ISSN No:-2456-2165

Salivary Osteocalcin as a Non Invasive Biomarker of Skeletal Maturation- A Cross Sectional Study

Sharath Kumar Shetty[1], Mahesh Kumar Y[2], Monica R[3]

1. Sharath Kumar Shetty- Professor & HOD, Department of Orthodontics & Dentofacial Orthopaedics, K.V.G Dental College & Hospital, Sullia, Karnataka, India
2. Mahesh Kumar Y- Professor, Department of Orthodontics & Dentofacial Orthopaedics, K.V.G Dental College & Hospital, Sullia, Karnataka, India
3. Monica R, Post Graduate Student, Department of Orthodontics & Dentofacial Orthopaedics, K.V.G Dental College & Hospital, Sullia, Karnataka, India

Abstract:-
Objective: This cross sectional study tested the hypothesis -salivary osteocalcin as a noninvasive biomarker of skeletal maturation. Methods: This cross sectional study included 50 subjects [25 males and 25 females] categorized into five CVMS based on lateral cephalographs and 2 ml unstimulated whole saliva samples taken from each patient on the same day. Salivary osteocalcin activity in unstimulated whole saliva were compared among five CVMS. The association between age and CVMS was also assessed. One-way analysis of variance (ANOVA) was used to compare salivary osteocalcin levels and cvms. Results:The highest value of salivary osteocalcin level was in CVM II [1.68±1.68] and lowest in CVM V[1.30±1.47].The comparison between salivary osteocalcin level with five CVM stages depicted insignificant results[p=0.869].However,there is a positive association between age and CVM stages[p=<0.01]. Conclusion: There was a negative correlation between the salivary osteocalcin levels and CVM stages Therefore,osteocalcin in saliva seem to be uncertain for skeletal growth assessment. More studies are needed to explain the precise role of salivary osteocalcin as predictor of skeletal maturation

I. INTRODUCTION

The present orthodontics primarily focus on the correction of malocclusion in the early stages, giving importance to position of teeth in the base of the bone and any discrepancy that can be corrected in any life time and these can be achieved by knowing the peak pubertal growth stage.[1]

In order to practise clinical orthodontics effectively, it is crucial to comprehend growth events. The diagnosis, treatment objectives, treatment planning, and final results of orthodontic treatment can all be significantly impacted by a patient's developmental stage.[2]

According to Ricketts, in order to benefit from growth, we must first understand its scale, direction, and time components. By combining one's understanding of size and direction with the moment of maximum growth, one may easily change orthodontics into a profession that focuses on "facial formation as well as tooth arrangement".[3]

There are different methods of assessing the skeletal maturity such as hand wrist radiographs, cervical vertebral maturation using lateral cephalogram, clinical and radiographical examination of different stages of tooth development[4]

In recent years, cervical vertebral maturation index using lateral cephalograms has become commonly used method for assessment of skeletal maturity because cervical vertebrae are readily visible on the lateral cephalogram which is routinely used in orthodontic practice[5]

Hassel and Farman conducted a study and developed a system of skeletal maturation determination using the cervical vertebrae [C2, C3, and C4]. They put forward six stages of cervical vertebral development[6]

Analysis of Salivary components markers for the process of diagnosis plays an important role in the field of medicine and dentistry.[7,8]

Bone-specific alkaline phosphatase (BSAP), osteocalcin, and N-terminal propeptide of type I procollagen (PINP) are markers specific for bone formation, while N-terminal telopeptide of type I collagen (NTX) and C-terminal telopeptide of type I collagen are markers specific for bone resorption (CTX)[9,10]

Bone and dentin contain osteocalcin, also known as bone gamma-carboxyglutamic acid-containing protein (BGLAP), which was first discovered as a calcium-binding protein in chick bone. It is a tiny (49 amino acid) noncollagenous protein hormone.[11]

Tulika Tripathi and colleagues performed a study to measure the levels of blood osteocalcin and insulin-like growth factor-1 (IGF-1) and relate them to stages of the cervical vertebral maturation index (CVMI). Across six CVMI stages, the data demonstrate a statistically significant association between blood IGF-1 and serum osteocalcin.[12]

To date, only a few studies have investigated salivary osteocalcin and their correlation with skeletal maturation Thus, the aim of the present study was to assess the levels of salivary osteocalcin and to compare it with CVMI stages.

