RESEARCH ARTICLE

Evaluation of Alkaline Phosphatase as Skeletal Maturity Indicator in Gingival Crevicular Fluid

Mridula Trehan[1], Chirag Patil[2]

Abstract

Introduction: The aim of this study was a comparison of the mean alkaline phosphatase (ALP) levels in gingival crevicular fluid (GCF) at distinct phases of skeletal maturity with the use of a hand-wrist radiograph and to analyze if GCF ALP levels can be used as a non-invasive biomarker for evaluation of skeletal maturity in patients undergoing orthodontic treatment.

Materials and methods: In this study, a standardized volume of 5 µL was collected from the subjects in the preadolescent, adolescent, and postadolescent phases from the mesial and distal embrasures of maxillary and mandibular central incisors after which a hand-wrist radiograph was obtained. Eppendorf tubes with buffer solution were used to transfer GCF to the laboratory for estimation of ALP level.

Results: The data collected were analyzed using Kruskal–Wallis and Mann–Whitney *U* test to obtain the ALP levels. Gingival crevicular fluid ALP levels were significantly higher in the adolescent stage. The site-wise comparison in the three groups show that there is a statistically insignificant difference between maxilla and mandible or between males and females.

Conclusion: It was concluded that the mean ALP levels were significantly increased in the adolescent phase in contrast with the pre- and post-adolescent stages. Gingival crevicular fluid ALP can be considered a promising diagnostic tool as a non-invasive biomarker of an adolescence growth spurt.

Keywords: Alkaline phosphatase, Biomarker, Gingival crevicular fluid, Hand-wrist radiograph, Skeletal maturity.

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Introduction

Preadolescent and adolescent patients reporting for orthodontic treatment have some amount of craniofacial growth remaining. Orthodontists should consider utilizing the remaining growth for successful treatment planning. For an individual, progression toward maturity can be assessed more readily with skeletal age compared with chronological age.[1]

Assessment of skeletal maturity is important as it has major clinical implications especially for the orthodontic treatment of patients who are in the growing age. For example, in patients with class II skeletal pattern and mandibular retrognathism, the functional appliance can be used for causing significantly greater mandibular growth when the treatment is carried out during the adolescent growth spurt.[2,3] Whereas for orthopedic treatment[4] of class III malocclusion and for rapid maxillary expansion (RME),[5,6] the treatment is more beneficial when it is carried out during the preadolescent growth phase. Therefore, it is very important to correctly identify the growth status of the subject for proper orthodontic diagnosis and treatment planning.

Skeletal maturity evaluation can be done with the help of various maturational indices.[7] Among these indices, the most common are the radiographic assessment of bone maturation which includes cervical vertebrae maturation index (CVMI)[8] and hand-wrist radiographic analysis.[9]

Novel prospects might be provided by biological markers[10] as they avoid invasive radiation exposure. Biomarkers denote agents which directly induce bone growth and remodeling. Research is being done to explore the role of biomarkers for skeletal maturity.

Gingival crevicular fluid (GCF)[11] is enriched with biomarkers derived from the serum and also from the interstitial fluid of periodontal tissues. The enzyme alkaline phosphatase (ALP) present in GCF is crucial for bone mineralization and has been suggested as a skeletal maturity indicator.[12]

Since serum ALP increases during puberty, it can be presumed that an increase in GCF ALP activity also occurs during an adolescent growth spurt.[13]

The present double-blind study aimed to evaluate the GCF ALP levels at distinct stages of skeletal maturity with hand-wrist radiographs and to determine if GCF ALP activity can be used as a non-invasive biomarker for evaluation of skeletal maturity in orthodontic patients.

Materials and Methods

The study was conducted on 60 subjects (33 boys and 27 girls) in the age range of 7–18 years. The subjects were divided into three groups using Bjork, Grave, and Brown[14] method of hand-wrist

