Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2024; 24:e230146
https://doi.org/10.1590/pboci.2024.047
ISSN 1519-0501 / eISSN 1983-4632

ORIGINAL ARTICLE

Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor Binding Protein-3 – Biomarkers for Skeletal Maturity Assessment in Class II Malocclusion

Arif Yezdani[1], Kesavaram Padmavathy[2], Kishore Kumar[1]

[1]Department of Orthodontics and Dentofacial Orthopedics, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.
[2]Department of Microbiology, Research Laboratory for Oral-Systemic Health, Sree Balaji Dental College & Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Corresponding author: Arif Yezdani **E-mail:** arifyezdani@yahoo.com

Academic Editor: Fátima Regina Nunes de Sousa

Received: July 26, 2023 / **Review:** September 14, 2023 / **Accepted:** December 07, 2023

How to cite: Yezdani A, Padmavathy K, Kumar K. Insulin-like growth factor-1 and insulin-like growth factor binding protein-3 – Biomarkers for skeletal maturity assessment in Class II malocclusion. Pesqui Bras Odontopediatria Clín Integr. 2024; 24:e230146. https://doi.org/10.1590/pboci.2024.047

ABSTRACT

Objective: To estimate the serum levels of non-radiologic biomarkers, Insulin-like Growth Factor-1 (IGF-1), and Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) to potentially identify the pubertal growth spurt in skeletal Class II malocclusion subjects. **Material and Methods:** Eighty subjects (M-38, F-42) with skeletal Class II malocclusion in the age range of 11-18 years were recruited for the cross-sectional study. Human serum IGF-1 and IGFBP-3 were quantitatively assessed by enzyme-linked immunosorbent assay, and the cervical stage (CS) was evaluated from a lateral cephalogram. **Results:** Gender-wise comparison of the mean serum IGF-1 levels revealed that the initial peak was detected at CS2 in both genders, [males (87.87 ng/mL), females (78.49 ng/mL)]. However, there was a cognizable difference in the second peak of the mean serum IGF-1 levels between males (CS5, 68.58 ng/mL) and females (CS4, 74.63 ng/mL). Mean IGFBP-3 serum levels in male subjects were high in CS4 (47.24 ng/mL) with a further spike in CS6 (50.54 ng/mL), and in female subjects, it was found to be highest in CS3 (51.95 ng/mL) and then in CS5 (49.68 ng/mL). **Conclusion:** Mean IGF-1 levels exhibited both sexes' prepubertal and late pubertal spikes. Mean IGFBP-3 levels revealed a pubertal and a late pubertal spike in both sexes, with an earlier growth trend observed specific to females compared to males.

Keywords: Malocclusion, Angle Class II; Growth and Development; Puberty; Cervical Vertebrae.

Introduction

Growth needs to be accurately assessed to correct developing skeletal jaw discrepancies. Genetic and environmental factors primarily affect the adolescent growth spurt. Since this spurt happens a little earlier in girls than boys, growth-related clinical decisions need to be implemented gender-wise [1]. Poor predictors of pubertal growth spurt are chronologic and dental age [2-3]. Skeletal maturity can also be assessed by MP3 stages on periapical x-ray films [4], hand-wrist radiographs [5-6], and cervical vertebral maturation (CVM) assessment on lateral cephalograms [7-8].

Radiographic exposure, complexity in identifying landmarks, inability to identify peak mandibular growth, high staging subjectivity, inter- and intra-observer variability, and failure to determine the end of growth spurt accurately were the inherent disadvantages of radiographic methods. Non-radiologic biomarkers seemed to address the above limitations, and thus, their use has been reported widely in the literature [9-11].

Insulin-like growth factors belong to a group of insulin-related peptides. It mediates the Growth Hormone (GH) function, mediating the linear growth-promoting effect and the pituitary GH protein anabolic effect. GH induces the liver to secrete IGF-1 and regulates paracrine production in many other tissues [12]. IGF-1 is also secreted by cartilaginous cells and other tissues [13]. IGF-1 actions were observed to be dependent and independent of GH during prepubertal growth, as during that period, it is directly stimulated by androgens. Animal experiments have reiterated that GH and IGF-1 are essential for pubertal growth spurt [14]. IGF-1 systemically and locally controls the bone's prenatal and postnatal longitudinal growth. The femoral head is less sensitive and responsive to IGF-1 than the condyle [15]. Experiments on mature rat condyles have shown that endochondral bone formation can be reactivated and stimulated with local injection of IGF-1 into their articular capsule [16]. Despite GH values being increased, deficiencies in IGF-1 values have been observed to cause retardation of growth.

IGF-1 serum levels in prepubertal subjects were reported to be unaffected by body mass index. It showed a pattern of a slow prepubertal increase, a steep rise circa puberty & a dip post-puberty [17]. An organism's growth and development depend on IGF-1 but also IGF-2 and IGF-1 receptors. While IGF-2 is only necessary prenatally, IGF-1, by exhibiting an endocrine vs. autocrine/paracrine function, is important both prenatally and postnatally. IGF-1 receptors were predominantly found in the posterosuperior and superior areas of the condyle cartilage and the condylars' fibrous articular surface. This led to the conclusion that the postnatally condylar cartilage growth and development were not GH-dependent but IGF-1-dependent [18]. Late pubertal stages exhibited higher levels. Compared with cervical vertebral maturational indicators (CVMI), a significant positive linear correlation was observed from pre to late stages of puberty with a contrasting negative one from late to post-pubertal [19]. Bone Alkaline Phosphatase & IGF-1biomarkers expressed increased levels at the 3rd stage of CVMI, with the mandibular growth pattern unaffected by changes in both markers [20].

In plasma, a large percentage of IGFs (99%) are combined as a complex with six binding proteins [21]. IGFBP-3 is the predominant binding protein among IGFBPs, regulating the half-life, bioavailability, and transport of IGF-1. IGFBP-3 carries 80% of IGF-1 in circulation and is regulated to a certain degree by it, though primarily by GH [22].

