Review Article

Biochemical markers as skeletal maturity indicators

ABSTRACT

Precise estimation of the stage of skeletal growth is essential for the formulation of accurate treatment planning and employing orthodontic intervention through functional orthopedic appliances for the shortest time possible yielding stable results. Along with clinical and radiological techniques, biochemical markers play an important role in the growth assessment for differential treatment application. Isolation and characterization of various systemic and local factors having a significant role in the growth process provided us the sight to tap their potential to be used as skeletal maturity indicators. Different methods for the assessment of biomarkers in use are enzyme-linked immunosorbent assay, radioimmunoassays, and immunoradiometric assays. These methods of assessment of biochemical markers are noninvasive and when interpreted correctly give useful information. This article presents an overview of various biomarkers under research for predicting skeletal growth.

Keywords: Diurnal, gingival crevicular fluid, growth, puberty

INTRODUCTION

One of the most important aims of an orthodontist is to resolve skeletal discrepancies by utilizing the growth potential of the patient so that most favorable results could be achieved in a short period of time.[1] Response to orthopedic treatment modalities is maximum during the peak of adolescent growth spurt. Thus, precise estimation of growth status at different time intervals is prerequisite for preparing a well-timed and suitable treatment plan for each patient.[2]

Various methods have been developed over the years in an attempt to precisely recognize the skeletal maturity status of an individual with high sensitivity and viability. These include height,[3,4] weight,[5] chronological age,[5-7] sexual maturation,[7] radiographic methods,[8-12] and biomarkers.[13-17]

A biomarker is defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease."[18] Different methods for the assessment of biomarkers used in various studies were enzyme-linked immunosorbent assay,[19] radioimmunoassays,[15,20] and immunoradiometric assays[21] and were found to be comparably accurate.[22]

Access this article online	
Website: www.orthodrehab.org	Quick Response Code
DOI: 10.4103/ijor.ijor_37_16	

Skeletal growth and maturation is the outcome of complex interaction of many genes, hormones, growth factors, and in addition environment. Role of growth factors in craniofacial growth regulation is well recognized.[23] This paper presents a review of various markers identified for prediction of skeletal growth.

GROWTH HORMONE

Growth hormone (GH) is an anterior pituitary hormone which was first isolated in 1956 by Li and Papkoff.[24] It chiefly functions in the growth and development of craniofacial structures. GH receptors in the mandibular condyle have both direct and indirect effects on tissues with indirect effects mediated by insulin-like growth factor-I (IGF-1), generated in the liver in response to GH.[25]

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How to cite this article: Tripathi T, Gupta P, Rai P. Biochemical markers as skeletal maturity indicators. Int J Orthod Rehabil 2017;8:60-6.

GH levels oscillate within a day with maximum release taking place shortly after sleep onset coinciding with slow-wave sleep.[26] After infancy, frequency and amplitude of GH pulses decrease. At puberty, amplitude of GH release (pulsatile pattern) increases and it was found to be highest at this stage of life. After puberty, GH secretion decreases with age by around 14% per decade. GH levels have been found to be 15% of pubertal levels at middle age. A gender variation is also seen with higher levels found in women. Maximum GH concentrations are reached in early puberty in girls and late puberty in boys.[27-30]

GH secretion is affected by multiple factors. GH accentuates with physical exertion, trauma, hypoglycemia, increased temperature, infection, some amino acids (leucine and arginine), and some drugs such as clonidine, L-dopa, and γ-hydroxybutyrate, whereas it decreases with disruption of hypothalamus-pituitary axis, in obesity, with carbohydrate-rich diet and intake of β_2-adrenergic agonists.[29,31]

GH has been measured in both serum and urine. Average urine levels are between 100 and 1000 times less than in blood. Hence, more sensitive assays are required to measure GH levels in urine.[31] Approximately half of all GH molecules in serum are attached to GH binding protein. This binding interferes with GH quantification by immunoassays. The use of different antibodies in immunoassays having affinity to specific GH isoforms yields varying results. Moreover, they do not provide reliable information about amount of biologically active functional GH in circulation.[32] Due to continual diurnal variation in GH levels, it is difficult to be identified accurately in daytime sampling as well as in obese and elderly participants.[29]

INSULIN-LIKE GROWTH FACTOR-I

IGF-1 is an effective growth-stimulating factor which mediates many GH functions.[19,20] Liver is the principal source of circulating IGF-1 though it is produced locally by many tissues.[29] IGF-1 was first detected in serum but can be quantified in saliva and urine. Salivary IGF-1 levels reflect serum levels, but precise quantification is difficult as salivary levels are <1% of serum levels. Moreover, gingival fluid and blood contamination may yield false results.[21,22]

