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Original article

Dentition phase and chronological age in relation to gingival crevicular fluid alkaline phosphatase activity in growing subjects

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ABSTRACT

Objective: Identification of skeletal maturation phases is of primary importance in terms of individual responsiveness to nearly all dentofacial orthopaedic treatments. In this regard, dentition phase and chronological age are still widely used to define the timing of and responsiveness to orthodontic treatments. Recently, gingival crevicular fluid (GCF) alkaline phosphatase (ALP) activity has been shown to be a reliable biomarker of skeletal maturation in growing subjects. Here, for the first time, circumpubertal dentition phases and chronological age were evaluated for correlations with GCF ALP activity, as a biomarker of skeletal maturation.

Materials and methods: Eighty-five healthy growing subjects (51 females, 34 males; mean age, 11.7 ± 2.3 years) were enrolled into this double-blind, prospective, cross-sectional-design study. Samples of GCF were collected from each subject at the mesial and distal sites of both of the central incisors, at the maxillary and mandibular arches. Their dentition phases were recorded as intermediate mixed, late mixed, or permanent. GCF ALP enzymatic activity was determined spectrophotometrically.

Results: The dentition phases showed median GCF ALP activities from 42.0 to 67.5 mU/sample. Although these were slightly greater for the permanent dentition, no significant differences were seen. Also, the chronological age did not correlate significantly with GCF ALP activity, and no significant differences were seen between maxillary and mandibular sites in any of the comparisons.

Conclusions: Assessment for treatment timing of dentofacial disharmonies in individual patients that require monitoring of their skeletal maturation phases should not rely on their circumpubertal dentition phase and chronological age.

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1. Introduction

Identification of the maturation phase that a growing subject has attained is necessary to make predictions relating to their subsequent developmental events. It is well established today that periods of accelerated growth, such as the pubertal growth spurt, can contribute dramatically to the correction of dentofacial disharmonies in individual patients.[1] Therefore, the diagnostic identification of the maturation phases is of primary importance in terms of individual responsiveness to nearly all dentofacial orthopaedic treatments.[1,2]

Several clinical parameters have been proposed towards evaluation of the maturation phase, with the most common procedures being radiographic methods based on analyses of the bones of the hand and wrist[3,4] and the cervical vertebrae.[2] Despite previous investigations reporting that skeletal maturity is poorly identified through dentition phase[3,5,6] and chronological age,[1,7,8] the use of these parameters to define individual timing and responsiveness to treatment is still widespread, in both clinical practice and research. To date, several studies, including randomised clinical trials, have compared the effectiveness of different orthopaedic treatments while basing the inclusion criteria on dentition phase or chronological age of the patients. For instance, the correction of skeletal class II by different treatment protocols has been investigated in subjects with mixed dentition[9–12] and late mixed dentition.[13] Other investigations have compared the outcomes of treatments performed at different dentition stages in both class II[14,15] and class III[16,17] patients. Similarly, skeletal effects of two class II treatment protocols have been compared in patients classified as being 10 to 14 years of age.[18] Moreover, class III treatment protocols have been evaluated in early treated patients who were defined as 5 to 10 years of age,[19] or more recently as having a mean age of 11.5 years.[20] A further recent randomised clinical trial evaluated skeletal effects of two palatal expansion procedures in patients with a mean age from 12 to 14 years.[21] Further studies have compared the effects of orthopaedic treatments across different age ranges, which were defined as early or late, with both class II[22] and class III[23] patients.

Gingival crevicular fluid (GCF) is a transudate, the molecular constituents of which derive mainly from serum, although its composition also depends on the local periodontal environment.[24] One of the first molecular constituents identified in GCF was alkaline phosphatase (ALP),[25] an enzyme required for bone mineralisation.[26] The GCF ALP activity of growing subjects has recently been shown to increase two-fold during puberty, as compared to pre-pubertal and post-pubertal stages, and thus GCF ALP activity has been proposed as a reliable biomarker of skeletal maturation in growing.[27]

The present prospective, double-blind study was aimed at evaluating possible correlations between the circumpubertal dentition phases and chronological age with skeletal maturation, as monitored through the GCF ALP activity.