Produced mainly by the liver, GH controls its synthesis and shows only minor fluctuations in its levels throughout the day; hence, it qualifies as a dependable measure of the production of GH. Compared to IGF-I or GH, which showed considerable variability, IGFBP-3 produced reproducible results during repeated testing, thus making it an interesting parameter for evaluating the GH-IGF axis. It was also less sensitive to GH regulation than IGF-1 [23]. Serum levels of IGFBP-3 increased from childhood to puberty, exhibiting a positive

correlation with birth weight [24]. IGFBP-3 is partly controlled by IGF-1 [25]. Animal experiments showed that IGFBP-3 was involved in mandibular adaptation during functional orthopedic therapy [26]. IGFBP-3 and IGF-1 concentrations primarily depend on age, nutrition & other factors.

In IGF-1 generation tests, compared to IGF-1, IGFBP-3 behaved as a more exact differentiator of those parameters dependent on GH [27]. An increase in IGF-1 in an active and free biological form in circulation was also commensurate with a molar ratio increase between IGFBP-3 and IGF-1. In children, IGFBP-3 serum levels were found to increase simultaneously with a concomitant increase in age with the attainment of the highest levels circa puberty [28]. IGFBP-3, IGF-1 and their ratio have also been reported in the literature as biomarkers with good potency for pubertal growth spurt assessment [29]. Thus, the study aimed to assess the IGF-1 and IGFBP-3 serum levels and correlate them with the cervical vertebral maturational stages to identify the pubertal growth spurt in subjects with skeletal Class II malocclusion.

Material and Methods

Study Design

The Institutional Ethics Committee of Sree Balaji Dental College and Hospital, Chennai, reviewed and approved this cross-sectional study (Approval No: SBDCH/IEC/08/2017/2 dated 25/10/2017). A systematic sampling method was adopted to recruit the study participants who reported to this teaching hospital's outpatient department of Orthodontics and Dentofacial Orthopedics. Sample size calculation was carried out based on the results of our pilot study. The formula: $n = (Z\alpha/2+Z\beta)2 *2*\sigma2 / d2$, with $\alpha = 0.05$ and $\beta = 0.20$ at 95% confidence and a power of 80% was used for the calculation of the minimum sample size using G*Power software Version: 3.1.9.4 (Franz Faul, Universitat Kiel, Germany). The minimum sample size required was 76, rounded off to 80 for the study.

A total of 80 subjects (Males = 38, Females = 42) who fulfilled the inclusion and exclusion criteria were recruited for the study. The inclusion criteria were patients with skeletal class II malocclusion of both genders in the age group of 11-18 years. The exclusion criteria were patients with bleeding disorders, growth abnormalities, endocrinal disorders, and a history of facial trauma/injury. Written informed consent was obtained from the study participant's parent/guardian before recruitment.

Data Collection

The complete case history of each patient was recorded along with information regarding the pubertal status of the subjects from the parents concerned. An extraoral and intraoral clinical examination and a clinical Visualized Treatment Objective (VTO) were performed. A lateral cephalogram was taken for the study subjects, and lateral cephalometric analysis was carried out to confirm the skeletal Class II pattern. Morphological evaluation of the 2nd, 3rd, and 4th Cervical Vertebrae (CV) was carried out by two independent investigators through visual inspection of the lateral cephalograms. Cervical Stage 1 was not identified in the age category considered. Two observers analyzed all the lateral cephalograms independently, and the CVM stages were identified per the method advocated by Baccetti et al. [30].

Sample Collection

Two mL of blood was collected aseptically by venipuncture, and serum was separated, aliquoted, and stored at -80°C until use. Quantitative estimation of the growth factors, Human IGF-1 and Human IGFBP-3 in the serum was performed by ELISA, (Human IGF-1(E-EL-H0086, Elabscience Biotechnology Inc., Houston,

TX, USA) and Human IGFBP-3 (E-EL-H0087, Elabscience Biotechnology Inc., Houston, TX, USA). The assays were performed as per the manufacturer's instructions. Standard Human IGF-1 and Human IGFBP-3 were included as positive controls. The standards were serially diluted to obtain a concentration gradient of 1.5625 to 100 ng/mL and 0.78125 to 50 ng/mL, respectively. The absorbance (optical density) was read at 450 nm using a microplate reader (Infinite M200 PRO, Tecan Trading AG, Seestrasse, Switzerland). The assay was performed in duplicates to minimize errors.

A standard curve was plotted, with the x-axis denoting standard concentration and the y-axis OD values. The IGF-1 and IGFBP-3 concentration in each patient's sample was calculated using the average absorbance value of each sample.

Statistical Analysis

It was carried out with SPSS (Version 24.0). Intra-observer (Observer 1 at 2-time points - 1A, 1B) and inter-observer (between observer 1A *Vs.* 2 and 1B *Vs.* 2) reliability of cervical vertebral maturation stages was measured using Kappa statistics.

Shapiro-Wilk test was used to assess the normality of the data on serum biomarkers. The hypothesis that IGF-1 and IGFBP-3 data were normally distributed was rejected. Hence, non-parametric tests were carried out. Statistical analysis for gender-wise comparison of IGF-1 and IGFBP-3 serum levels at each cervical stage and in the intervals of the cervical stages was carried out using the Kruskal-Wallis test. For intergroup comparisons, the Mann-Whitney U test with Bonferroni's correction was done ($p < 0.05$ was considered statistically significant). Spearman's correlation coefficient was used to assess the correlation between age and serum biomarkers.

Results

Based on the assessment of the lateral cephalogram of the study participants (n=80), the cervical stages were classified as CS2 (n=12), CS3 (n=13), CS4 (n=19), CS5 (n=23) and CS6 (n=13). Kappa measurement of agreement between Observer 1A *Vs.* 1B (intra-observer) was 98.4% in the classification of cervical stages; the inter-observer agreement between Observer 1A *Vs.* Observer 2 had 96.8%, whereas there was 98.4% agreement between Observer 1B *vs.* Observer 2.

