounced. In conclusion the growth hormone deficient children after substitution showed acceleration in dental maturity and thus dental age.

## ***Hand wrist radiographs***

***Hellman (1928)***[39] used the total length of the digits and the width and length of phalanges to supplement his inspection roentgenograms of skeletal maturation.

In the same year Howard produced one of the earliest atlases of skeletal maturation and described the importance of unusual rates of skeletal maturation in orthodontic practice.

***Flory (1936)***[19] published an atlas for male and female patient's showing the sequence of ossification of different bones of hand and wrist.

***Todd T.W. in (1931)***[12] (as stated by ***Bogdon G.J. (1974***)[12] started a longitudinal study by taking a series of periodic hand and wrist radiographs of growing children in Cleveland , Ohio USA. Unfortunately Professor Todd died in 1938 after publishing the initial data of his study in 1937. After his death the study was continued and ***William Greulich & Idell Pyle***[12, 19] compiled the Radiographic atlas of skeletal development of hand and wrist, which was published in 1950 and revised in 1959. The atlas contains standards, which were developed on the basis of skeletal age as opposed to chronological age. By taking a hand and wrist radiograph of an individual

and comparing it to the standards on Greulich and Pyle atlas, one is able to determine the skeletal age of that individual. If the skeletal age is accurately known, one can then predict whether or not a potential for further growth exists for that individual.

***Nanda R.S. (1955)***[62] in a longitudinal study of several facial dimensions, formulated distance and velocity curves to illustrate the changes he observed. He found a general circumpubertal increase in growth velocity through the timing of both the onset and the peak rates of growth were different for various dimensions of the same child. He stated that facial growth precedes general body height by approximately nine months during the pubertal growth spurt period.

***Bambha J.K. and Nanda P.V. (1963)***[6] studied the skeletal maturation and adolescent growth of the face in twenty-two boys and twenty eight girls born in Denver. Their study showed an association between the skeletal maturation and the facial growth at the two extremes. The individual who tend to mature later have greater facial growth, thus supporting Nanda's study. He concluded that it was possible to predict the time of onset of adolescent growth spurt in face from that of acceleration of growth in body height. Growth spurt in body height precedes that of face.

***Hunter (1966)***[43] challenged the findings of both Nanda and Bambha. He concluded that maximum facial growth was coincident with maximum growth in height in the majority of subjects in his study. They stated that the measurement articulare to pogonion in mandible exhibited the most consistent relationship with growth in height through out adolescence.

***Bjork A., Helm.S. (1967)***[11] in longitudinal study on Danish children report that, in the hand, at puberty, the only centre which ossify consistently is the metacarpopharyngeal sesamoid of the thumb. The sesamoid at this joint is said to appear in nearly all persons. The sesamoid was ossified on an average 12±2.1 months before maximum pubertal growth for girls and 9±1.4 months before the boys. A close association between the age at maximum growth in body height and age at ossification of ulnar metacarpophalangeal sesamoid occurred and it ossified one year before the maximum pubertal growth. Onset of ossification of the sesamoid therefore indicated the maximum pubertal skeletal growth was imminent or has been reached. They stressed the importance of ossification of ulnar sesamoid as an indicator maturity marker.

***Chapman S.M (1972)***[14] conducted short longitudinal and cross sectional study to relate the ossification status of metacarpophalangeal joint of the thumb with accelerated increase in statural height of adolescent males and

females. He suggested using standard size dental film to assess the developmental status of the 1st metacarpophalangeal joint. He concluded that onset of ossification of the sesamoid take place at the time of adolescent spurt in statural height begins. Commencement of epiphyseal- diaphyseal fusion of proximal phalanx is found to mark completion of maturational event.

***Bergersen E.O. (1972)***[7] investigated to relate skeletal maturity as estimated by hand-wrist radiographs to the facial adolescent pubertal growth spurt and standing height. The sample consists of semiannual hand film and standing height data and yearly lateral cephalometric radiographs on 23 males from birth to maturity. Seven linear facial dimensions were studied. They concluded that a significant correlation exists between the onset of male adolescent spurt represented by total face height, the Y-axis, mandibular length and standing height. And metacarpal sesamoid is also significantly correlated with onset of the male adolescent growth spurt in the face and in standing height.

***Pileski R.C.A, et al (1973)***[68] investigated whether the presence or absence of sesamoid bone could provide clinically useful information concerning the onset of peak velocity in mandibular growth at adolescence. The study was done on 108 females and 91males from the serial experimental group of

Burlington Orthodontic Research center. Results showed that mean appearance of sesamoid bone precede mean maximum mandibular velocity by 0.72years in males and 1.09 years in females. However, the peak mandibular velocity occurred before appearance of sesamoid bone in 25-37% of males and 19.5% of females. This finding was quite contrary to that of Bjork and Helm (1967) who found that sesamoid bone never appeared after peak growth in body height. Pileski et al concluded that there is a lack of sufficient correlation to enable any form of prediction to be made, concerning peak mandibular velocity from the 1st appearance of sesamoid.

***Sarcar S, et al (1974)***[78] investigated 304 school going children ranging 6½ years to age levels of boys and girls. They concluded the sequence of appearance of carpal bones was different in both sexes, all the carpal bones appeared significantly earlier in girls than in boys and there was no significant difference between the right and left hand of the same individual. The sequence of appearance of different carpal bones among boys were capitate, hamate, triquetral, lunate, scaphoid trapezium, trapezoid and scaphoid capitate were present before the age of 3 years in all cases on both sexes.

***Grave K.C. and Brown T (1976)***[32] conducted a study on 88 aboriginal children (52 boys and 26 girls) selected from a longitudinal growth study

that has been in progress since 1961 to provide more extensive series of ossification taking place in the hand and wrist skeleton around time of puberty. Fourteen ossification events in the hand and wrist were studied which were divided into 3 stages the acceleration phase, peak growth velocity and deceleration. Possibility of use of these indicators was also discussed.

***Grave K.C. and Brown T. (1979)***[33] published four case reports to further emphasize the importance of carpal radiographs as diagnostic aids and to predict growth potential around puberty.

***Houston W.J.B, Miller J.C. & Tanner J.M. (1979)***[42] in a mixed longitudinal study of 64 boys and 49 girls from Harpenden growth studying the age group of 8-16 years correlated certain osseous events with peak velocity to predict timing of adolescent growth spurt. They differentiated between "bone age" and ossification event. According to them bone age described by earlier workers Bjork and Helm (1967), Bowden (1971), Helm (1971), Pileski (1973), Grave and Brown (1976) were based on appearance of bone on standardized radiographs annually. However, an ossification event, which is the change over from one bone stage to the next requires serial radiographs taken 6 monthly intervals during puberty and annually of all the carpals, metacarpals, phalanges,

radius, and ulnar developing within few years of puberty were rated according to skeletal maturity criteria (Tanner et al). They observed that the reliability of prediction using the osseous event as indicated by confidence limits is still so low that ossification events are of limited value.

***Singer J (1980)***[81] published a paper to help clinician to examine certain stages of growth for rapid reliable use of hand wrist film in orthodontic practice to determine the maturation status of patients. Six stages of hand-wrist development were described in the prediction of adolescent growth spurt.

***Smith R.J. (1980)***[84] questioned the diagnostic value of hand –wrist radiograph among females. He concluded that use of hand wrist film may be routinely indicated for adolescent males the available literature does not justify this radiographic exposure in female patients lacking obvious developmental pathosis.

***Bishara S.E., et al (1981)***[10] conducted a study on 20 boys and fifteen girls 5-17 years to examine the changes in mandibular dimension and relationship as they relate to standing height, which is one indicator of skeletal maturation. They conducted that timing of mandibular changes in size and relationship are not accurately predictable, from the changes in standing height. With the available methods of prediction, it is impossible to

accurately estimate the timing as well as magnitude of change. Treatment of anteroposterior discrepancies should be initiated as soon as orthodontist believe that treatment is indicated rather than wait for pubertal spurt, since the presence magnitude and timing of such events in any on person are highly unpredictable.

***Jamison J. E., et al (1982)***[44] in their further study, again stressed that timing of pubertal spurt is highly unpredictable.

***Fishman L.S. (1982)***[25] is an extensive longitudinal and cross sectional study of more than 1400 records derived a system of evaluation of hand-wrist radiographs. It was found that a system of skeletal maturation assessment (SMA) offers an organized and simple approach to assess the level of skeletal maturation. The system uses four stages of bone maturation, which are found at six anatomical sites located on the thumb, 3rd finger, 5th finger and radius. Eleven skeletal maturity indicators (SMI) were identified that can be applied directly in clinically diagnosis. He evaluated the interrelationships between the 11 SMI scores and growth rate in among both sexes. Percentage of levels was also established for statural height, maxilla and mandible. He observed a sexual difference between males and females in the age of onset and progression of adolescent skeletal maturation. Girls showing earlier maturation age than males. Both maxilla and mandible

achieved their maximum growth rate is greater than statural height. The study demonstrated that the system maturational assessment provides a progression scale of maturation levels through a series of readily identified skeletal maturity indicators (SMI) and the percentage of growth completed that can be directly applied in clinical orthodontics.

***Hagg U. and Taranger J. (1982)***[36] examined 212 Swedish children from birth to adulthood including a representative proportion of early, average and late maturing subjects. Skeletal development of hand and wrist was assessed in four bones were chosen according to Bjork, But a great number of epiphyseal stages were used in the study in order to obtain indicators of shorter duration, which are more informative than those of longer duration. They observed that the peak and end, but not the beginning of the pubertal growth spurt could be determined by means of indicators taken from skeletal development of the hand and wrist and pubertal development (voice change and menarche). They concluded that the maturation indicators of skeletal development are of limited value for prediction of pubertal growth, since these indicators that were closely related to pubertal growth event occurred closed to or after pubertal growth spurt.

***Demirjian.A, et al (1985)***[21] did a study on longitudinal data of fifty girls between 6-15 years of age obtained from Montreal Human Growth Research

center reported that peak height velocity, precedes menarche by approximately 1 year. These findings were seen in accordance with that of Tanner T.M. (1962) and Andersen et al (1975).

***Lewis A.B et al (1985)***[55] made measurement on serial radiographs of 34 boys and 33 girls enrolled in the Fels longitudinal study and found that mandibular height velocity and the appearance of ulnar sesamoid. Difference in facial and general body growth spurt was found in agreement with previous reports of Nanda (1955), (1956) and Bambha (1961).

***Letik H.R., et al (1987)***[53] conducted a study on 20 females and 19 male subjects from the files of the Bolton Brush foundation to investigate whether skeletal age assessment using I, II and III fingers of hand are as valid as those using whole hand. Two maturity indicators, the sesamoid and epiphyseal, diaphyseal stages of ossification were evaluated. The results showed that the two significant amounts with the 3 fingers assessments being slightly more advanced than the hand-wrist assessments. The maximum deviation occurred during the time of epiphyseal diaphyseal fusion when growth is nearing its completion and therefore they are of no clinical importance. The advantage being that the three fingers can be incorporated in the lateral cephalometric radiograph.