2. Materials and methods

2.1. Study population and design

Enrolment was for subjects seeking orthodontic treatment who had never undergone such treatment before. Signed informed consent was obtained from the parents prior to the start of the study, and the protocol was reviewed and approved by the Ethical Committee of the local University. The following enrolment criteria were observed: i) aged between 7 and 18 years; ii) presence of intermediate, late mixed, or early permanent phases of dentition; iii) good general health, with absence of any nutritional problems; iv) no use of anti-inflammatory or antibiotic drugs in the month preceding the start of the study; v) probing depth values not exceeding 4 mm in the whole dentition, and 3 mm in the anterior sextants; vi) no radiographic evidence of periodontal bone loss at a dental panoramic radiograph examination; and vii) a full-mouth plaque score (FMPS) and a full-mouth bleeding score (FMBS) $\leq$25%. The subjects were scheduled for enrolment at a first clinical examination; subsequently, during a second visit 7 to 10 days prior to GCF collection, they underwent a session of professional supragingival and subgingival scaling, and also received repeated oral hygiene instructions. Moreover, on the days between the professional scaling and the GCF collection, the subjects were asked to rinse twice a day with a 0.012% chlorhexidine mouthwash, and they were not allowed to take any anti-inflammatory or antibiotic drugs. Subsequently, at the last clinical session when GCF was collected for ALP activity determinations, the patient clinical parameters were recorded, while dental panoramic radiographs and lateral cephalograms were taken at the same session, immediately after GCF collection. A total of 92 subjects were screened, out of which 85 were enrolled in the study: 51 females and 34 males (mean age, 11.7 $\pm$ 2.3 years; range, 7.7-16.9 years).

2.2. Assessment of dentition phase

The assessments of the dentition phase were carried out according to the following definitions[5]: i) intermediate mixed dentition, when the permanent incisors and first molars had fully erupted, with the presence of deciduous teeth in the buccal region (deciduous canine and first and second molars); ii) late mixed dentition, when any of the deciduous canines and molars had exfoliated, with eruption of any permanent canines and premolars; and iii) early permanent dentition, when all of the permanent teeth were present (possible presence of second molars; absence of third molars). These assessments of the phases of dentition were performed by a single operator by intra-oral evaluation (LC), as well as on dental casts and dental panoramic radiographs when needed.

2.3. Clinical monitoring and GCF collection procedures

The intra-oral clinical examinations were performed by a single operator (GP) on four sites per each maxillary and mandibular central incisor (mesial, distal, medio-buccal and medio-palatal/ lingual), as previously described.[28] Briefly, this consisted of recording the presence of supragingival plaque

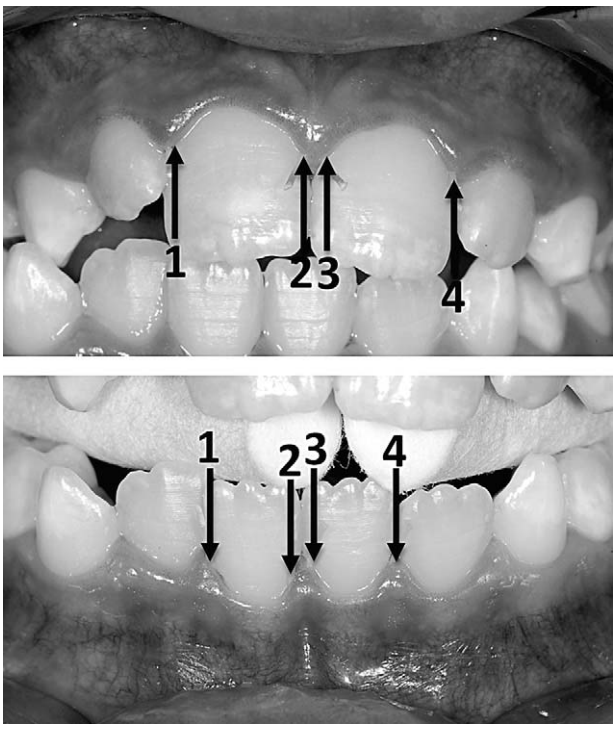

Fig. 1 – The GCF collection. Four samplings were performed at the mesial and distal aspects of each of the maxillary and mandibular central incisors.