A gender-wise comparison of IGF-1 serum levels at each cervical stage (CS) is depicted in (Table 1). Mean IGF-1 was highest in CS2 in males (87.87 ± 7.53 ng/mL) and females (78.49 ± 21.93 ng/mL). Nevertheless, there was a difference in the second peak in IGF-1 between males (CS5: 68.58 ± 43.47 ng/mL) and females (CS4: 74.63 ± 30.10 ng/mL). A statistically significant difference was not observed concerning the mean IGF-1 serum levels in both males (p=0.286) and females (p=0.465) (Table 1). Intergroup comparisons of IGF-1 serum levels with different cervical stages using the Mann-Whitney U test with Bonferroni's correction revealed no statistical significance in both genders (p>0.05). A Boxplot representing the median and IQR of IGF-1 at different cervical stages is shown in (Figure 1).

Table 1. Gender-wise IGF-1(ng/mL) serum levels distribution at each cervical stage.

Cervical Stages	No of Subjects	Mean Age (Years)	Mean IGF-1 (ng/mL) ± SD	Standard Error	95% CI	
					LL	UL
Male Group - IGF-1(ng/mL) serum levels in males (n=38) at each cervical stage*						
CS 2	8	11.50	87.87 ± 7.53	2.66	81.57	94.17
CS 3	10	13.40	57.45 ± 40.87	12.92	28.21	86.69
CS 4	9	13.89	51.96 ± 46.69	15.56	16.07	87.84

CS 5	4	16.75	68.58 ± 43.47	21.73	-.59	137.74
CS 6	7	16.71	53.72 ± 36.34	13.73	20.11	87.33
Female Group - IGF-1(ng/mL) serum levels in females (n=42) at each cervical stage**						
CS 2	4	12.25	78.49 ± 21.93	10.96	43.60	113.38
CS 3	3	12.67	27.52 ± 55.71	32.16	-110.88	165.91
CS 4	10	13.40	74.63 ± 30.10	9.52	53.10	96.16
CS 5	19	15.79	52.84 ± 43.47	9.46	32.96	72.72
CS 6	6	16.00	45.93 ± 43.15	17.61	.65	91.21

CS: Cervical Stage; IGF-1: Insulin-like Growth Factor-1; CI: Confidence Interval; LL: Lower Limit; UL: Upper Limit; Kruskal-Wallis Test: *p=0.286 (NS); **p=0.465 (NS)

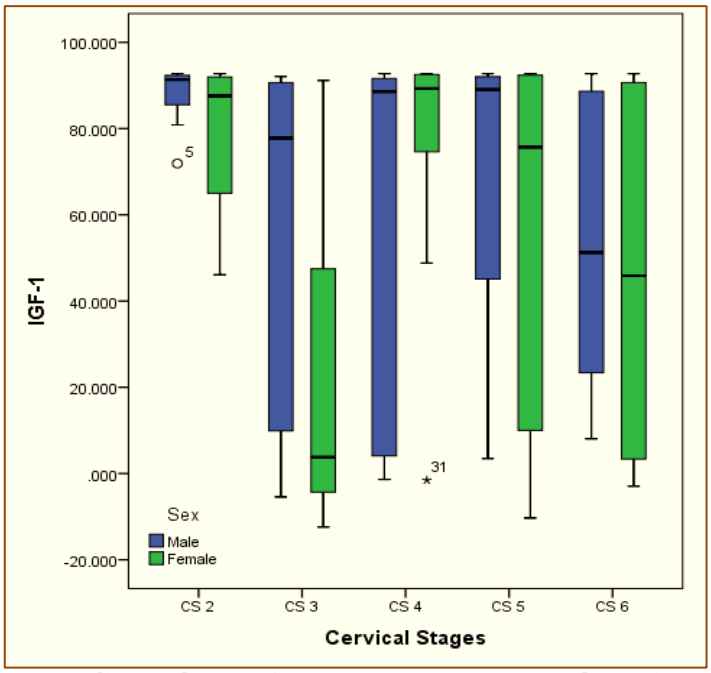

Figure 1. Median and IQR of serum IGF-1 levels at each cervical stage.

Gender-wise comparison of IGF-1 serum levels in the four intervals of CS is depicted in (Table 2). It revealed that the highest mean IGF-1 levels were 70.97 ± 33.91 ng/mL in CS2-CS3 in males, while it was highest in the CS3-CS4 stage (63.76 ± 40.30 ng/mL) in females, though not statistically significant (Table 2). Evaluation of the intergroup comparisons of IGF-1 serum levels with different CS intervals using the Mann-Whitney U test with Bonferroni's correction revealed that none of the variables were statistically significant (p>0.05).

Table 2. Gender-wise distribution of IGF-1 (ng/mL) serum levels at each interval of cervical stage.

Cervical Stage Intervals	No of Subjects	Mean Age (Years)	Mean IGF-1 (ng/mL) ± SD	SEM	95% CI for Mean		Median	IGF - 1 (ng/mL)	
					LL	UL		Min.	Max.
Male Group - IGF-1 (ng/mL) serum levels at each interval of cervical stage*									
CS2 - CS3	18	12.56	70.97 ± 33.91	7.99	54.11	87.83	88.95	-5	93
CS3 - CS4	19	13.63	54.85 ± 42.57	9.77	34.33	75.37	78.53	-5	93
CS4 - CS5	13	14.77	57.07 ± 44.60	12.37	30.12	84.02	88.58	-1	93
CS5 - CS6	11	16.73	59.12 ± 37.62	11.34	33.85	84.40	86.69	3	93
Female Group - IGF-1 (ng/mL) serum levels at each interval of cervical stage**									
CS2 - CS3	7	12.43	56.64 ± 44.91	16.98	15.11	98.18	83.93	-12	93
CS3 - CS4	13	13.23	63.76 ± 40.30	11.18	39.41	88.11	87.66	-12	93
CS4 - CS5	29	14.97	60.35 ± 38.68	7.18	45.64	75.07	77.67	-10	93
CS5 - CS6	25	15.84	51.18 ± 40.90	8.18	34.29	68.06	67.31	-10	93

CS: Cervical Stage; IGF-1: Insulin-like Growth Factor-1; CI: Confidence Interval; LL: Lower Limit; UL: Upper Limit; Kruskal-Wallis test: *p=0.816 (NS); **p=0.916 (NS).

