



# Evaluation and Correlation of Salivary Bone-Specific Alkaline Phosphatase Level with Skeletal Age

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## Abstract

**Background:** This study aimed to measure bone-specific alkaline phosphatase (B-ALP) levels in saliva and determine how they relate to different stages of skeletal development, as assessed by hand-wrist X-rays using the Hagg and Taranger method. Since orthodontic treatment is closely linked to an individual's growth, the development of the jaws, face, and overall body, and how they affect the alignment of teeth, the suggestion to use biomarkers to evaluate an individual's skeletal maturity has emerged as a promising approach. Unlike traditional radiographic methods, which are subjective and based on morphology, biomarkers provide objective indicators related to the patient's physiology, and they do not involve radiation exposure or magnification errors. The main goal of this study was to measure B-ALP levels in saliva and examine their correlation with varying degrees of skeletal maturity. In a positive correlation, the total ALP present in saliva could be a valuable biological indicator in growing patients.

**Methods:** Thirty patients were randomly selected for the study based on the inclusion criteria: Age of the individual: 9–19 years, with good general health and no nutritional issues. A sample of unstimulated whole saliva was collected using a passive drooling method to estimate levels of the bone alkaline phosphatase using an enzyme-linked immunosorbent assay (ELISA) kit. After saliva collection, hand-wrist radiographs were immediately obtained and manually traced onto 50-micron-thick lead acetate tracing sheets using an 0.5-mm lead pencil. The hand-wrist radiographs were then categorized into five groups, which involved analyzing the morphology of the hand-wrist radiographs: group SO (prepubertal), group S (pubertal onset), group MP3G (peak pubertal), group DP3 (pubertal deceleration), and group RJ (growth completion).

**Results:** There were significant differences between the B-ALP levels between different skeletal ages. The salivary B-ALP values of the group MP3 were significantly higher than those of groups SO and RJ. The mean salivary B-ALP levels consistently increased from the group SO to the group MP3, followed by a gradual decrease.

**Conclusion:** Salivary alkaline phosphatase activity was significantly higher during the peak pubertal period (group MP3) compared to both the pre-peak (group SO) and post-peak (group RJ) values. This finding suggests that salivary bone alkaline phosphatase can be used as an additional indicator alongside conventional skeletal maturation indicators to assess pubertal development.

**Keywords:** Alkaline Phosphatase, Puberty, Saliva, Skeletal Age, Skeletal Maturity.



## Background

Orthodontics examines how jaw, facial, and body development impact tooth alignment. It aims to prevent and correct abnormal growth. Any deviation in craniofacial growth can lead to malocclusion, categorized as dental or skeletal (1). Understanding normal growth and spotting deviations is essential. Growth involves self-multiplication, size increase, and variability. Specific growth periods, like growth spurts, differ by gender (1). Understanding these growth phases is crucial for orthodontic treatment, especially when skeletal disharmonies occur. Analyzing x-rays, particularly cephalometric x-rays and hand-wrist radiographs, aids in evaluating skeletal maturity for orthodontic cases (2,3). Various methods, such as Fishman's bone maturity stages and Lamparski's cervical vertebrae assessment, have been crucial in understanding skeletal age (4-9). Biomarkers, like bone-specific alkaline phosphatase (B-ALP), are gaining significance in determining skeletal maturity (10,11). They offer advantages over traditional radiographic methods by reflecting physiological aspects, though osteocalcin's significance is debated (12). Saliva, a non-invasive diagnostic fluid, also contains ALP and has advantages in collection and diagnosis (13,14). Serum B-ALP levels peak during puberty, aiding bone growth assessment (15). However, its correlation with saliva during growth phases remains uncertain. Biomarkers offer a promising, less invasive way to predict skeletal maturity and could potentially reduce radiation exposure, benefiting orthodontic patients. Salivary ALP could serve as a valuable biomarker in growing patients if proven to correlate.

## Aim

To determine salivary bone-specific alkaline phosphatase (B-ALP) levels and correlate them with various skeletal ages as assessed by hand-wrist radiographs using the Hagg and Taranger method.