***Fishman L.S (1987)***[26] in a mixed longitudinal study evaluated that the maturational pattern of 4000 data records both cross- sectional and longitudinal. The data records associated with each of the eleven SMI's for each sex were statistically evaluated to establish mean associated standard deviation from those mean values. Chronological age values deviating by one standard deviation or more were considered either late or early, relative to their respective level of maturation. He was of the opinion that this allows for the immediate typing of the individual as being maturationally early, average, or late.

***Moore R.N et al (1990)***[61] assess the relevance of hand wrist radiograph to craniofacial growth and clinical orthodontists was from the records of 47 girls and 39 boys from the Bolton Brush data base. The hand wrist radiographs were scored by Tanner –Whitehouse TW2RUS method of skeletal maturity assessment. The result of the study indicated that statural height and hand wrist skeletal maturation in both sexes are significantly related. However the relationship between acceleration and deceleration in growth of the specific craniofacial dimensions and statural height skeletal maturity were not deemed clinically significant to prediction.

***Silveira A.M., Fishman L.S., (1992)***[80] in a study on 34 adolescent females (11-19 years) and 36 adolescent males (12-22 years) categorized the

individuals by skeletal maturation into early, average and late maturation groups based upon Fishman's SMA method of assessment from hand wrist radiographs. The rates of mandibular and maxillary growth relative to the stages of pubertal growth were measured. The results showed that the late maturing individuals showed larger growth increments as compared to average and early maturing individuals. Difference in incremental growth between maxilla and mandible during last stages of pubertal growth was noted with mandible growing significantly than maxilla.

***Kopecky G.R. and Fishman L.S. (1993)***[48] evaluated 17 boys and 24 girls aged to 9-17 years who were clinically diagnosed to have class II division 1 malocclusion with mid face prognathism and who were treated with Kloehn type cervical headgear. Skeletal and dental maturational periods and compared with their optimum treatment timing for maximum response. Results showed that timing of cervical headgear treatment on the basis of skeletal maturation is a more statistically significance means obtaining the maximum desirable orthopedic effect than chronological age. More favourable results were demonstrated during maturational periods that were associated with a higher degree of incremented growth velocity.

***Revela. B. and Fishman L.S. (1994)***[71] conducted a study to determine whether a positive correlation exists between adolescent maturational

development and the approximation of the midpalatal suture. Maturational evaluation of the approximation of the midpalatal suture was accomplished by examining hand-wrist radiographs with Fishman's system of skeletal maturational assessment of skeletal (SMA). Results showed that there is increase in amount of sutural approximation (fusion) as the SMI stages progressed through adolescence. It was suggested to accomplish maxillary expansion before SMI level 9 as the percentage of approximation as significantly less. However the ideal time to initiate orthopedic expansion is during the early maturational age.

***Abdel –Kader H.M. (1998)***[1] from a clinical study on orthodontic patients aged 1-15 years suggested a simple method to assess the pubertal growth spurt stages by recording MP3 stages advocated by Hagg and Taranger (1982) with the dental periapical radiograph and standard X-ray machine. He concluded that high degree, of clarity of radiographs, low patient radiation exposure and simplicity of the method as a sensitive technique in a dental clinic.

***Abdel-Khader H.M.,*** [2] in a further study ***(1999)*** evaluated the reliability of using digital dental radiography in recording two growth indicators, the adductor sesamoid and MP3 stages. Results showed that with exposure time 5 times less than used in conventional approach a high contrast radiographic

image without any distortion can be viewed on the screen of the computer monitors. Different image manipulation like zooming and measurements, comparisons with patient's radiographic images of same patient or with other patients was also feasible. Abdel-Kader recommends the technique as if provides the highest quality image with less X-ray exposure.

***Suda .N, et al (2000)***[85] in a clinical study to examine the relationship between bone age and effect of reverse pull head gear (RPH) treated 60 Japanese patients (30 males and 30 females) with Class III malocclusion. Bone age was appraised by the TW2 method with hand wrist radiographs. The forward movement of maxilla and increase in palatal length were larger in the bone age based younger male reverse pull head gear subgroup than in the bone age based older male-reverse pull headgear group, the forward movement of the maxilla and increase in the palatal length showed significant inverse correlation with the bone age, but not with the chronological age. Concluding that bone age is useful clinical indicator to determine the effective treatment plan with reverse pull head gear.

## ***Cervical Vertebrae***

Vertebral bodies as derived from mesenchyme which undergo chondrification at seventh intrauterine week and ossification at 9th intrauterine week. In the new born child the vertebral body ossification centers are ovoid as in the lateral cephalogram. Vertebral growth in the vertical dimension takes place from the cartilaginous layers on the superior and inferior surfaces of each vertebra and is equal at both of these surfaces. ***Gooding C.A., Neuhauser E.B.D. (1965)*** [30].

***Bick E.M., Copel J.W. (1950)***[8] reported that longitudinal growth of the vertebral body takes place by means of true epiphyseal cartilage plates, like longitudinal growth in the metaphysis of long bones. Hence the body of the vertebra is subjected to same deforming forces that influence the growth of long bones elsewhere in the body. We may interpret from the above conclusion that cervical vertebrae may be used to represent the general body growth.

**Lampraski (1972)**[52] utilized the cervical vertebrae and found them to be reliable and as valid as the hand – wrist radiographs for assessing skeletal age. He developed a series of standards for assessment of skeletal age for both males and females by using 5 vertebrae (2nd to 6th).This method has the

advantage of eliminating the need for an additional radiographic exposure, since the vertebrae are already recorded on lateral cephalometric radiograph.

Although Lampraski's study showed that cervical vertebrae were reliable and valid as the hand wrist radiograph for assessment of skeletal age , the 1st reported study to correlate the stages of vertebral maturation to mandibular growth changes during puberty was reported by ***O'Reilly, M.T. and Yenniello G.J. (1988)***[66] who studied 13 Caucasian females (9-15 years) derived from Bolton Broadbent growth study in Cleveland to investigate the relationship of the stages of cervical vertebral maturation (Lampraski) to growth changes in mandible. Results showed that cervical vertebral stages of maturation are related to statistically significant increase in mandibular length corpus length and ramus height, during puberty. The vertebral stages 1 through 3 occur in accelerative growth phase with stages 2&3 occur most frequently in the year preceding the maximum increment of mandibular growth. Stages 4 through 6 were observed to occur during decelerative phase of growth after peak velocity.

**Hellsing E. (1991)**[40] studied a sample of 107 children divided into age groups 8, 11 and 15years of age respectively and 22 adults. The statural height and the length of the vertebral body measured form lateral skull radiographs, were studied. Statural height was significantly correlated with

the variables for vertebral growth of 8 and 11 years where as there was no correlation at 15 years of age among the children who had passed the pubertal peak height. It was concluded that the development of the vertebrae showed similarities with earlier reported skeletal maturity without the need for hand roentgenograms.

***Mitani H. and Sato .K (1992)***[58] examined the timing of mandibular growth during puberty and related it to the growth of several other bones like cervical vertebrae and hand bones to standing height. Sample consisted of 33 Japanese girls from 9-14 years of age. They concluded that mandibular growth rate defined from other growth rates. The timing of maximum growth velocity of the mandible varied more widely than the timing of maximum growth velocity of the other parameters measured, and the total amount of mandibular growth did not correlate to any other measurement. Mitani and Sato concluded that orthodontists should take cognizance of unpredictable nature and variation in timing and amount of mandibular growth in treatment planning.

**Hassel.B and Farman A.G. (1995)**[38] reviewed that lateral cephalometric and hand wrist radiographs of 11 groups of 20 males and 10 females (220 subjects ) aged from 8-18 years from Bolton Brush growth Centre at Case Western Revenue University. They modified Lampraski's method of

cervical vertebrae assessment and developed cervical vertebrae maturation index (CVMI) by using lateral profiles of 2nd, 3rd, 4th cervical vertebrae. They correlated CVMI index with skeletal maturation indicator (SMI) developed by Fishman (1982) from hand wrist radiograph. It was concluded that CVMI is reliable method to assess the potential for future adolescent growth.

***Garcia Fernandez P. et al (1998)***[29] conducted a comparative study on 113 patients (50 males, 63 females) to determine whether cervical vertebrae maturation would correlate with the maturation indicated by hand wrist x rays in Mexican population. The hand wrist radiographs were evaluated with system developed by Fishman and cervical vertebral development of the sample were evaluated by Hassel and Farman's modification of Lampraski's criteria.

These two methods were selected, because they provide the information regarding the percentage of adolescent growth that has occurred and for predicting the future growth. Moreover six stages of CVMI can be easily compared with SMI's by Fishman. They concluded that there is no significant difference between the two techniques in assessing skeletal maturation in Mexican population.

***Franchi L, Baccetti T. Mc Namara JA .Jr. (1999)***[27] evaluated the skeletal and dentoalveolar changes induced by acrylic Herbst therapy of class II malocclusion. One of the requirements of the study was to have a control group, which should be homogeneous with the stage of skeletal maturity and should be comparable to treated group. They advocated the use of cervical vertebrae as suggested by O' Rielly and Yannello for evaluation of skeletal maturity. Bacetti,T Franchi.L Toth L.R. and Mc Namara.JA.Jr.(2000) studied 79 patients treated by twin block therapy to evaluate skeletal and dentoalveolar changes in two groups of subjects with class II disharmony treated at different stages of skeletal maturity (before and during the pubertal peak growth) to define optimal treatment timing. Skeletal maturation was determined on the basis of stages of cervical vertebral maturation according to Lampraski. Results indicated that optimal timing for twin block therapy of class II disharmony is during or slightly after the onset of pubertal peak in growth velocity. They emphasized the importance of biological evaluation of skeletal maturity in individual patients to be treated with functional appliances.

***Franchi L. Bacetti. T, Mc Namara JA.Jr. (2000)***[28] did a study on 24 individuals (15 females and 9 males) from files of university of Michigan to analyze the validity of 6 stages of cervical vertebrae maturation in 24

subjects. Results showed that the greatest increment in mandibular and craniofacial growth occurred during the interval from vertebral stage 4 (CVS 3 to CVS 4) when peak in statural height also occurred. It was concluded that cervical vertebrae method is an appropriate method for the appraisal of mandibular skeletal maturity in individual patients on basis of single cephalometric observation without additional radiographic exposure. Franchi et al concluded that the accuracy of cervical vertebrae method in detection of onset of pubertal growth spurt in mandibular growth provides helpful indications concerning treatment during timing of mandibular deficiencies.

## ***Miscellaneous***

**Joffe (1964)**[45] found frontal sinus enlargement to the associated with prognathic subjects

**Tanner (1962)**[87] found that the annual height (stature) growth increment ion children reached a plateau at 16 years I boys and 14 years in girls and it was thought that these are the ages at which frontal sinus enlargement increased. These findings are later supported by Brown, Molleson and Chenn (1984). This suggests that the increase in the sinus size follow the trend in growth ion bone length very closely.