A gender-wise comparison of serum IGFBP-3 levels at each CS is depicted in (Table 3). In males, a gradual increment in mean IGFBP-3 levels was observed from CS2 to CS4, with a subsequent decline in CS5 and an appreciable increase in CS6. The highest mean IGFBP-3 level was noted in CS6 (50.54 ± 9.65 ng/mL) in males and CS3 (51.95 ± 18.73 ng/mL) in females. However, a statistically significant difference was not observed in the mean IGFBP-3 levels at each stage in both males (p=0.211) and females (p=0.711) (Table 3). Intergroup comparisons of serum IGFBP-3 levels with different CS evaluated using the Mann-Whitney U test with Bonferroni's correction revealed no statistical significance in males and females (p>0.05). A Boxplot representing the median and IQR of IGFBP-3 at different cervical stages is shown in (Figure 2).

Table 3. Gender-wise distribution of IGFBP-3(ng/mL) serum levels at each cervical stage.

Cervical Stages	No of Subjects	Mean Age (Years)	Mean IGFBP-3 (ng/mL) ± SD	Standard Error of Mean	95% CI for Mean	
					LL	UL
Male Group - IGFBP-3 (ng/mL) serum levels in males (n=38) at each cervical stage*						
CS 2	8	11.5	35.03 ± 11.65	4.12	25.29	44.76
CS 3	10	13.4	36.74 ± 23.22	7.34	20.13	53.35
CS 4	9	13.9	47.24 ± 13.23	4.41	37.08	57.41
CS 5	4	16.8	41.72 ± 17.00	8.50	14.66	68.78
CS 6	7	16.7	50.54 ± 9.65	3.65	41.62	59.47
Female Group - IGFBP-3 (ng/mL) serum levels in females (n=42) at each cervical stage**						
CS 2	4	12.3	39.47± 25.88	12.94	-1.72	80.66
CS 3	3	12.7	51.95± 18.73	10.81	5.42	98.49
CS 4	10	13.4	47.63± 14.33	4.53	37.38	57.88
CS 5	19	15.8	49.68± 17.07	3.92	41.45	57.91
CS 6	6	16.0	38.10± 20.03	8.18	17.08	59.12

CS: Cervical Stage; IGFBP-3: Insulin-like Growth Factor Binding Protein-3; Confidence Interval; LL: Lower Limit; UL: Upper Limit; Kruskal-Wallis test: *p=0.211 (NS); **p=0.711 (NS).

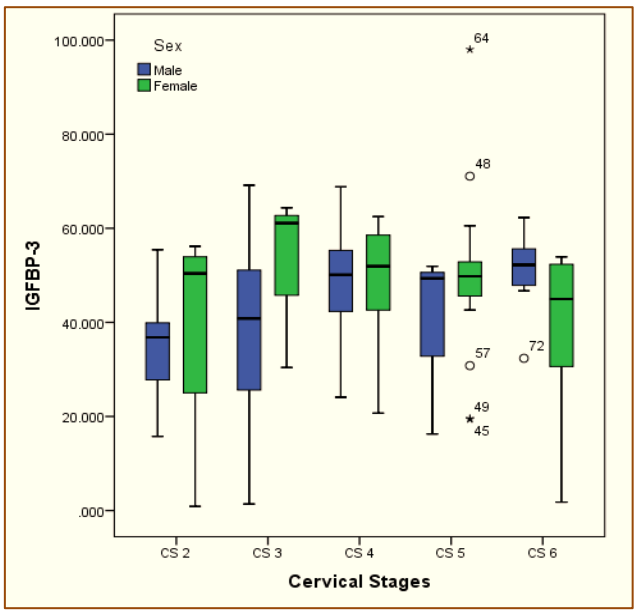

Figure 2. Median and IQR of serum IGFBP-3 levels at each cervical stage.

A gender-wise comparison of serum IGFBP-3 levels in the four intervals of CS is depicted in (Table 4). In males, a gradual increase in the mean IGFBP-3 levels was observed from CS2-CS3 (males: 35.98 ± 18.50 ng/mL; females: 44.82 ± 22.28 ng/mL) up to CS5-CS6 in both genders (males: 47.33 ± 12.75 ng/mL; females: 46.90 ± 18.10 ng/mL). However, it was statistically not significant (p>0.05) (Table 4). Also, intergroup

comparisons of serum IGFBP-3 levels with different intervals of CS by Mann-Whitney U test with Bonferroni's correction showed no statistical significance (p>0.05).

Table 4. Gender-wise distribution of IGFBP-3 (ng/mL) serum levels at each interval of cervical stage.

Cervical Stage Intervals	Mean IGFBP-3 (ng/mL) ± SD	SEM	95% CI for Mean		Median	IGFBP-3 (ng/mL)	
			LL	UL		Min.	Max.
Male Group - IGFBP-3 (ng/mL) serum levels at each interval of cervical stage*							
CS2 - CS3	35.98 ± 18.50	4.36	26.78	45.18	36.81	1	69
CS3 -CS4	41.72 ± 19.40	4.45	32.37	51.07	45.78	1	69
CS4 - CS5	45.54 ± 14.00	3.88	37.08	54.00	49.38	16	69
CS5 - CS6	47.33 ± 12.75	3.84	38.77	55.90	49.38	16	62
Female Group - IGFBP-3 (ng/mL) serum levels at each interval of cervical stage**							
CS2 - CS3	44.82 ± 22.28	8.42	24.21	65.43	51.69	1	64
CS3 - CS4	48.63 ± 14.70	4.08	39.74	57.51	53.90	21	64
CS4 - CS5	48.97 ± 15.95	2.96	42.90	55.04	49.94	19	98
CS5 - CS6	46.90 ± 18.10	3.62	39.43	54.37	49.79	2	98

CS: Cervical Stage; IGFBP-3: Insulin-like Growth Factor Binding Protein-3; Confidence Interval; LL: Lower Limit; UL: Upper Limit; Min.: Minimum; Max.: Maximum; Kruskal-Wallis test: *p=0.237 (NS); **p=0.829 (NS).