➢ *Aim and Objectives of the study*

1. To estimate the osteocalcin levels in whole unstimulated saliva.
2. To estimate the cervical vertebral maturation using lateral cephalogram
3. To compare the salivary osteocalcin levels with cervical vertebral maturation stages

II. SUBJECTS AND METHODS

A total of 50 individuals reporting to KVG dental college and hospital,sullia who are in the age group between 9-18years were included in this cross-sectional study after patient's consent. The study was approved by the institutional review board.[IECKVGDCH/SS31/2022-23]

The sample was divided into the following 5 subgroups according to the different skeletal maturity stages as assessed Hassel and Farman. The staging is described as below

Stage 1 (INITIATION): At this stage, Inferior borders of C2, C3, and C4 should be flat, wedge shaped, and tapering of the superior vertebral borders posterioanteriorly.

Stage 2 (ACCELERATION): At this stage, the Inferior borders of C4 was flat, in the inferior borders of C2 and C3 concavities started developing, the bodies of C3 and C4 were nearly rectangular in shape.

Stage 3 (TRANSITION): At this stage, in the inferior borders of C2 and C3, distinct concavities will be seen, in the inferior border of C4 concavity started developing, the bodies of C3 and C4 were rectangular in shape.

Stage 4 (DECELERATION): At this stage, distinct concavities will be seen in the inferior borders of C2, C3, and C4, the vertebral bodies of C3 and C4 will be more square in shape.

Stage 5 (MATURATION): At this stage, more accentuated concavities will be seen in the inferior borders of C2, C3, and C4 and the bodies of C3 and C4 would be nearly square to square in shape.

Stage 6 (COMPLETION): At this stage, deep concavities will be seen in the inferior borders of C2, C3, and C4 and the bodies of C3 and C4 will be square or will be greater in vertical dimension than in horizontal dimension.

The criteria for sample selection were based on the following:

➢ *Inclusion criteria:*

- Patients aged 9-18 years.
- Patients with healthy periodontium
- Patients before initiation of orthodontic /orthopaedic/functional appliance treatment

➢ *Exclusion criteria*

- Previous history of fixed orthodontic appliance treatment
- Patients already undergoing orthodontic treatment with fixed/removable/functional appliance
- History of systemic diseases affecting growth and development
- Patients under any medication.
- History of xerostomia
- Patients with periodontal diseases
- Patients with bone disorders

III. COLLECTION OF DATA

A total of 50 subjects reporting K.V.G. Dental College and Hospital, Sullia were be included in this study, based on eligibility criteria cited above. The subjects eligible at screening visit were scheduled for a second visit and were instructed to avoid food and fluid ingestion or chewing gums for 90 minutes before saliva collection. From the subjects, who fulfilled the above instruction, 2ml of un-stimulated saliva were collected in plastic vials and stored immediately in an ice box before sending to laboratory. The Salivary samples were analysed for osteocalcin levels using enzyme-linked immunosorbent assay (ELISA). .Pre-treatment lateral cephalograms of these participants were collected on the same day and assessed for CVM stages

IV. STATISTICS

Data were analysed using SPSS software version 21.The P value set at <0.05. The association between salivary osteocalcin levels and stages of skeletal maturity using CVMI was determined by ANOVA test with Bonferroni correction used for post-hoc comparisons

V. RESULTS

The basic characteristics and distribution of study showed mean age at 12.47±2.45 years and salivary osteocalcin level at 1.47±1.31ng/ml.Categorical variables were expressed in frequency and percentage males and females were 50% each. Cervical vertebral maturation stage was divided into I-V in which CVM I was 10%,CVM II was 26%,CVM III was 30%,CVM IV was 26%,CVM V was 8%[table 1]

Table 1:Basic Characteristics

Variables		**Mean±SD**	
Age[years]		**12.47±2.45**	
Salivary osteocalcin level[ng/ml]		**1.47±1.31**	
	Responses	**Frequency**	**Percentage**
Sex	Male	25	50%
	Female	25	50%
Cervical vertebral maturation stage	I	5	10%
	II	13	26%
	III	15	30
	IV	13	26%
	V	4	8%

