Salivary Bone-specific Alkaline Phosphatase as Predictor of Puberty Phase

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Abstract

The identification of the puberty phase is crucial, as it enhances the efficiency and effectiveness of malocclusion treatment. The observation or evaluation of bone morphology developments and changes through radiographs, known as the skeletal maturation method, is the most valid puberty phase indicator. Since this method requires radiographic exposure, a new, non-invasive prediction method for the puberty phase is needed. This study aimed to analyze the potential of the level of salivary bone-specific alkaline phosphatase (BALP), chronological age, and Body Mass Index (BMI) percentile as predictors of the puberty phase.

The cross-sectional study included 136 subjects categorized into three phases of puberty based on Bacceti's Cervical Vertebrae Maturation System. Salivary BALP level was determined using a commercial enzyme-linked immunosorbent assay kit.

Multinomial logistic regression analysis produced a predictive model with a correct classification rate of 78% in the pre-peak phase, 57.7% in the peak phase, and 81.4% in the post-peak phase. The puberty phase, especially at pre and post-peak, can be predicted with salivary BALP level, chronological age, and BMI percentile.

Introduction

Identification of the puberty phase is essential to determine the optimal time for malocclusion treatment.[1-8] When performed during puberty, interventions for malocclusions can have increased efficiency and effectiveness. For example, growth modification for class III skeletal malocclusions results in excellent outcomes when performed during the pre-peak phase of puberty. Orthopedics treatment of class II skeletal malocclusion has an optimum effect on mandible growth when performed at the peak phase of puberty. In contrast, orthognathic surgery should be performed when the post-peak phase of puberty has been completed.[9-12]

The evaluation of hand and wrist bone

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morphology using radiographs (skeletal maturation method) is the most valid puberty phase indicator.[13,14] However, in orthodontics, this method is rarely applied due to additional X-ray radiation exposure and it is complex to analyze.[15] The lateral cephalogram as a routine radiograph for orthodontic diagnosis can be used to assess skeletal maturation through the images of cervical vertebral bone, an approach the Cervical Vertebrae Maturation System (CVMS) method.[9] Several studies have shown that CVMS is a valid, reliable method for determining skeletal maturity during a circumpubertal growth spurt.[10] However, some studies have questioned the CVMS as an index of skeletal maturity due to methodological issues or safety concerns.[15,16] Therefore, efforts have been carried out to find reliable, reproducible, and non-invasive indicators of skeletal maturity in individual subjects. The CVMS method consists of six stages of skeletal maturation: Stages one and two show the pre-peak phase of puberty; stages three and four occur during the peak phase of puberty; and the post-peak phase of puberty occurs at stages five and six.[17]

The cellular process of bone growth

results in the release of biochemical molecules into the blood; these are known as bone metabolism biomarkers, which consist of bone formation and resorption biomarkers.[18]Bone-specific alkaline phosphatase (BALP) is a specific and sensitive bone formation biomarker due to its function in the bone mineralization process.[19-22] BALP levels detected in human saliva and correlate significantly with that in serum levels. At puberty, its levels increase sharply, implying that this biomarker is an indicator of skeletal growth.[21,23]

The use of saliva as a diagnostic fluid offers some advantages over blood. Saliva collection procedures are preferable to those for serum because the formers are painless and non-invasive. Also, it is possible to collect multiple times from the same individual. Moreover, saliva collection does not require personnel training, and it is easier to manipulate and store.[24,25] It is, therefore, possible that salivary BALP can be used as a reliable, reproducible, and non-invasive biomarker for phase of puberty.[26-28]

The literature shows that phase of puberty is associated with parameters such as chronological age, secondary sexual signs, formation stages, dental eruptions, and Body Mass Index (BMI). Although there is wide individual variation during the puberty phase, chronological age and BMI have the advantage of being accessible and inexpensive in terms of application.[29-32]

The objective of this study is to analyze the potential of the salivary BALP level, chronological age, and BMI percentile to predict puberty phase.

Materials and methods

This cross-sectional study was conducted on 136 Indonesian children (64 boys and 72 girls). A signed informed consent and assent were obtained from the subjects and their parents before enrollment in the study, and the protocol was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, University of Indonesia. The following enrollment criteria were observed: 1) age between eight and 18 years; 2) good general health with no nutritional problems; and 3) good oral hygiene.