## Objectives

- To identify and estimate the B-ALP levels in whole unstimulated saliva at various skeletal ages
- To correlate B-ALP levels with different stages of skeletal development.

## Null hypothesis

There is no correlation between bone-specific alkaline phosphatase salivary levels and skeletal age.

## Methods

Thirty patients referring to the Department of Orthodontics & Dentofacial Orthopaedics, Inderprastha Dental College & Hospital, Sahibabad, Ghaziabad, India, for orthodontic treatment were included in the study.

## Inclusion criteria

- ❖ Age of the individual: 9–19 years
- ❖ Good general health, no nutritional issues
- ❖ Good oral hygiene

## Exclusion criteria

- ❖ Individuals with systemic diseases, such as vitamin D metabolism disorders, parathyroid, growth and thyroid hormone disorders, renal impairment, and diabetes mellitus impacting the growth
- ❖ Individuals having taken any medications that affected bone metabolism in the last six months, such as vitamin preparations and calcium supplements.

## Materials

- Eppendorf tubes (Figure 1)
- Elisa kit (Figure 2)
- Hand wrist radiographs (Figure 3)
- Lead acetate tracing sheets (50 µm in thickness) (Figure 4)
- Lead pencil (0.5 mm) (Figure 5)



Figure 1. Eppendorf tubes



Figure 2. ELISA kit



Figure 3. Hand-wrist radiograph



Figure 4. Lead acetate tracing sheets (50  $\mu$ m)



Figure 5. Lead pencil (0.5 mm)

### Procedural steps

Patients referred to the Department of Orthodontics and Dentofacial Orthopaedics, Inderprastha Dental College and Hospital, requiring fixed orthodontic treatment, 30 patients were included in the study based on the inclusion and exclusion criteria. After the patients had been selected, they were informed about the study, and if they agreed with the protocol, they signed the consent form.

A sample of unstimulated whole saliva was collected using the passive drooling method to estimate salivary levels of bone alkaline phosphatase (salivary B-ALP) (Figure 6). The collected samples were stored in an ice box before being sent to the biochemistry laboratory. In the laboratory, the samples underwent centrifugation to separate the sediments in saliva. The salivary bone alkaline phosphatase levels were analyzed using the enzyme-linked immunosorbent assay (ELISA) kit.

The hand-wrist radiographs were obtained immediately following saliva collection. They were manually traced onto tracing sheets composed of 50- $\mu$ m-thick lead acetate using an 0.5-mm lead pencil (Figure 7). Furthermore, the hand-wrist radiographs were classified into five groups using Hagg and Taranger's proposed assessment method, which involves morphological analysis of hand-wrist radiographs.

Group SO (prepubertal) consisted of 6 patients who exhibited the absence of ossification of the ulnar sesamoid in the metacarpophalangeal joint of the first finger.

Group S (pubertal onset) comprised 6 patients who demonstrated the presence of ossification of the ulnar sesamoid in the metacarpophalangeal joint of the first finger.

Group MP3G (peak pubertal) included 6 patients who displayed thickening of the epiphysis

of the middle phalanx of the third finger (MP3), which formed a sharp edge distally on one or both sides while also covering the metaphysis.

Group DP3 (pubertal deceleration) consisted of 6 patients who showed complete fusion between the epiphysis and metaphysis of the distal phalanx of the third finger (DP3).

Group RJ (growth completion) comprised 6 patients who exhibited the completion of fusion between the epiphysis and metaphysis of the distal epiphysis of the radius (R).

**Assessment of salivary bone alkaline phosphatase (salivary B-ALP)**

Saliva samples were collected and stored at 4°C before being sent to the biochemistry laboratory. The samples were then analyzed using an enzyme-linked immunosorbent assay (ELISA) kit to measure the level of salivary B-ALP.

