Journal of Contemporary Orthodontics 2024;8(3):281–289

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Journal of Contemporary Orthodontics

Journal homepage: https://www.jco-ios.org/

Original Research Article

Appraisal of skeletal maturity indicators with respect to non-invasive biomarkers

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ARTICLE INFO

Article history:
Received 20-03-2024
Accepted 02-04-2024
Available online 27-08-2024

Keywords:
Salivary IGF-1
Salivary ALP
CVMI stages
Skeletal growth
Cephalometrics
Growth assessment
Biomarker
Non-invasive biomarkers
Mandibular base length

ABSTRACT

Background: An orthodontist prefers to begin treatment during the pubertal growth spurt which is is a promising time in terms of treatment outcome. Non-invasive salivary biomarkers are being investigated as they may be helpful in predicting skeletal age. This study was conducted to evaluate the correlation between salivary insulin-like growth factor-1, salivary alkaline phosphatase, cervical vertebrae maturation stages and chronological age in the assessment of skeletal age during the growth period. This study also aims to find associations with mandibular base length and maxillary base length.

Materials and Methods: Total 80 eligible subjects aged 7 to 21 years were selected and divided into two gender-specific groups (41 males, 39 females). A further subdivision was made on the basis of six cervical vertebral maturation stages from the lateral cephalogram. The chronological age was given for each subject, and The maxillary base length and the length of the mandibular base was calculated from the impressions on the lateral cephalogram. Saliva samples were collected from each subject to determine salivary IGF-1 and ALP levels using an enzyme-linked immunosorbent assay (ELISA). The data obtained was statistically analysed using SPSS (20.0) software.

Results: It was found that the mean IGF-1 activity in saliva was highest at CVMI stage 3 and the highest mean ALP levels in saliva occurred in males at CVMI stage 3 and in females at CVMI stage 4.

Conclusions: Mean salivary ALP and IGF-1 levels correlate well with CVMI stage 3. Salivary ALP levels show a correlation with chronological age and could also be used as a diagnostic model to predict cervical stage.

Clinical Relevance: The diagnostic levels of certain biomarkers that appear in human saliva are representative of an individual's circumpubertal growth spurt. The collective pattern of increase and decrease in the sample during the circumpubertal stages is essential to study for orthodontic diagnosis and treatment planning.

1. Introduction

The time of onset of puberty varies from person to person depending on gender, generation and population. Knowledge of the relationship between pre-pubertal, pubertal and post-pubertal growth stages and other bases of physical maturation can be of some value in many ways and in with context of caring for the growing child, for example, in knowing whether an individual has reached or passed the pubertal growth spurt.[1]

The maturation pattern of the cervical spine in 6 stages for the assessment of skeletal age made an additional hand wrist radiography unnecessary.[2] Hassel and Farman 1995[3]

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https://doi.org/10.18231/j.jco.2024.042

modified the Lamparski method by observing only the morphologic changes from C2 to C4 to assess the remaining part of growth (Figure 1]. It is a suitable and an accurate method that eliminates the need for additional hand wrist radiography unnecessary.[4,5]

Biomarkers in saliva have the advantage that invasive blood sampling can be avoided where it is difficult to perform. Studies in mice have shown that IGF-I is essential for pubertal growth spurt and ALP plays a role in bone mineralization. An increase in IGF-I levels is evident during longitudinal condylar bone growth.[6] Studies on IGF-1 have shown that it plays an important role in the regulation of prenatal and postnatal longitudinal bone growth both systemically and locally and mediates the function of growth hormone.[7] In contrast to GH, IGF-1 levels do not fluctuate throughout the day.[8] Studies have quantified salivary IGF-I levels at different stages of quantitative cervical maturation (QCVM) and assessed the potential role of salivary IGF-1 levels in the assessment of skeletal maturation. Salivary IGF-I levels were found to be lowest in the quantitative CVM skeletal stages previously associated with the acceleration phase of mandibular growth. The highest levels were found in the high velocity phase, followed by a gradual decline.[9]

ALP is conveniently derived from saliva and functions during bone mineralization; its role is understood during osteoblastic differentiation by examining the phasic expression of genes. ALP is expressed early in development in tissues, bone and calcifying cartilage.[10,11] ALP levels have been found to increase in saliva,[12,13] gingival crevicular fluid (GCF)[14] and serum[15] during puberty.