**Rossouw P.E., et al (1991)**[76] studied to access whether a large frontal sinus size could be correlated with excessive mandibular growth. A sample of 103 cephalogram consisting of 53 class I growth patterns and 50adult skeletal class III growth patterns and female white subjects were analyzed as advocated by Ricketts et al to assess abnormal mandibular growth. The frontal sinus was expressed in square millimeters and measured on digitizer connector to a micro computer. The results indicate that there is a significant correlation between maxillary length, mandibular length, symphysis width, condylar length and frontal sinus size or a lateral cephalogram. The frontal

sinus can possibly be used as an additional indicator when one is predicting mandibular growth.

**Ruf .S. Pancherz.H (1996)**[77] did a study to find the possibility of predicting the stage of somatic maturity by analyzing frontal sinus growth on viewed on lateral head films. The study was performed on53 adolescent boys. The results revealed that if the only prediction was whether the pubertal growth maximum has passed, the precision of the body height peak was to predicted the method accuracy is lower (approximately 55%). They concluded that this prediction procedure may deliver important information with respect to the person's stage of somatic development when two lateral head films are available spaced approximation1-2 years apart.

## ***Dehydroepinadrosterone sulphate***

***Hopper.B. R and Yen S.S.C (1975)***[41] evaluated the circulating dehydroepiandrosterone and dehydroepiandrosterone sulphate levels during and prior sexual maturation in 76 boys and 65 girls as well as adult male and female measured by radioimmunoassay (RIA). There was a progressive and parallel increase in serum DHEA and DHEAs concentrations in boys and adult male levels were reached earlier were reached for DHEA than for DHEAS. From age 8- adult male there was a 2-6 fold increase in DHEA. 7.7 fold in DHEAS. The rise in DHEA in girls is not in parallel fashion. There was an abrupt increase in 11 and 12 years of age. Adult female range was reached age 12 for DHEA in girls and by age 15 for DHEAs

***SizonenkoP.C. and Paunier.L.(1975)***[82] studied the plasma dehydroepiandrosterone and testosterone levels in104 normal boys aged 7-14 years (bone age 5-15 years). Plasma DHEA levels rose at 7 years and a further increase was seen at 12 years of age. In relation to bone age DHEA increased at bone age of 5 years and then at 7 years. Further increase was seen at 11 years and 12 years of bone age. Increase in plasma testosterone was noted at bone age 13. In 123 normal girls 6-13 years bone age 5- 15 years, first increase was seen in 6 years of age. Further increase was seen between 9 and 10 years, then between 10and 11 years. The increase in

DHEA was seen before the increase in the gonadotropins. The elevation of DHEA prior to the signs of puberty suggests that DHEA may play a role in the maturation of the hypothalamus pituitary complex.

***Sizonenko P. C., et al (1976)***[83], conducted a longitudinal study of plasma dehydroepiandrosterone sulphate (DHEAS) and dehydroepiandrosterone were made in girls aged 7 years and 10 years during 3 years and 6 months intervals and similarly for 8 yeas and 11 year old boys with Addison's disease. Significant rise in DHEA and DHEAS is seen in the four groups. In female patients with premature adrenarche with higher plasma levels of DHEAS were found when compared to the normal levels of similar chronological age and bone age. Decreased concentration of dehydroepiandrosterone was found in Addissson's disease.

***Bing. C et al (1988)***[9] Serum dehydroepiandrosterone sulphate (DHEAS), estradiol, luteinizing hormone(LH), and follicle stimulating hormone (FSH) were measured in school girls 7-16 years old. A significant DHEAS increment is found at chronological age of 13 in girls with earlier menarche than girls having later menarche. Serum DHEAS levels also correlated with bone age, height, weight, subcutaneous fat and pubertal stages. Results suggested that adrenal androgens might be involved in the invitation of puberty and female maturation.

***Joseph Ghafari et al (1995)***[47] correlated anthropometric and biochemical measures of general growth with facial and occlusal changes during early treatment of class II division 1 malocclusion. DHEAS and osteocalcin, indicator of bone turnover were used as biochemical measures at time intervals for predict mandibular growth. They considered that these biochemical measures may not increase the accuracy of growth depiction by physical measures alone.

**Christian H et al (1997)**[17] investigated the effects of adrenal androgens dehydroepiandrosterone sulphate on human osteoblastic cells in vitro. There was no resultant qualitative difference between the adrenal androgens and gonadal androgens on human osteoblastic cell metabolism in vitro. Both were stimulatory as regards cell proliferation and differentiated functions, but the gonadal androgens were more potent than adrenal androgens. They exert their mitogenic effects through androgen mediate mechanisms; stimulate the action of alkaline phosphatase through TGF β expression.

***Sulcova.J et al (1984)***[86] studied the serum levels of dehydroepiandrosterone in subjects of either sex from birth to 100 years. DHEAS levels declined rapidly during the first year of life and was maintained a minimum up to 5 years, then increased significantly from 6 to7 years and reached maximum levels in women at 24 years and in men at about 30 years. DHEA levels are

minimum for girls between 5-7 years and for boys between 5-9 years. Then a significant rise began and reached maximum in women and men at about 20 years. In men it declined up to 80 years. In women it declined during next 15 years and from approximately 30 years of age again significantly.

***Kulick Rechberger B, et al (2000)***[51] investigated the serum concentrations of dehydroepiandrosterone sulphate in relation to serum levels of follicle stimulating hormone, estradiol, insulin like growth factor (IGF_I) and height and weight velocity in girls during puberty were studied in 113 girls. The mean serum concentration of DHEAS, FSH, estradiol and IGF-1 increase constantly throughout puberty while the level of cortisol remains same. positive correlation also was found between the DHEAS and estradiol and IGF-1 concentration. No correlation was found between DHEAS and height velocity. It was concluded that DHEAS might play an important role in puberty.

***Remer .T et al (2003)***[73] Proximal radial bone and urinary steroid hormone were analysed cross sectionally in 205 healthy children and adolescents. Positive adrenarchial effects on radial diaphyseal bone were observed. Positive effects of C19 steroid on bone strength, strain index was found Periosteal circumference (PC), cortical density, cortical area, bone mineral content, bone strength strain index (SSI), and forearm cross-sectional muscle

area were determined with peripheral quantitative computed tomography (pQCT) at the proximal radial diaphysis in healthy children and adolescents. It was found that there was a significant influence of muscularity, but not of hormones, on periosteal modeling (PC) before the appearance of pubic hair (prepubarche). Similarly, no influence of total cortisol secretion (C21) was seen on the other bone variables. However, positive effects of C19 on cortical density, cortical area, bone mineral content and SSI-reflecting, at least in part, reduction in intracortical remodeling-were observed in prepubarchal children after muscularity or age had been adjusted for. This early adrenarchal contribution to proximal radial diaphyseal bone strength was further confirmed for all cortical variables (except PC) when, instead of C19 and C21, specific dehydroepiandrosterone metabolites were included as independent variables in the multiple regression model. They concluded that especially the prepubarchal increase in adrenal androgen secretion plays an independent role in the accretion of proximal radial diaphyseal bone strength in healthy children.

***Remer .T et al (2004)***[74] evaluated the urine levels of major glucocorticoid metabolites cortisol, sum of adrenarchal dehydroepiandrosterone and its metabolites and its intermediate 16-hydroxylated metabolites(DHEA and M)and 5- adrostene- 3β-7βdiol, in a cross- sectional study in 1hour urine

samples of 109 healthy boys and girls, aged 6-13 years. The steroid profiling was done by gas chromatography- mass spectrometer. Total and trabecular volumetric bone mineral densities, bone mineral content (BMC) and bone strength strain index were determined with peripheral quantitative computed tomography at the distal forearm. Significant associations with the metaphyseal radius were seen for grip force, age, or BMI depending on gender and bone variable analyzed was seen. DHEA&M did not contribute to the explanation of the variance of any bone variable. However, hermaphrodiol positively explained a significant part of variation of bone mineral densities, and BMC ($p < 0.01$) in girls. Significantly negative associations with all bone variables were seen in boys for cortisol. It was concluded that the steroid hormones, cortisol and hermaphrodiol, in their physiological ranges, but not the adrenarche marker DHEA&M, appear to associate with metaphyseal bone in a sex-dependent manner during childhood.

**Tung Y.C, et al (2004)**[89] investigated the change in serum dehydroepiandrosterone sulfate and androstenedione concentration during childhood on 577 healthy children with ages ranging from 5 days to 12 years were conducted. It was found that serum levels of adrenal androgens change dramatically during childhood. Serum concentrations of DHEAS are good

marker of adrenal androgens production, because gonadal androgens may interfere with serum concentration of androstenedione. The onset of adrenarche occurred between the ages of 6 and 8 years which was 1-3 years earlier than onset of puberty.

***Richard J.A and William E.R (2004)***[72] in a review article refers adrenarche to the onset of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) production from the adrenal zona reticularis that can be detected at round 6 years of age. The result of adrenarche is pubarche or the development of axillary and pubic hair that occurs in both girls and boys at about age 8. The physiological triggers of adrenarche and the role of DHEAS remain speculative. However the biochemical pathways that leads to the production of DHEAS is well characterized.

**Bongfiglio .D et al (2004)**[13] evaluated dietary calcium intake, the bone mineral density, together with the serum levels of androstenedione, dehydroepiandrosterone , dehydroepiandrosterone sulphate, testosterone, estradiol, the apparent free fractions of testosterone and estradiol, osteocalcin, parathyroid hormone and 25-hydroxyvitamin D in 50 pre-menarcheal girls from highest and lowest end of calcium intake distribution of a large population. It was found that despite similar chronological age of

the high calcium intake and low intake premenarcheal groups, the low intake group had lower bone age, delayed puberty and lower circulating adrenal androgens. Of interest, in girls who had low calcium intake had increased levels of parathyroid hormone. In all the premenarcheals DHEA, testosterone, and apparent free fraction of testosterone positively correlated with bone age and with bone density at both radial sites. Hence it was concluded that low calcium intake and reduced levels of adrenal androgens, leading to decreased bone age and delayed pubertal development indicate a link between calcium intake, the hormonal milieu, and skeletal maturation.

**Adachi M and Takayanagi R. (2006)**[3] stated that androgens that androgens have a major role in the growth and the maintenance of both cancellous and cortical bone mass in men. Androgen receptor is expressed in osteoblasts, osteoclasts and bone marrow stromal cells. Androgens have been shown to regulate the expression and activity of several cytokines and growth factors, and control the homeostasis in bones. Dehydroepiandrosterone (DHEA) has a protective effect against osteoporosis in women after menopause through the intracrine mechanism in osteoblasts, in which DHEA is converted in to estrogen through the aromatase activity.

# *Materials & Methods*

## ***Material and methods.***

### Subjects:

Sixty individuals between the age group of 7-30 years were selected randomly for the study from people reporting to Tamil Nadu Government Dental College, Chennai. The inclusion criterion was that they were individuals without clinical signs of any systemic and local disease and had clinically class I occlusion. Subjects with malocclusion and those who were suffering from any chronic illness or under any medication were excluded from the study.

