Insulin-like Growth Factor I as a Skeletal Maturity Indicator

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ABSTRACT

Introduction: Accurate determination of skeletal maturity and remaining growth is crucial to many orthodontic, orthognathic and dental implant timing decision. Cervical vertebral stages and hand-wrist radiographs are currently used to identify peak mandibular bone growth. These are highly subjective techniques that not only involve radiographic exposure but also lack the ability to determine the intensity of growth spurt and the end of the growth. Insulin-like growth factor I (IGF-I) is a circulating growth hormone-dependent factor whose level correlates with sexual maturity and is used to diagnose growth hormone deficiency and excess. IGF-I level was correlated with cervical skeletal maturity and would be highest at cervical stages that correspond to the greatest amount of facial growth.

Materials and methods: Blood sample of 90 patients between the age group of 10 and 25 years of both sexes and their lateral cephalogram were taken.

Results: The results showed that the IGF-I levels were low at prepubertal levels, (CS 1, CS 2, CS 3). It increased gradually, peaking during puberty, showing maximum values (CS 4, CS 5), which later dropped to reach prepubertal values (CS 6).

Conclusion: Blood spot IGF-I correlates well with skeletal age as determined by radiographic techniques. IGF-I can act as a potential skeletal maturity indicator without the hazard of additional radiographic exposure.

Keywords: Cervical vertebrae, IGF-I, Skeletal maturity.

INTRODUCTION

Developmental status of a child is usually assessed in relation to events that take place during the progress of growth. Thus, chronological age, dental development, height and weight measurements, sexual maturation characteristics and skeletal age are some of the biological indicators that have been used to identify stages of growth. The use of dental age as an indicator for maturity is simple but not so accurate because of wide variations in time of eruption of teeth due to the influence of local and environmental factors. Accurate determination of skeletal maturity and remaining growth is crucial for many orthodontic, orthopaedic, orthognathic and dental implant timing decisions. Chronological age and dental age do not exactly correlate with sexual maturity. Cervical vertebrae stages and hand-wrist radiographs are currently used to identify peak mandibular bone growth.

Using hand-wrist radiographs to assess skeletal maturity was first described by Todd in his 1937 publication. The routine use of hand-wrist radiographs has recently been questioned due to ethical issues. Additional radiation exposure is the primary concern. Hand-wrist radiographs have been shown to be poor predictors of the end of mandibular growth. Residual facial growth is of particular importance to relapse from orthopedic and orthognathic surgical corrections, as well as osseointegrated dental implant placement.

Since lateral cephalometric radiographs are routinely taken for orthodontic patients, using the cervical vertebrae from these radiographs to assess skeletal maturity has been especially appealing to orthodontists. These are highly subjective techniques that not only involve radiographic exposure but also lack the ability to determine the intensity of the growth spurt and the end of growth.

In 1957, insulin-like growth factor I (IGF-I) was discovered by Salmon and Daughaday. Insulin-like growth
factor I is a circulating growth hormone-dependent factor whose level correlates with sexual maturity.

Insulin-like growth factor I is measurable in serum (in which it was first detected) as well as in urine and saliva. Salivary IGF-I levels reflect its levels in the plasma. However, salivary IGF-I levels are extremely low: less than 1% of serum levels. This makes accurate measurements difficult.1

IGF-I mediates most of the physiological actions of Growth Hormone on bone growth. It is reported that IGF-I rapidly activates bone turnover, as it is the major factor that affects linear bone growth. There is significant correlation between sex hormones and IGF-I. The estradiol levels of girls and testosterone levels of boys differed significantly between pubertal stages and increased with the proceeding stages. In both the sexes, serum IGF-I levels were significantly correlated with sex steroid levels.3

It was reported that mean serum IGF-I (somatomedins) concentrations increased slowly in prepubertal children, with a further steep increase during puberty; after puberty, a subsequent continuous fall in circulating IGF-I levels were apparent through adulthood.4