The ELISA kit used a sandwich assay format. Antibodies to salivary B-ALP were first coated onto the wells of an ELISA plate. Then, 100 µL of saliva

was added to each well of the plate. The plate was incubated at room temperature for 2 hours, allowing the salivary B-ALP to bind to the antibodies in the wells. Next, 100 µL of a secondary antibody was added to each well of the plate. This antibody was linked to an enzyme that would react with a substrate to produce a colored product. The plate was incubated for another 2 hours, allowing the secondary antibody to bind to the salivary B-ALP-antibody complexes in the wells. Then, 100 µL of the enzyme substrate was added to each well of the plate. The plate was incubated for the specified time, according to the manufacturer’s instructions, typically 30 minutes. During this time, the enzyme in the secondary antibody would react with the substrate to produce a colored product. The absorbance of the colored product in the wells was measured using a spectrophotometer (Figure 8), and the results were expressed in units per liter (U/L). Salivary B-ALP levels were then subsequently correlated to skeletal maturity stages.



Figure 6. Unstimulated whole saliva collection



Figure 7. A) Hand-wrist radiograph. B) Hand-wrist radiograph traced to assess the skeletal maturity stage





Figure 8. Spectrophotometer

**Results**

The means and standard deviations of salivary B-ALP (U/l) levels were calculated for each group based on skeletal age. The comparison of salivary B-ALP values between the different skeletal groups was performed using one-way ANOVA (Table 1). Subsequent intergroup analysis was performed using Tukey tests to examine the differences between the groups (Table 2). Salivary B-ALP levels

were notably higher in group MP3, followed by group S, group DP3, and group SO, and were at their minimum in group RJ. These findings are also illustrated graphically in Figure 9, where group MP3 displayed the highest peak at 122.5 U/l, while group RJ exhibited the lowest level at 70.03 U/l.

Significant differences were found between groups SO and MP3 (P=0.001) and between groups MP3 and RJ (P=0.001). Additionally, statistically significant differences were observed between subgroups SO and S (P=0.010) and subgroups S and RJ (P<0.01). No significant differences were seen between groups SO and S (P=0.01), SO and DP3 (P=0.14), SO and RJ (P=0.99), S and MP3 (P=0.87), S and DP3 (P=0.87), and MP3 and DP3 (P=0.21).

Moreover, the overall correlation table demonstrated that salivary alkaline phosphatase activity was significantly higher during the peak pubertal period (group MP3) compared to both the pre-peak (group SO) and post-peak (group RJ) values. This finding suggests that salivary bone alkaline phosphatase can be used as an additional indicator alongside conventional skeletal maturation indicators to assess pubertal development.

Table 1. ALP activity levels in different groups

GROUPS	N	ALP mean	Standard deviation	P-value
SO	6	72.6500	3.40869	< 0.001
S	6	112.2667	5.25761	
MP3	6	122.5000	6.13210	
DP3	6	98.8000	11.63320	
RJ	6	70.0333	9.07960	

