THE SALIVARY BONE-SPECIFIC ALKALINE PHOSPHATASE IN RELATION TO PUBERTAL GROWTH PHASE IN INDONESIAN CHILDREN

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ABSTRACT

Objective: Objective of the study was to evaluate the level of salivary bone-specific alkaline phosphatase (BALP) during the pubertal growth phases in Indonesian children.

Methods: The study conducted on 64 boys and 72 girls who were age group of 8-18 years old were randomly selected. Salivary BALP level were estimated using enzyme-linked immunosorbent assay commercial kits and pubertal growth phase were assessed using cervical vertebral maturation (CVM) method according to Baccetti. Mean salivary BALP was compared based on pubertal growth groups by analysis of variance test.

Results: Test revealed no significant differences between groups with p=0.312. The highest mean of salivary BALP level was found in the prepeak pubertal growth phase (CVM Stage 1-2), and the lowest one was detected in the postpeak pubertal growth phase (CVM Stage 5-6).

Conclusion: The using of salivary BALP as a biomarker for pubertal growth prediction is questionable.

Keywords: Salivary, Bone specific alkaline phosphatase, Pubertal growth, Cervical vertebral maturation.

INTRODUCTION

Identification of the growth phase, with particular regard to the onset of the pubertal growth phase, has major clinical implications when dealing with orthodontic treatment in growing subjects, especially when there are skeletal disharmonies [1,2]. In orthodontics, the classic method of assessing pubertal growth phase is through observation of skeletal maturity with the most common being the radiography-based, hand-wrist analysis, and cervical vertebral maturation (CVM) methods [3-6]. Those methods are mainly morphological and subjective, while new possibilities might be offered by biomarkers (biochemical markers) due to they represent agents that are involved directly in bone growth and remodeling. The use of biomarkers has been proposed very recently as a new aid in assessing individual skeletal maturity, with the advantage of being related to the physiology of the patient and of avoiding the use of radiations. The very scarce data reported to date include molecular constituents from the serum, such as insulin-like growth factor 1 or from the gingival crevicular fluid (GCF), such as alkaline phosphatase (ALP) [7,8].

Traditionally, total serum ALP activity has been used as a biomarker for bone formation. The changes in total serum ALP activity roughly reflect the changes in serum bone specific ALP (BALP) levels in healthy children. However, the same is not necessarily true in other clinical groups because of the changes in liver-specific ALP isoforms caused by the underlying disease state, or drug treatment, often affects the total serum ALP activity. Measurement of BALP in the serum provides a more specific assessment of the growth status of bone in normal conditions [9]. Several studies have shown that regardless of gender, levels of BALP in serum appear to reach peak values during peak puberty and decrease markedly in postpeak puberty. It has hence been proven that serum BALP levels fluctuate with puberty, but all of them have assessed BALP levels in serum and not many studies have been carried out in saliva [9-11]. It is only recently that whole saliva has gained significant recognition as a biologic sample to detect BALP levels [12]. Research has shown that salivary BALP 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 BALP have been measured in correlation with orthodontic tooth movement [14]. However, no study has correlated salivary BALP levels with growth status of an individual.

Most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. Saliva is a mirror of the body and reflects normal and disease state and its use as a diagnostic fluid has many merits over serum. Whole saliva can be collected non-invasively, and by individuals with limited training, including the patient. No special equipment is needed for collection of the fluid. Valuable for children, since collection of the fluid is associated with fewer compliance problems. Further, analysis of saliva may provide a cost-effective approach for the screening of large populations [15,16].

With regard to determination whether the salivary BALP represents a non-invasive biomarker of the pubertal growth phase, therefore, the aim of this study was to evaluate the level of salivary BALP during the pubertal growth phases in Indonesian children.

METHODS

Study population and design

This cross-sectional was conducted in the city of Jakarta, Indonesia, on 136 (64 boys and 72 girls) Indonesian children. A signed of informed accent and consent was obtained from the subjects and their parents before enrolment into the study, and the protocol was reviewed and approved by the Ethical Committee of Faculty of Dentistry, Universitas Indonesia. The following enrolment criteria were observed: (1) Age between 8 and 18 years, (2) good general health with the absence of any nutritional problems, (3) no use of anti-inflammatories or antibiotics in the month preceding entry to the study, and (4) good of oral hygiene.