The major change in mandibular growth was observed at circumpubertal age, as the cartilaginous part of the condyle is present. In studies, the biomarkers B-ALP and IGF-1 were evaluated as indicators of skeletal maturity and compared with the length of the mandible, measured from the condyle to the gonion.[16]

The disadvantage of intra-examiner reproducibility, the subjective perception of the individual clinician, the difficulty of cephalometric radiographic grading due to noise in the radiographs, the complexity in identifying landmarks and borderline individuals, the lack of prediction of residual mandibular growth[9], which is influenced by morphology and posture, calls into question the reliability of CVMI. With current conceptual development, quantitative assessment of skeletal maturation can be performed by salivary biomarkers, gingival crevicular fluid (GCF) biomarkers and urinary biomarkers.[17]

Based on the role of biomarkers and other evidence mentioned above, the present study will investigate the most highly correlated biomarker and its role in young adolescent subjects in relation to their individual skeletal growth stages as well as any sexual dimorphism. We will also examine the correlation between maxillary basal length and mandibular basal length in relation to non-invasive salivary biomarkers so that a more reliable and least variable parameter for growth and development can be identified. Our hypothesis is that both salivary IGF-1 and ALP follow circumpubertal growth phases, one or both of which must be relating closely to either chronological age or cervical maturation stages, or both.

2. Materials and Methods

This investigation was a cross-sectional study conducted on 80 eligible subjects of North Indian origin, aged 7 to 21 years, who presented to the outpatient clinic of the Department of Orthodontics and Dentofacial orthopedics, Faculty of Dental Sciences, King George's Medical University, Lucknow, Department of Preventive and Paediatric Dentistry, Faculty of Dental Sciences, King George's Medical University, Lucknow and in collaboration with the Centre for Advanced Research, King George's Medical University, Lucknow.

Ethical approval was obtained from the Ethics Committee of King George's Medical University, Research Cell. Informed consent was obtained from each subject prior to the commencement of the study.

The subjects who participated in this study were healthy individuals with no systemic diseases, were not pregnant and breastfeeding, had no gingivitis, and were not undergoing orthodontic treatment. Subjects who willingly participated in the study by completing the informed consent form were included in the study.

A standardised lateral head cephalometric radiograph was taken for each subject in a natural head position and the subjects were divided into groups based on the classification of the 6 cervical vertebrae according to Hassel and Farman 1995. At the time of radiography, subjects were instructed to maintain centric occlusion with relaxed lips, which was recorded by the same technician. Radiographs were randomly numbered after collection, and to avoid bias, two examiners (GP and DS) were blinded to the subject's details and therefore final staging of cervical vertebrae was done. The superior, inferior, posterior, and anterior margins of the second, third, and fourth cervical vertebrae were traced for cervical staging. Saliva samples were collected for analysis of salivary IGF-1 and ALP levels. Subjects were not allowed to eat, drink or take any medication 1 hour prior to sample collection. Unstimulated total saliva was collected between 9 and 12 am. Subjects were asked to rinse their mouth 10 minutes before sample collection. Then the subjects were instructed to pool the saliva in the floor of the mouth by holding the tongue to the palate. The saliva collected in the first minute was discarded. Thereafter, the pooled saliva was collected by passive drooling from the lower lip into 1.5 mL microcentrifuge tubes.

Samples were stored in sealed plastic bags in a refrigerator compartment and sent to the Centre for

Advanced Research, King George's Medical University, Lucknow, for subsequent testing. The samples were centrifuged at 10000 rpm at 4 degrees for 20 minutes until a pellet like sedimentation was seen at the bottom of the tube. The supernatant was then collected and stored at -80°C until analysis. The enzyme immunoassay for insulin-like growth factor-1 (sIGF-1) and alkaline phosphatase (sALP) in saliva was performed using the FineTest^{TM} kit (Wuhan Fine BioTech Co. Ltd., China) and then analysed using a spectrophotometer at 405 nm at room temperature. The variables of the study are as follows:

1. Chronological age
2. Salivary Insulin like Growth Factor -1 (sIGF-1 in pg/ml)
3. Salivary Alkaline Phosphatase (sALP in ng/ml).
4. Mandibular base length (Go-Pog in mm)[Fig 2]
5. Maxillary base length (PNS-Point A in mm)[Fig 2]

2.1. Statistical analysis

The data obtained in this way was subjected to statistical analysis using the Windows software SPSS (version 20.0, Chicago, Inc., USA). The results were expressed in mean ± standard deviation (SD) and percentages. The categorical variables were analyzed using the Student t-test and the chi-square test. Continuous variables were analyzed using an unpaired t-test between groups and a one-way analysis of variance (ANOVA) to calculate the within-group comparison. Correlations were analyzed using Spearman's Rho, and dependent variables were analyzed using linear regression. Six models were used to predict CVMS using multinomial logistic regression analysis. All statistical tests were two-sided, with the p-value <0.05 considered statistically significant.

3. Results and Observations

The data obtained was found to be normally distributed; therefore, an unpaired t-test was used to compare the mean sALP and sIGF-1 levels between the two groups at each stage (Table 1). Mean sIGF-1 levels increased at stage 3 and 4 in both Group A and Group B, with females (297.92±136.88pg/ml) having higher levels than males (245.41±135.63pg/ml) at stage 3, but these were not statistically significant. In group B (females), mean sIGF-1levels (220.37±179.01pg/ml) increased again in stage 5. Mean sALP levels increased during stage 3 and 4 in both group A and group B, followed by higher levels during stage 2 and 5 and a decrease during stage 1 and 6 (Table 1,Figure 3 A & B)

The maxillary base length was found to increase steadily from stage 1 to stage 6. There was a statistically significant difference (p=0.013) between the maxillary base length of Group A (male) and Group B (female) during stage 6. The mandibular base length steadily increases across CVM

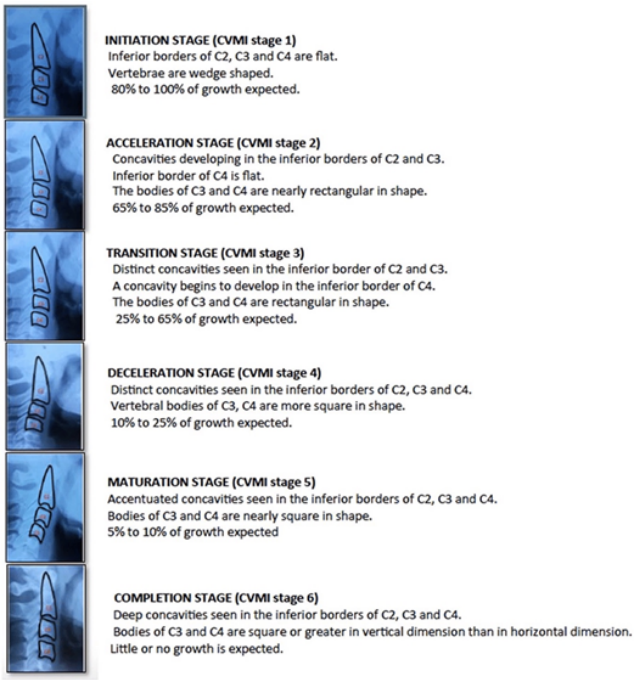

Figure 1: Cervical vertebrae maturation stages by hasseland farman 1995.

