Review Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2017;6:3:56-64.

Biomarkers: A New Era of Skeletal Maturity Assessment with Changing Trends from Radiographic Imaging to Non-Invasive Methods

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ABSTRACT

Aim: Biomarkers have been hailed as a paradigm shift for assessment of skeletal maturity in children and young adolescents. In this article the aim was to critically appraise the literature with regards to various skeletal maturity indicators with much emphasis on paradigm shift from radiographic methods to non-invasive biomarkers.

Materials and Method: Critical review of literature where various skeletal maturity indicators have been used in growth assessment of children and adolescents reported.

Results: Most of the studies published showed that skeletal maturation staging can be widely used as an approach to predict timing of pubertal growth, to estimate growth velocity and proportion of growth remaining. Skeletal growth in mixed and early permanent dentition offers an advantage when growth modification procedures are needed.

Conclusion: Biomarkers mark the new revolution in skeletal maturity assessment. They are found to be highly correlating with existing method (Cervical vertebrae and Middle phalanx of third finger). Biomarkers render new possibilities towards non – invasive and simpler methods.

Keywords: Biomarkers, Dehydroepiandrosterone(DHEA), Dehydroepiandrosterone(DHEAS), Cervical Vertebrae, Dental maturation, MP3.

INTRODUCTION

The growth of skeletal system shows an underlying symmetry. The skeletal development of one part of the body must be bearing resemblance to the development of another part.[1] Growth is influenced by many factors from birth till adulthood, including chromosomal aberrations, endocrine abnormalities, bone and cartilage abnormalities, chronic disorders of the main organs etc. Furthermore, hormone secretion also plays an important role in development and maturation of the patient. Growth is mainly affected by the hormones secreted by the pituitary and thyroid gland, adrenocortical and sex hormones.

In the assessment of maturation of the child, knowledge and understanding of growth events is of prime importance. The maturational development of an individual comprises of overall biologic progression of an individual throughout the life. At the time of life when an individual is

Received: Feb. 11, 2017: **Accepted:** Apr. 11, 2017
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pISSN 2278-0076
eISSN 2321-9270

growing, indicators of the level of maturational development provide the greatest measures for evaluating the biologic age and associated timing of skeletal growth.[2] Adolescence is a time in an individual's life wherein various skeletal changes occur in different parts of the body. The characteristic growth pattern of a child shows a growth rate that decreases from birth, with a minor mid growth spurt at approximately six to eight years of age followed by minimum growth changes during the pre-pubertal age and then a pubertal growth spurt.[3]

Although growth events usually proceed in a sequence which is fairly predictable, their timing is quite variable among different individuals. Treatment during the phase of intensive or accelerated growth can have a significant impact on the correction of dentofacial deviations and also on the betterment of facial appearance. Determining the biological timetable of an individual and recognizing the phases of accelerated and intense growth can give important information associated with the treatment planning and retention procedure.[4]

ASSESSMENT OF DEVELOPMENTAL STATUS OF CHILD

The maturity of a child is usually assessed by various events that take place during the period of growth. The developmental status of a child may be best assessed by physiological parameters such as peak growth velocity, voice changes in boys, menarche in girls, dental development and skeletal ossification.[3,5,6] Physical stature, peak height velocity, and growth charts were used as methods of assessing growth previously. All these methods assessed growth in relation to chronological age. Less variation exists in the timing of developmental events if a maturational time scale is used. These methods followed the usage of skeletal maturation as an indicator of physical development and maturation, which ultimately led to the use of radiographic method to determine skeletal maturation.[7]

RADIOGRAPHIC ASSESSMENT

The use of radiographic analysis to estimate skeletal maturation stage is a universally used method for assessing the timing of pubertal growth and for estimating growth velocity and residual growth.[8] While, the use of the cervical spine [9, 10] and frontal sinus [11] to assess the skeletal maturation has been reported, it is generally determined by using ossification stages in the hand wrist radiographs. The quantity of different types of bones available in the area, [12-20] the ossification onset of the ulnar sesamoid [21-26] provides better options to evaluate the skeletal maturation.