Saliva collection procedures

The subjects were scheduled for enrolment at their first clinical examination; subsequently, during a second visit 7-10 days before saliva collection; they underwent a session of professional supra-gingival and sub-gingival scaling and also received repeated oral hygiene instructions.

The subjects received detailed information about the collection protocol, such as the timing of sample collection to ovoid circadian effect (all samples were collected in the morning at 9), the exclusion of tooth brushing prior to sample collection, and the instruction to avoid food and fluid ingestion or chewing gum for at least 30 minutes before collection, and mouth cleansing with distilled water. Finally, their lateral cephalograms were taken and saliva was collected using passive drooling method.

Measurement of total protein and BALP

Saliva sample that has been collected was stored in an ice box before sending to the biochemistry laboratory, where they were centrifuged to separate the precipitates in saliva. The samples were 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.

Total protein was a non-specific measure of the total amount of all proteins present in a solution. It is used to examine the changes in overall protein secretion in biologic fluids or to look for differences in the ratio of specific proteins to total protein that occur in response to physiological changes. Measurement of total protein was used to normalize concentrations of various salivary proteins in different samples since concentrations can vary significantly in response to stimulation or alterations of saliva flow [17-20].

Measurement of total protein and BALP was determined using a microplate reader (iMark™, Microplate Absorbance Reader; Bio-Rad, Hercules, California, USA) set to the optical density (OD) 595 nm and 450 nm, respectively [21]. For BALP, the enzyme-substrate reaction was terminated by the addition of a sulfuric 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 coefficient of variation were <10%. No significant cross-reactivity or interference between BALP and analogs was reported.

Appraisal to pubertal growth phase

Pubertal growth spurt was assessed using the CVM method on lateral cephalograms according to the definition as described by Baccetti [5]. Briefly, these were defined as follows:

- Stage 1: When the lower borders of the second, third and fourth vertebrae (C2, C3 and C4) are flat and the bodies of C3 and C4 are trapezoid in shape. CS1 occurs at least 2 years before the pubertal growth spurt;
- Stage 2: When only the lower border of C2 is concave and the bodies of C3 and C4 are trapezoid. CS2 occurs 1 year before the growth spurt;
- Stage 3: When the lower borders of both C2 and C3 have concavities and the bodies of C3 and C4 are either trapezoid or rectangular horizontal in shape. CS3 marks the ascending portion of the growth spurt;
- Stage 4: When the lower borders of C2-C4 have concavities, and the bodies of both C3 and C4 are rectangular horizontal. CS4 marks the descending portion of the growth spurt;
- Stage 5: When the lower borders of C2-C4 have concavities and at least one of the bodies of C3 and C4 is square. CS5 occurs 1 year after the growth spurt;
- Stage 6: When the lower borders of C2-C4 have concavities and at least one of the bodies of C3 and C4 is rectangular vertical. CS6 occurs at least 2 years after the growth spurt.

The CVM observer was blinded of the result of biochemical assay of the subjects. After the stage of CVM was assessed, then the subjects were divided into 3 clusters by pubertal growth phase, i.e.: Prepeak (before the pubertal growth spurt; Stage 1 and 2), peak (during the pubertal growth spurt; Stage 3 and 4), and post-peak (after the pubertal growth spurt; Stage 5 and 6) groups [5]. Salivary BALP levels were then subsequently compared according to the pubertal growth phase.

Statistical analysis

Descriptive statistics were used to summarize the distribution of all measurements. The intraexaminer agreement of the CVM categories was tested with Kappa statistic. The balancing of the groups (pubertal growth phases) by sex was tested by Chi-square analysis. An analysis of variance and post hoc Tukey honest significant difference test were used to compare salivary BALP levels with pubertal growth phase. A $p < 0.05$ was used for rejection of the null hypothesis. The data were analyzed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The distribution of age and salivary BALP as the mean±standard deviation were 13.53 years ± 2.72 and 825.90 pg/ml ± 530.99, respectively. Kappa statistic for the intraexaminer agreement of the CVM appraisal showed a value of above 0.90. The distribution of gender was similar among the groups compared (p=0.143). The age of the subjects clustered according to the CVM stage and pubertal growth phases were shown in Tables 1 and 2.