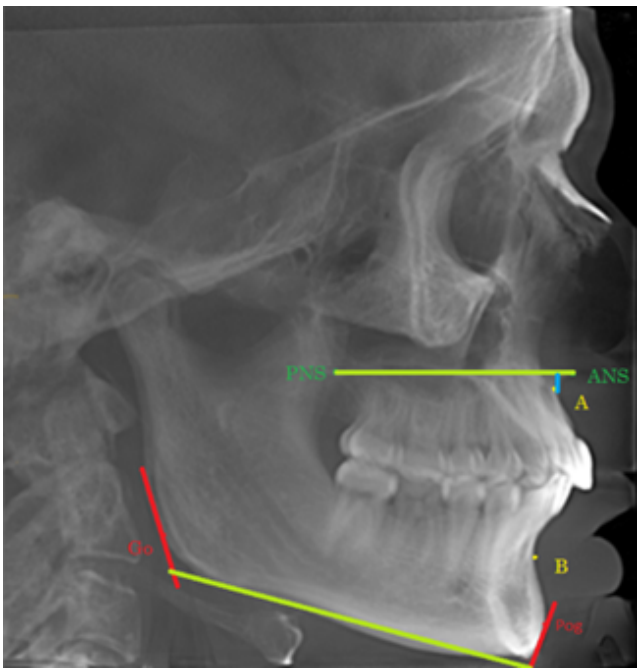

Figure 2: PNS-Pt A is Maxillary base length (posterior nasal spine to Point A) and Go-Pog is Mandibular base length (Gonion-Pogonion) in mm [47].

Table 1: Comparison of mean, standard deviation and p-value ofchronological age, sIGF-1, sALP, among CVMI stages between Group A (Male) andGroup B (Female).

CVMI Stages	Gender	Age (Years) Mean±SD	p-value	sIGF-1 (pg/mL) Mean±SD	p-value	sALP (ng/mL) Mean±SD	p-value
Stage-1	Group A N=7	9.14±2.41	0.857	206.65±71.68	0.711	1.65±0.84	0.775
	Group B N=6	9.33±0.82		193.09±54.08		1.78±0.75	
Stage-2	Group A N=7	10.42±2.43	0.294	206.52±77.15	0.974	1.70±0.43	0.251
	Group B N=5	9.10±1.24		208.06±86.83		2.04±0.54	
Stage-3	Group A N=7	12.42±1.27	0.597	245.41±135.63	0.484	2.96±1.81	0.251
	Group B N=7	11.85±2.47		297.92±136.88		2.09±0.61	
Stage-4	Group A N=7	15.00±1.41	0.039*	226.89±96.18	0.651	2.08±0.54	0.472
	Group B N=7	13.28±1.38		202.88±97.56		2.33±0.71	
Stage-5	Group A N=7	16.71±2.28	0.032*	113.15±25.63	0.015*	1.87±0.40	0.885
	Group B N=7	14.28±1.38		220.37±179.01		1.83±0.60	
Stage-6	Group A N=6	19.16±0.75	0.974	144.95±63.15	0.989	1.38±0.16	0.193
	Group B N=7	19.14±1.35		145.44±50.78		1.54±0.24	

*Significance level was set at P <0.05.

Table 2: Comparison of Mean, Standard deviation and p-value of Chronological age, mandibular length, maxillary length among CVMI stages between Group A (Male) and Group B (Female).

CVMI Stages	Gender	Age (Years) Mean±SD	Mandibular length (mm) Mean±SD	p-value	Maxillary length (mm) Mean±SD	p-value
Stage-1	Group A	9.14±2.41	58.42±3.20	0.808	35.57±3.95	0.318
	Group B	9.33±0.82	58.00±2.82		37.33±1.21	
Stage-2	Group A	10.42±2.43	63.71±2.98	0.052	38.57±4.89	0.284
	Group B	9.10±1.24	59.60±3.50		41.72±4.54	
Stage-3	Group A	12.42±1.27	63.35±4.24	0.997	41.42±5.06	0.718
	Group B	11.85±2.47	63.36±5.52		40.57±3.40	
Stage-4	Group A	15.00±1.41	70.28±3.81	0.046*	42.14±4.59	0.940
	Group B	13.28±1.38	65.28±4.57		41.96±4.29	
Stage-5	Group A	16.71±2.28	68.71±5.58	0.169	40.85±2.54	0.296
	Group B	14.28±1.38	64.71±4.61		42.28±2.36	
Stage-6	Group A	19.16±0.75	69.50±3.27	0.025*	46.00±2.44	0.013*
	Group B	19.14±1.35	62.71±5.96		42.71±1.60	

Table 3: Overall comparison of sIGF-1 and sALP among the CVMIstages (ANOVA).

