Serum and urine insulin-like growth factor-1 as biochemical growth maturity indicators

Mohita Sinha,[a] Tulika Tripathi,[b] Priyank Rai,[c] and Santosh Kumar Gupta[d]
New Delhi, India

Introduction: Biochemical markers are agents directly involved in bone growth and remodeling and can be quantitatively evaluated from various biologic fluids. The aim of this study was to assess the changes in the levels of insulin-like growth factor-1 (IGF-1) in serum and urine as a growth maturity indicator and to compare them with the cervical vertebral maturation radiographic stages. **Methods:** The study was conducted with 72 female subjects aged 8 to 20 years. Cervical vertebral maturation stages, and serum and urine IGF-1 levels were recorded for all subjects, and the subjects were equally divided into the 6 cervical vertebral maturation groups. Median values of IGF-1 for each stage of cervical vertebral maturation were calculated and statistically compared with those of the other stages. **Results:** The levels of serum and urine IGF-1 at stage 4 of cervical vertebral maturation were significantly higher than those from the other stages ($P < 0.01$). Stage 4 corresponded to a mean age of 13.67 years. A significant correlation was observed between serum and urine IGF-1 ($P < 0.001$). **Conclusions:** Urine IGF-1 follows the growth curve similar to serum IGF-1. Thus, urine IGF-1 may be regarded as a promising noninvasive tool for growth assessment. Further research is necessary to validate these results in a different population and with a larger sample. (Am J Orthod Dentofacial Orthop 2016;150:1020-7)

Identification of skeletal maturity—ie, the growth phase—with particular regard to the onset of the pubertal growth spurt, has major clinical implications when dealing with orthodontic treatment in growing subjects, especially when there are skeletal disharmonies.[1,2]

The use of the cervical vertebrae has the advantage of not requiring an additional radiograph.[3] However, the assessment of the maturational stage depends on the subjective evaluation and perception of the clinician, thereby questioning its repeatability and validity.[4]

Newer possibilities might be provided with biochemical markers representing agents that are directly involved in bone growth and remodeling.[5] Levels of biochemical markers of bone formation and resorption change with longitudinal bone growth and remodeling, and these changes are related to the pubertal stages.[6] Biomarkers have the advantage of avoiding invasive x-ray exposure. They can be measured from various biologic fluids such as blood, saliva, and urine, thereby overcoming the subjectivity associated with radiographs.

Insulin-like growth factor-1 (IGF-1) mediates most of the physiologic actions of growth hormones and is the major effector of bone growth.[7] IGF-1 accelerates growth, differentiation, and substrate synthesis activities in the osteoblasts and chondroblasts.[8]

IGF-1 is measurable in serum[9,10] (in which it was first detected) as well as in urine[11] and saliva.[12] Studies conducted on serum IGF-1 have reported that its levels in children and adolescents follow a pattern that is closely related to the pubertal growth curve: low in the prepubertal stages followed by a sharp increase at puberty and returning to lower baseline values after pubertal growth.[6,10,13,14]

Hizuka et al[11] were the first to demonstrate IGF-1 in urine, and they stated that the quantity of IGF-1 in urine was altered in patients with either growth hormone excess or growth hormone deficiency. Although IGF-1 was initially detected in urine about 27 years ago and has been shown to be lower when compared with that of

[a]Postgraduate student, Department of Orthodontics and Dentofacial Orthopaedics, Maulana Azad Institute of Dental Sciences, New Delhi, India.
[b]Professor and head, Department of Orthodontics and Dentofacial Orthopaedics, Maulana Azad Institute of Dental Sciences, New Delhi, India.
[c]Professor, Department of Orthodontics and Dentofacial Orthopaedics, Maulana Azad Institute of Dental Sciences, New Delhi, India.
[d]Director and professor, Department of Biochemistry, Maulana Azad Medical College, New Delhi, India.
All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
Address correspondence to: Mohita Sinha, 4th Floor, Department of Orthodontics and Dentofacial Orthopaedics, Maulana Azad Institute of Dental Sciences, New Delhi, 110002, India; e-mail, sinha_m7@yahoo.co.in.
Submitted, August 2015; revised and accepted, April 2016.
0889-5406/$36.00

http://dx.doi.org/10.1016/j.ajodo.2016.04.028

serum under healthy conditions, no investigation has evaluated the possibility of using this urinary parameter as a clinical diagnostic aid in dentistry and orthodontics.[15,16]

The aims of this study were to assess the changes in the levels of IGF-1 in serum and in urine as growth maturity indicators and to compare them with the cervical vertebral maturation index (CVMI) radiographic stages. Furthermore, we intended to study the relationship between serum and urine IGF-1.

