Identification of Bone-Specific Alkaline Phosphatase in Saliva and its Correlation with Skeletal Age

Abstract

Context: Bone-specific alkaline phosphatase (B-ALP), a product of osteoblasts, is a bone formation marker whose serum levels fluctuate with puberty and adolescence. **Aims:** This study aims to assess B-ALP levels in saliva and correlate it with different skeletal maturity stages of hand-wrist radiographs using Hagg and Taranger method. **Settings and Design:** Observational study and cross-sectional design. **Subjects and Methods:** Total sample comprised of 90 individuals, right hand-wrist radiographs, and 2 ml unstimulated whole saliva samples taken from each patient on the same day. The hand-wrist radiographs were traced and staged into 5 subgroups (18 individuals each) according to Hagg and Taranger method. **Statistical Analysis Used:** One-way analysis of variance (ANOVA) and Tukey's multiple *post hoc* test. **Results:** The comparison of salivary B-ALP values between the different skeletal subgroups using one-way ANOVA depicted statistically significant results ($P = 0.0003$). Pairwise comparison using Tukey's multiple *post hoc* procedures showed that salivary B-ALP levels were comparatively higher in subgroup 3 and that the difference between subgroups 1 and 3 ($P = 0.0109$) and subgroups 3 and 5 ($P = 0.0014$) was statistically significant. **Conclusion:** B-ALP could be successfully identified and quantitatively estimated in saliva and showed significant correlation with different skeletal age subgroups as determined by Hagg and Taranger method.

Keywords: *Bone-specific alkaline phosphatase, puberty, saliva, skeletal age, skeletal maturity*

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Introduction

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. In humans, ALP is present in all tissues throughout the body. Humans show 3 isoenzymes of ALP. Bone-specific ALP (B-ALP) is an isoenzyme of ALP and is a product of osteoblasts which are involved in the process of osteoid mineralization.[1]

Bone is a specialized connective tissue continuously undergoing remodeling as a result of coordinated activity of osteoblasts and osteoclasts. It is a cyclic process occurring at the cellular level, and these cells are called as basic multicellular units. The activity of these cellular units can be measured biochemically by determining the presence of certain markers of bone turnover in body fluids such as serum, urine, and saliva.[2] Serum levels of B-ALP and osteocalcin are currently the most convincing bone formation markers.[3-6] Several studies have shown that regardless of gender, levels of bone turnover markers in serum appear to reach peak values during midpuberty and decrease markedly in late puberty.[1,5,7] It is hence proven that serum B-ALP levels fluctuate with puberty and adolescence.

Studies have been carried out to assess levels of B-ALP isoenzyme in the body to investigate its age-related changes,[1] to assess its proportional relationship with bone volume and bone density at different stages of sexual development,[5] or to assess its levels during orthodontic tooth movement,[6] but all of them have assessed B-ALP levels in serum or gingival crevicular fluid.[8,9] It is only recently that whole saliva has gained significant recognition as a noninvasive biologic sample to detect B-ALP.[10-12] Research has shown that salivary B-ALP levels when assessed in rats reflected similar patterns of change to those evaluated in serum under normal, increased, or diminished condition of systemic bone turnover.[13] Salivary levels of B-ALP have been measured in correlation with orthodontic tooth movement;[14] however, no study has correlated salivary B-ALP levels with growth status of an individual.

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Growth and maturational status of an individual can be best evaluated relative to different stages of physiologic maturity rather than correlating it with chronologic age because the latter is not a reliable indicator.[15,16] Physiologic maturity is best estimated by the maturation of one or more tissue systems, such as somatic, sexual, skeletal, or dental. Skeletal maturity assessment involves visual inspection of the developing bone and their initial appearance or sequential ossification and related changes in shape and size. Skeletal maturity has been assessed by using different techniques, but hand-wrist radiograph method of assessment of skeletal age is considered to be the most reliable.[17-25] Several methods of assessing hand-wrist radiographs have appeared in the literature since 1900s. The method suggested by Hagg and Taranger is based on the sequential ossification of four different hand-wrist bones for skeletal age estimation.[26,27]

The present study aims to assess the salivary B-ALP levels and correlate it with different skeletal ages as assessed by hand-wrist radiographs using Hagg and Taranger method[26,27] and projecting saliva as a potential noninvasive tool for assessment of skeletal age.

Aims and objectives

1. Identification and estimation of B-ALP levels in whole unstimulated saliva at different skeletal ages
2. Correlating the levels of B-ALP with different stages of skeletal development as assessed by Hagg and Taranger method.