(PL+), gingival bleeding within 15 s after probing (BOP+), and the probing depth (PD). Contamination of the GCF was minimised by recording the PL+ before carefully cleaning the tooth with a sterile curette, then by collecting GCF and subgingival plaque from the isolated area, and finally by recording the PD and BOP+. The GCF collection was performed at both the mesial and distal sites on each of the central upper and lower incisors. Briefly, #25 standardised sterile paper strips (Inline; Torino, Italy) were inserted 1 mm into the gingival crevice and left *in situ* for 60 s (Figure 1).[29] The four samples from the same dental arch, as maxillary or mandibular, were pooled. The GCF samples were transferred to plastic vials and immediately stored at -80 °C until analysed.

2.4. *Enzymatic activity determination*

The biochemical assays were performed by a single operator (GP) who was blinded to the dentition phase and chronological age of the subjects. The four GCF samples from either the maxillary or mandibular sites were resuspended 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 samples were then incubated at 37 °C (±<0.1 °C fluctuations) for 2 h, during which time ALP hydrolyses p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate. The reactions were then stopped by adding 5 μl 3 M NaOH, and the rate of increase in absorbance was read with a spectrophotometer at 405 nm[30]. For each analysis, a control was used that consisted of the reagent and the Tris buffer without a sample. By using 18.45 mM as the p-nitrophenol absorptivity, the absorbance was converted into enzyme activity units (1 unit = 1 mmol of p-nitrophenol released per minute at 37 °C) and was expressed as total activity in mU/sample.

2.5. *Sample size calculation*

Sample size of at least 22 subjects for each of the phase groups was set to detect an effect size coefficient[31] for the GCF ALP activity of 0.8 between any two of them, with an alpha set at 0.01 and a power of 0.8. An effects size of at least 0.8 which is regarded as to a 'large effect',[31] i.e. a clinically relevant correlation necessary for a potential diagnostic tool to be accurate.[32]

2.6. *Statistical analysis*

The following analyses were carried out considering the maxillary and mandibular sites of each patient as the statistical unit. The significance of the differences in chronological age among the subjects clustered according to the dentition phase was evaluated by one-way analysis of variance (ANOVA). The balancing of the sex distribution among the subjects clustered according to the dentition phase was tested by chi-squared analysis. A Kruskal-Wallis test and one-way ANOVA were used to assess the significance of the differences in the %PL+ and %BOP+ and mean PD, respectively, among the different dentition phases. A Wilcoxon rank sum test and a paired t-test were used to assess the significance of the differences of the %PL+ and %BOP+ and mean PD, respectively, between the maxillary and mandibular sites, within each dentition phase, excluding those subjects with different dentition phases between the maxillary and mandibular arches. Finally, the significance of any correlation between both maxillary and mandibular GCF ALP activities and chronological age in the whole group was assessed using the Spearman rho coefficient. A p value <0.05 was used for rejection of the null hypothesis.

3. Results

The chronological ages of the subjects clustered according to either their maxillary or mandibular phases of dentition are shown in Table 1. Significant differences were seen for both the maxillary and mandibular dentitions ($p < 0.001$). In contrast, the distribution of the sexes was not significantly different among the compared groups (not shown).

The pooled maxillary and mandibular %PL+, %BOP+ as medians (25th;75th percentile) were 12.5 (0;25.0) and 6.3 (0;12.5), respectively. The pooled maxillary and mandibular PD as mean ± SD was 1.6 ± 0.3. No significant differences were seen among the dentition phases, and none of these clinical parameters showed significant correlations with chronological age (not shown).