Serum IGF-1 levels demonstrate GH status, high in acromegaly and low in GH deficiency.[33] Serum IGF-1 levels tend to peak whenever there is accelerated growth in the body whether during pubertal growth spurt, adrenarche,[34] residual mandibular growth,[35] condylar hyperplasia,[36] or tumorous growth occurring in the body.[37] IGF-1 levels were found to be low in protein malnutrition, impaired cognitive function, hypothyroidism, coeliac disease, anorexia nervosa, and liver disorders.[38-40]

A study on mice revealed that GH and IGF-I, but not IGF-II, are necessary for the pubertal growth spurt. Rise in IGF-I causes longitudinal bone growth in the condyle with no effect on their histologic pattern.[35]

Its serum levels follow similar pattern to pubertal growth curve with a surge during puberty reaching highest levels at 16 years, which reduces by more than 80% with advancing age.[29] Studies have been conducted to correlate biochemical markers with skeletal growth status using the modified cervical vertebral maturation method of Baccetti *et al*.[8] It was observed that females have an earlier and shorter growth spurt showing sharp spike up to cervical staging (CS) 3 and rapid decline in IGF-1 levels up to CS6. Males, on the other hand, experience a later and longer growth spurt denoted by a steady increase in IGF-1 levels from CS1 to CS4 followed by a slow decline to CS6 with a relative plateau phase extending from CS3 to CS5.[41]

Both Juul *et al*. and Sinha et al. have reported prepubertal rise to reach peak levels during pubertal stage followed by postpubertal decline in humans.[42,43] Masoud *et al*. in Saudi population and Ishaq *et al*. in Egyptians showed similar results but with peak reached at CS5 and CS4, respectively.[35,44] Gupta *et al*. observed peak serum values at 14.08 years in males coinciding with CS4 stage and in female participants at CS3 at a mean age of 12.04 years.[41] Brabant *et al*. reported higher mean peak IGF-1 values in females as compared to males during adolescence.[45] Peak IGF-1 levels in Danish and Chinese girls were found at 14.5 years and 12–14 years and at 15.5 years and 14–16 years in boys, respectively.[35,44] Similar results with peak serum IGF-1 levels were found 1 year earlier in girls as compared to boys in Turkish population.[44]

A study showed IGF-1 to be better indicator of mandibular condyle growth as compared to long bones, but no correlation could be obtained between mandibular growth and IGF-1 levels as mandibular growth continues even after radiographic skeletal maturity. Among all methods of measurement, blood spot IGF-1 measurement, which is a contemporary method, less invasive with relatively prolonged sample stability at room temperature, and reported good correlation with serum IGF-1.[35]

IGF-1 is a better marker for estimating growth status as IGF-1 levels may not decline in obese individuals opposite

to GH. Furthermore, its levels do not vary throughout day unlike GH.[29]

PARATHYROID HORMONE-RELATED PROTEIN AND INDIAN HEDGEHOG PROTEIN

Parathyroid hormone-related protein (PTHrP) was originally established as the primary mediator of humoral hypercalcemia of malignancy.[46] PTHrP is synthesized at the periarticular ends of bones and affects adjacent chondrocytes carrying PTHrP receptors to retain their proliferation potential and slow down differentiation. Chondrocytes distant from the influence of PTHrP, however, differentiate and secrete Indian hedgehog protein (Ihh), which triggers further PTHrP release. This feedback system thus determines the width of the zone of chondrocyte proliferation. Ihh is secreted by prehypertrophic and hypertrophic chondrocytes after cessation of chondrocyte proliferation potential.[47] Both factors have been described in condylar cartilage even after the initiation of functional appliance therapy.[14]

PTHrP expression showed no significant variations during growth in rats,[48] whereas PTHrP expression showed 5-fold increase after mandibular advancement.[14] Increased levels of Ihh and PTHrP expression have been reported in early pubertal stages than in later stages of human growth plate by Kindblom *et al*.[49] However, Hussain *et al*. showed peak PTHrP levels at CS5 stage (late pubertal stage) with lower levels in early pubertal stages. Although stimulation of PTHrP and Ihh releases sustain growth during adolescence, varying results were obtained in regard to correlation of PTHrP levels with skeletal maturation during puberty and hence using PTHrP to predict skeletal growth accurately is not substantiated by sufficient evidence.[14]

DEHYDROEPIANDROSTERONE/DEHYDROEPIANDROSTERONE SULFATE

Dehydroepiandrosterone (DHEA) and its sulfated conjugate dehydroepiandrosterone sulfate (DHEAS) are steroid hormones secreted from adrenal gland during adrenarche, which is a period of 3 years before puberty. They stimulate gonadostat (pituitary and hypothalamus together) to initiate puberty.[17] In peripheral tissues, DHEA and DHEAS act as precursors of androgens and estrogens and hence, preventing the loss of androgens and estrogens into the circulation.[50,51] They speed up growth and proliferation of epiphyseal cartilage and increase the GH activity.[17]