MATERIAL AND METHODS

This cross-sectional clinical study was conducted on female subjects aged 8 to 20 years who came to the out patient department at the Department of Orthodontics and Dentofacial Orthopaedics, Maulana Azad Institute of Dental Sciences, New Delhi, India. Approval for the study was obtained from the research ethical committee of the institute. The subjects and their parents were informed about the research plan through a bilingual patient information sheet, and written informed consent was obtained for collection of blood and urine samples and appropriate radiographs. Selection of the subjects was done on the basis of the following inclusion criteria: absence of systemic (acute or chronic) disease, growth abnormality, or bleeding disorder; no history of chronic medication; and no history of any trauma or surgery in the area of the cervical vertebrae.

The sample size estimation performed at the 5% level of significance ($\alpha = 0.05$) with power of 80% showed that a minimum of 10 subjects per CVMI group was necessary. With further inclusion of a 20% attrition rate, the total number of subjects finally evaluated was 72, with 12 per CVMI group.

Personal information and history were recorded, and standardized lateral cephalograms were taken of all subjects in natural head position for assessing the cervical vertebral maturation stages. The criteria of Hassel and Farman[17] were used to evaluate the cervical vertebral radiographic morphology. These criteria involve an evaluation of the morphologic features of the cervical vertebral bodies restricted to those that are visible on the lateral cephalogram even when a protective collar is worn.[1,17] The purely subjective assessment is easy to learn and simple to apply clinically.[4] Hassel and Farman's assessment is based on the change in shape of the vertebral bodies, mainly the height-width ratio and the appearance of the inferior concavity. These features have been shown to progress during ontogeny in a caudal direction, from stages 2 to 6.[18]

To reduce the subjective variability in CVMI stage assessment, 2 examiners (M.S., T.T.) reviewed the 72 radiographs. They were blinded to each subject's age and pubertal status to ensure interexaminer reliability. The same examiners revaluated the radiographs a week later for intraexaminer reliability.

For the subjects, 5 mL each of blood and random morning midstream urine samples were collected on the day of the radiographs. The blood samples were centrifuged to separate the serum from blood. The urine and serum samples were then pipetted out separately using individual tips, into plastic Eppendorf tubes (Eppendorf, Hamburg, Germany), which were stored in a sealed plastic box in a deep freezer at −80°C until assay. On the day of the assay, all the samples were brought to room temperature, and the urine samples were ultracentrifuged. During the entire period of sample collection, utmost care was taken to prevent multiple freeze-thaw cycles before the final assay procedure.

Measurements of serum and urine IGF-1 levels were made using human IGF-1 ELISA (RayBiotech, Norcross, Ga) kits. The kit uses an in-vitro sandwich enzyme-linked immunosorbent assay for the quantitative measurements of IGF-1 in serum, plasma, cell culture supernatants, and urine, with an antibody specific for human IGF-1 coated on a 96-well plate. The kit has a coefficient of variation for intra-assay reproducibility of less than 10%. We constructed the standard calibration curves for the ranges corresponding to serum and urine IGF-1 values. By testing duplicate samples, we estimated the average errors across 18 observations to be about 0.15 ng per milliliter for urine IGF-1 and about 15 ng per milliliter for the serum IGF-1. The urine IGF-1 assay was followed by creatinine estimation using an auto analyzer, to rule out any renal disorder.

Statistical analysis

The kappa statistic was used to measure the interexaminer and intraexaminer reliabilities. The Kruskal-Wallis test was used to compare the mean ranks of serum IGF-1 (ng/mL) and urine IGF-1 (ng/mL) across the different stages of the CVMI. The Mann-Whitney U test was used for pair-wise comparisons between the values of each study variable (serum IGF-1, urine IGF-1) across all the possible combinations of CVMI stages. The Spearman correlation coefficients were determined between serum IGF-1 and urine IGF-1 levels. All statistical analyses were performed using SPSS software (version 17; SPSS, Chicago, Ill).

RESULTS

We calculated the interexaminer kappa statistics for both weeks and found the average to be 0.89. We computed the average intraexaminer kappa statistic to be 0.91, thus showing no statistically significant difference between the examiners' readings.