The mean serum levels of IGF-1 and IGFBP-3 at different age intervals are depicted in (Figure 3). Spearman's correlation coefficient between age and the serum biomarkers was statistically insignificant.

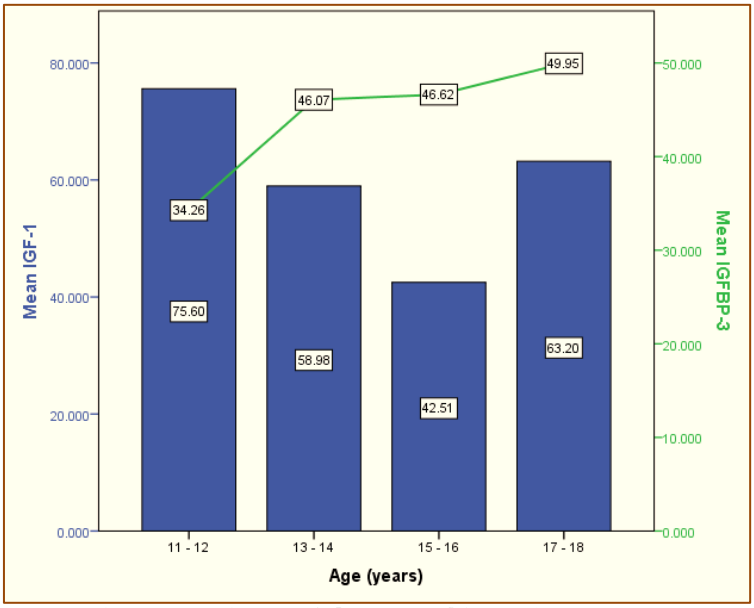

Figure 3. Age-wise comparisons of the serum biomarkers, IGF -1 & IGFBP – 3.

Discussion

Facial bones and jaws are subject to dramatic changes in growth along with an increased linear growth rate during an adolescent growth spurt. In this context, the quest for a potential biochemical maturity indicator to accurately determine the child's maturational stage assumes prime importance. Cervical vertebrae (CV) as reliable indicators of skeletal maturity have been widely reported in the literature by many authors [31]. However, CV, hand-wrist radiograph, and MP3 are rift with a few limitations in that they have questionable reliability and validity and are highly subjective techniques to identify peak mandibular growth. Non-radiologic biomarkers have been reported to accurately determine the skeletal maturity of an individual and detect the peak in pubertal growth spurt with less ambiguity. Among the many biochemical markers available, IGF-1 and

IGFBP-3 were chosen for the study as studies of IGF-1 and IGFBP-3 to predict optimal treatment timing of functional malocclusions in particular skeletal Class II malocclusion subjects have yet to be reported in the literature.

CVM assessment for functional appliance therapy in Class II skeletal jaw discrepancies has been extensively reported [32-34]. Franchi et al. [35] believed that treatment timing in mandibular deficiencies directly depends on the mandible's growth spurt in sync with pubertal onset and that this spurt could be well assessed with cervical vertebral maturational indicators (CVMI).

IGF-1 can be measured in serum, saliva, and urine [36,37]. Determination of GH status is done by measurement of IGF-1 in serum as fluctuation of its levels does not occur all along the day as has been observed with GH levels. IGF-1 can be estimated in saliva, but its low salivary concentration and issues like contamination of saliva with blood or gingival fluid preclude its widespread use in practice. Another minimally invasive technique with excellent correlation with serum IGF-1 is blood spot IGF-1 measurements wherein the collected samples remain stable for up to 2 weeks at room temperature [38,39].

Reportage of estimation of serum IGF-1 and IGFBP-3 levels with the use of various types of assays with different skeletal maturity assessment methods have been observed to be rift with gender bias, wide age range as also the chosen study subjects were a concocted mix of standard and malocclusion subjects. Our study was unique in that only skeletal Class II malocclusion subjects were considered in the age range of 11-18 years, with an almost equal number of males and females spanning CS2 to CS6. The ELISA method using the Sandwich-ELISA principle was chosen in our study as studies have shown that other methods, such as IGF-1 with radioimmunoassays (RIA), however, did not measure the quantitative serum IGF-1 content accurately [40]. Moreover, the hazards of handling and preparing the radioactive antigen used in RIA are eliminated. No special sample preparation was done before the ELISA method, as any predilution done to the samples would have led to increased assay sensitivity. In our study, we used the lateral cephalogram to assess the CVM stages, as it was a routine radiograph used in orthodontics for diagnosis and treatment planning.

Our data showed that the IGF-1 serum concentrations in males showed two peaks, an increased one in CS2 followed by another though a little less intense in CS5, and that in the females too, two peaks were observed, one in CS2 and another less intense in CS4. The interval CS2-CS3 exhibited a rise in the mean serum IGF-1 concentrations in males followed by CS5-CS6, whereas, in females, a peak was observed in the interval CS3-CS4 followed by a dip in CS5-CS6. This was consistent with other studies that showed a late pubertal peak in serum IGF-1 levels [41,42]. Our results were in tune with another study where, in the advancing pubertal stage, an increase in IGF-1 plasma levels was observed [43]. Our results, too, correlated with those of yet another study where the IGF-1 levels were found to be increased in early puberty with a subsequent decline in late puberty [44]. However, our results in males contradicted that of another study where the mean IGF-1 levels were found to increase in CS3 and CS4 [45]. Our results also differed from those of two other studies, where the mean values were higher in CS5 in males and CS3 in females [46] and CS4 in males and CS3 in females [47]. Peak IGF-1 levels vary according to ethnicity and race, too. In Egyptians, it was observed to peak in females at CS3 & in males at CS4 [48]. Our results showed that at CS5-6, when growth has traditionally been accepted as complete, IGF-1 levels were still high, which indicates that they could still serve as good indicators of residual mandibular growth. Serum IGF-1 level estimation is reliable and valid as it does not exhibit a diurnal variation and does not show a decline in obese individuals.