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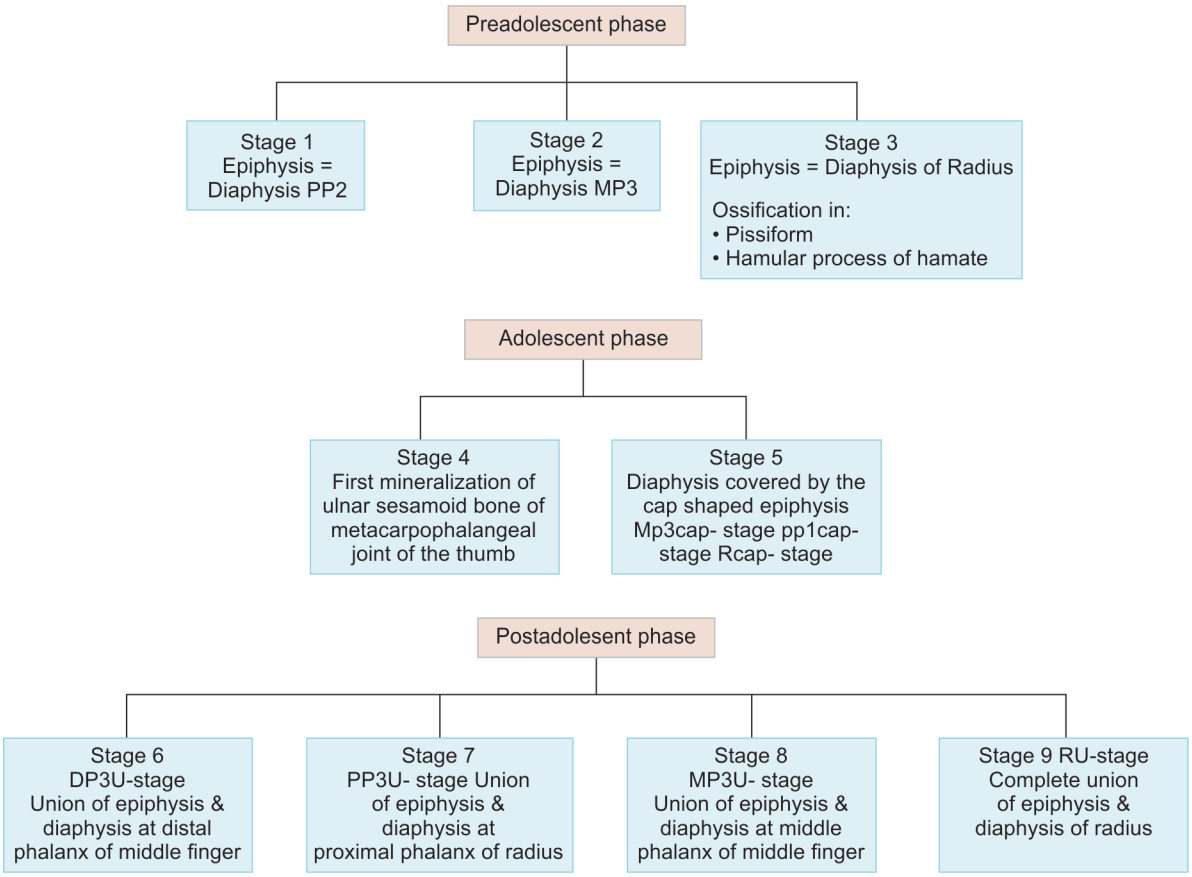
radiographs, i.e., preadolescent, adolescent, and postadolescent groups. Each group consisted of 20 subjects. The preadolescent phase consisted of stages from 1 to 3, the adolescent phase with stages 4 and 5, and the postadolescent phase included stages from 6 to 9. The subjects were randomly selected, to avoid any gender bias, from patients visiting the Department of Orthodontics and Dentofacial Orthopedics.

For GCF sample collection, the selected site was dried and isolated with the help of cotton rolls following which 5 µL GCF was collected from the mesial and distal embrasures of maxillary and mandibular central incisors by placing the pipette extra crevicularly over the site. Eppendorf tubes with buffer solution were used to send the samples to the laboratory for evaluation of ALP level by spectrophotometry, where it was stored at −80°C.



The inclusion criteria included subjects with no health or nutritional issues. It was also ensured that the subjects were not on any medications during the course of the study.

Subjects who were undergoing orthodontic treatment or had been treated before and those with compromised oral hygiene conditions were excluded from the study.

At the first clinical examination, the subjects were explained about the investigation. An informed consent was obtained from the parents of the subjects. The Institutional Ethical Committee clearance was obtained for the study. A session of professional supra-gingival and subgingival scaling was carried out at the second visit which was 7 to 10 days before GCF collection. In this interim period, the subjects were asked to use 0.012% chlorhexidine mouthwash twice daily and to abstain from taking any medications.