It is reported that IGF-I rapidly activates bone turnover and insulin-like growth factor-binding protein 3 (IGFBP-3), the major binding protein of IGF-I that has a direct role in the endocrine regulation of bone metabolism. In puberty, both total IGF-I and IGFBP-3 serum levels increase. Furthermore, the molar ratio between IGF-I and IGFBP-3 increases in puberty, suggesting that free IGF-I increases in puberty when growth velocity is high.5

Insulin-like growth factor I (IGF-I) is a mediator for growth hormone (GH) that plays an essential role in both local and systemic regulation of bone growth. Recent studies have shown that the condylar cartilage is highly sensitive to the changes in IGF-I concentrations. Several growth studies demonstrated that serum IGF-I levels reflect serum GH levels but without the fluctuation involved with the latter. Therefore, IGF-I levels have been used by endocrinologists to diagnose GH disturbances. Serum IGF-I levels have also been related to chronologic age and sexual maturity stages, and have been shown to peak late in puberty. This peak is mainly due to the stimulation of GH secretion by adrenal and gonadal steroids.6

Blood spot IGF-I measurement is a relatively new, minimally invasive technique and has an excellent correlation with regular serum IGF-I. In addition, the samples are stable at room temperature for up to 2 weeks. IGF-I levels can also be measured from salivary samples. The disadvantage was that there was variation of the values throughout the day.7

In this study, an attempt was made to correlate mean IGF-I levels and cervical vertebrae stages, to assess the IGF-I levels in the prepubertal, pubertal and postpubertal orthodontic patients, to correlate IGF-I levels to skeletal maturity by using the cervical vertebral stages and to compare the mean IGF-I levels at each of the stages, to identify if there is any gender variation in the IGF-I values among the different CS. Also, a hypothesis was made that IGF-I levels would also correlate with cervical stages that corresponds to the greatest amount of facial growth.

**MATERIALS AND METHODS**

Blood spot samples of 90 individuals were collected of both the sexes between age group of 10 and 25 years, and their corresponding lateral cephalograms were obtained. The samples were divided into four groups based on the age, which were between 10-13 years, 14-17 years, 18-21 years and 22-25 years. Cervical vertebrae was divided into six stages: CS 1 to CS 6, which were initiation, acceleration, transition, deceleration, maturation and completion stages. Out of the 90 individuals, 49 (55%) were females and 41 (45%) were males.

Patients included in the study were the ones to begin orthodontic treatment, patients undergoing orthodontic treatment, and post-treatment follow-up cases. Exclusion criteria included patients with systemic illness (liver and kidney disease), growth abnormality and bleeding disorders. As IGF-I is synthesized in the liver and metabolized in the kidney, patients with liver and kidney disorders were not included in the study.

Informed consent was taken from the patient. Parent’s or guardian’s blood sample was collected using sterile lancet, under aseptic precaution using ‘S and S 903 filter paper’. Samples were stored at −20° in a deep freezer. Samples were sent for laboratory evaluation of IGF-I level by radioimmunoassay. Active IGF-I ELISA kit was used for immunoassay.

The CS (cervical stage) technique as described by Baccetti et al was used to stage the cervical vertebrae. In this technique, the second, third and fourth cervical vertebrae were examined. Curvatures were defined when the depth of curvature was greater than 1 mm. Linear correlations were performed to determine the IGF-I trends relating to the various cervical skeletal maturation stages.8

IGF-I enzyme-linked immunosorbent assay (ELISA) was used for the quantitative measurement of IGF-I in serum or plasma. It is an enzymatically amplified ‘one-step’ sandwich-type immunoassay. The assay included an extraction step in which IGF-I was separated from its binding protein in serum.

**Extraction Procedure**

The samples were labelled with Laboratory IDs and a template was made. The dried blood spots (DBSs) are punched into the round-bottomed microplate. To the dried blood spots,
225 ml of extraction solution was added and, after shaking it well, was incubated for 60 minutes at room temperature. During the extraction period, fifty microliters of neutralizing solution was added to all tubes labeled for neutralization. After neutralization, 100 ml of sample dilutents was added to another dilution microplate. Fifty microliters of neutralized extract was added to the corresponding dilution microplate and shook well.