HAND WRIST RADIOGRAPHS

The hand-wrist radiograph is recognized to be the most standardized and accepted method of skeletal maturation assessment.[19, 22, 27, 28] Though skeletal growth follows the same pattern in almost everybody, an important consideration to be remembered is that the initiation, duration and amount of growth vary considerably among individuals during puberty. Some individuals achieve skeletal maturation early with a relatively short pubertal growth spurt; whereas in others it occurs with a prolonged pubertal growth spurt.[27]

METHODS OF HAND WRIST RADIOGRAPH ASSESSMENT

Assessment of skeletal maturity using hand wrist radiographs is described by a number of methods. There are two general approaches for assessment of the hand-wrist radiograph. The first method includes comparison methods of **Greulich and Pyle and Tanner et al**. In 1959, **Greulich and Pyle** [16] developed a radiographic atlas for hand and wrist region based on skeletal age. They used an atlas as a standard of comparison, which is composed of plates of typical hand wrist radiographs. The corresponding bones in the atlas are compared with the patient's each bone. The average of all ages corresponds to the mean age of the individual. This approach is often shortened to a gross assessment in clinical practice, to find the finest match of the individual with one of the plates. The **Tanner et al.** [14] method is based on comparison of an individual's skeletal maturity with that of a child of same age and sex.

The second method of the hand wrist radiograph assessment comprises of specific indicators to relate skeletal maturation to the

pubertal growth curve. This approach is based on the maturation evaluation of the individual rather than on mean values. Several skeletal maturity indicators have been described in the literature which includes the calcification of the adductor sesamoid, hamate's hook, and staging of the middle phalanx of the third finger.[16,17,19-21,22,23,25, 26,29-31,32]

Bowden[12] described a graphical approach to assess skeletal maturation analysing the pubertal growth curve using the different bones like ulnar, radius, carpal, metacarpals, phalanges, and the sesamoid bone. This method helps in approximation of the relative growth velocity and percentage growth remaining. Furthermore, Fishman's [4,12,31,32] system of assessment of skeletal maturation is based on 11 discrete skeletal maturity indicators (SMI) that are located on six anatomic sites on the thumb, third finger, fifth finger, and radius. It consists of graphs and tables to assess an individual's relative growth rate and total adolescent growth completed which are independent of chronological age.

However, owing to the ALARA Principle (As low as reasonable achievable) and concerns about radiation safety, the routine use of hand radiographs has been questioned. [2,33,34] Various reasons such as: additional radiation exposure and expense to the patient need to be considered while assessing the skeletal maturation. Also, as the regions of the skeleton differ in their development; hand wrist is not always a representative as it forms a small component of the skeletal system, and it interprets only the amount of the growth but not the direction of the growth. In addition, prediction of onset of pubertal growth spurt using hand wrist radiographs has not been indicated in the British Orthodontic Society Guidelines.[35]

Medical and Dental field universally, in their effort to reduce radiographic exposure due to imaging techniques, resorting to alternative methods. This lead to a trend of acquiring additional data, for research, from existing radiographic records to overcome iatrogenic radiation exposure. With this background a lateral cephalogram is investigated as an alternate to determine skeletal maturation. Cephalometric radiograph is considered as a valid method to estimate vertebral bone age. It can be used with the same assurance as hand wrist radiographs to evaluate skeletal maturity, thus avoiding the need for an additional radiograph. The technique's simplicity and ease of use encourages this method to assess skeletal maturation. The cervical vertebrae can be easily seen on lateral cephalometric radiographs, which are routinely recorded for most orthodontic treatment planning. Determination of skeletal maturation from dimensional measurements and anatomical changes of cervical vertebrae will facilitate orthodontic evaluation. Thus, eliminating the need for hand wrist films, and therefore decrease radiation exposure.

CERVICAL VERTEBRAE MATURATION(CVM)

A system was developed by Hassel and Farman[7] (as shown in Table 1) to assess the skeletal maturation using the cervical vertebrae. The difference in shapes of cervical vertebrae at each level of skeletal development provides a means to determine the skeletal maturity of an individual. The vertebral bodies of C3 and C4 become taller as skeletal maturity progressed from wedge more square in shape. The inferior vertebral borders remain flat when immature, and they become concave when mature. The inferior vertebral borders show curvatures in sequence from C2 to C3 to C4 as the skeleton matured.

Lamparski [7] in 1972 utilized cervical vertebrae and found it to be more reliable and valid than hand wrist radiograph for assessing the skeletal age, further followed by Hassel and Farman. Using cervical vertebra bone age, one may obtain a detailed and objective manner to assess the maturity and thereby improve orthodontic diagnosis and therapeutic decisions. The technique is simple and easy to use which encourages more clinicians to use this method to assess skeletal maturation. Also, this method is considered as reliable as the hand wrist radiograph.

However, this can be further cut down by employing of the Intra Oral Periapical Radiograph (IOPA) for assessing the digital maturation. It produces high quality radiographic images with exposure time 5 times less than usual, faster and easier patient doctor communication and an archive that could be easily generated when compared to other methods of radiographic assessment.