CVMI Stage	Age (Years) Mean±SD	sIGF-1 (pg/mL) Mean±SD	sALP (ng/mL) Mean±SD
Stage 1	9.23±1.78	200.39±61.94	1.71±0.77
Stage 2	9.8±2.0	207.16±77.39	1.84±0.48
Stage 3	12.1±1.9	271.67±133.71	2.53±1.37
Stage 4	14±1.6	214.88±93.90	2.20±0.61
Stage 5	15.5±2.2	166.76±134.86	1.84±0.48
Stage 6	19.1±1.06	145.22±54.32	1.46±0.21
p-value	<0.0001	0.030	0.009

p value issignificant at the 0.05 level
ns:non-significant
*p < 0.05, **p < 0.01, ***p < 0.001

Table 4: Independent variable: Chronological age

Gender		Beta	p-value
	(Constant)		.000
	sIGF-1 (pg/mL)	-.002	.986
Male	sALP (ng/mL)	-.230	.035*
	Mandibular base length (mm)	.596	.000
	Maxillary base length (mm)	.340	.004
	(Constant)		.812
	sIGF-1 (pg/mL)	-.129	.431
Female	sALP (ng/mL)	-.218	.168
	Mandibular base length (mm)	.172	.319
	Maxillary base length (mm)	.244	.163

*Significance level was set at P <0.05.

Table 5: Association of 6 multinomial models to determine bestfit model to predict cervical stage.

Effect	R^2 MF	value
CA	0.412	<0.0001*
IGF	0.048	0.017
sALP	0.070	0.001
CA+sALP	0.422	<0.0001
CA+sIGF-1	0.412	<0.0001
CA+sALP+sIGF-1	0.449	<0.0001

Table 6: Spearman correlation in values of sIGF-1 with mandibular base length, and sALP with mandibular base length during CVMI Stage3, Stage 4, Stage 5

Spearman Correlation	Stage	Stage 3	Stage 4	Stage 5
	sALP vs Mandibular length	0.374*	0.363*	0.309*
	sIGF-1 vs mandibular length	.119	-.317*	-.173

Spearman correlation coefficient r>0.5 signifies strong positive correlation

Table 7: Association between sALP and sIGF-1 with Chronological age range among CVMI Stage 3, CVMI Stage 4 and CVMI Stage 5.

Age (Years)				sIGF-1 (pg/mL)	sALP (ng/mL)
8-9 Years N=11	Spearman's rho	sIGF-1 (pg/mL)	Correlation Coefficient	1.000	0.482*
		sALP (ng/mL)	Correlation Coefficient	.482	1.000
10-11 Years N=11	Spearman's rho	sIGF-1 (pg/mL)	Correlation Coefficient	1.000	-.391*
		sALP (ng/mL)	Correlation Coefficient	-.391	1.000
12-13 Years N=15	Spearman's rho	sIGF-1 (pg/mL)	Correlation Coefficient	1.000	.147
		sALP (ng/mL)	Correlation Coefficient	.147	1.000
14-15 Years N=16	Spearman's rho	sIGF-1 (pg/mL)	Correlation Coefficient	1.000	-.200
		sALP (ng/mL)	Correlation Coefficient	-.200	1.000

Spearman correlation coefficient r>0.5 signifies strong positive correlation

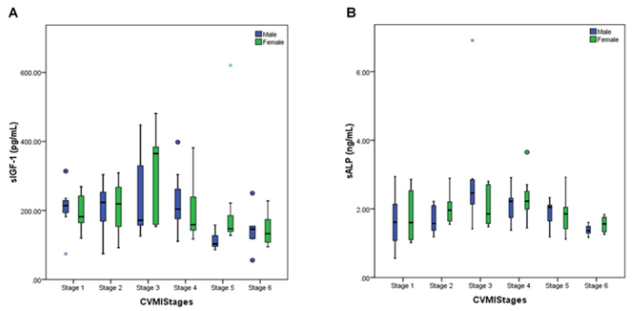

Figure 3: Level of salivary insulin growth factor (sIGF) andsalivary alkaline phosphatase (sALP) level. **A.** Comparison of Mean, Standard deviation andp-value of Chronological age, sIGF-1, among CVMI stages between Group A (Male)and Group B (Female). The mean sIGF-1 levels werefound to increase during stage 3 and stage 4 in both Group A and Group B, butfemales reported higher levels than males during stage 3, though statisticallyinsignificant. In Group B (Females) mean sIGF-1levels again increase duringstage 5 **B.** Comparisonof Mean, Standard Deviation and p-value of Chronological age, sALP among CVMIstages between Group A (Male) and Group B (Female). The mean sALP levels werefound to increase during stage 3 and stage 4 in both Group A and Group Bfollowed by higher levels during stage 2 and stage 5 and decreasing duringstage 1 and stage 6