ISSN No:-2456-2165

The comparison of Age and CVM stages which was done using one-way ANOVA test depicted statistically significant results[table 2]

Table 2:Comparison Of Age And Cvm Stage

		AGE[years]	P value
CVM STAGE	I	9±0.63	<0.01**
	II	10.69±0.75	
	III	12.6±0.63	
	IV	14.07±0.75	
	V	16±0.81	

*P<0.05

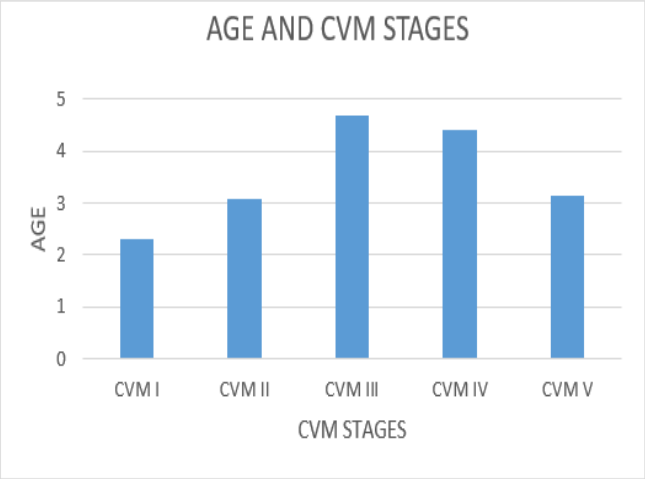

Fig 1: Comparison of Age and CVM stages

Table 3 :Comparison Between Salivary Osteocalcin Levels And Cervical Vertebral Maturation Stages

		Salivary osteocalcin level[ng/ml]	P value
CVM STAGE	I	1.34±1.20	0.869
	II	1.68±1.68	
	III	1.52±1.23	
	IV	1.36±1.45	
	V	1.30±1.47	

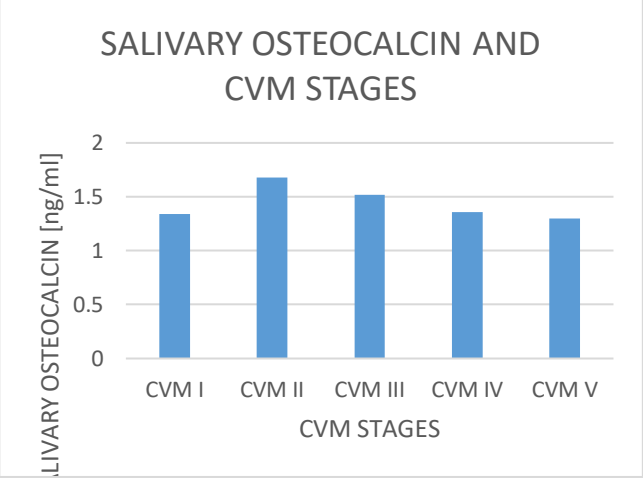

Fig 2: comparison of salivary osteocalcin and CVM stages

The comparision of salivary osteocalcin level and CVM stages were done using ANOVA test and they were not statistically significant. The highest value of salivary osteocalcin is seen in CVM II[1.68±1.68] and lowest value is seen in CVM V[1.30±1.47]

VI. DISCUSSION

The findings of this cross-sectional investigation showed a favourable relationship between cervical vertebral maturation stages and chronological age. [table 3]. This result is in line with research by Nora Alhazmi et al, Litsas and Lucchese et al, and others.[13,14]

According to the study's findings, CVM II (1.68 ng/ml) had the highest salivary osteocalcin levels, followed by CVM III (1.52 ng/ml) and CVM IV (1.36 ng/ml). The two CVMs with the lowest values were CVM I (1.30 ng/ml) and CVM V (1.34 ng/ml)

The comparison between salivary osteocalcin levels and CVM stages were done using ANOVA test and the results were not statistically significant.