**Assay Procedure**

The microtitration strips to be used were marked. Twenty microliters of each standard, control and diluted unknown extract were pipetted to the appropriate wells.

The antibody enzyme conjugate solution was prepared by diluting the IGF-I antibody enzyme conjugate concentrate with the assay buffer. One hundred microliters of antibody enzyme conjugate solution were added to each well using a dispenser. The wells were incubated shaking at a fast speed (500-700 rpm) on an orbital microplate shaker, for 2 hours at room temperature. It was aspirated and each well was washed with the wash lotion using an automatic microplate washer. It was then blot dried by inverting the plate on absorbent microplate washer. One hundred microliters of the TMB chromogen solution were added to each well using a semi-automatic dispenser. The wells were incubated, shaking at a fast speed (500-700 rpm) on an orbital microplate shaker, for 10 minutes at room temperature. One hundred microliters of the stopping solution to each well were added using a semi-automatic dispenser.

The absorbance of the solution in the wells within 30 minutes was read, using microplate reader set at 450 nm. Analysis of variance (ANOVA) and posthoc test was used to compare mean IGF-I levels and corresponding cervical maturation stages. The proportion was compared using Chi-square test of significance. In all the above tests, p-value less than 0.05 was taken to be statistically significant. The data were analyzed using statistical package for social science (SPSS, V 10.5).

**RESULTS**

The results showed that the IGF-I levels were low at prepubertal levels (CS 1, CS 2, CS 3), it increased gradually, peaking during puberty showing maximum values (CS 4, CS 5), which later dropped to reach prepubertal values (CS 6). The mean values of IGF-I (ng/ml), in CS 1 stage,  

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IGF-I: Insulin-like growth factor I; CS: Cervical staging

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was 108.24 ng/ml. In CS 2 stage, the mean IGF-I level was 142.80 ng/ml. In the CS 3 stage, it was 146.88%, in CS 4 the mean IGF-I level increased to 220.74 ng/ml and it further increased to 270.98 ng/ml in CS 5. In CS 6 stage, the mean IGF-I level had decreased, showing values of 119.66 ng/ml (Table 1).

Comparison of mean IGF-I (ng/mg) according to CS stage by gender were as follows: in males, it was 100.94 ng/ml in CS 1 stage, 130.36 ng/ml in CS 2, 148.69 ng/ml in CS 3, 218.04 ng/ml in CS 4 stage, 301.38 ng/ml in CS 5 and 120.61 ng/ml in CS 6 stage, while in females, the mean IGF-I levels were 117.01 ng/ml in CS 1, 144.87 ng/ml in CS 2, 144.78 ng/ml in CS 3, 222.32 ng/ml in CS 4, 248.88 ng/ml in CS 5 and 118.62 ng/ml in CS 6 (Table 2).

**DISCUSSION**

IGF-I levels are low at the prepubertal stages of cervical development. There was an increase in IGF-I levels from CS3 to CS4, and maximum at CS5 stage. There was a decline between CS5 and CS6 stages. Mean IGF-I levels at CS4 and CS5 were significantly higher than at the other stages. These findings can be compared with the study done by Mohamed Masoud in which the mean IGF-I level in CS 1 was 182.0074 microgram/L. In CS 2, it was 212.1 microgram/L. In CS 3, it was 208.68 micrograms/L and in CS 4, the mean IGF-I levels were 335.89 micrograms/L, in CS 5, it was 406.82 micrograms/L and 199.75 micrograms/L in CS 6. In addition to radiation exposure and the subjectivity of staging the X-rays, an inherent disadvantage of hand-wrist radiographs and cervical vertebra staging is that the final stage of development does not necessarily indicate the completion of growth, especially mandibular growth. Several studies have shown that mandibular growth continues after radiographic skeletal maturity.