The GCF ALP activities clustered according to dentition phase of the subjects is shown in Table 2. These GCF ALP activities were generally similar, with median values from 42.0 mU/sample (mandibular sites, late mixed dentition) to 67.5 mU/sample (maxillary sites, permanent dentition). For both the maxillary and mandibular sites, no significant differences were seen for GCF ALP activity among the dentition phases, in spite of a slight increase in GCF ALP activity in the early

Table 1 – Age of the subjects according to their dentition phase (n = 85).

Dentition phase	No	Maxilla		No	Mandible	
		Mean ± SD (years)	Min-Max (years)		Mean ± SD (years)	Min-Max (years)
Intermediate mixed	23	9.4 ± 1.2	7.7-11.4	20	9.1 ± 1.0	7.7-10.9
Late mixed	23	11.5 ± 1.8	8.5-15.7	23	11.2 ± 1.5	8.5-15.2
Permanent	39	13.3 ± 1.7	10.7-16.9	42	13.3 ± 1.8	10.7-16.9
Diff		***			***	

No, number of subjects; **Min**, minimum age; **Max**, maximum age; **Diff**, significance of the differences among the dentition phases: ***p < 0.001.

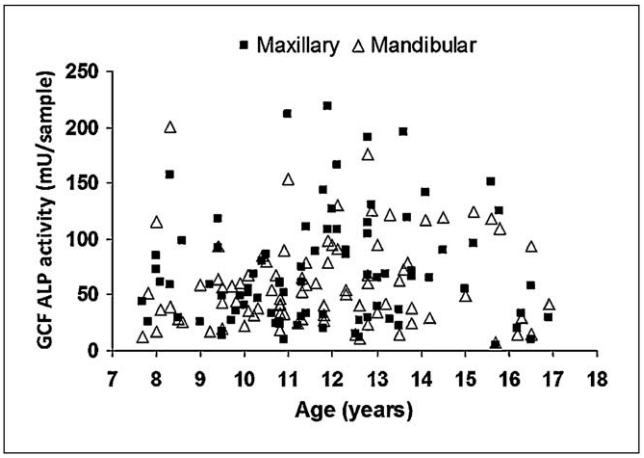

Fig. 2 – The GCF ALP activities of the sampling sites according to chronological ages. GCF ALP activities (mU/sample) of the maxillary (closed squares) and mandibular (open triangles) sites according to chronological ages of the subjects (n = 85). Spearman rho values: maxillary sites, 0.101; mandibular sites, 0.105 (p > 0.3).

permanent dentition. Moreover, for 65 subjects showing the same maxillary and mandibular dentition phases, no significant differences were seen in GCF ALP activity between the two dental arches within each dentition phase (not shown).

The correlation of GCF ALP activity with chronological age is shown in Figure 2. For both the maxillary and mandibular sites, no significant correlations were seen, with rho values of 0.101 and 0.105, respectively (p > 0.3).

4. Discussion

The present study investigated possible relationships between either circumpubertal dentition phase or chronological age and GCF ALP activity, as a biomarker of skeletal maturation. These data obtained demonstrated that neither of these clinical parameters correlated significantly with the biochemical, GCF ALP activity, parameter.

When considering GCF formation,[33] three potential sources can be deemed responsible for GCF ALP activity changes: i) serum ALP (as a systemic factor); ii) maxillary/ mandibular growth (as a local skeletal factor); and iii) dental permutation (as a local dentoalveolar factor). Dental permutation cannot be considered as a process of bone growth, although tooth eruption can have effects on the local metabolism of the alveolar bone. However, the present study was not designed to evaluate whether dental eruption *per se* can have a local impact on GCF ALP activity, but rather to determine whether the overall phase of dental permutation correlates with growth, as recorded through GCF ALP activity as a biomarker. Therefore, subjects with early mixed dentition were not enrolled in the trial, and the GCF sampling was performed at the central incisors that, like the lateral incisors, were fully erupted. In this manner, no direct continuity between the sampling sites and eruption areas was encountered.