IGF-1 has been reported to bind to specific IGFBPs, specifically IGFBP-3, which GH regulates. IGFBP-3 controls the bioavailability and half-life of IGF-1. Our findings showed that in males, two peaks were

observed, one in CS4 and another steep increase in CS6, whereas in females, it showed a rise in CS3 and CS5. The same scenario was observed in CS4-5 and CS5-6 intervals in males and CS3-4 and CS4-5 in females. Our results were in concert with the findings of the Blumsohn et al. [43] study, reiterating that a rise in IGFBP-3 plasma levels was observed with the advancing pubertal stage. Our data also correlated with another study where IGFBP-3 was increased during sexual maturation, with a pubertal peak around 15 years of age [49]. In our research, the IGFBP-3 mean serum levels in female subjects increased more than in male subjects, in concurrence with the findings of another study on healthy Turkish children [50]. IGFBP-3 produced reproducible results during repeated testing, thus making it an interesting parameter for evaluating the GH-IGF axis, as it was also less sensitive to GH regulation than IGF-1. However, it needs to be emphasized that IGF-1 & IGFBP-3 serum concentrations depend to a great extent on nutrition, age, and other factors.

Almalki [51], in his study, highlighted the noninvasive approach of salivary estimation of IGF-1 and IGF-1/IGFBP-3 molar ratio in conjunction with CVMI in estimating the adolescent growth spurt. Recent studies have opined that salivary Vitamin D binding protein and IGF-1 increased in CS3 and CS4 stages and that an optimal threshold of 3.96ng/mL of the latter had better diagnostic accuracy in differentiating pubertal and non-pubertal subjects [52].

Isoforms of IGF-1 with four amino acid polymorphisms with decreased biological activity are reported to occur. The ELISA kit used in this study is of questionable reliability in detecting IGF-1 mutants. Hence, the presence of the IGF-1 reduced-function mutants, if any, in the study subjects could be underestimated. Immunoassays are entirely dependent on the operator's skills, experimental environments, different lab equipment, the validity of the products, and transportation and storage conditions of the reagents of the kits. All these factors could lead to technical & qualitative risks. Longitudinal studies are therefore recommended to highlight the use of these biomarkers to estimate the intensity and timing of the growth spurt precisely. Tabulating reference ranges of IGF-1 & IGFBP-3 serum levels at different CS in different populations could also be considered a future research study. As CVM is not a perfect rating system, the quest for a valid and reliable non-radiologic biomarker assumes even greater importance in the present scenario of skeletal maturity assessment.

Conclusion

Cervical vertebral staging using lateral cephalogram has long been considered a reliable radiologic indicator of skeletal maturity. However, the non-radiologic biochemical markers IGF-1 and IGFBP-3 have also emerged as potential biomarkers of the adolescent growth spurt. Mean IGF-1 serum levels increased in females' prepubertal stage and pre- and post-pubertal levels in males. Meanwhile, mean IGFBP-3 serum levels increased in both genders, reiterating that both could serve as potential biomarkers of pubertal growth assessment. Class II skeletal patterns can be corrected in both genders in the prepubertal stage (CS2) and with the judicious use of fixed functional appliances even up to the late post-pubertal stage (CS5-CS6).

Authors' Contributions

AY	https://orcid.org/0000-0001-5172-1885	Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review and Editing, Supervision and Project Administration.
KP	https://orcid.org/0000-0002-0790-1464	Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Review and Editing, Supervision and Project Administration.
KK	https://orcid.org/0000-0002-8600-0954	Validation, Visualization, Supervision, and Project Administration.