During the last clinical visit, before the collection of GCF, the periodontal status of subjects was reassessed. The GCF was collected with the help of microcapillary pipettes (Fig. 1), after which a hand-wrist radiograph of the patient's left hand (Fig. 2) was obtained.

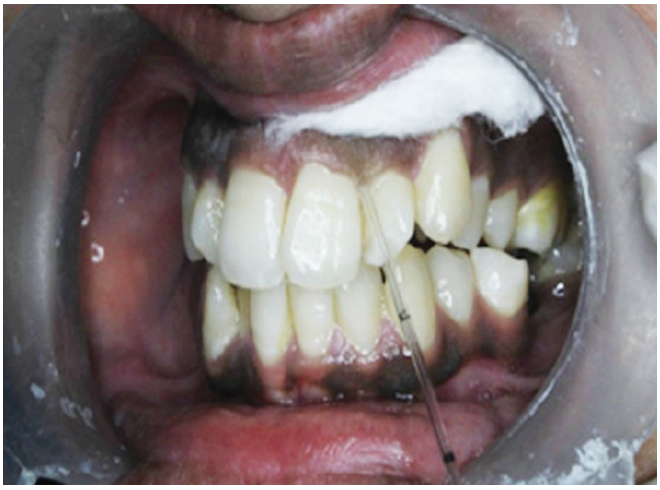


Fig. 1: GCF collection with microcapillary pipette

Biochemical Assays

A single operator who was double-blinded for the study performed the biochemical assays of the subjects. In 200 µL buffer containing 100 mM Tris and 20 mM $MgCl_2$ (pH 9.8 ± 0.1) and 6 mM *p*-nitrophenol phosphate, the GCF samples were resuspended. An incubation at 37°C (±<0.1_C fluctuations) for 2 hours was performed, whereby the ALP in the samples hydrolyzes the *p*-nitrophenyl phosphate to *p*-nitrophenol and inorganic phosphate. By adding 5 µL 3 M NaOH, the reactions were halted and the rates of increase in absorbance were read with a spectrophotometer at 405 nm wavelength. The relevant control for each analysis consisted of the reagent and the Tris buffer without the sample. Using 18.45 as the *p*-nitrophenol mM absorptivity, the absorbance was converted into International units per liter enzyme activity units (1 unit = 1 mmol of *p*-nitrophenol released per minute at 37°C).[15]

Statistical Analysis

The Kruskal–Wallis test and Mann–Whitney *U* test were used for intergroup comparison of GCF ALP activity between the three growth phases. The Chi-square test was used for both site-wise and gender-wise comparison of GCF ALP levels between the three groups (pre-adolescence, adolescence, and post-adolescence).

Results

Gingival crevicular fluid ALP levels rise sharply in the adolescence stage to a mean of 24.7 IU/L and return to a mean of 15.25 IU/L in the postadolescent stage. So, GCF ALP levels were significantly increased in the adolescence stage than pre- and post-adolescence stages (Table 1).

There was a statistically significant difference in GCF ALP levels among the three groups in the maxilla as well as in the mandible (Table 2 and Figs 3 and 4).

A statistically significant difference in GCF ALP levels was seen in both maxilla and mandible, between the preadolescent and adolescent groups. However, the difference between pre-adolescent and postadolescent groups among maxilla and mandible was statistically insignificant (Table 3).