Our results showed that IGF-I levels were still relatively high in many subjects, who were at CS 6 and had supposedly completed their growth. In that stage, we found that IGF-I levels were negatively correlated with time since the onset of puberty. Thus, IGF-I might be a good indicator of residual mandibular growth. Further studies of longitudinal data are underway to evaluate this.

Optimal treatment timing in orthodontics and dentofacial orthopedics can be assessed and determined by skeletal maturation. It has been advocated that orthopedic treatment of Class III malocclusion for maxillary protraction is more effectively performed at the prepubertal stage than at puberty. Thus, if maxillary protraction is indicated, treatment should be performed before CS stages I and II. At these stages, the IGF-I level is lower.

Pubertal growth begins at CS III which correlates with the beginning of increase in IGF-I levels. Between CS III and CS IV, there is a rapid increase in IGF-I levels. Peak mandibular growth occurs between CS III and CS V when the IGF-I levels are the maximum.

IGFBP-3 is a carrier for IGF-I, meaning that IGF-I binds IGFBP-3, creating a complex whose combined molecular weight and binding affinity allow the growth factor to have an increased half-life in serum. Without binding to IGFBP-3, IGF-I is cleared rapidly through the kidney, due to its low molecular weight. But when bound to IGFBP-3, IGF-I evades renal clearance. Also, since IGFBP-3 has a lower affinity for IGF-I than IGF-I has for its receptor, IGFR, its binding does not interfere with IGF-I function. For these reasons, an IGF-I/IGFBP-3 combination approach was approved for human treatment. IGF-I has also been shown to be effective in animal models of stroke when combined with Erythropoietin. Both behavioral and cellular improvements were found.

IGF-I administration is used for the treatment of retarded growth or growth failure.

IGF-I hormonal therapy has a pathogenic role in cancer. Studies have shown that increased levels of IGF lead to increased growth of existing cancer cells. Studies have shown chronic IGF-I administration leads to mitochondrial dysfunction and reduced cell viability.

Insulin-like growth factor I is a mediator for growth hormone (GH) that plays an essential role in both local and systemic regulation of bone growth. IGF-I levels have been shown to depend on GH before puberty. However, during puberty, IGF-I levels can also be independent of GH because IGF-I production can be directly stimulated by androgens.

Recent studies have also shown that the condylar cartilage is highly sensitive to changes in IGF-I concentrations. Several growth studies demonstrated that serum IGF-I levels reflect serum GH levels but without the fluctuation involved with the latter. Therefore, IGF-I levels have been used by endocrinologists to diagnose GH disturbances. Serum IGF-I levels have also been related to chronologic age and sexual maturity stages, and have been shown to peak late in puberty. This peak is mainly due to the stimulation of GH secretion by adrenal and gonadal steroids.

The advantages of using IGF-I immunoassay method for assessment of skeletal maturity over other radiographic techniques was accuracy. Inter-examiner and intra-examiner variations are ruled out, which is seen with the radiographic techniques. Additional radiographic exposure to the patient is avoided. Residual mandibular growth can be accurately assessed using this technique.
The disadvantages include difficulties in storage and transport of the samples to the laboratory for analysis. The patient cooperation for collection of sample among the younger children is difficult. Also this technique is not very cost effective.

CONCLUSION

• Blood spot IGF-I correlates well with skeletal age as determined by radiographic techniques.
• Blood spot IGF-I levels are low in the prepubertal cervical stages.
• They rise sharply to their peak during pubertal CS stages, and then decline to prepubertal levels.
• IGF-I can act as a potential skeletal maturity indicator, without the hazard of additional radiographic exposure.
• Longitudinal data are needed to confirm the usefulness of this technique to accurately determine the timing of a patient’s growth spurt and to determine whether IGF-I levels are good predictors of residual mandibular growth.
• In most of the CS stages, the mean IGF-I values were higher in females compared to males, but not significantly higher.

REFERENCES