The selected subjects were further divided in to three groups. Each group containing 10 males and 10 females. The division in to three groups was based on the stages of skeletal maturation assessed with the help of hand-wrist radiograph by the method given by Bjork (1972), Grave and Brown (1976). Subjects with hand-wrist stages before appearance of adductor sesamoid were categorized as pre pubertal (group I, photograph 4). Those from the stage of appearance of adductor sesamoid up to the fusion of epiphysis and diaphysis of the radius were categorized as pubertal (group II, photograph 5). Subjects whose radiographs shows complete fusion of the epiphysis and diaphysis of the radius were categorized as adult (group III photograph 6).

**Protocol method:**

The subjects were explained about the purpose of the study and an informed consent was obtained from them (Annexure 2). The protocol of this study was presented at the regular meeting of the Institutional Ethical Committee, Madras Medical College Chennai and the committee's approval was obtained (Annexure 1).

Subjects were clinically examined and the following details about the patients were recorded and included in the specially designed Proforma**.**

1. Name
2. Age
3. Sex
4. Father's name
5. Address
6. Height
7. Weight
8. Previous medical history.
9. Extra oral examination
10. Intra oral examination
11. Serum dehydroepiandrosterone sulphate concentration

12. Hand wrist radiograph interpretation.

**Hand wrist radiographs:**

60mA X-ray machine with 40-45 KV, 12-16 mAs was used for taking the hand wrist radiographs. The film is 8×10inch, extra oral Kodak (T-Mat) blue base film.

**Patient positioning for the hand wrist radiograph**:

- The film in the cassette was placed on the table along with its long axis parallel with the long axis of the hand.
- Subjects were seated on an adjustable stool with his/her left forearm resting on the table. Hand placed on the table, palm of the hand downward and fingers straight. The hand was placed on the film so as to include the lower end of the radius and ulna.
- The centre of the ray was perpendicular to the centre of the film.
- Distance between the hand and X-ray source was fixed at variable distances depending on the age of the subject which can be adjusted from the machine.

Radiographs were evaluated in a dark room on cephalometric table with posterior illumination and traced on an acetate tracing paper. Evaluation of

the hand –wrist radiographs was done by Bjork, Grave ad Brown (1976) method.

**Estimation of Serum of Dehydroepiandrosterone sulphate**

Serum dehydroepiandrosterone sulphate is measured by quantitative Enzyme Linked Immunosorbent Assay method (ELISA). Dehydroepiandrosterone sulphate in the sample competes with the horse radish peroxidase dehydroepiandrosterone sulphate (enzyme linked antigen) for binding in to the limited member of anti dehydroepiandrosterone sites on the micro plate. The estimation of the hormone is calculated by a series of standards set by the manufacturer.

The kit used for determination of serum dehydroepiandrosterone sulphate supplied by Diametra CE, Italy.

The reagents and materials supplied in this kit are as follows:

1. DHEAS standards 6×(1 bottle =1ml)
2. Serum diluent (1 bottle). The serum diluent consists of a phosphate buffer.25mM and pH 7.4.
3. Conjugate (1 bottle -12ml).
4. Coated micro plate (1 micro plate breakable) containing antidehydro epiandrosterone sulphate IgG.
5. TMB substrate (1 bottle) 12ml-containing H2O2 -0.25mg/ml.

**Method:**

About 2.5ml of venous blood is drawn using a sterile syringe and needle from each subject. The blood sample is left in the stand vertically half an hour for the serum to separate and then centrifuged. The separated serum is transformed into eppendorf tubes and stored in ultra deep freeze at about -20ºC.

At the time of procedure the samples are brought to room temperature and are then only used for the test.

Contents in the serum diluent bottle are diluted to 100ml with distilled water or deionized water in a suitable storage container.

**Preparation of the standards:**

Dehydroepiandrosterone sulphate concentration in the serum is calculated based on a series by a set of standards. There are 6 standards. Concentration of the standards are 50 times lower than the value reported in the normal reference range because in the method the samples are diluted 1/50 while the standards are not diluted.

Concentrations of the standards to be entered in the instrument calculations are

| S0 | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| 0 | 0.1 | 0.4 | 1.0 | 4.0 | 10.0 |

20µl of serum is added to the 1ml of the serum diluent. Then 30µl of each of the diluted sample is added to each of the wells and 30µl of the standards are also added onto the wells, two wells for each standard. Now conjugate is added to each well and then incubated for one hour at 37ºC, after which the TMB substrate is added and then incubated in dark at 22-28ºC for 15 minutes. After the maximum color change has occurred the enzyme reaction is stopped and the absorbances are determined. The color developed in this reaction is yellow in color and its intensity is inversely proportional to the concentration of DHEAs in the sample. Then the absorbances are read at 450nm in a semi automated ELISA reader.

**Calculation of the results:**

The mean absorbances for each of the standards are plotted on a graph against the concentrations of the standards given above (X-axis – concentration of the standard, Y-axis – absorbent values). The resulting curve is the standard curve (graph 1). The final value for each subject is calculated by finding the X-axis value on the curve against their corresponding absorbent value(Y- axis value).

## ***Armamentarium***

### ***For clinical Examination***

1. Mouth mirror
2. Explorer
3. Sterile disposable latex gloves

### ***For hand-wrist radiographs.***

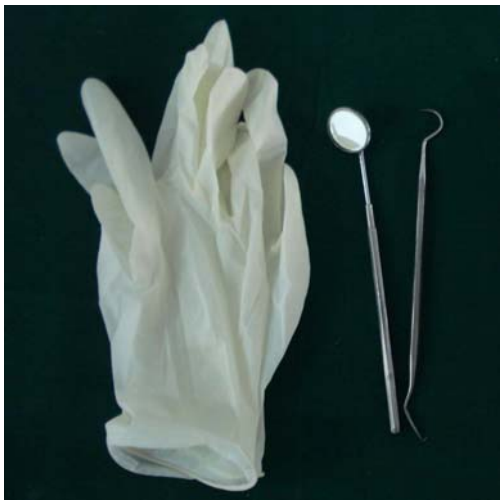
1. Kodak T Mat Blue base film (8×10”).
2. 60mA X-ray machine.( GE company)
3. X-ray illumination box.
4. Tracing sheet.
5. 4H pencil.

### ***For measurement of serum DHEAs***

1. 5ml disposable syringe and needle ( Hindustan syringes and medical equipments Ltd)
2. Test tubes
3. Centrifuge machine. (Labline medical Equipments, Gujarat, India.)
4. Eppendorf tubes.
5. Deep freezer at -20ºC (Blue star, India).
6. DHEAs ELISA kit (Diametra CE Italy).
7. Semi automated ELISA reader (Bio-Rad, California, USA).