Figs 2A and B: Hand-wrist radiograph

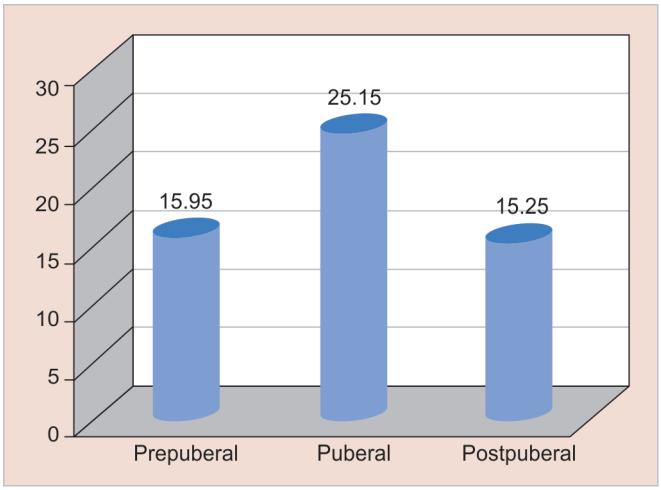


Fig. 3: Intergroup comparison among maxilla

Table 1: Descriptive data of the study groups

	N	*Minimum*	*Maximum*	*Mean*	*Std. deviation*
Prepubertal	20	10.00	21.00	15.275	3.30
Pubertal	20	20.00	29.00	24.7	2.1
Postpubertal	20	10.00	20.00	15.25	3.09

Table 2: Kruskal–Wallis test

	N	*Mean*	*Std. deviation*	*p*
Intergroup comparison among maxilla				
Pre-pubertal	20	15.95	3.83	0.004 (S)
Pubertal	20	25.15	1.98	
Post-pubertal	20	15.25	3.35	
Total	60	18.78	5.5	
Intergroup comparison among mandible				
Pre-pubertal	20	14.6	2.702	0.005 (S)
Pubertal	20	24.25	2.22	
Post-pubertal	20	15.25	2.98	
Total	60	18.03	5.15	

Test applied: Kruskal–Wallis test, *S* = significant

A statistically insignificant difference among preadolescent, adolescent, and postadolescent groups in maxillary and mandibular sites (Table 4 and Figs 5 to 7).

A statistically insignificant difference in males and females among maxilla and mandible in preadolescent, adolescent, and postadolescent groups (Table 5 and Fig. 8).

Discussion

The amount of mandibular growth taking place during treatment determines the success of treatment of many orthodontic problems. The skeletal age is considered to be more reliable and precise than the chronological age in assessing the progress of an individual toward maturity.[16]

Table 3: Mann–Whitney *U*-test for multiple comparisons (intergroup)

		Mean difference	*Sig.*
Multiple comparisons (intergroup) among maxilla			
Pre-pubertal	Pubertal	−9.02	0.003 (S)
	Post-pubertal	0.7	1.000
Pubertal	Post-pubertal	9.9	0.004 (S)
Multiple comparisons (intergroup) among mandible			
Pre-pubertal	Pubertal	−9.65	0.005 (S)
	Post-pubertal	−0.65	1.000
Pubertal	Post-pubertal	9.00	0.004 (S)

Test applied: Mann–Whitney *U*-test, *S* = significant

Traditionally, both cervical vertebrae and hand-wrist radiographs are utilized to estimate the timing of mandibular growth spurt. According to Ball et al.[17] cervical vertebral maturation stages cannot predict the onset of peak in mandibular growth and should be used with other methods of biologic maturity assessment. In contrast to the cervical vertebrae method, bone age assessment using hand-wrist radiographs shows good reproducibility and high reliability. Moreover, hand wrist radiograph involves no exposure to vulnerable tissues like the thyroid gland.[18]

The radiograph of the hand wrist has been the most often used area of the skeleton for estimation of skeletal age as many centers in this area of skeleton change at distinct times and rates. The information from a hand-wrist radiograph has been used in several ways to evaluate the skeletal age of the child.[19]

Hence, we used hand-wrist radiographs for the assessment of skeletal maturation and correlated it with GCF ALP levels. Since there is a rise in the GCF ALP activity during periodontal inflammation; it is of utmost importance that the periodontal condition of the subjects should be good.[20]

As compared with serum, GCF ALP analysis offers several advantages from a clinical point of view in that it is a very simple, rapid, and non-invasive procedure that can be performed in a clinical setting, even in the case of multiple GCF collections.[21] The ALP activity can be determined through routine and inexpensive laboratory analyzes that are easily available.