All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

Financial Support

None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

References

[1] Profitt WR, Fields HW. Contemporary Orthodontics. 3rd ed. St. Louis: Mosby-Year Book's; 2000.
[2] Fishman LS. Maturational patterns and prediction during adolescence. Angle Orthod 1987; 57(3):178-193.
[3] Chertkow S. Tooth mineralization as an indicator of the pubertal growth spurt. Am J Orthod 1980; 77(1):79-91. https://doi.org/10.1016/0002-9416(80)90226-2
[4] Rajagopal R, Kansal S. A comparison of modified MP3 stages and the cervical vertebrae as growth indicators. J Clin Orthod 2002; 36(7):398-406.
[5] Singer J. Physiologic timing of orthodontic treatment. Angle Orthod 1980; 50(4):322-333.
[6] Fishman LS. Radiographic evaluation of skeletal maturation. A clinically oriented method based on hand-wrist films. Angle Orthod 1982; 52(2):88-112.
[7] Baccetti T, Franchi L, McNamara JA Jr. An improved version of the cervical vertebral maturation (CVM) method for the assessment of mandibular growth. Angle Orthod 2002; 72(4):316-323.
[8] Hassel B, Farman AG. Skeletal maturation evaluation using cervical vertebrae. Am J Orthod Dentofacial Orthop 1995; 107(1):58-66. https://doi.org/10.1016/s0889-5406(95)70157-5 Erratum in: Am J Orthod Dentofacial Orthop 1995; 107(6):19.
[9] Juul A, Flyvbjerg A, Frystyk J, Müller J, Skakkebaek NE. Serum concentrations of free and total insulin-like growth factor-I, IGF binding proteins -1 and -3 and IGFBP-3 protease activity in boys with normal or precocious puberty. Clin Endocrinol 1996; 44(5):515-523. https://doi.org/10.1046/j.1365-2265.1996.711531.x
[10] Johansen JS, Giwercman A, Hartwell D, Nielsen CT, Price PA, Christiansen C, et al. Serum bone Gla-protein as a marker of bone growth in children and adolescents: Correlation with age, height, serum insulin-like growth factor I, and serum testosterone. J Clin Endocrinol Metab 1988; 67(2):273-278. https://doi.org/10.1210/jcem-67-2-273
[11] Kindblom JM, Nilsson O, Hurme T, Ohlsson C, Sävendahl L. Expression and localization of Indian hedgehog (Ihh) and parathyroid hormone related protein (PTHrP) in the human growth plate during pubertal development. J Endocrinol 2002; 174(2):R1-6. https://doi.org/10.1677/joe.0.174r001
[12] Laron Z. The Somatostatin-GHRH-GH-IGF-1 Axis. In: Merimee T, Laron Z, eds. Growth Hormone, IGF-1and Growth: New Views of Old Concepts. Modern Endocrinology and Diabetes, Vol.4. London-Tel Aviv: Freund Publishing House Ltd; 1996.
[13] D'Ercole AJ, Applewhite GT, Underwood LE. Evidence that somatomedin is synthesized by multiple tissues in the fetus. Dev Biol 1980; 75(2):315-328. https://doi.org/10.1016/0012-1606(80)90166-9
[14] Mohan S, Richman C, Guo R, Amaar Y, Donahue LR, Wergedal J, et al. Insulin-like growth factor regulates peak bone mineral density in mice by both growth hormone-dependent and -independent mechanisms. Endocrinology 2003; 144(3):929-936. https://doi.org/10.1210/en.2002-220948
[15] Delatte M, Von den Hoff JW, Maltha JC, Kuijpers-Jagtman AM. Growth stimulation of mandibular condyles and femoral heads of newborn rats by IGF-I. Arch Oral Biol 2004; 49(3):165-175. https://doi.org/10.1016/j.archoralbio.2003.09.007
[16] Suzuki S, Itoh K, Ohyama K. Local administration of IGF-I stimulates the growth of mandibular condyle in mature rats. J Orthod 2004; 31(2):138-143. https://doi.org/10.1179/146531204225020436
[17] Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jørgensen K, et al. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: Relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 1994; 78(3):744-752. https://doi.org/10.1210/jcem.78.3.8126152
[18] Visnapuu V, Peltomäki T, Rönning O, Vahlberg T, Helenius H. Growth hormone and insulin-like growth factor I receptors in the temporomandibular joint of the rat. J Dent Res 2001; 80(10):1903-1907. https://doi.org/10.1177/00220345010800100801
[19] Masoud M, Masoud I, Kent RL Jr, Gowharji N, Cohen LE. Assessing skeletal maturity by using blood spot insulin-like growth factor I (IGF-I) testing. Am J Orthod Dentofacial Orthop 2008; 134(2):209-216. https://doi.org/10.1016/j.ajodo.2006.09.063

[20] Tripathi T, Gupta P, Rai P, Sharma J, Gupta VK, Singh N, et al. Longitudinal evaluation of the association between Insulin-like growth factor-1, bone specific alkaline phosphatase and changes in mandibular length. Sci Rep 2019; 9(1):11582. https://doi.org/10.1038/s41598-019-48067-7

[21] Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr Rev 1999; 20(6):761-787. https://doi.org/10.1210/edrv.20.6.0382

[22] Kanety H, Karasik A, Klinger B, Silbergeld A, Laron Z. Long-term treatment of Laron type dwarfs with insulin-like growth factor-1 increases serum insulin-like growth factor-binding protein-3 in the absence of growth hormone activity. Acta Endocrinol 1993; 128(2):144-149. https://doi.org/10.1530/acta.0.1280144

[23] Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB. Serum levels of insulin-like growth factor I (IGF-I) and IGF-binding protein 3 reflect spontaneous growth hormone secretion. J Clin Endocrinol Metab 1993; 76(6):1610-1616. https://doi.org/10.1210/jcem.76.6.7684744

[24] Baxter RC. Insulin-like growth factor binding proteins in the human circulation: A review. Horm Res 1994; 42(4-5):140-144. https://doi.org/10.1159/000184186

[25] Froesch ER, Hussain MA, Schmid C, Zapf J. Insulin-like growth factor I: Physiology, metabolic effects and clinical uses. Diabetes Metab Rev 1996; 12(3):195-215.

[26] Hajjar D, Santos MF, Kimura ET. Mandibular repositioning modulates IGFBP-3, -4, -5 and -6 expression in the mandibular condylar cartilage of young rats. Biorheology 2006; 43(3,4):311-321.

[27] Blum WF, Cotterill AM, Postel-Vinay MC, Ranke MB, Savage MO, Wilton P. Improvement of diagnostic criteria in growth hormone insensitivity syndrome: Solutions and pitfalls. Pharmacia Study Group on Insulin-like Growth Factor I Treatment in Growth Hormone Insensitivity Syndromes. Acta Paediatr Suppl 1994; 399:117-124. https://doi.org/10.1111/j.1651-2227.1994.tb13303.x

[28] Juul A, Dalgaard P, Blum WF, Bang P, Hall K, Michaelsen KF, et al. Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: The relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. J Clin Endocrinol Metab 1995; 80(8):2534-2542. https://doi.org/10.1210/jcem.80.8.7543116

[29] Jain N, Tripathi T, Gupta SK, Rai P, Kanase A, Kalra S. Serum IGF-1, IGFBP-3 and their ratio: Potential biochemical growth maturity indicators. Prog Orthod 2017; 18(1):11. https://doi.org/10.1186/s40510-017-0165-1

[30] Baccetti T, Franchi L, McNamara JA Jr. The cervical vertebral maturation (CVM) method for the assessment of optimal treatment timing in dentofacial orthopedics. Sem in Orthod 2005; 11(3):119-129. https://doi.org/10.1053/j.sodo.2005.04.005

[31] McNamara JA Jr, Franchi L. The cervical vertebral maturation method: A user's guide. Angle Orthod 2018; 88(2):133-143. https://doi.org/10.2319/111517-787.1