***Photograph 1***

***Armamentarium for clinical examination***



***Photograph 2***

***Patient positioning for hand wrist radiograph***

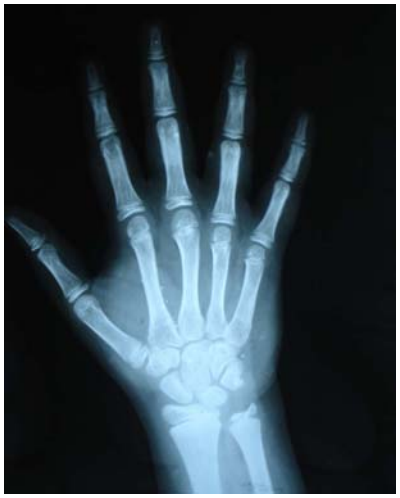
***Photograph 3***

***Extra oral  8×10” X-ray film***

***Hand –wrist radiographs***

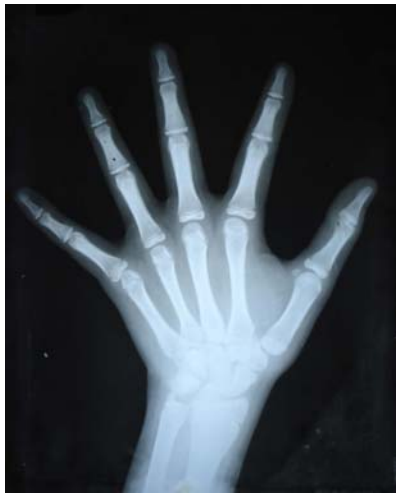
***Photograph 4***

***Group I***



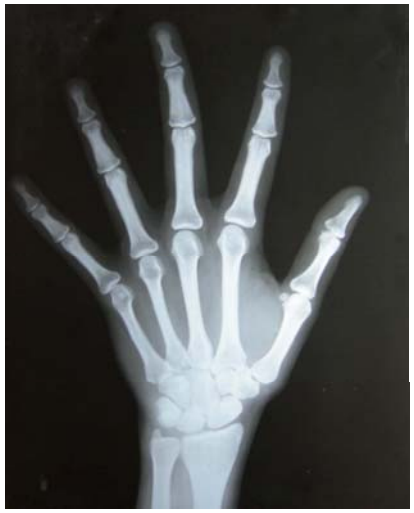
***Photograph 5***

***Group II***



***Photograph 6***

***Group III***

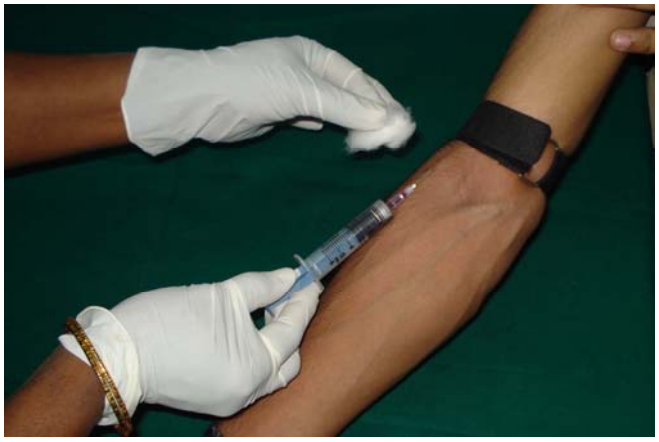


***Photograph 7***

***Armamentarium for serum collection***


***Photograph 8***

***Blood sample collection***

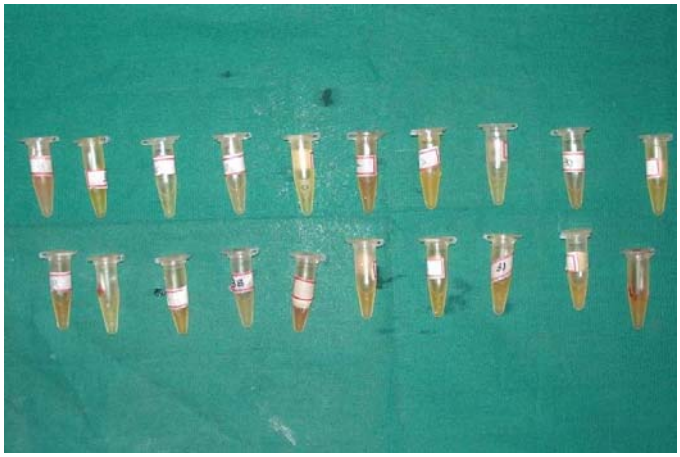


***Photograph 9***

***Centrifuge***

***Photograph 10***

***Serum samples in eppendorf tubes***



***Photograph 11***

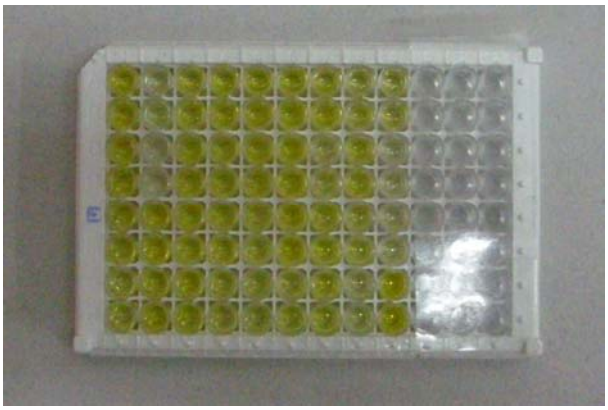
***DHEAs  ELISA kit***


***Photograph 12***

***Wells in ELISA Kit***

***Photograph 13***

***Serum sample after the color change in ELISA***

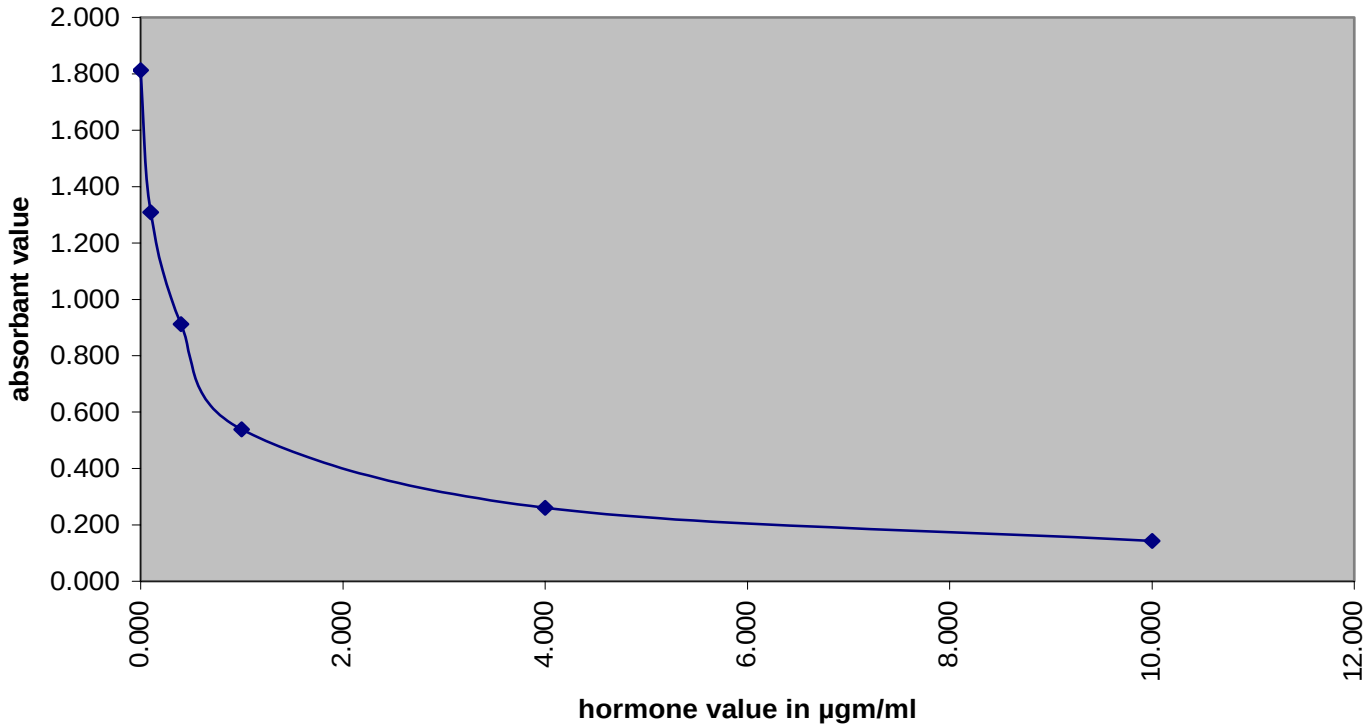


***Photograph 14***

***Semi Automated ELISA reader***

***Graph 1***

***Standard curve (DHEAs ELISA)***



# Results

## ***Results***

Sixty subjects between the age group of 7-30 years, clinically belonging to class I occlusion grouped as pre pubertal, pubertal and adult (20 subjects in each group; based on skeletal maturation) were tested for serum dehydroepiandrosterone sulphate. The skeletal maturation level was assessed by hand wrist radiographs using the method given by Bjork (1972), Grave and Brown (1976). The results were analyzed statistically.

The measured serum DHEAs values for each group are tabulated (Table I, II, and III). The statistical method 'ANOVA' was used to compare the mean hormone value of the three groups. Student's't' test was used to compare the sex difference in each group. A generalized logistic model was proposed to find out the probabilities for each of the hormone level to be in group I, II or III.

### ***Interpretation of the results***

The results showed that the mean hormone value in each of the group was found to be 0.43µgm/ml (group I), 2.17µgm/ml (group II) and 4.60µgm/ml (group III).The standard deviation for each of the groups was 0.28, 0.92, and 1.34 respectively. In ANOVA the observed p value is significant at 1% level. Hence, there was a significant difference between the means of the hormone levels between group I, II and III (table IV).

By using ANOVA the 95% confident interval for mean for the hormone values in each group was found and the range is given as follows.

Group I – 0.2993 to 0.5627µgm/ml

Group II -1.7388 to 2.60002µgm/ml

Group III- 3.9732-5.2268µgm/ml

From the results of student 't" test it was inferred that there was no significant sex difference in the hormone value with in each group (TableV). In the group I the p value was 0.489, 0.998 for group II and 0.276 for group III. It is inferred that there was no difference in the hormone values between male and female in each group at a particular stage of skeletal maturation. A bar chart (Graph 2) was used to pictorially represent the sex differences in the hormone value in each group.

The distribution of the hormone levels in the three groups was pictorially represented by scatter diagrams (Graphs 3, 4, and 5).

The generalized logistic model throws probabilities for each of the hormone level to be in Group I, II or III. The results are expressed in the graphs 6,7and 8 for group I, group II and group III respectively.

Graph 6 shows that 95% of data with less than 0.6 µgm/ml of hormone level belongs to group I with more than 0.9 probability.

Graph 7 graph shows 65% of the cases can be predicted to have bone maturation belonging to group II for hormone level between 1.5 and 2.22µgm/m with probability level of 0.85.

Graph 8 shows that 70% percentage of the cases can be predicted to have bone maturation belonging to group III of an adult for hormone level 4.26µgm/ml with probability greater than 0.85. The concordance values for each of the groups were found to be 99.18%, 93.38%, 97.18%. High concordance value indicates the accuracy of the predicted probabilities (Table VI).

## Group I

## Table I

***Serum DHEAs concentration of subjects belonging to GroupI***

| Serial no | Age | sex | Hormone value µgm/ml |
|---|---|---|---|
| 1 | 10 | M | 0.2 |
| 2 | 10 | M | 0.35 |
| 3 | 10 | M | 0.36 |
| 4 | 11 | M | 0.45 |
| 5 | 11 | F | 0.48 |
| 6 | 12 | M | 0.4 |
| 7 | 12 | M | 0.58 |
| 8 | 10 | F | 0.6 |
| 9 | 12 | F | 0.24 |
| 10 | 10 | F | 0.44 |
| 11 | 9 | F | 0.12 |
| 12 | 11 | M | 0.24 |
| 13 | 7 | M | 0.18 |
| 14 | 8 | M | 1.48 |
| 15 | 8 | M | 0.52 |
| 16 | 11 | F | 0.5 |
| 17 | 10 | F | 0.44 |
| 18 | 13 | F | 0.44 |
| 19 | 10 | F | 0.35 |
| 20 | 9 | F | 0.25 |

## Group II

## Table II

***Seruum DHEAs concentration of subjects belonging to group II***

| Serial no: | Age | Sex | Hormone value µgm/ml |
|---|---|---|---|
| 1 | 11 | M | 1.58 |
| 2 | 9 | M | 1.24 |
| 3 | 15 | M | 1.88 |
| 4 | 11 | M | 1.64 |
| 5 | 15 | M | 1.76 |
| 6 | 13 | F | 2.38 |
| 7 | 16 | M | 1.69 |
| 8 | 14 | M | 0.48 |
| 9 | 12 | F | 2 |
| 10 | 17 | M | 3.58 |
| 11 | 13 | F | 3.6 |
| 12 | 17 | M | 4.4 |
| 13 | 17 | M | 3.4 |
| 14 | 14 | F | 2.2 |
| 15 | 12 | F | 1.78 |
| 16 | 13 | F | 1.88 |
| 17 | 12 | F | 1.54 |
| 18 | 13 | F | 2.2 |
| 19 | 13 | F | 2.22 |
| 20 | 13 | F | 1.9 |

## Group III

## Table III

***Seruum DHEAs concentration of subjects belonging to group III***

| Serial no | Age | sex | Hormone value µgm/ml |
|---|---|---|---|
| 1 | 30 | F | 4.84 |
| 2 | 25 | M | 1.98 |
| 3 | 25 | M | 7.64 |
| 4 | 26 | M | 5.72 |
| 5 | 25 | F | 3.72 |
| 6 | 24 | M | 6.74 |
| 7 | 26 | F | 4.64 |
| 8 | 30 | M | 4 |
| 9 | 22 | M | 6.84 |
| 10 | 23 | F | 4.84 |
| 11 | 22 | F | 4.52 |
| 12 | 22 | F | 3.8 |
| 13 | 23 | F | 4.24 |
| 14 | 18 | M | 2.84 |
| 15 | 16 | F | 3.24 |
| 16 | 22 | M | 4.54 |
| 17 | 24 | F | 4.28 |
| 18 | 22 | F | 4.54 |
| 19 | 26 | M | 4.4 |
| 20 | 25 | M | 4.64 |

## Table IV

**ANOVA to test the significance in serum DHEAsbetween the three groups**

| Groups | Hormone value | | p value** |
|---|---|---|---|
| | Mean | SD | |
| Pre- pubertal | 0.43 [a] | 0.28 | |
| Pubertal | 2.17 [b] | 0.92 | <0.00l |
| Adult | 4.60 [c] | 1.34 | |