Table 1: Developmental stages of cervical vertebrae (Hassel and Farman method):[7]

STAGE	DESCRIPTION	PERCENTAGE OF GROWTH REMAINING
CVMI 1 (INITIATION)	The inferior borders of C2, C3, and C4 are flat in this stage. The vertebrae are wedge shaped, and the superior vertebral borders are tapered from posterior to anterior.	80%–100% of adolescent growth remains to be completed
CVMI 2 (ACCELERATION)	Concavities develop on the inferior borders of C2 and C3. The inferior border of C4 is flat. The bodies of C3 and C4 are nearly rectangular in shape.	65%–85% of adolescent growth remains to be completed
CVMI 3 (TRANSITION)	Distinct concavities are seen in the inferior borders of C2 and C3. A concavity is beginning to develop in the inferior border of C4. The bodies of C3 and C4 are rectangular in shape	25%–65% of adolescent growth remains to be completed.
CVMI 4 (DECELERATION)	Distinct concavities are seen in the inferior borders of C2, C3, and C4. The vertebral bodies of C3 and C4 are becoming squarer in shape	10%–25% of adolescent growth remains to be completed
CVMI 5 (MATURATION)	More accentuated concavities are seen in the inferior borders of C2, C3, and C4. The bodies of C3 and C4 are square or nearly square in shape. .	5%–10% of adolescent growth remains to be completed.
CVMI 6 (COMPLETION)	Deep concavities are seen in the inferior borders of C2, C3, and C4. The bodies of C3 and C4 are square or have greater vertical dimension than horizontal dimension	Little or no adolescent growth remains to be completed

Hagg and Taranger[2,27,36-38] noted that the stages of ossification of middle phalanx of the third finger (MP3) follow the pubertal growth spurt. Five stages of pubertal growth spurts from beginning to end can be represented by five MP3 stages. These stages of maturation can useful to measure skeletal age as well as developmental status of a child. Alternatively, it can be used to assess the chronological age of a child with unknown birth history. Digital radiograph of the MP3 stages is definitely a simple, valid, easy on the pocket technique for the assessment of skeletal maturity. Also, as it can be done chair side within seconds, it is considered to be more convenient and faster method than other radiographic imaging technique.

Many researchers have agreed that this simple method has high reliability and could be used as an alternative method to assess skeletal maturity of growing children. All these studies revealed highly significant association between MP3 stages in determining skeletal age. The radiographic interpretation of MP3 stages is usually done by method of Hagg and Taranger's [27] to which E3/4 stage was added later by **Leite et al** [28] as shown in Table 2.

DENTAL MATURATION

Tooth maturation is a constant process and is considered to be an alternative method to determine skeletal maturity. Dental maturity can be approximated by the number of erupted and

unerupted teeth, stages of dentition (deciduous, mixed or permanent); tooth calcification, degree of tooth structure, stages of crown formation of developing teeth and stages of root formation of all erupted teeth.

Dental eruption may be influenced by local factors such as ankylosis, early or extraction of the deciduous tooth, impacted teeth and crowding of the permanent teeth. However, the method can only be used during relatively short periods as no teeth emerge during some periods of life. There are basically two types of techniques for evaluation of dental maturation and correlating it to skeletal maturation-one may use Atlas approach while the other method is Scoring systems. Altas approach involves comparison of radiographs of jaws consisting of tooth mineralization stages which are compared with standard tables, figures, charts or radiographs provided by the respective authors in the form of atlas. Method using Atlas approaches are Schour and Massler, Nolla, Andersen et al. and Moorreees et al.[39-46]

Technique using Scoring systems include Demirjian[47] and Haavikko[48] methods. These techniques also require jaw radiographs but they restrict the number of teeth included in the analysis. The teeth are assessed for crown and root formation, and the results are interpreted into the dental age by reference tables. Out of all above methods Demirjian method is easy and accurate so, most widely accepted method for dental maturation.

Several studies have outlined the relationship between maturational stages of individual teeth and skeletal maturity.[2, 47, 49-54] Many researchers have assessed the developmental stages of maxillary canine as a tool for skeletal maturity assessment and it was found it to be correlating with CVMI stages and MP3 stages. Most of the studies concluded that there exists a strong correlation present between canine calcification and skeletal maturity between MP3 staging and CVMI. Other studies have taken either mandibular canine or third molar for dental age assessment.[5,50,51] But these two parameters exhibit some drawbacks of their own. Apex closure of mandibular canine occurs by the age of thirteen years wherein growth in most children extends up to the age of 16-17 years. Third molars on the other hand are the most commonly missing teeth in human dentition, which makes it an unreliable parameter for age assessment.[50,52]

BIOMARKERS

Biomarkers have gained important recognition globally in research field in assessment of skeletal maturation as they represent agents involved in bone growth and remodelling. The main advantage of biomarkers is that they avoid radiographic exposure to the patient. Serum Alkaline phosphate(ALP), [55] ALP in gingival crevicular fluid, proteins in gingival crevicular fluid,[56] Serum Parathyroid hormone related protein (PTHrP),[57] Dehydroepiandrosterone(DHEA) and Dehydroepiandrosterone Sulphate(DHEAS) [58] had been used to explore their role in assessment maturation of an individual. Recently biomarkers have also been correlated with the dentition phase.