Subjects and Methods

A total of 90 individuals reporting to the SDM College of Dental Sciences and Hospital, Dharwad were included in this cross-sectional study after patient's consent. The study was approved by the institutional review board, and all the procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 2000. The sample was divided into the following 5 subgroups (18 subjects each) according to the different skeletal maturity stages as assessed by Hagg and Taranger. Subgroups of the total sample are:
1. Subgroup S_0 (Prepubertal): Absence of ossification of ulnar sesamoid of the metacarpophalangeal joint of the first finger – 18 individuals
2. Subgroup S (Pubertal onset): Presence of ossification of ulnar sesamoid of the metacarpophalangeal joint of the first finger – 18 individuals
3. Subgroup MP3-G (Peak pubertal): The sides of the epiphysis of the middle phalanx of the third finger (MP3) show thickening and also cap the metaphysis, forming a sharp edge distally at one or both sides – 18 individuals
4. Subgroup DP3 (Pubertal deceleration): Fusion of the epiphysis and metaphysis of the distal phalanx of the third finger (DP3) is completed-18 individuals
5. Subgroup R-J (Growth completion): Fusion of the epiphysis and metaphysis of the distal epiphysis of the radius (R) is completed – 18 individuals.

The criteria for sample selection were based on the following:

Inclusion criteria

1. Healthy controls (both males and females) in the age group of 6–19 years.

Exclusion criteria

1. Individuals having systemic disease affecting growth such as disorders of Vitamin D metabolism, parathyroid, growth and thyroid hormone, renal impairment, and diabetes mellitus
2. Individuals under any medication that could affect bone metabolism during the previous 6 months, for example, vitamin preparations and calcium supplements
3. Individuals with a history of xerostomia
4. Individuals undergoing fixed orthodontic or functional orthopedic treatment.

Collection of data

Hand-wrist radiographs along with unstimulated whole saliva samples were collected in plastic vials (2 ml each) for each patient on the same day. Saliva samples were assessed for B-ALP levels using enzyme-linked immunosorbent assay (ELISA) (Human Bone Alkaline Phosphatase ELISA kit, Kinesis Dx, Los Angeles, USA) [Figure 1]. The hand-wrist radiographs were traced by a single investigator staged into the predetermined subgroups [Figure 2]. Manual tracings of the hand-wrist radiographs were made on 50 microns (thickness) lead acetate tracing sheets (Garware Polyster Ltd., Nasik) using 0.3 mm lead pencil (Staedtler, Germany). The investigator was blinded about each patient's age, pubertal status, and salivary B-ALP levels. All the hand-wrist radiographs were traced by the same investigator.

Statistical analysis

The method error was calculated using Dahlberg formula by repeating the tracings and was not statistically significant.[28] Statistics was carried out using the Statistical Package for Social Sciences (Version 16, SPSS, Chicago, IL, USA). Mean and standard deviation of salivary B-ALP (units/liter) according to the different subgroups was calculated. The

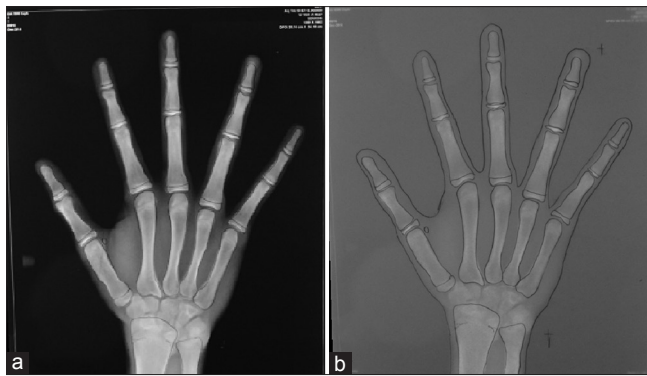**Figure 1:** Human bone-specific alkaline phosphatase enzyme-linked immunosorbent assay kit used in the study

Figure 2: (a) Right hand-wrist radiograph taken for each individual. (b) Right hand- wrist radiograph traced to assess the skeletal maturity stage

Figure 3: Mean bone-specific alkaline phosphatase levels in units per liter with confidence intervals for each of the subgroups

Kolmogorov–Smirnov test showed normality of distribution for the measurements used in the study; therefore, parametric statistics was used. Comparison between the five subgroups with respect to salivary B-ALP was done using one-way analysis of variance (ANOVA). Pairwise comparison between the different subgroups with B-ALP was carried out by Tukey's multiple *post hoc* procedures. The significance level was established at $P < 0.05$.

Results

Mean and standard deviation of salivary B-ALP (U/l) levels in each skeletal age subgroup was calculated [Figure 3]. The comparison of salivary B-ALP values between the different skeletal subgroups using one-way ANOVA test depicted statistically significant results [Table 1]. The results obtained with Tukey's multiple *post hoc* procedures showed that salivary B-ALP (U/l) levels were comparatively higher in subgroup 3 and that the difference between subgroups 1 and 3 and subgroups 3 and 5 was statistically significant. Statistically significant differences were also seen between subgroups 1 and 2 and 2 and 5 [Table 2].