** indicates significance at 1% level.
Different alphabets indicate significance at 5% level.

**Table V**

***Studetnt's' t test to find the significance in serum Dheas between the sexes.***

| Groups | Sex | Mean ± SD | p value |
|---|---|---|---|
| Group I | Male | 0.48±0.38 | 0.489 |
| | Female | 0.39±0.14 | |
| Group II | Male | 2.17±1.21 | 0.998 |
| | Female | 2.17±0.56 | |
| Group III | Male | 4.93±1.81 | 0.276 |
| | Female | 4.27±0.53 | |

p >0.05 indicates statistically insignificant

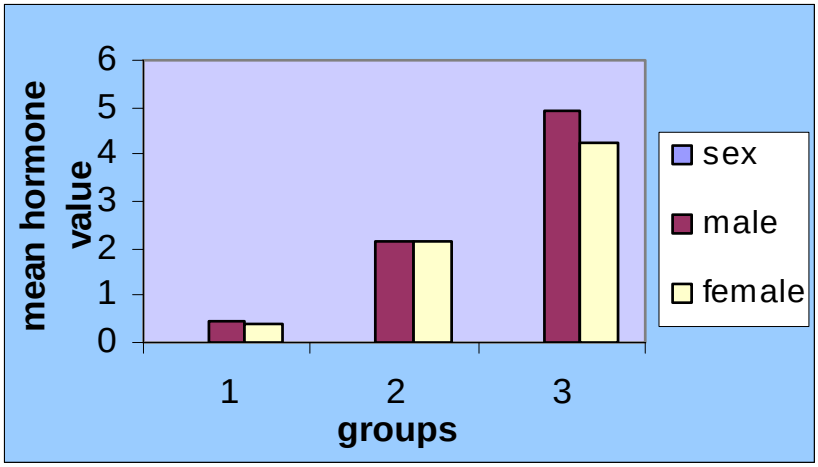
**Table VI**

***Concordance values for the three groups***

| Group | Concordance in % |
|---|---|
| GroupI : | 99.18 |
| GroupII : | 93.38 |
| GroupIII : | 97.18 |

***Graph 2***

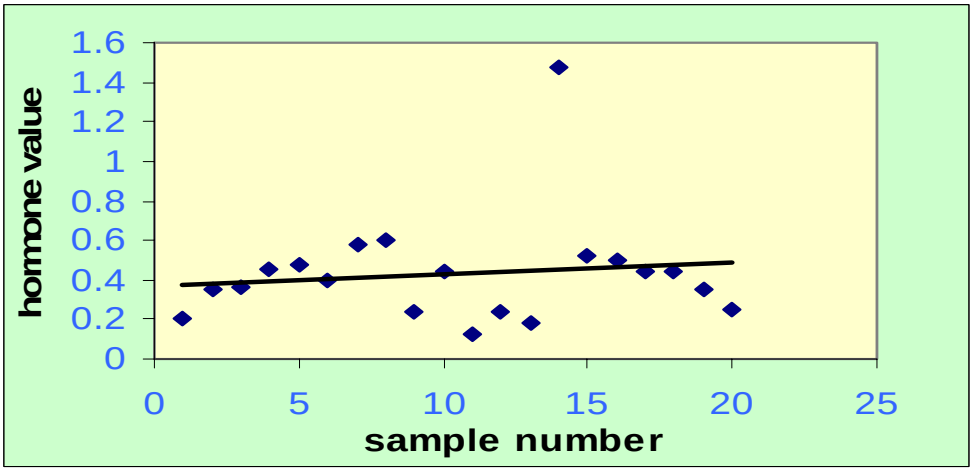
***Bar graph to show sex differences in hormone value in each group***




***Graph 3***

**Group I**

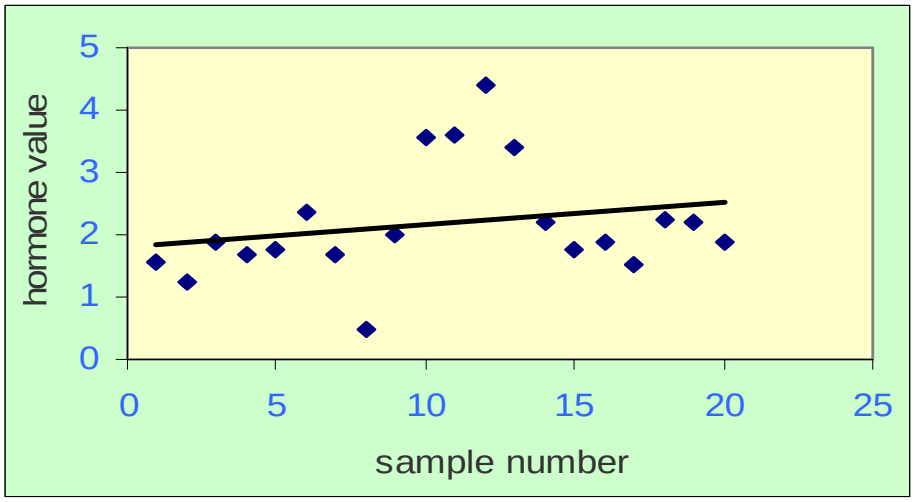
***Scatter diagram depicting the distribution of hormone values in groupI***



***Graph 4***

***Group II***

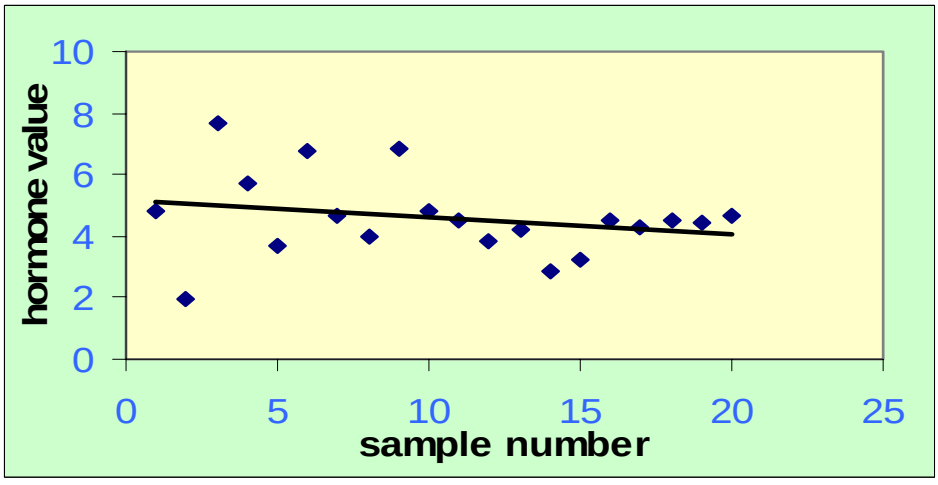
***Scatter diagram depicting distribution of hormone values in GroupII***




**Graph 5**

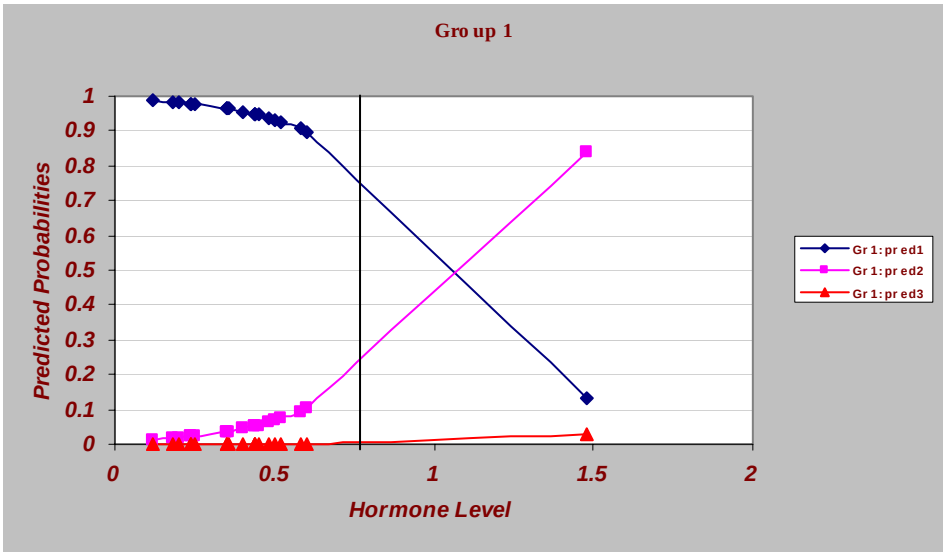
**Group III**

***Scatter diagram depicting the distribution of hormone value in GroupIII***



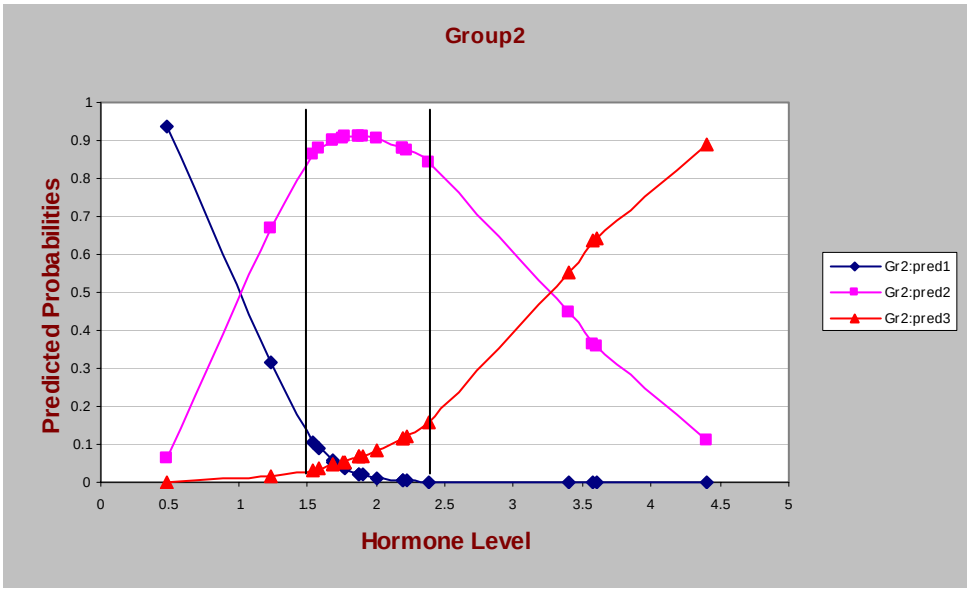
## *Graph 6*

## *To show the predicted probabilities in Group I*



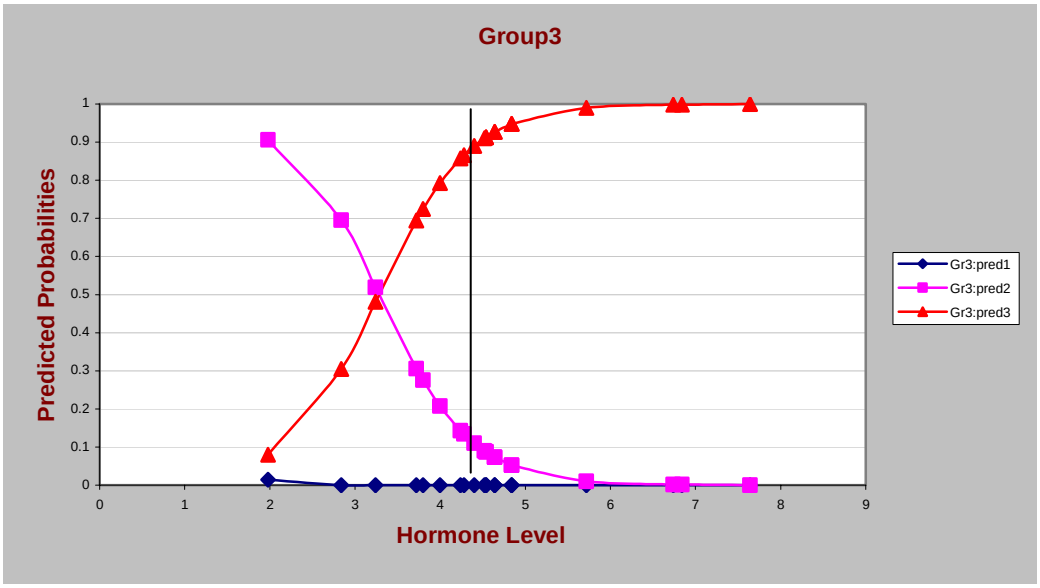

## *Graph 7*

## *To show predicted probabilities in Group II*



## *Graph 8*

## *To show predicted probabilities in group III*



# Discussion

## ***Discussion***

Time the fourth dimension in orthodontics is very much crucial in planning growth modification therapy. For growth prediction to be successful there must be adequate growth remaining. Accurate growth prediction of the growth spurts is required for planning growth modification therapy. Among the growth spurts adolescent growth spurt is more important because the physical changes at adolescence significantly affect the face and dentition. There is acceleration in the growth of the face and the jaws at the same time during the pubertal growth spurt. **Nanda in 1955**[62] found a general circumpubertal increase in growth velocity by noting the timing of both the onset and the peak rates of growth.