In this study, subjects were divided into three groups by using Bjork, Grave, and Brown[14] method of hand-wrist radiograph

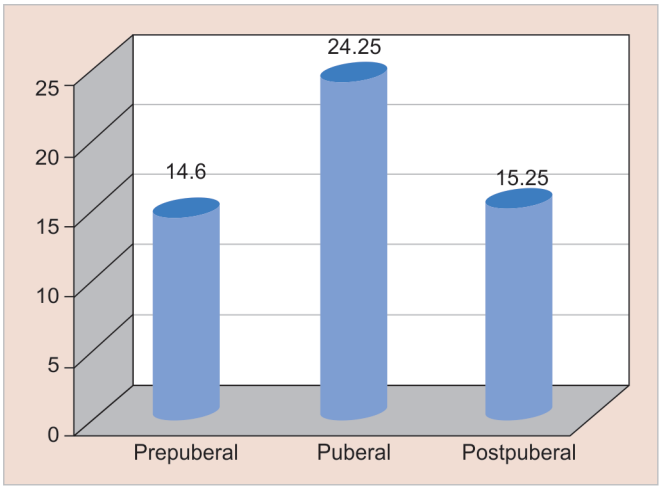


Fig. 4: Intergroup comparison among mandible

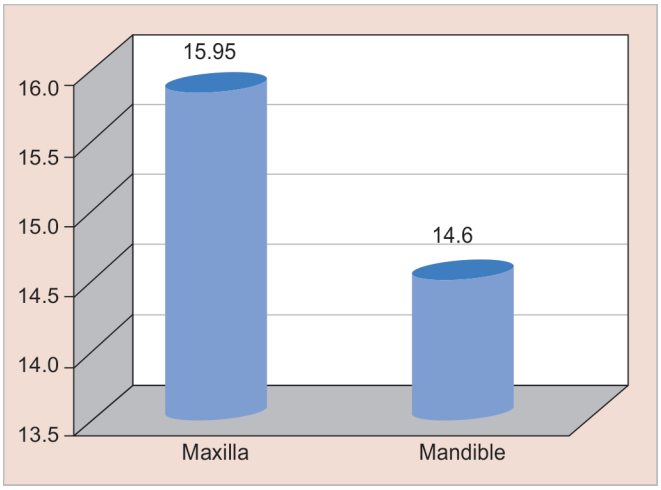


Fig. 5: Site-wise comparison of pre-pubertal growth

Table 4: Mann–Whitney *U* test for site-wise comparison

	N	*Mean*	*Std. deviation*	*Mean differences*	*p*
Site-wise comparison of pre-pubertal growth					
Maxilla	20	15.95	3.83	1.35	0.206
Mandible	20	14.6	2.702		
Site-wise comparison of pubertal growth					
Maxilla	20	25.15	1.98	0.9	0.18
Mandible	20	24.25	2.22		
Site-wise comparison of post-pubertal growth					
Maxilla	20	15.25	3.35	0.00	1.00
Mandible	20	15.25	2.98		