The level of salivary BALP according to the growth phase can be seen in Table 3. We found that the mean level of BALP decreased

Table 1: Age of the subjects in the different groups according to CVM stages

CVM stages	n	Age (year)	
		Mean±SD	95% CI
1	20	10.55±0.98	10.09-11.02
2	22	11.16±1.20	10.62-11.69
3	14	12.10±1.65	11.15-13.06
4	37	13.91±2.23	13.16-14.66
5	25	15.89±1.66	15.20-16.57
6	18	16.81±0.53	16.54-17.08

n: Number of subjects in each group (Sample size=136), SD: Standard deviation, CI: Confidence interval, CVM: Cervical vertebral maturation

Table 2: Age of the subjects in the different groups according to pubertal growth phase

Pubertal growth phase	n	Age (year)	
		Mean±SD	95% CI
Prepeak	41	10.87±1.15	10.51-11.23
Peak	52	13.36±2.23	12.74-13.99
Postpeak	43	16.27±1.38	15.85-16.70

n: Number of subjects in each group (Sample size=136), SD: Standard deviation, CI: Confidence interval

Table 3: Level of salivary BALP in the different groups according to pubertal growth phase

Pubertal growth phase	n	Salivary BALP (pg/ml)		p
		Mean±SD	95% CI	
Prepeak	41	931.90±643.14	728.90-1134.90	0.312
Peak	52	782.28±451.10	656.69-907.87	
Postpeak	43	777.58±499.52	623.84-931.31	

n: Number of subjects in each group (Sample size=136), SD: Standard deviation, CI: Confidence interval, BALP: Bone-specific alkaline phosphatase

with pubertal growth phase. It was also found that the association between salivary BALP and pubertal growth phase was not significant (p=0.312).

DISCUSSION

This study investigated the possible association between salivary BALP levels and pubertal growth phase. To date, there has been no study investigated the salivary BALP in relation to pubertal growth phase that was assessed with CVM method. One of the main reasons for the application of the method was that the analysis of CVM was performed on the lateral cephalogram, a type of radiographic used routinely in orthodontic examination.

When mean chronologic age of each CVM stage of this study were compared with Baccetti's [5], the result showed that the Indonesian children reached each developmental stage later than white children. This result was in agreement with the previous by Soegiharto *et al.* [22].

The relation of GCF and salivary ALP total activity to growth phases had been studied. The authors found the highest concentration at peak pubertal growth phase and lower at others (pre- and post-peak) pubertal growth phase [1,23]. They concluded that ALP total activity in GCF and salivary could be used as adjunct methods in identification of pubertal growth phase. In human, however, tissue non-specific (liver, bone, and kidney), intestinal, and placental ALP isoforms had been identified. These sources could contribute to total ALP activity, where interpretation of the result will become even more difficult without fractionation of these ALP isoforms. Although separating the intestinal and placental ALPs was relatively easy, it was much more difficult to distinguish between BALP and liver ALP because these two isoforms were the products of a single gene and differ only with respect to posttranslational glycosylation [9]. For that reason, we measured the level of salivary BALP by using ELISA which has lower cross-reactivity with the other forms of ALP. We found that the mean level of salivary BALP decreased with pubertal growth phase and no significant differences were found between the groups. A potential cause related to this result was perhaps non-serum sources of BALP that can detectable in GCF from diseased periodontal sites and local bone remodeling processes [24,25].

Saliva has a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones that originate from multiple local and systemic sources. However, its use as a diagnostic fluid has been hindered, mainly because of our lack of understanding of the biomolecules present in saliva, combined with the lack of high-sensitivity detection systems. As a diagnostic medium, saliva has disadvantages such as the fact that it would not always reliably reflect the concentrations of these molecules in serum. Salivary composition can also be influenced by the method of collection and the degree of stimulation of salivary flow [26].

CONCLUSION

This study did not find differences of salivary BALP level during the pubertal growth phases in Indonesian children. As a matter of fact, the use of salivary BALP as a biomarker for pubertal growth prediction was questionable. There is a need to construct further study exclusively for salivary BALP. Moreover, to explore other salivary biomarker indicating pubertal growth spurt is also becoming more important. To date, the radiographic methods such as CVM may be considered as the most preferred method for pubertal growth prediction in orthodontics.

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