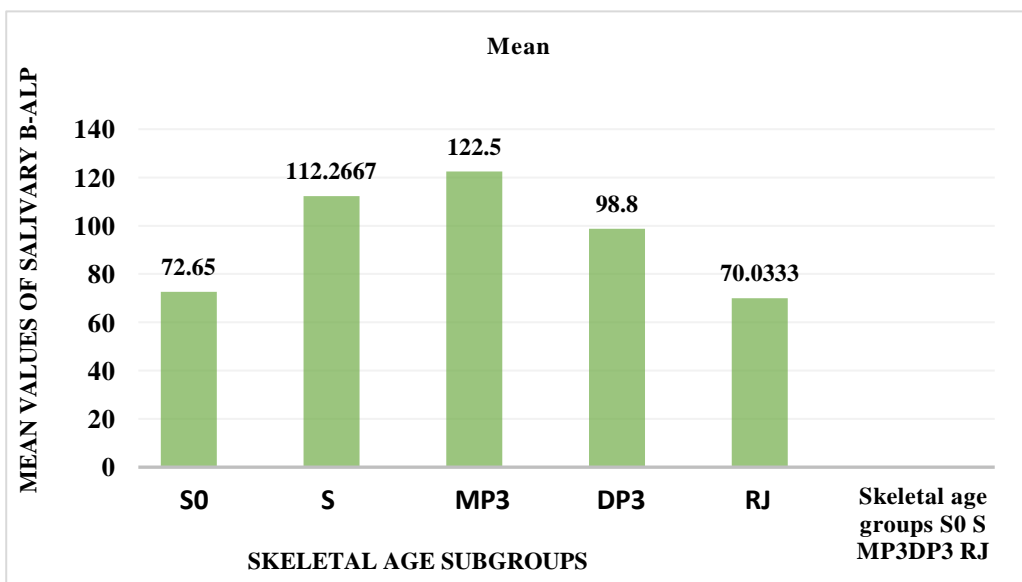


Figure 9. Mean levels of bone-specific alkaline phosphatase, measured in units per liter, along with confidence intervals for each subgroup

**Table 2.** Inter-group comparison of ALP activity

Groups	SO	S	MP3	DP3	RJ
SO	-	0.010	0.010	0.146	0.999
S (P)	0.010	-	0.877	0.728	0.005
MP3 (P)	0.001	0.877	-	0.219	0.001
DP3 (P)	0.146	0.728	0.219	-	0.091
RJ (P)	0.999	0.005	0.001	0.091	-

P-value based on post hoc analyses using Tukey test\*

= statistically significant (p<0.05)

## Discussion

This study evaluated and correlated the salivary bone-specific alkaline phosphatase levels (B-ALP) with skeletal age by identifying and estimating the B-ALP levels in whole unstimulated saliva at various skeletal ages and correlating B-ALP levels with different stages of skeletal development using the Hagg and Taranger method in patients undergoing orthodontic treatment.

This study categorized the patients into different groups based on the assessment of skeletal maturity stages outlined by Hagg and Taranger using x-rays of the hand and wrist. These stages included prepubertal, pubertal onset, peak pubertal, pubertal deceleration, and growth completion (7). The study then conducted valid comparisons between skeletal maturity stages and ALP to examine their relationship.

According to the study's findings, comparing the group MP3 (MP3) to subgroups 1 (SO) and 5 (RJ), salivary B-ALP values were significantly higher in the group MP3 (MP3). Additionally, the salivary B-ALP levels demonstrated a steady rise in mean value from subgroup SO to subgroup MP3, followed by a constant decline. The study's findings are consistent with previous research on how serum B-ALP levels relate to Tanner's developmental stage and chronological age. The normal range for serum alkaline phosphatase is 20–140 U/L. ALP for the subgroup SO in our study ranged from 40 to 103 U/L, showing less growth in the individual.

The subgroup MP3 was considered peak pubertal because increased growth will occur during puberty. According to Scammon's growth curve, body tissues generally accelerate during puberty. The ALP range for the subgroup MP3 of our study was 55–137 U/L, indicating increased individual growth, consistent with the findings of Tarvade et al. (2015), who discovered that salivary ALP levels were the highest at the G stage of MP3.

Subgroup RJ was considered post-peak because the growth would have reached its apex. The ALP range for the subgroup RJ was 30–58 U/L, indicating that the individual had completed growth. The

findings of our study revealed that salivary ALP activity was higher in the subgroup MP3 (peak) and lower in groups SO (pre-peak) and RJ (post-peak), which corresponds to skeletal stages, suggesting a positive relationship between skeletal maturation and salivary alkaline phosphatase levels.

ALP values are highly significant when correlated with the skeletal age, so when growth estimation is required, they can be used alone or in conjunction with hand-wrist radiographs during treatment, particularly in CI II cases with headgear therapy and in CI III cases with chin cup therapy, or to evaluate the prognosis of the treatment early on during routine fixed appliance treatment.

The results correlate well with the standard radiographic maturity indicator, so estimating the salivary ALP, when necessary, could help reduce radiation exposure for developing individuals more completely.

This study also demonstrated that the non-invasive method of assessing skeletal maturation with salivary ALP could replace the most frequently used supplemental hand-wrist radiographs for growth assessment.