Discussion

The results obtained in this cross-sectional study demonstrated that the salivary B-ALP values were significantly higher in subgroup 3 (MP3) as compared to subgroup 1 (So) and subgroup 5 (R-J). Furthermore, the salivary B-ALP levels showed a steady increase in mean value from subgroup So to subgroup MP3, followed by a steady decline thereafter. The resultant values obtained in the study are consistent with the studies conducted on serum B-ALP levels correlated with Tanner's physical stages and chronologic age.[1,2,5,7]

Subgroup MP3 according to the stages enumerated by Hagg and Taranger which showed the highest mean values of salivary B-ALP also marked the peak of pubertal growth spurt.[26] The subgroup So (prepubertal) and subgroup R-J (growth completion) have shown significantly lower values as compared to subgroup MP3 (peak pubertal) which shows that the
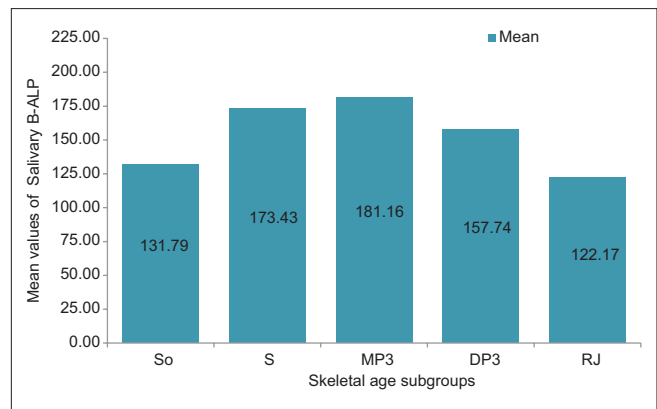
Table 1: Comparison of five subgroups (So, S, MP3, DP3, and RJ) with bone-specific alkaline phosphatase by one-way analysis of variance

Sources of variation	Mean sum of squares	F	P
Between groups	11,850.57	6.0062	0.0003*
Within groups	1973.07		
Total			

*$P<0.05$

Table 2: Pairwise comparison of five groups (So, S, MP3, DP3, and RJ) with bone-specific alkaline phosphatase levels by Tukey's multiple *post hoc* procedures

Group	So	S	MP3	DP3	RJ
Mean	131.79	173.43	181.16	157.74	122.17
SD	29.19	41.08	49.72	57.43	39.45
So	-				
S (*P*)	0.0469*	-			
MP3 (*P*)	0.0109*	0.9850	-		
DP3 (*P*)	0.4078	0.8265	0.5131	-	
RJ (*P*)	0.9663	0.0074*	0.0014*	0.1247	-

SD=Standard deviation, *$P<0.05$

values of salivary B-ALP are comparatively lower before and at the end of pubertal growth spurt. Similar results were obtained by Tsai *et al*. when serum B-ALP was correlated to chronologic age.[7]

The values obtained in the study showed a high range of standard deviation which could have been because of great individual variation with regard to skeletal maturation. It could also have occurred because of the cross-sectional nature of the study. A longitudinal study with a larger sample size to get standardized values of salivary B-ALP at different stages of skeletal development with less variation would be better suited to overcome this limitation. A comparative study to assess the changes in the values of B-ALP simultaneously in serum and saliva at different stages of pubertal development would also give a broader picture.

This study had aimed to detect B-ALP levels in saliva and to correlate it with the skeletal age and to project it as a noninvasive tool for assessment of skeletal growth. The results showed that there was a significant relationship between stages of skeletal maturity and salivary B-ALP levels. Salivary B-ALP levels can be used as a tool for estimation of skeletal age and maturity is the question. Definitely the use of saliva is a noninvasive tool when compared to serum/blood spot investigations and can be used for patients and as an adjunct to the conventional skeletal maturity indicators, such as the hand-wrist and cervical vertebrae maturation index whenever in doubt. However, to solely rely on salivary B-ALP as tool for growth assessment, studies with a large sample size are needed to establish baseline values.

Conclusion

1. B-ALP can be identified and quantitatively estimated in saliva at different stages of skeletal age as assessed by hand-wrist radiographic method (Hagg and Taranger)
2. A significant correlation exists between salivary B-ALP (U/l) levels and different skeletal age groups as determined by hand-wrist radiographic method (Hagg and Taranger). Salivary B-ALP values show a significantly higher value at the peak of pubertal growth (MP3 stage) with significantly lower levels at prepubertal (So stage) and Growth completion (R-J stage) periods

Therefore, the study concludes that salivary B-ALP can be used as a noninvasive tool for assessment of skeletal maturity as an adjunct to conventional skeletal maturity indicators.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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