Both were found to be different for various dimensions of the same child. This increased growth in the body height precedes that of the face **(Bambha et al 1963**[6]**)**. Their study showed an association between the skeletal maturation and the facial growth at the two extremes. The individuals who tend to mature early with advanced skeletal age have an early adolescent facial growth spurt, whereas the children with retarded skeletal maturation tend to mature later. He concluded that it was possible to predict the time of onset of adolescent growth spurt in face from that of acceleration of growth spurt in body height which precedes that of face.

Major events in dentofacial development that occur during adolescence include exchange from mixed dentition to permanent dentition, acceleration in overall rate of facial growth and differential growth of jaws.

To identify these growth spurts it is essential to assess the growth status. There are numerous methods for assessing growth status. Earlier methods included physical stature, peak height velocity, growth charts. The main drawback of these methods is measuring growth in relation to chronological age. The draw back of growth chart includes is that it is representation of a population of well nourished children of United States and may not be applicable to other racial population. The growth curves may be less applicable to adolescence because growth during adolescence is linked temporarily to onset of puberty[19].

This was later followed by using skeletal maturation as an indicator of physical development and maturation. This led to the roentgenographic method of finding skeletal maturation. Many bones of the body were used such as carpals, the femur, the elbow joint, the shoulder joint and the skull were used for measuring skeletal maturation. **Ranke[70] (1896)** (was the first one to study skeletal development progress by means of hand wrist radiographs. Later **Hellman (1927)[39], then Todd (1931)[12], Flory (1936)[19], and Greulich and Pyle (1950)[12]** compiled the Radiographic atlases of

skeletal development of hand and wrist. The atlases contain standards, which were developed on the basis of skeletal age as opposed to chronological age.

However these atlases determine skeletal age as opposed to chronological age. But the event of skeletal maturation is closely related to adolescent growth spurt. It was found that the chronological age is a poor indicator of skeletal maturation (**Bjork et al 1967[11], Grave K.C et al 1976[32], Fishman L.S. 1979[24], Fishman L.S. 1982[25], Smith R.J. 1980[84], Suda et al 2000[85], Hagg and Taranger 1982)**. In contrary **Engstrom et al (1983)[23]** reported correlation between lower third molar development, skeletal maturation and chronological age. **Nanda R.S.(1960)[63], Bambha et al (1959)[5], Krebs et al (1964).Gupta et al (1973)[35], Rodney et al[75]** reported that there is less association between the time of tooth eruption and skeletal maturation. In contrary **Coutinho et al 1993** stated that canine calcification can be used as a tool for associating with skeletal maturation. It was then **Bjork, Grave and Brown (1976)[32], Singer (1980)[81], Fishman (1982)[25], Hagg and Taranger (1982)[36], Leitik et al (1987)[53],** who associated the ossification events of the hand wrist bones with the pubertal growth spurt. Bones from all over the body such as the carpals, the femur, the elbow joint, the shoulder joint, and the skull can be used for the purpose of assessing skeletal maturation. But hand wrist proved to be a more effective method

because of numerous centers of ossification which are undergoing changes at different times and rates. Although prediction with the hand wrist radiographs improves as the growth spurt approaches, there are limitations for early prediction and serial observations are required which will increase the radiation exposure. After the hand wrist radiographs cervical vertebrae were used for assessment of skeletal maturation **(Lampraski et al 1972[52], Hellsing (1991)[40], Hassel and Farman (1995)[38], Garcia Fernandes et al (1998)[29], Franchi et al (1999)[27,] Franchi et al (2000)[28].** The advantage of using cervical vertebrae is that they can be visualized in a lateral cephalogram without any need of additional radiation exposure. There are some disadvantages with cervical vertebrae which includes 1) difficulty in visualization of the subtle changes in the vertebrae, 2) difficulty in visualization due to improper neck posture while taking radiograph, and 3) blocking out of cervical vertebrae due to the use of thyroid collar (**Letik et al (1987)[53]**.

All of these skeletal maturity indicators are associated with the pubertal growth spurt. The major development change of adolescence is maturation of the reproductive system, which is a complex process, also results in a brief growth spurt termed as adolescent growth spurt. The earliest study about adolescent growth spurt started in **1759** when (**Gueneau de**

**Montbeillard**)[60] began a18 year study on his son to determine the early increments of growth. The adolescent growth spurt is characterized by an increased growth rate in practically all of the bones and all of the muscles of the body. Woodside in his study 1959[19]in his study of the Burlington group, points out that growth spurts are really possible. The first peak usually occurs around 3 years of age. The second peak is from 6-7 years in girls and 7-9 years in boys. The third peak is 11-12 years in girls and 14-15 years in boys. The whole event of puberty is due to the circulating pool of various hormones in the body and the central nervous system. Pubertal maturation is initiated by developments in the CNS that result in increased secretion of gonadotropin releasing hormone (GnRH), gonadotropins (FSH and LH), sex steroids, growth hormone (GH) and somatomedins C. The gonadotropins and the sex steroids are necessary for the production of secondary sexual characteristics. There is a considerable variation in timing of the adolescent growth spurt between boys and girls. Generally girls mature 2 years earlier than boys.

Adolescence in girls can be divided into three stages, based on the extent of sexual development[69]. The first stage which occurs at about the beginning of the physical growth spurt (appearance of the breast buds, early stages of pubic hair development), stage II after 1 year of stage I during which peak

velocity of growth occurs. The third stage 1-1½ years after stage II is marked by the onset of menstruation (menarche).

The stages of sexual development in boys are very difficult to specify starting with stage1 with "fat spurt" marked by gain in body weight and increase in the size of the scrotum. Stage II begins 1 year after the stage I shows the beginning of height spurt, followed by stage III (8-12 months) marked by peak velocity in body height. Stage IV which occurs15-24 months after stage III is difficult to pinpoint and it is marked by the end of spurt of growth in height. **Hagg and Taranger (1982)[36]** suggested that voice change in puberty (pitch) can be used as a reliable indicator for pubertal growth spurt, but the main draw back in this method is the difficulty for the clinician in diagnosis.

The neuroendocrine role in onset of puberty starts with the maturation of hypothalamus pituitary complex called as gonadostat. This event is termed as adrenarche. The event is heralded by the significant increase in the secretion of the androgen dehydroepinadrosterone and its conjugate dehydroepiandrosterone sulphate **(Parker and Mehesh 1978[19], Richard J.A and William E.R.2004[72])**. Richard et al stated that adrenarche refers to the onset of dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEA-S) production from the adrenal zona reticularis that can be detected at

around 6 years of age. The phenotypic result of adrenarche is pubarche or the development of axillary and pubic hair that occurs in both girls and boys at about age 8. The phenomenon of adrenarche is unique to human beings and to some Old World primates, and a reversal of adrenarche appears to occur in the ageing process. Premature and exaggerated adrenarche can be indicative of future onset of adult diseases, thus increasing the clinical relevance of adrenarche. The physiological triggers of adrenarche and the role(s) of DHEAs remain speculative**.** After the pubarche is the gonadarche which marks the secretion of the gonadal steroids which includes the testosterone and estrogen.

DHEA is a sex steroid secreted by the adrenal cortex. DHEAs is the sulfated conjugate of DHEA. Its concentration is 100-1000 fold greater than DHEA. DHEA shows diurnal variation whereas DHEAs does not. DHEA has a rapid metabolic clearance rate where as DHEAs does not have a rapid metabolic clearance. The serum levels of this hormone is significantly high in the neonate, after which there is a fall in the level, and there is sudden rise in the value from 7 years in girls and 8 years of age in males and gradually increase until it reaches a adult value **( Hopper and Yen 1975[41]).** It was reported that adrenal androgens appear to be transformed in to estrogens in peripheral fatty tissue which in turn causes maturation of the gonadostat.

This occurs approximately at the age of 6-7 years of age. This early rise in adrenal androgens was termed adrenarche and this occurs approximately two years prior to pubertal growth acceleration **(Parker and Mehesh 1977[19], Sizonenko and Paunier 1975[82]).**

Dehydroepinadrosterone sulphate is also found to increase bone mineral density, maintain the cancellous bone and cortical bone mass, protective action in osteoblast (**Bing C et al .1988[9], Bongfiglio D, et al. 2004[13], Adachi M and Takayanagi R. 2006[3])**. **Christian K H et al (1997)[17]** stated that DHEA and DHEAs have similar actions like dehydrotestosterone on human osteoblast cell metabolism. They exert their mitogenic influence on osteoblast through androgen receptor mediated mechanisms and stimulate the action of alkaline phosphatase activity through TGF β expression.

In this study serum dehydroepiandrosterone sulphate levels of 60 healthy individuals were categorized as pre pubertal, pubertal and adult based on skeletal maturation assessed by **Bjork, Grave, and Brown method (1976)**.The sample for the hormone investigation was taken from venous blood. Serum dehydroepiandrosterone sulphate was chosen rather than dehydroepiandrosterone because DHEA shows diurnal variation whereas DHEAs does not show such pattern. The results obtained clearly show that serum concentration of hormone for the three groups were statistically

significant. There was a progressive rise in the serum concentration as the skeletal maturation progressed almost reaching a maximum value after the complete fusion of the epiphysis and the diaphysis of the radius.

Earlier **Hopper and Yen (1975)[41], Sulcova Hill et al (1997)[86]**, studied thse serum concentrations of DHEA and DHEAs at different age groups found that were a progressive increase in the serum concentration from 8 years and abrupt rise in the value from 11-12 years in females and the adult value was reached by 15 years. Since in this study the groups were categorized based on the stages of skeletal maturation we could see very low value in the pre pubertal group and higher values in the pubertal group and the highest values in the adult value. In each group there was no significant difference in the mean hormone values between the sexes. It is inferred that there is no difference in the hormone values between the sexes at a particular stage of skeletal maturation. High concordance values were obtained for each group which suggested the accuracy of the predicted probabilities for the hormone value to be in a particular group.