Previous studies have shown elevated levels of salivary ALP during rapid growth periods, adolescence, and puberty, possibly due to hormonal and growth-related changes (15). However, these studies often involve invasive procedures that patients and parents may object to. For example, collecting gingival crevicular fluid (GCF) for ALP measurement is cumbersome (16). Saliva collection, on the other hand, is non-invasive and easily performed. It consistently shows increased ALP levels during puberty. However, due to underlying conditions, the correlation between total serum ALP activity and serum bone-specific ALP (B-ALP) levels may vary in clinical groups.

Tarvade et al. (13) observed elevated levels of salivary ALP during the period of rapid growth. Similarly, Cabras et al. (14) reported increased protein levels in saliva during adolescence, which could be linked to hormonal and growth-related changes. Several other studies, including those by Christesen (15), Takimoto et al. (16), and Insoft et al. (17), have demonstrated an elevation in serum

ALP levels during puberty. However, due to the invasive nature of these procedures, patients and parents often express objections. Baccetti and Perinetti (18) found an increase in GCF ALP levels during puberty, but the collection of GCF is a cumbersome process. Nonetheless, various studies have consistently shown increased salivary ALP during puberty, making saliva collection a non-invasive and easily performed alternative. Wijaya et al. (19) discovered that the changes in total serum ALP activity generally reflect the alterations in serum bone-specific ALP (B-ALP) levels in healthy children. However, this correlation may not hold in other clinical groups due to the impact of underlying diseases or drug treatments on liver-specific ALP isoforms, which often affect the overall serum ALP activity.

The research by Wijaya et al. (19) did not reveal any variances in the levels of salivary B-ALP throughout the various stages of pubertal growth in Indonesian children. Consequently, the effectiveness of salivary B-ALP as a predictive biomarker for pubertal growth is questionable. The authors suggest additional studies specifically focusing on salivary B-ALP concerning disease conditions or the impact of drug treatments, as these factors can frequently influence the overall activity of ALP in the bloodstream.

According to the current study, salivary alkaline phosphatase can be conclusively regarded as a sign of skeletal maturation. Using a non-invasive, concise, and easy procedure that causes the patient the least discomfort while maintaining the validity of the desired results, this study opens up new possibilities for growth maturation assessment.

## Conclusion

The pubertal growth phase has many clinical implications, particularly when there are skeletal disharmonies. They must be diagnosed precisely with the developing malocclusion, whether skeletal or dental, as soon as possible. The orthodontic treatment regime's preventive and interceptive modalities are essential in dealing with these adverse clinical circumstances. The patient's skeletal age must be determined before beginning the appropriate treatment for these conditions.

Biomarkers have recently been proposed as a promising aid in assessing individual skeletal maturity, with the advantage of being a true indicator pertaining to the patient's physiology. In contrast, traditional radiographic methods are morphologically subjective, with radiation exposure and magnification errors.

This study aimed to determine whether salivary alkaline phosphatase could be used as a non-invasive biomarker to assess skeletal maturation.

Salivary samples were collected for this study, and alkaline phosphatase activity was measured using an ELISA kit. It was correlated to Hagg and Taranger's skeletal maturity stages. For intergroup comparisons, salivary alkaline phosphatase and skeletal bone age were analyzed using ANOVA followed by post hoc Tukey tests. Subgroups SO, S, MP3, DP3, and RJ had mean values of 72.65, 112.26, 122.5, 98.8, and 70.03 U/L, respectively. The findings revealed a significant relationship between salivary alkaline phosphatase and the skeletal age method. The highest mean ALP values were observed during the peak stages of pubertal development. The comparisons within groups were statistically significant as well.

Hence, salivary alkaline phosphatase could be a different approach for evaluating the skeletal maturation of growing individuals because of the relatively simple sample collection procedure, non-invasiveness, and radiation avoidance as advantages over conventional radiographic techniques used in orthodontic diagnosis.