DHEA is influenced by hypothalamic pituitary adrenal (HPA) axis and its levels increase in response to stress.[52] It also exhibits diurnal rhythm with highest levels in the morning and lowest in the late evening.[53] Circulating DHEA is weakly bound to albumin or sex hormone-binding globulin.[54,55] Unbound DHEA enters saliva through intracellular mechanisms, and the serum-saliva correlation has been reported to be high.[56,57]

Serum DHEAS levels are 100–1000 times greater than DHEA[17] due to greater half-life, slower clearance, and are strongly bound to albumin than DHEA and hence, do not exhibit diurnal variation unlike DHEA and yield more valid results.[50,53,58] DHEAS is a charged molecule,[57] and it is suggested to be actively transported through salivary membranes through organic anion transport polypeptides.[59] Salivary levels of DHEAS are found to be <0.1% of plasma levels.[57] Since serum DHEAS levels are 250 and 500 times higher than DHEA in women and men, respectively,[50] DHEAS salivary levels are high enough to be measurable. Its salivary levels decrease with increase in saliva flow rate.[57] Salivary estimation of DHEAS has been found to be unreliable.[17]

Serum DHEA levels peak around 20–30 years of age, then decrease to reach 20%–30% of peak level by the age of 70–80.[50] Serum DHEAS levels are high in newborn, after which the levels decline.[17] According to study by Apter *et al*., in girls, serum DHEA showed increase between 7.5 years to 12.5 years followed by a plateau up to 15.5 years of age, and then a continuous increase till 18.5 years while in boys, a progressive increase in DHEA was seen from 8.5 years to 12.5 years of age followed by rapid increase till 18.5 years.[60] Peaks were described in few studies with first peak in DHEAS concentration occurring between 6 and 8 years of age in both sexes and second peak at 11 years for females and 13 years in males. Srinivasan and Premkumar reported gradual rise in serum concentration at initiation of maturation and reached peak values after complete fusion of epiphysis and diaphysis of radius. Their findings revealed a significant association of DHEAS with skeletal maturation in relation to early maturation of girls than boys.[17] Although DHEAS has been reported to be a better indicator of skeletal maturity than DHEA, its validity and reliability to estimate the growth status needs to be established.

TESTOSTERONE, ANDROGENS, AND ESTROGENS

Testosterone (T) and estradiol (E_2) are the main circulating sex steroids acting on human male bone tissue. Testosterone is produced from the Leydig cells in the testis while the estradiol forms from aromatization of the androgens by aromatase.[61]

Estrogens reduce bone resorption by means of both direct and indirect effects on osteoclasts and act on osteoblasts, by inhibiting their apoptosis.[62] Threshold value of serum

estrogen to produce its effects lies in the suggested range of 15–25 pg/mL.[63] Androgen exerts direct effects through stimulation of androgen receptors and indirect effects through aromatization of androgens into estrogens,[64] which stimulate estrogen receptors (ERs) of nuclear receptor family, ERα and ERβ, and they both are expressed by human epiphyseal chondrocytes.[65] The role of GH and IGF-1 on skeletal growth could be even indirect through estrogens stimulation of GH and IGF-1 secretion.[63]

Sex steroids prepare the immature bone to develop in terms of size, structure, bone mineral density, and proportions to finally achieve skeletal maturity. Estrogens continue bone remodeling in adulthood with decline associated with bone loss from adult to aging life.[63] Androgen effects cannot be generalized for different species as a species difference exists in the regulation of skeletal changes.[66]

Serum E_2 was significantly higher and bone age more advanced in obese boys compared with healthy boys at the same pubertal stage. This could be due to the excess of adipose tissue in obese boys, which accounts for increased aromatization of androgens into estrogens and for the advancement of bone age.[67]

Serum E_2 increases simultaneously with T levels during puberty where estrogen in early puberty is associated with growth plate lengthening and during late puberty inhibits chondrocyte proliferation and stimulates chondrocyte differentiation, thus inducing the progressive ossification of the growth plate and its final disappearance.[63]

Commercially available assays are low in accuracy and reproducibility. Hence, at present, serum E_2 is not currently part of the work-up used for the clinical diagnosis.[63]