Chronological age was calculated manually in months; weight and height were measured by scales and a stature meter (SMIC type ZT-120). The measurements were then used to calculate BMI percentile. The subjects were scheduled for enrollment at their first clinical examination; subsequently, during a second visit seven to ten days before saliva collection, they underwent a professional supra-gingival and sub-gingival scaling session and received a review of oral hygiene techniques.

The subjects received detailed information about the collection protocol, all samples collected in the morning at 9), the exclusion of tooth brushing before sample collection, and the instruction to avoid food and fluid ingestion or chewing gum for at least 30 min before collection, and mouth cleansing with distilled water. Saliva collected using a passive drooling method.

Saliva sample was stored in an ice box before sending to the biochemistry laboratory, where they were centrifuged to separate the precipitates in saliva. The samples analyzed for total protein content and BALP by using Bradford Assay Kit (Thermo Fisher Scientific, USA) and Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Elabscience, China), respectively, following the manufacturer's instructions.

Measurement of total protein and BALP were determined using a microplate reader (iMark[TM], Micro-plate Absorbance Reader; Bio-Rad, Hercules, California, USA) set to the Optical Density (OD) 595 nm and 450 nm, respectively. For BALP, the enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turns yellow. The OD value was proportional to the concentration of BALP. The concentration of BALP in the samples was calculated by comparing the OD of the samples to the standard curve. The minimum detectable dose of BALP was 46.875 pg/mL with a coefficient of variation were <10%. No significant cross-reactivity or interference between BALP and analogs was reported.

Puberty phase was assessed using the CVMS method on lateral cephalogram according to the definition as described by Baccetti.[9] The CVMS observer blinded of the result of biochemical assay of the subjects. After the stage of CVMS was assessed, then the subjects were divided into 3 clusters by puberty phase, i.e., pre-peak (CVMS stage 1 and 2), peak (CVMS stage 3 and 4), and post-peak (CVMS stage 5 and 6) groups.[10]

The data were analyzed using SPSS software version 17.0 (SPSS Inc., Chicago, IL,

USA) was used to perform the statistical analysis. The significance of the differences in the sex distribution was tested by chi-square analysis. Chronological age, level of salivary BALP, and BMI percentile among the experimental groups were tested using Brown- Forsythe followed by Tamhane's posthoc test and One-way Analysis of Variance (ANOVA) followed by a Tukey's post-hoc test for pairwise comparisons, respectively. The multivariate analysis was multinomial logistic regression with the puberty phase (pre-peak and peak where are post-peak acts as a reference) as the dependent variable.

Results

The result of bivariate analysis between the tested variable and puberty phase is summarized in table 1.

Based on the bivariate test, the variables that can be included in the multinomial logistic regression analysis were the chronological age, salivary BALP levels and BMI percentile(Table 2).

The validation of the model to predict the puberty phase through correct classification rates can be seen in table 3. The validation of data resulted in the correct classification rates of 78% for prediction of pre-peak phase and 57.7% for prediction of peak phase; whereas the rate for the post-peak phase was 81.4%. Based on the Nagelkerke value, the coefficient determinant value (pseudo-R-Square) from the resulting modeling is 0.675. The R- Square indicates that the ability of the independent variable to explain the dependent variable is 67.5 % or in other words, it can be assumed that 32.5% of the other factors outside independent variables can explain the dependent variables.

Variable	Puberty phase			Diff. (p value)
	Pre-peak	Peak	Post-peak	
Gender	22/19	30/22	19/24	
BMI	29.73 (28.1)	47.85 (32.2)	50.72 (32.27)	.41
Salivary BALP	1001.71 (594.97)	834.80 (411.90)	691.16 (334.97)	.003
Chronological Age	130.51 (13.80)	160.42 (26.85)	195.32 (16.61)	.011 <.001

Table 1. Gender, BMI, salivary BALP and chronological age of the subjects (n = 136) in the different growth phase groups. Gender is presented as number of cases; BMI (percentile), salivary BALP (pg/mL), and chronological age

(months) as mean (SD). Diff., significance of the differences among the groups (p <0.05).