## References

1. Proffit WR, Fields Jr HW, Sarver DM. Contemporary orthodontics. Elsevier Health Sciences. 2006.
2. Rakosi T. An atlas and manual of cephalometric radiography. Lea & Febiger.1982.
3. Premkumar S. Textbook of craniofacial growth. JP Medical Ltd. 2011.
4. Fishman LS. Radiographic evaluation of skeletal maturation: a clinically oriented method based on hand-wrist films. The Angle Orthodontist. 1982;52(2):88-112. PMID: 6980608 doi: 10.1043/0003-3219(1982)052<0088:REOSM>2.0.CO;2
5. Hägg U, Taranger J. Skeletal stages of the hand and wrist as indicators of the pubertal growth spurt. Acta Odontol Scand. 1980;38(3):187-200. doi: 10.3109/00016358009004719 PMID: 6932165
6. Hägg U, Taranger J. Maturation indicators and the pubertal growth spurt. Am J Orthod. 1982;82(4):299-309. PMID: 6961802 doi: 10.1016/0002-9416(82)90464-x
7. Lamparski DG. Skeletal age assessment utilizing cervical vertebrae. Master's thesis, University of Pittsburgh, Pittsburgh. 1972:164.
8. O'Reilly MT, Yanniello GJ. Mandibular growth changes and maturation of cervical vertebrae--a longitudinal cephalometric study. Angle Orthod. 1988;58(2):179-84. PMID: 3164596 doi: 10.1043/0003-3219(1988)058<0179:MGCAMO>2.0.CO;2
9. Hassel B, Farman AG. Skeletal maturation evaluation using cervical vertebrae. Am J Orthod Dentofacial

- Orthop. 1995;107(1):58-66. doi: 10.1016/s0889-5406(95)70157-5 PMID: 7817962
10. Masoud M, Masoud I, Kent Jr RL, 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):209-16. PMID: 18675202 doi: 10.1016/j.ajodo.2006.09.063
  11. Perinetti G, Contardo L. Gingival crevicular fluid alkaline phosphatase activity in relation to pubertal growth spurt and dental maturation: a multiple regression study. South European Journal of Orthodontics and Dentofacial Research. 2016;3(1):6-11. doi: 10.5937/sejodr3-15217
  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. Prog Orthod. 2017;18(1):30. PMID: 28967046 doi: 10.1186/s40510-017-0184-y
  13. Tarvade SM, Ramkrishna S, Sarode S. Salivary alkaline phosphatase-a biochemical marker for growth prediction. Indian J Basic Appl Med Res. 2015;4(3):17-22.
  14. Cabras T, Pisano E, Boi R, Olianias A, Manconi B, Inzitari R, Fanali C, Giardina B, Castagnola M, Messana I. Age-dependent modifications of the human salivary secretory protein complex. Journal of proteome research. 2009;8(8):4126-34.
  15. Christenson RH. Biochemical markers of bone metabolism: an overview. Clin Biochem. 1997;30(8):573-93. PMID: 9455610 doi: 10.1016/s0009-9120(97)00113-6
  16. Takimoto K, Deguchi T, Mori M. Histochemical detection of acid and alkaline phosphatases in periodontal tissues after experimental tooth movement J Dent Res. 1968;47(2):340. PMID: 5238774 doi: 10.1177/00220345680470022601
  17. Insoft M, King GJ, Keeling SD. The measurement of acid and alkaline phosphatase in gingival crevicular fluid during orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 1996;109(3):287-96. PMID: 8607474 doi: 10.1016/s0889-5406(96)70152-x
  18. Perinetti G, Baccetti T, Contardo L, Di Lenarda R. Gingival crevicular fluid alkaline phosphatase activity as a non-invasive biomarker of skeletal maturation. Orthod Craniofac Res. 2011;14(1):44-50. PMID: 21205168 doi: 10.1111/j.1601-6343.2010.01506.x
  19. Wijaya H, Kusdhany LS, Redjeki S, Soegiharto BM. The salivary bone specific alkaline phosphatase in relation to pubertal growth phase in Indonesian children. Asian J Pharm Clin Res. 2017;10(5):389-92. doi: 10.22159/ajpcr.2017.v10i5.17752