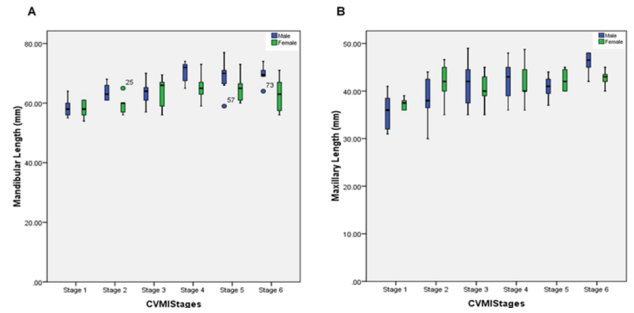

Figure 4: Mandibularand maxillary lengths. **A.** Comparison of Mean,Standard Deviation and p-value of Chronological age, mandibular base lengthamong CVMI stages between Group A (Male) and Group B (Female). Mandibularbase length is found to steadily increase across CVM stages in both GroupA(Male) and Group B (Female) **B**. Comparisonof Mean, Standard Deviation and p-value of Chronological age, maxillary baselength among CVMI stages between Group A (Male) and Group B (Female). Maxillarybase length was found to steadily increase from stage 1 to stage 6

stages for both Group A (male) and Group B (female), with a statistically significant difference found between Group A and Group B values during stage 4 (p=0.046) and stage 5 (p=0.025). (Table 2), (Figure 4 A & B)

The results of one-way analysis of variance showed that there was a significant difference in the total levels of sALP and sIGF-1 between the different CVM stages for both biomarkers (Table 3).

Linear regression analysis was performed to find a dependent variable and its association with chronological age (years) as an independent variable based on gender. It shows a positive association with sALP in men, which is statistically significant ($p < 0.05$) with a p-value of 0.035. In women, no association with chronological age is found (Table 4).

Multinomial logistic regression was used to determine the most appropriate model for predicting cervical maturation stage. Table 5 shows R^2 MF for six multinomial logistic regression models, each using one or more independent variables. The CA, CA+sALP, CA+sIGF-1, CA+sALP+sIGF-1 models were compared, and it was found that the McFadden's R^2 value is 0.412, 0.422, 0.412, and 0.449, respectively, which is greater than 0.3, indicating a reasonable model fit. Among these, CA+sALP+sIGF seems to be the best model for predicting cervical maturation stage (Table 5).

Spearman's Rho correlation showed that sALP correlated negatively with mandibular length in stage 3 and 4 and positively with mandibular length in stage 5, which is a weak correlation overall (Table 6). sIGF-1 did not correlate with either maxillary or mandibular length.

Association of sALP and sIGF-1 with chronological age range of 8-9 years (n=11), 10-11 years (n=11), 12-13 years (n=14), 14-15 years (n=15) was derived using Spearman's Rho. There is a moderately positive correlation with sALP and in the age range of 8-9 years (n=11) with a correlation coefficient of r=0.482, but not with sIGF-1. In the age range of 10-11 years (n= 11), there is a negative correlation with sALP with a correlation coefficient of r= -0.391, but not with sIGF-1. In the other age groups of 12-13 years and 14-15 years, no statistically significant correlation was found either with sIGF-1 or sALP (Table 7).