Pubertyphase	Predictor Variable	Odds ratio	95% Confidence Interval
Pre-peak	Chronological age	0.874	0.838, 0.912
	Salivary BALP	1.002	1.001, 1.004
	BMI percentile	0.979	0.955, 1.002
Peak	Chronological age	0.942	0.921, 0.964
	Salivary BALP	1.001	0.999, 1.002
	BMI percentile	0.994	0.977, 1.010

Table 2. Multinomial logistic regression predicting puberty phase from the chronological age, salivary BALP levels and BMI percentile.
*The reference category was post-peak, † Salivary BALP level (pg/ml), ‡ Chronological age (months)

	Prediction			
	Pre-peak phase	Peak phase	Post-peak phase	Correct Classification Rates
Pre-peak phase	32	9	0	78.0%
Peak phase	12	30	10	57.7%
Post-peak phase	0	8	35	81.4%

Table 3. Validation of prediction model through correct classification rates

Discussion

In this study, BALP levels were examined because of their more specific characteristics, i.e., to measure bone metabolism during the span of growth compared to total ALP activities. Perinetti et al. who reported that the highest of ALP activity in the GCF was during the peak of a puberty growth spurt.[12] Tobiume et al. also stated that the peak of serum bone ALP activity occurred during infancy and puberty, indicating high bone metabolism during these periods.[21] The level and activity of an enzyme are two different things. The increased levels of enzyme will not directly increase the activity of the enzyme due to the influence of environmental factors such as temperature and the degree of acidity to enzyme activity.[33] This study is the first to analyze the relationship between salivary BALP levels and the phase of puberty. Therefore, the comparison was made to studies in the field of pediatric medicine, which has used the levels of BALP serum against the phase of puberty. The results showed that there was a tendency to have the same pattern i.e., the decreasing levels with the increasing phase of puberty.[22, 34, 35] It is possible that this pattern can be connected to the

role of ALP plays in bone mineralization which occurs at the early stages of bone tissue formation. The role of ALP isoenzymes in the mineralization can be examined through the genetic expression during the osteoblast differentiation and calcification of growth-plate cartilage. In both processes, the expression of ALP appears in the early stages.[36,37]

Most literature states that chronological age cannot represent the phase of puberty because there is a wide age variation towards the skeletal maturation stage.[38-40] However, this study did not demonstrate a wide variety of chronological age for each phase of puberty based on CVMS method. Our finding shows that chronological age has a significant positive association with the puberty phase. Therefore, chronological age can contribute to the salivary BALP in predicting the phase of puberty.

The influence of gender on skeletal maturation or the phase of puberty has been verified by other research and literature.[13,15] Gender dimorphism is also evident in the subject of this study, in which the girls have reached the phase of puberty earlier compared to the boys. Although there was a slightly different proportion between several phases, however, the results of this study have indicated a balanced proportion of gender.

The literature shows different results and contradictions and some studies have concluded that there is an effect of BMI towards the skeletal maturation but there is also the opposite.[41,42] The results of this study indicate that BMI percentile has a significant relationship with the phase of puberty so it is estimated that it can contribute to BALP saliva in order to predict the phase of puberty.

Multinomial logistic regression yielded a probability for predicting the puberty phase with a predictor of chronological age; salivary BALP levels; and BMI percentile. To date, no published studies have investigated the puberty phase prediction from a combination of chronological age, salivary BALP, and BMI percentile. Based on the validation results, these three variables can be used to predict the puberty phase primarily at the pre-peak and post-peak phases, whereas to predict peak phase, the accuracy is less adequate. These data potentially can be used by a clinician to identify the pre-peak phase of puberty. This phase is critical in determining the optimal timing of orthodontic treatment,

especially for modifying the growth of jaws because the effectiveness of this treatment is minimal after the peak phase of puberty.

In the future, some suggestions can be put forward based on the results of this study, which is to confirm the result of this study through another subject group. Also, further study is needed to compare the BALP levels between saliva and GCF. Moreover, it is recommended to research analyzing the relationship between BALP levels and total ALP activities on both saliva and GCF.

Conclusion

The puberty phase can be predicted as a non-radiographic, non-invasive and objective way by using salivary BALP levels, chronological age, and BMI percentile. The most accurate predictor of puberty phase is the pre-peak and post-peak phases. Salivary BALP levels are potential biomarkers of puberty phase. The levels are high at the pre-peak phase and decrease with the increasing phase of puberty.

Acknowledgements

Declaration of Interest

None to declare.

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