The diagnostic use of GCF ALP activity in orthodontics has been proposed previously.[29,34] In addition, skeletal maturation effects on GCF ALP activity have been reported very recently.[27] In particular, during the pubertal growth spurt, GCF ALP activity showed an up-to-two-fold increase, as compared to subjects at the pre-pubertal or post-pubertal phases.[27] As it has been shown that local maxillary/ mandibular growth does not influence GCF ALP activity in growing subjects, this would thus be due to GCF ALP serum levels.[27] However, no previous evidence has been provided for any relationships between dental permutation and GCF ALP activity. Similarly, there are no data on correlations between chronological age and GCF ALP activity. However, this information would be useful to further define the roles of these clinical parameters for the assessment of individual skeletal maturity in growing

Table 2 – The GCF ALP activity in the maxillary and mandibular sites according to their dentition phase (n = 85).

Dentition phase	Maxillary		Mandibular	
	Median (25th;75th per) (mU/sample)	Mean (mU/sample)	Median (25th;75th per) (mU/sample)	Mean (mU/sample)
Intermediate mixed	58.5 (31.4; 85.2)	60.0	43.5 (27.5-62.4)	54.7
Late mixed	48.9 (26.4; 89.5)	64.5	42.0 (27.8-78.9)	56.0
Permanent	67.5 (29.7; 108.9)	77.6	59.5 (31.5-93.8)	63.5
Diff	NS		NS	

Diff, significance of the differences among the phases of the dentition; **NS**, no statistically significant difference.

orthodontic patients, and eventually to establish any clinical implications for treatment planning.

Since GCF ALP activity increases during periodontal inflammation,[25,30] local tissue health is necessary to exclude any possible unwanted bias. In the present study, all of the enrolled subjects received a session of professional scaling and showed an optimal periodontal state, with a very low number of sites PL+ or BOP+, and a mean PD below 2 mm. In particular, the clinical conditions were similar among the subjects of the compared groups, and between the maxillary and mandibular sites within each group (not shown). Other possible bias in the GCF analysis might arise from inter-subject variability, which will have been reduced here by including multiple collection sites in each of the maxilla and mandible.

As shown in Table 1, although when clustered according to dentition phase, the differences in the mean ages of the three groups of subjects were significant, the corresponding ranges were notably large, confirming that dental permutation correlates poorly with chronological age.[5] This is particularly important when referring to individual patients. Indeed, tooth eruption can be dependent on several environmental factors, such as loss of teeth and tooth crowding.[3,5,6,35]

In the present study, the GCF ALP activities across the three dentition phases were similar, with no statistically significant differences seen, although a slight increase with the permanent dentition was recorded, as compared to the intermediate and late mixed dentitions (Table 2). The GCF ALP activity also showed notably large ranges in each of the datasets. A previous report showed that only intermediate mixed dentition can be considered to reliably correlate with pre-pubertal growth phase.[5] However, for a subject with late mixed or permanent dentition, any relationships with skeletal maturity lost significance.[5] This can explain the slightly greater GCF ALP activity seen for the permanent dentition group, where it is most likely that several subjects in this group were monitored here during their pubertal growth spurt. The present data are also consistent with previous evidence reporting tooth eruption as a process with intrinsic variability, in terms of the timing, when compared to skeletal maturation.[3,5,6] Even the relationships between dental maturity, rather than eruption, and skeletal maturity have shown inconsistent data, with both noted[36] and non-significant[37] correlations reported. However, the only study based on a diagnostic performance analysis showed that the timing of the onset of the growth spurt is not provided by dental maturation phases.[38]

The present evidence can also explain the different effectiveness of orthopaedic treatments in class II subjects that has been seen in studies enrolling patients according to the presence of a mixed dentition phase,[9,12] or according to a reliable indicator of skeletal maturity.[39] In the former studies, the non-significant skeletal effects seen when comparing the 1-phase and 2-phase treatment protocols[9,12] might thus be attributed to the use of an unreliable indicator, i.e. the dentition phase, for the onset of the pubertal growth spurt.[39]