Test applied: Mann–Whitney *U* test

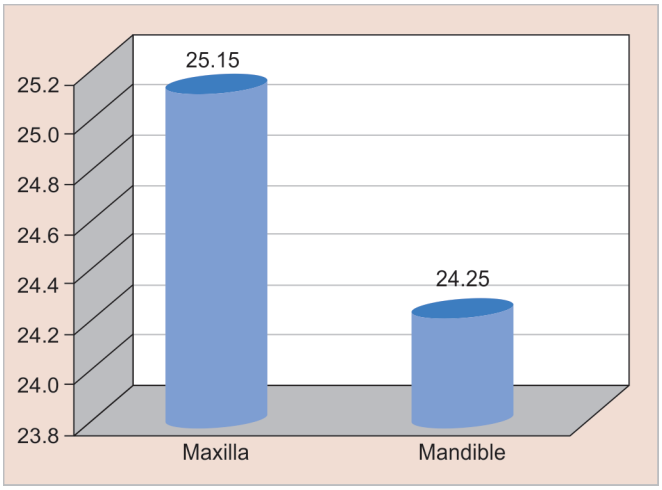


Fig. 6: Site-wise comparison of pubertal growth

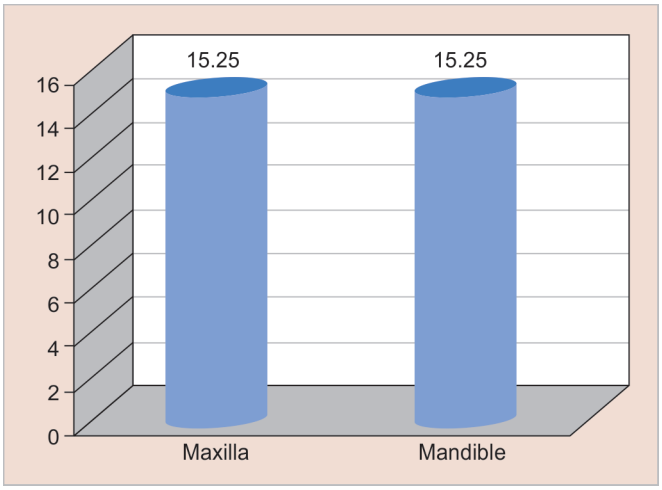


Fig. 7: Site-wise comparison of post-pubertal growth

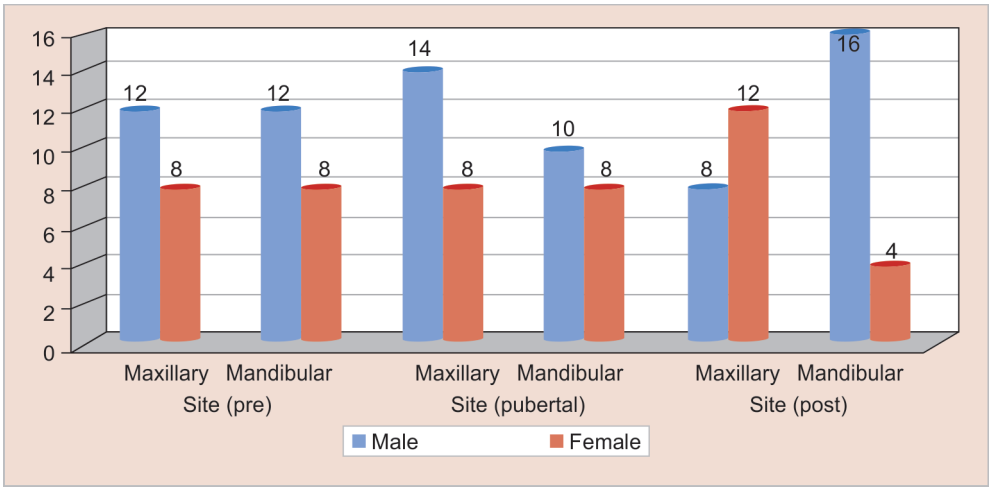


Fig. 8: Gender-wise comparison among mandible and maxilla

Table 5: Chi-square test for genderwise comparison

	Pre	*Pubertal*	*Post*
Gender-wise comparison among maxilla			
Male	12 (60)	11 (55)	10 (50)
Female	8 (40)	9 (45)	10 (50)
Total	20 (100)	20 (100)	20 (100)
p value	0.18	0.4	0.31
Gender-wise comparison among mandible			
Male	12 (60)	11 (55)	10 (50)
Female	8 (40)	9 (45)	10 (50)
Total	20 (100)	20 (100)	20 (100)
p value	0.24	0.604	0.37

Test applied: Chi-square test

assessment, i.e., preadolescent, adolescent, and postadolescent groups.

When the GCF ALP activity was assessed in relation to the stages of skeletal maturation, increased activity was seen for both maxilla and mandible at the adolescent stage compared with pre- and post-adolescent stages. These differences were statistically significant which are in accordance with the previous studies conducted by Perinetti et al.[21,22]

The results of this study revealed that the GCF ALP levels were low in the preadolescent phase, increased markedly to their peak in puberty, and again decreased in the postadolescent phase to approach the preadolescent levels. Gingival crevicular fluid ALP levels were lowest in the postadolescent phase. This is consistent with previous studies[21,22] that show GCF ALP levels increase in puberty whereas a decline in late adolescence.

In this study, there was a statistically insignificant difference in GCF ALP levels among males and females both in the maxilla and mandible ($p > 0.005$). This is in accordance with the previous study conducted by Perinetti et al.[22]

The limitations of the study are that it is a cross-sectional investigation with limited sample size. Hence, a longitudinal study with an increased sample size should be conducted to enhance the precision and confirm the adequacy of GCF ALP activity as a skeletal maturity indicator.

Conclusion

The mean GCF ALP levels were significantly higher in the adolescent phase when compared with the pre- and postadolescent stages.

There was a statistically insignificant difference in GCF ALP levels between the maxillary and mandibular sites or between males and females. Gingival crevicular fluid ALP appears to be a reliable diagnostic tool as a non-invasive biomarker of the pubertal growth spurt.

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