With such a plethora of methods for predicting pre pubertal and pubertal growth acceleration available, the clinician is not in dearth of methods. Enquiries about physiological growth changes like secondary sexual characteristics may be embarrassing for both the physician and patient.

Repetitive exposure to X-rays may be a radiation hazard, especially in growing individual. The evaluation of hormones on the other hand has more mathematical significance as repetitive test over the years can be charted for assessment and prediction and such tests do not have any long term consequences.

Dehydroepiandrosterone and dehydroepiandrosterone sulphate is considered as the marker of adrenarche (an event occurs two years prior to the pubertal growth acceleration), its pro active nature on bone growth, and its correlation with the stages of hand wrist radiographs it can be considered as an indicator of skeletal maturation to assess growth status during adolescence.

# ***Annexure 1***

# ***Ethical Approval Letter***

**INSTITUTIONAL ETHICAL COMMITTEE**
**Government General Hospital & Madras Medical College,**
**Chennai – 600 003, India.**
**Off. Ph. No. 044-25305000**
**Fax: 044-25305115**

Ref. No.: 12299 / P&D / Ethics / Dean / GGH / Chennai, dated July 19th, 2007

**Title of the Work:** Reliability of Serum Dehydroepiandrosterone sulphate as an indicator of skeletal maturation – a comparative study

**Principal Investigator:** Dr. S. Bhadrinath

**Department:** Orthodontics and dentofacial orthopedics, MMC, Chennai

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on **July 19th 2007**, at the conference hall of the Dean, Tower Block I, GGH, Chennai.

The members of the committee, the secretary, and the chairman are pleased to
- approve the proposed work mentioned above, submitted by the principal investigator /
- ~~consider the proposed work but advised for revision and resubmission~~

The principal investigator and their team are directed to adhere the guidelines given below:

01. You should get detailed informed consent from the patients / participants and maintain confidentiality.
02. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
03. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
04. You should not deviate form the area of the work for which I applied for ethical clearance.
05. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
06. You should abide to the rules and regulations of the institutions(s).
07. You should complete the work within the specific period, and if any extension of time is required, you should apply for permission again and do the work.
08. You should submit the summary of the work to the ethical committee on completion of the work.
09. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

Secretary,
IEC, GGH, Chennai.

Chairman,
IEC, GGH, Chennai.

Dean,
GGH & MMC, Chennai.

# ***Annexure 2***

# ***Informed consent***

**Realibility of Serum dihydroepiandeosterone sulphate (DHEAS) as an indicator of Skeletal maturation – a comparative study.**

**ஒப்புதல் படிவம்**

இந்த பல் சீரமைப்பு சிகிச்சை சம்மந்தமான பல் மருத்துவ ஆய்வின் செய்முறை மற்றும் முக்கியத்துவம் பற்றி முழுதாக எனக்கு விளக்கப்பட்டுள்ளது. என் ஐயப்பாடுகளும் விளக்கப்பட்டுள்ளது. இது பரிசோதனைக்கான ஆய்வு மட்டுமே, சிகிச்சைக்காக அல்ல என தெரிவிக்கப்பட்டுள்ளது. இந்த ஆய்விலிருந்து எச்சமயத்திலும் விலகிக் கொள்ள எனக்கு முழு உரிமை உண்டு என்பதையும் தெரிந்து கொண்டேன். இப்படி விலகிக்கொள்வதால் என் சிகிச்சையோ மற்ற உரிமைகளோ பறிக்கப்படமாட்டாது என்பதையும் அறிந்து கொண்டேன். இந்த ஆய்வில் கலந்து கொள்ள என் முழு சம்மதத்தையும் சுயமான விருப்பத்துடன் தெரிவிக்கிறேன்.

தேதி :

கையொப்பம்.

**Consent Form**

The aim of the present study, its procedure and importance has been explained to me. All my doubts regarding the study has been clarified. I know that it is not a part of treatment and I have all the rights to restrain from the study at any time and it has also been explained that this will not restrict me from availing any treatment at any time. I hereby give my full consent in my conscious state to participate in this study.

Date

Signature.

## Annexure 3

## Proforma

**Reliability of serum dehydroepiandrosterone sulphate as an indicator of skeletal maturation – a comparative study.**

Name: Age: Sex:

Father's Name:

Father's Occupation:

Address:

Medical history:

**Clinical examination**:

Extra oral examination:

Height: weight:

Body type:

Facial type:

Profile:

FMA:

Intra oral examination:

Anteroposterior relationship: molar relation: right:

left:

Transverse relationships: Vertical relationships:

Blood investigations: Bleeding time: Clotting time:

Serum dehydroepiandrosterone sulphate level:

Hand wrist radiograph interpretation:

## ***Annexure 4***

## ***Maturation indicators of the hand bones for determining skeletal age Bjork (1972), Grave and Brown (1976)***

Presence of 9 developmental stages was given by Bjork (1972), Grave and Brown (1976). The ossification events are localized in the area of the phalanges, carpal bones and radius (R). Growth stages of the fingers are assessed according to the relationship between the epiphyses and diaphyses . There are three stages of ossification of the phalanges.

First stage:

Epiphysis shows same width as diaphysis.

Second stage:

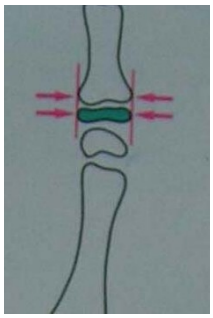
Capping stage – the epiphysis surrounds the diaphysis like a cap.

Third stage:

U Stage – bony fusion of the epiphysis and the diaphysis.

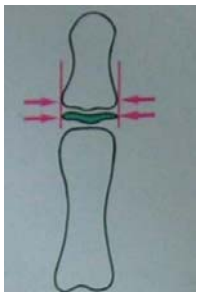
For assessment of maturity in the area of phalanges, fingers 1-5 beginning with the thumb are labeled.

First stage of maturation: PP2 stage.



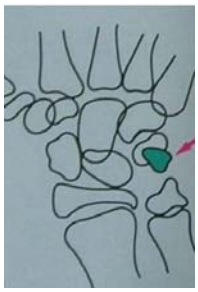
The epiphysis of the proximal phalanx of the index finger (PP2) has the same width as the diaphysis. This stage occurs approximately 3 years before the peak of the pubertal growth spurt.

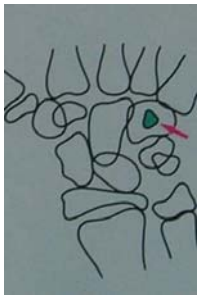
Second stage: MP3 stage.

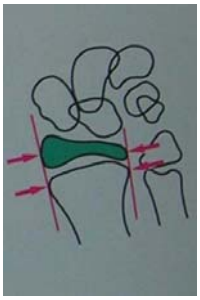


Epiphysis of the middle phalanx of the middle finger (MP3) is of the same width as the diaphysis.

Third stage: Pisi, H1, R stage.







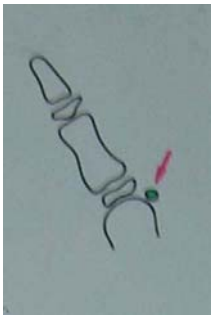
This stage of development can be identified by thee distinct ossification areas; these show individual variations but appear at the same time during the process of maturation.

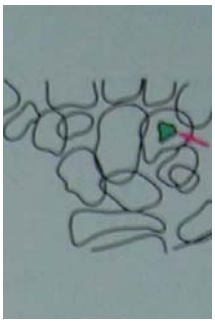
Pisi stage – visible ossification of the pisiforme.

H1 stage- Ossification of the hamate prodess of the haatum.

R- stage - same width epiphysis and diaphysis of the radius.

Fourth stage: S and H2 stage.



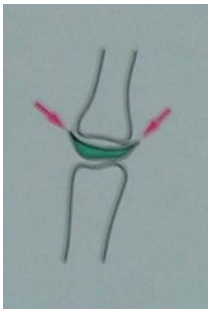
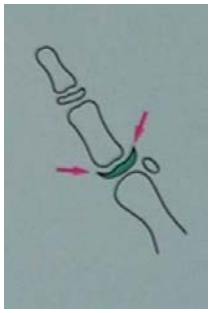
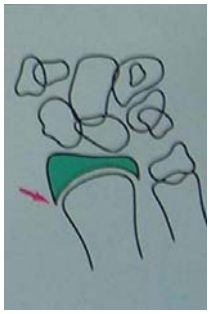


S- stage- First mineralization of the ulnar sesamoid bone of the meta-carpophalangeal joint of the thumb.

H-stage – Progressive ossification of the hamular process of the hamtum.

The fourth stage is reached before the or at the beginning of the pubertal growth spurt.

Fifth stage: MP3cap, PP1cap and Rcap stage.

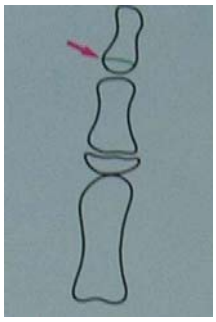
During this stage the diaphysis is covered by the cap-shaped epiphysis.

In MP3 cap stage, the process begins at the middle phalanx of the third finger.

In PP1 cap stage at the proximal phalanx of the thumb.

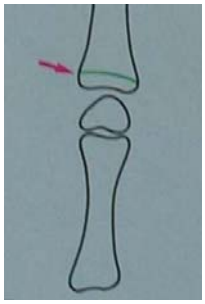
In the R cap stage at the radius. This stage of ossification marks the peak of the pubertal growth spurt.

Sixth stage: DP3u stage



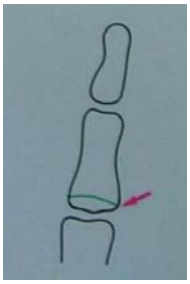
Visible union of the epiphysis and diaphysis at the distal phalanx of the middle finger (DP3).

Seventh stage: PP3u stage.



Visible union of the epiphysis and diaphysis at the proximal phalanx of the little finger (PP3).

Eighth stage: MP3u stage.



Union of the epiphysis and diaphysis of the middle phalanx of the middle finger clearly visible (MP3).

Ninth stage: Ru stage.

Complete union of the epiphysis and diaphysis of the radius. The ossification of the all the hand bones is completed and skeletal growth is finished.

# Summary & Conclusion

## ***Summary and Conclusion***

The usefulness and reliability of serum dehydroepiandrosterone sulphate as an indicator of skeletal maturation with that of hand wrist radiographs was studied in sixty subjects including both males and females between the age group 7-30 years.

The impact of mean serum dehydroepiandrosterone sulphate concentration was determined and assessed with hand wrist radiographs. The following conclusions were drawn.

1. Males and females having same level of skeletal maturation have similar serum dehydroepiandrosterone sulphate concentration.
2. Serum dehydroepinadrosterone sulphate concentration can be used as indicator of skeletal maturation

The present study is a cross-sectional study with relatively small sample size. A longitudinal study with a larger sample size would give us a better picture and open future venues.

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