Human saliva can be easily obtained by non-invasive techniques. It contains many components of interest for screening, diagnosis and monitoring which include steroid and other non-peptide hormones, therapeutic drugs, drugs of abuse and antibodies. Saliva can be used as a diagnostic specimen not only to obtain information more economically and efficiently than serum, but also to provide information not readily available from serum testing.[59] The prime advantage of saliva is that it offers stress-free and real-time repeated sampling where blood collection is unwanted or complicated. It is well suited for paediatric and psychobiological studies. In addition, no special preparation or equipment is needed and subjects can conveniently collect samples themselves, if required. Also, salivary steroid levels reflect the circulating level of free steroid rather than total circulating levels.[60]

The main advantage of hormonal assessment is that multiple sample collection can be done in relative short interval of time when analysing the peak growth of an individual whereas one needs to take series of radiographs to assess the same when radiographic measures are used. However it can be argued that it is possible to obtain maximum information from pre -existing radiographs taken for routine orthodontic purpose such as a pre-treatment diagnostic

orthopantomogram may be assessed for the calcification stages of canine; a lateral cephalogram for assessment of cervical vertebrae maturation etc.

DHEA AND DHEAS

DHEA and DHEA-S are important endogenous steroid hormones produced from the adrenal gland.[61] They are known as neurosteroids as they are synthesized *de novo* in central nervous system.[62] DHEAS is the immediate metabolite of DHEA and the concentration of DHEAS is 100 to 1000-fold more than DHEA. Hence, measurement of DHEAS levels is more consistent and suitable when compared with measurement of DHEA. Both DHEA and DHEAS are believed to be the markers of adrenarche. They play an important role in bone growth and their correlation with the stages of hand wrist radiographs makes them a possible indicator of skeletal maturation in the assessment of growth status during adolescence.[58]

The concentrations of DHEA and DHEAS show variation with age.[63] DHEA is basically formed by placenta and hence, the foetus is exposed to it. Hormonal levels are high in early months of life which decline until 5 years of age and then rise rapidly from 7 years of age in girls and around 9 years in boys until it reaches the maximum value. This period of life is called as Adrenarche. However, it is considered as a separate entity from puberty as gonadropins and oestrogen does not have any effect on DHEA and the two events are not correlated over time.[64]

Precise assessment of the hormonal levels may be a practical diagnostic tool for determining growth status as these biomarkers do not show diurnal and nocturnal fluctuations, unlike other hormone levels. Moreover, minor gender hormonal differences can also be evaluated in cases where sexual dimorphism exists and when the clinician is in dilemma. It is measurable in serum and saliva. As salivary hormone levels reflect the serum hormone levels showing parallel patterns, measurement of steroid levels in saliva can be used as a useful diagnostic tool.

The collection methods of DHEA and DHEAS include passive drooling and cotton salivette. An important consideration in the saliva collection method exists due to the difference in hormone recovery from saliva which can vary by collection device. Specifically, hormones such as sex steroids and DHEA that are detectable in saliva are collected through the passive drool method because they adhere to the collection device and provide either falsely positive or negative results.[65-67]

A significant increase in the secretion of DHEA and its conjugate DHEAS marks the onset of puberty. Both of these hormones are present in circulation approximately 3 years prior to puberty. These hormones potentiate the action of growth hormone (GH) by stimulating the proliferation of epiphyseal cartilage. Also, DHEAS is found to promote bone deposition by means of osteoblasts through androgen receptor- mediated mechanisms and stimulate the alkaline phosphatase activity through tumour growth factor β expression to exert their mitogenic influence. Christain et al stated that DHEA and DHEAS have actions similar to dehydrotestosterone on human osteoblast metabolism. As hormones play a primary role in the initiation of puberty and are found to correlate with skeletal maturation, they might serve as a possible indicator of skeletal maturation in the assessment of growth status during adolescence.[58]

CONCLUSION

Accurate assessment of maturational stage should be an essential part of both diagnosis and treatment. Different authors had stated different methods in an attempt to determine the best indicator of maturity. Every method has its own advantages, disadvantages and limitation over the other method.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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