A study conducted by Georgios Kouvelis concluded that alkaline phosphatase and osteocalcin levels in saliva and GCF were not statistically correlated to the maturation stages. [15]

Additionally, a study by Katharina Kerschan-Schindlet et al. revealed no significant link between bone mineral density and the corresponding bone turnover indicators in saliva and a substantial correlation between serum osteocalcin and bone mineral density.[16]

This study was consistent with the above mentioned studies.[15,16] However , a study conducted by Tulika Tripathi et al ,found a statistically significant correlation between serum IGF-1 and serum osteocalcin across six CVMI stages ($P < 0.01$][12]

A longitudinal study with a bigger population would have provided standardised salivary osteocalcin levels at different stages of skeletal development instead of this cross-sectional study's small sample size and cross-sectional design. A comparison research comparing the levels of serum and salivary osteocalcin at various CVM phases would have produced more conclusive findings.

VII. CONCLUSION

The levels of salivary osteocalcin and CVM stages had a negative correlation. Therefore, the value of osteocalcin in saliva for measuring bone growth appears to be questionable. The specific function of salivary osteocalcin as a predictor of skeletal maturation requires further research.

REFERENCES

[1]. Moore RN, Moyer BA, DuBois LM. Skeletal maturation and craniofacial growth. American Journal of Orthodontics and Dentofacial Orthopedics. 1990 Jul 1;98(1):33-40
[2]. Todd TW. Atlas of skeletal maturation: part 1: hand. London: Kimpton. 1937
[3]. Ricketts RM. Facial and denture changes during orthodontic treatment as analyzed from the temporomandibular joint. American Journal of Orthodontics. 1955 Mar 1;41(3):163-79
[4]. Songra G, Mittal TK, Williams JC, Puryer J, Sandy JR, Ireland AJ. Assessment of growth in orthodontics. Orthodontic Update. 2017 Jan 2;10(1):16-23
[5]. Zhao XG, Lin J, Jiang JH, Wang Q, Ng SH. Validity and reliability of a method for assessment of cervical vertebral maturation. The Angle Orthodontist. 2012 Mar;82(2):229-34.
[6]. Hassel B, Farman AG. Skeletal maturation evaluation using cervical vertebrae. American Journal of Orthodontics and DentofacialOrthopedics. 1995 Jan 1;107(1):58-66
[7]. Seibel MJ. Molecular markers of bone turnover: biochemical, technical and analytical aspects. Osteoporosis International. 2000 Dec 1;11:S18.
[8]. Mora S, Pitukcheewanont P, Kaufman FR, Nelson JC, Gilsanz V. Biochemical markers of bone turnover and the volume and the density of bone in children at different stages of sexual development. Journal of bone and mineral research. 1999 Oct;14(10):1664-71.
[9]. Delmas PD. Biochemical markers of bone turnover for the clinical investigation of osteoporosis. Osteoporosis International. 1993 Jan;3(1):81-6
[10]. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiological reviews. 1989 Jul 1;69(3):990-1047.
[11]. Magnusson P, Häger A, Larsson L. Serum osteocalcin and bone and liver alkaline phosphatase isoforms in healthy children and adolescents. Pediatric research. 1995 Dec;38(6):955-61.
[12]. 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. Progress in Orthodontics. 2017 Dec;18(1):1-8.
[13]. Alhazmi N, Trotman CA, Finkelman M, Hawley D, Zoukhri D, Papathanasiou E. Salivary alkaline phosphatase activity and chronological age as indicators for skeletal maturity. The Angle Orthodontist. 2019 Jul;89(4):637-42
[14]. Litsas G, Lucchese A. Dental and chronological ages as determinants of peak growth period and its relationship with dental calcification stages. The open dentistry journal. 2016 Apr 8;10(1).
[15]. The role of alkaline phosphatase and osteocalcin in saliva and gingival crevicular fluid as indicators of skeletal maturity in children (Doctoral dissertation,).
[16]. Kerschan-Schindl K, Boschitsch E, Marculescu R, Gruber R, Pietschmann P. Bone turnover markers in serum but not in saliva correlate with bone mineral density. Scientific Reports. 2020 Jul 14;10(1):1-8