4. Discussion

The present cross-sectional observational study was conducted on 80 healthy subjects of North Indian origin with the aim of evaluating salivary concentrations of the biomarkers insulin-like growth factor-1 (sIGF-1) and alkaline phosphatase (sALP) as indicators of circumpubertal stages in adolescent individuals to assess skeletal maturation and to find correlations with chronological age, mandibular base length and maxillary base length. The variables were also examined in males and females. Subjects were required to be healthy individuals free of systemic disease

or pregnancy. Clinically healthy subjects who met the inclusion and exclusion criteria to exclude any confounding variable. Those undergoing treatment were excluded to avoid bias, as fluctuations and oscillations in marker levels are prevalent during orthodontic treatment, which could lead to false quantifications of the proposed parameters. In this way, standardisation of sampling was achieved. The evaluation of cervical spine maturation stages using the method described by Hassel and Farman provides a convenient assessment by looking at the shape and concavities in the lower part of CV2, CV3 and CV4 seen in the lateral cephalogram.

Saliva was preferred as a sample because it reflects serum levels and can be used as a potential tool to calibrate skeletal age. It is easy to obtain, available in sufficient quantity and far less invasive than GCF and serum.[13,15–21]

A correlation has been observed between growth spurts and biomarker levels peaking at the same time. When biomarkers are used, radiation exposure can be avoided and they are directly involved in bone growth and remodeling.[22,23] The proposed biomarkers were quantified using the Enzyme Linked Immunosorbent Assay. This immunological test is highly sensitive and is considered the gold standard among immunoassays.[24]

The correlation between chronological age and maturation of the cervical vertebrae has been demonstrated.[25] A significant difference is observed between the chronological age and the skeletal age determined with the CVMI.[26] Insulin-like growth factor-1 (IGF-1), which regulates the action of growth biomarkers, was originally identified as a liver-derived “sulfation factor".[27] IGF-1 mediates growth hormone function and plays an important role in the systemic and local regulation of prenatal and postnatal longitudinal bone growth. Liver, bone and intestine are to some extent the main sources of sALP in serum (> 80%). It is controversial whether sIGF-1 has a direct effect on bone, so it seemed appropriate to include sALP in our study to add to the current knowledge.[28] ALP is more bone-specific and can also withstand multiple freeze-thaw cycles and prolonged freezer storage.[29] study have shown that mandibular growth continues even after skeletal maturity as seen on radiographs.[16] In this study, a similar assessment of mandibular growth and its relationship to CVMI stages is made.

sIGF-1 activity is highest at stage 3, showing a similar pattern to previous studies[30–33]. In our study (Table 1& Figure 3 A), at stage 3 females had higher mean sIGF-1 levels than males (297.92±136.88 pg/ml and 245.41±135.63 pg/ml, respectively), which is consistent with a previous study.[34] In our study, a high standard deviation was observed at stage 3. This could be due to large individual differences in terms of skeletal maturation and has also been noted by other authors[35,36] and in previous studies.[6,9] It has also been observed that the increase in the mean value of sIGF-1 in stage 6 males could be a marker of residual mandibular growth as well as their prolonged growth spurt.[6] Sexual dimorphism is also clearly visible here. The mean value of sIGF-1 increases in both subgroups during the pubertal growth phase, which has also been found in previous studies.[9,37,38]

The mean sALP value in saliva (Table 1 & Figure 3 B)was highest in group A (males) at stage 3 (2.96±1.81 ng/ml) followed by stage 4 (2.08± 0.54 ng/ml), while in group B (females) the highest mean value was observed at stage 4 (2.33±0.71 ng/ml) followed by stage 3 (2.09±0.61ng/ml).

This pattern shows that the highest concentration is reached in the pubertal phase and at the same time a sexual dimorphism can be observed. The pattern observed in group B (female) is consistent with the general results of previous studies[39]. The pattern observed in group A (male) is consistent with previous studies.[16] The results of our study support the findings demonstrating higher sALP activity in the pubertal phase of skeletal maturation compared to the pre- and post-pubertal phases, which has also been found in previous studies[13,16,22,32,40–44] There is an insignificant difference in sALP levels between males and females at stage 3 and 4, indicating that there is no difference in sALP levels at the threshold of the pubertal growth spurt. Such a result has been reported previously.[16] The mean sALP levels show no statistically significant difference ($p > 0.05$) between pre-pubertal, pubertal and post-pubertal stages which is analysis consistent with previous studies.[45]

Table 3 hows a statistically significant difference in the mean values of sIGF-1 between all stages. Our data show the pattern that the second highest overall mean value of sIGF-1 is observed in stage 4 and there is a drastic decrease in stage 5 and 6, which was similarly found in other studies[9] and is statistically significant with a p-value (0.003). A similar pattern was also found in other work[22,39] in relation to GCF ALP.