[32] Baccetti T, Franchi L, Toth LR, McNamara JA Jr. Treatment timing for Twin-block therapy. Am J Orthod Dentofacial Orthop 2000; 118(2):159-170. https://doi.org/10.1067/mod.2000.105571

[33] Wong RW, Alkhal HA, Rabie AB. Use of cervical vertebral maturation to determine skeletal age. Am J Orthod Dentofacial Orthop 2009; 136(4):484.e1-6; discussion 484-485. https://doi.org/10.1016/j.ajodo.2007.08.033

[34] Cericato GO, Luiz de Freitas PH, Bittencourt MAV, Paranhos LR. Reliability, efficacy and reproducibility of the cervical vertebrae maturation index (CVMI). Biosci J 2015; 31(1):319-325. https://doi.org/10.14393/BJ-v31n1a2015-26901

[35] Franchi L, Baccetti T, McNamara JA Jr. Mandibular growth as related to cervical vertebral maturation and body height. Am J Orthod Dentofacial Orthop 2000; 118(3):335-340. https://doi.org/10.1067/mod.2000.107009

[36] Hizuka N, Takano K, Tanaka I, Asakawa K, Miyakawa M, Horikawa R, et al. Demonstration of insulin-like growth factor I in human urine. J Clin Endocrinol Metab 1987; 64(6):1309-1312. https://doi.org/10.1210/jcem-64-6-1309

[37] Costigan DC, Guyda HJ, Posner BI. Free insulin-like growth factor I (IGF-I) and IGF-II in human saliva. J Clin Endocrinol Metab 1988; 66(5):1014-1018. https://doi.org/10.1210/jcem-66-5-1014

[38] Diamandi A, Khosravi MJ, Mistry J, Martinez V, Guevara-Aguirre J. Filter paper blood spot assay of human insulin-like growth factor I (IGF-I) and IGF-binding protein-3 and preliminary application in the evaluation of growth hormone status. J Clin Endocrinol Metab 1998; 83(7):2296-2301. https://doi.org/10.1210/jcem.83.7.4923

[39] Schütt BS, Weber K, Elmlinger MW, Ranke MB. Measuring IGF-I, IGFBP-2 and IGFBP-3 from dried blood spots on filter paper is not only practical but also reliable. Growth Horm IGF Res 2003; 13(2-3):75-80. https://doi.org/10.1016/s1096-6374(03)00004-2

[40] Daughaday WH, Mariz IK, Blethen SL. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: A comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. J Clin Endocrinol Metab 1980; 51(4):781-788. https://doi.org/10.1210/jcem-51-4-781

[41] Mitchell ML, Hermos RJ, Schoepfer A, Orson JM. Reference ranges for insulin-like growth factor-1 in healthy children and adolescents, determined with filter-paper blood specimens. Clin Chem 1990; 36(12):2138-2139.

[42] Kanbur-Oksüz N, Derman O, Kinik E. Correlation of sex steroids with IGF-1 and IGFBP-3 during different pubertal stages. Turk J Pediatr 2004; 46(4):315-321.

[43] Blumsohn A, Hannon RA, Wrate R, Barton J, al-Dehaimi AW, Colwell A, et al. Biochemical markers of bone turnover in girls during puberty. Clin Endocrinol 1994; 40(5):663-670. https://doi.org/10.1111/j.1365-2265.1994.tb03019.x

[44] Gupta S, Jain S, Gupta P, Deoskar A. Determining skeletal maturation using insulin-like growth factor I (IGF-I) test. Prog Orthod 2012; 13(3):288-295. https://doi.org/10.1016/j.pio.2011.09.006

[45] Jain S, Jain S, Deoskar A, Prasad VS. Serum IGF-1 levels as a clinical tool for optimizing orthodontic treatment timing. Prog Orthod 2013; 14:46. https://doi.org/10.1186/2196-1042-14-46

[46] Gupta S, Deoskar A, Gupta P, Jain S. Serum insulin-like growth factor-1 levels in females and males in different cervical vertebral maturation stages. Dental Press J Orthod 2015; 20(2):68-75. https://doi.org/10.1590/2176-9451.20.2.068-075.oar

[47] Tripathi T, Gupta P, Rai P, Sharma J, Gupta VK, Singh N. Osteocalcin and serum insulin-like growth factor-1 as biochemical skeletal maturity indicators. Prog Orthod 2017; 18(1):30. https://doi.org/10.1186/s40510-017-0184-y

[48] Ishaq RA, Soliman SA, Foda MY, Fayed MM. Insulin-like growth factor I: A biologic maturation indicator. Am J Orthod Dentofacial Orthop 2012; 142(5):654-661. https://doi.org/10.1016/j.ajodo.2012.06.015

[49] Elmlinger MW, Kühnel W, Weber MM, Ranke MB. Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). Clin Chem Lab Med 2004; 42(6):654-664. https://doi.org/10.1515/CCLM.2004.112

[50] Bereket A, Turan S, Omar A, Berber M, Ozen A, Akbenlioglu C, et al. Serum IGF-I and IGFBP-3 levels of Turkish children during childhood and adolescence: Establishment of reference ranges with emphasis on puberty. Horm Res 2006; 65(2):96-105. https://doi.org/10.1159/000091301

[51] Almalki A. Association of salivary IGF and IGF/IGFBP-3 molar ratio with cervical vertebral maturation stages from pre-adolescent to post-adolescent transition period - A cross-sectional exploratory study. Int J Environ Res Public Health 2022; 19(9):5172. https://doi.org/10.3390/ijerph19095172

[52] Gv V, Tripathi T, Rai P, Mahajan B, Kanase A. Association, diagnostic accuracy and optimal threshold of salivary IGF-1 and vitamin DBP levels for estimation of pubertal growth spurt: A cross sectional study. Int Orthod 2023; 21(3):100786. https://doi.org/10.1016/j.ortho.2023.100786