CORTISOL

Cortisol is the major glucocorticoid released from the adrenal cortex and is controlled by HPA axis. Cortisol stimulates GH production and secretion.[68,69] Cortisol follows a circadian rhythm,[70] reaches a peak in early morning and lowest levels at night.[71] Cortisol showed a seasonal effect, with the acrophase (peak time) occurring earlier in spring than in summer. Cortisol acrophase occurred later in the day for boys than for girls during later puberty.[72] Cortisol levels increase with stress, pain, illness, trauma, and in obese people. There is increased cortisol production in Cushing's syndrome and adrenal tumors and decreased cortisol production in adrenal insufficiency (e.g., Addison's disease) and adrenocorticotropic hormone deficiency.[73] Due to its low molecular weight and lipophilic nature, minute amounts enter saliva through intracellular mechanisms. Salivary cortisol levels are uninfluenced by salivary flow rate or salivary enzymes.[57] Studies have reported high correlations between serum and salivary cortisol levels.[74] Cortisol levels show sharp rise at pubertal spurt and gradual postpubertal increase with aging.[70] Study by Apter *et al*. showed postmenarche concentrations being significantly higher than premenarche levels in females, whereas in boys, a decrease was seen up to 12.5 years of age and an increase occurred from 16.5 years onward.[60]

All studies conducted so far could not correlate cortisol with skeletal growth status with sufficient evidence, and longitudinal studies are required with better methods of measurement, which are more sensitive and reliable.

ALKALINE PHOSPHATASE

Alkaline phosphatase (ALP) is a membrane-bound enzyme attached to glycosylphosphatidylinositol moieties located on the outer cell surface. Normally, in adults, 50% of total serum ALP is derived from liver and 50% from bone, whereas in children and adolescents, 90% of the ALP activity is bone specific. To accurately predict skeletal growth, detection of bone-specific ALP is required. However, due to cross-reactivity between liver and bone ALP, these assays may yield false-positive results. Moreover, patients with liver disease may aggravate this problem.[75]

As ALP is a marker for osteoblastic activity, its levels are higher at the time of growth as compared to the levels after the growth cessation. ALP increases up to age of 14 years in boys and 11 years in girls and rapid falls after that in both sexes to reach adult levels by 20 years in boys and 18 years in girls.[76]

Studies have been done which recorded the highest levels at infancy and puberty.[77] Christenson, Takimoto, and Insoft reported an increase in serum ALP levels during puberty[78] while Perinetti et al have reported two-fold peak increase in gingival crevicular fluid ALP levels.[79] Study correlating greatest common factor ALP, circumpubertal dentition phase, and chronological age could not produce significant association among these variables.[80] Tarvade *et al*. found a significant correlation of salivary ALP with MP3 skeletal maturation stages. They reported peak levels of salivary ALP in girls as well as in boys correlated with G stage of MP3 at the age of 13.[78] Krabbe *et al*. reported that bone mineral content and serum ALP were negatively correlated.[76] Thus, further studies are required to validate its use as a potential and reliable biomarker for estimating growth status.

OSTEOCALCIN

Osteocalcin, also known as bone γ-carboxyglutamic acid (Gla) protein, is Vitamin K-dependent protein of the bone.[81] It is produced by osteoblasts, odontoblasts, and hypertrophic chondrocytes and binds to hydroxyapatite.[82] Following release from osteoblasts, larger part is integrated into extracellular bone matrix and smaller part is released into circulation, available for detection by immunoassays.[75] Furthermore, upon osteocalcin breakdown, Gla is excreted in urine, which is also a substrate for assays for growth estimation.[81] Diurnal variation is also seen where osteocalcin levels fell during the morning, rose in the afternoon and early evening, and reached a peak nocturnally.[83] Osteocalcin elevates in primary hypoparathyroidism, remains low in untreated hypoparathyroidism, but normal in hypoparathyroidism (including pseudohypoparathyroidism) during Vitamin D treatment.[84] Its level increased significantly with age, body weight, height, and bone age until age 12–13 years in girls and 14–15 years in boys.[85] According to Kirmani *et al*., serum osteocalcin increased early in puberty and peaked at 14 years of age but declined after the age of 14 years.[86] Osteocalcin is a potential biomarker, which can predict growth status with the development of more sensitive assays.

CONCLUSION

Ossification sequence and timing of the skeletal maturity with radiographic growth indicators show polymorphism and sexual dimorphism, which limit their clinical use. Moreover, ethical issues concerning additional radiographic exposure have resulted in profound research with biochemical markers to employ them to assess growth status of an individual. Short half-life, pulsatile secretion, diurnal variation, and effects of environment on secretion of hormones have perplexed the scientific world. In addition, gingival fluid is liable to be contaminated with blood or saliva, resulting in inaccurate results. Urinary samples demand greater patient cooperation as it would be embarrassing for the patient and contamination of sample can also occur. Moreover, not all biomarkers can be estimated with any single assay and not all assays give similar results with any particular biomarker. It is hard to standardize any particular value for a biomarker at a particular growth phase due to prevailing variation and cross-sectional studies cannot provide sufficient data.

Hence, longitudinal studies with better measurement assays are required for accurate assessment of growth status using biochemical markers.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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