The post-pubertal activity of sALP was the lowest, followed by the pre-pubertal, similar to the results of a previous study.[16]

Using linear regression analysis, an association between sALP and chronological age was found in males, but further studies are needed to confirm this [Table 4]. Of the 6 multinomial models (Table 5), although CA+sALP+sIGF-1 is the best model for predicting CVMS, the model with the second best fit CA+sALP is better for clinicians per se. In addition to traditional techniques, skeletal assessment can be improved by new tools such as biomarkers. This study is in line with previous work.[39,46]

It was found that sALP correlates only weakly with mandibular length. In agreement with previous studies,[16] a positive correlation was found between sALP and mandibular base length (Table 6) at stage 3, 4 and 5.

No strong correlation was found with growth changes in maxillary base length. There is a negative correlation between mandibular base length and sIGF-1 at stage 4 with a correlation coefficient of -0.317, which is a weak correlation, but no correlation was observed at stage 3 and stage 5, which is consistent with previous studies.[6] Since no correlation was observed between stage 3, 4 and 5 with sIGF-1 activity, this result is consistent with a previous study.[28]

sALP was found to correlate moderately with chronological age. This finding is consistent with previous studies[12,15] sIGF-1 does not correlate with chronological age (Table 7).

The results show that recruiting a specific population is a tedious task and that a larger sample size is required when conducting further studies. Since sIGF-1 is only a one percent quantification compared to serum or blood, further studies using blood or serum as samples could be conducted to find more correlations. One can also include a larger number of biomarkers to find correlations that could be clinically relevant. Since saliva as a sample is non-invasive, the results can be correlated with invasive analysis using blood as a sample.

5. Conclusions

The levels of biomarkers (sIGF-1 and sALP) was found to be higher in both men and women and correlate well with CVMI stage 3. Salivary ALP was found to be dependent on chronological age and thus showed both an association and sexual dimorphism with a p-value of 0.035 in men. Salivary IGF-1 levels peak in the corresponding age range of 12±1.9 years at CVMI stage 3 in both males and females and could be a potential diagnostic measure to predict pubertal growth spurt. sIGF-1 could serve as a marker for residual growth spurt as the level increases at CVMI stage 6 and shows a significant difference compared to CVMI stage. The sALP concentrations with their mean values peaked at stage 3 in males and at stage 4 in females, indicating a sexual dimorphism. The highest mean value of sALP was found in CVMI stage 3 with 2.53±1.37 ng/ml at the age range of 12±1.9 years. Mandibular length was found to correlate weakly with sALP levels. The values quantified with this study could serve as an estimate of the clinical range at which pubertal peak growth predominates.

6. Abbreviations

1. sIGF-1: Salivary Insulin-like growth factor-1
2. sIGF: Salivary alkaline phosphatase
3. CVMS: Cervical vertebrae maturation stages
4. Go-Pog: mandibular length
5. PNS- Point A: maxillary length
6. ELISA: Enzyme- linked immunosorbent assay
7. ANOVA: Analysis of variance
8. SPSS: Statistical Package for the Social Sciences

7. Compliance with Ethical Standards

Ethical approval was obtained from the institutional ethical committee, Research Cell, King George Medical University, Lucknow, Uttar Pradesh, India. The ethical approval number of the study is Ref code VII-PGTSC-2A/P26. No photograph of the patient is used in this study.

7.1. Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

8. Source of Funding

None.

9. Conflict of Interests

The authors declare that there is no conflict of interests.

10. Acknowledgments

The authors are grateful to the Vice Chancellor, King George's Medical University (KGMU) Lucknow, for the encouragement for this work. Authors Gyan P. Singh and Shailendra K. Saxena contributed equally as first author. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Cite this article: Kumar S, P. Singh G, K. Singh G, Shastri D, K. Saxena S. Appraisal of skeletal maturity indicators with respect to non-invasive biomarkers. *J Contemp Orthod* 2024;8(3):281-289.