It has been reported that boys usually enter their pubertal stages at 12-13 years of age, and that girls usually reach their pubertal growth spurt at 14-16 years of age.[8] Consistent with this, it has been shown more recently that 9-year-old boys and 14-year-old girls are most likely in their pre-pubertal and post-pubertal stages, respectively.[7] However, chronological age shows wide variations when correlated with the maturation phases during adolescence,[1,3,8] with a consequent low diagnostic performance for the detection of the onset of the adolescent peak.[7]

The present results are in line with this evidence, where there were no significant correlations of both maxillary and mandibular GCF ALP activities with chronological age, with rho values of 0.101 and 0.105, respectively (Figure 2). Similar enzymatic activities were also recorded for the maxillary and mandibular sites (Table 2), confirming previous findings of no relevant local basal bone growth influence on GCF ALP activity.[27] Moreover, even the pooled maxillary and mandibular GCF ALP activities showed no significant correlations with chronological age (not shown). These results thus show very little biological correlation between chronological age and individual skeletal maturation, as monitored through GCF ALP activity, at least in the circumpubertal age range.

5. Conclusions

In growing subjects, the dentition phase and chronological age do not show significant correlations with the skeletal maturation phases, as monitored through the GCF ALP activity. Therefore, treatment of dentofacial disharmonies in individual patients that requires the monitoring of individual skeletal maturation phases in the circumpubertal term should not rely on the two clinical parameters of dentition phase and chronological age.

Conflict of interest

The authors have reported no conflict of interest.

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Riassunto

Obiettivo: *L'identificazione della fase di maturazione scheletrica è di primaria importanza in termini di risposta individuale a quasi tutti i trattamenti di ortopedia dento-facciale. In questo contesto, la fase della dentizione e l'età cronologica sono ancora apiamente utilizzate nel definire il timing e la responsività al trattamento ortodontico. Recentemente, l'attività della fosfatasi alcalina (ALP) del fluido crevicolare gengivale (GCF) è stata mostrata essere un affidabile biomarker di maturazione scheletrica in soggetti in crescita. In questo studio, per la prima volta, le fasi di dentizione e l'età circumpuberali sono state studiate alla ricerca di correlazioni con l'attività dell'ALP del GCF, usata come biomarker di maturazione scheletrica.*
Materiali e metodi: *Ottantacinque soggetti in crescita (51 femmine, 34 maschi; età media 11.7 ± 2.3 anni) sono stati inclusi in questo studio trasversale, porspettico e a doppio cieco. I campioni di GCF sono stati raccolti da ogni soggetto nei siti mesiali e distali di entrambi gli incisivi centrali, superiori e inferiori. La fase della dentizione è stata registrata come mista intermedia, mista tardiva e permanente.*

L'attività dell'ALP del GCF è stata determinata spettrofotometricamente.
Risultati: *Le fasi della dentizione hanno mostrato delle mediane di attività dell'ALP del GCF tra 42.0 to 67.5 mU/campione. Nonostante l'attività è stata lievemente più alta per la dentizione permanente, differenze significative non sono state riscontrate. Ancora, l'età cronologica non si è correlata significativamente con l'attività dell'ALP, e nessuna differenza significativa è stata vista tra i siti mascellari e mandibolari.*
Conclusioni: *La determinazione individuale del timing per il trattamento delle disarmonie dento-facciali, in pazienti che richiedono il monitoraggio della maturazione scheletrica, non dovrebbe fare affidamento sulle fasi di permuta e l'età cronologica circumpuberali.*

Résumé

Objectif: *L'identification de la phase de maturation squelettique revêt une importance fondamentale en termes de réponse individuelle à presque tous les traitements d'orthopédie dento-faciale. Dans ce cadre, la phase de denture et l'âge chronologique constituent encore des éléments phares pour définir le timing et la réactivité au traitement orthodontique. Récemment, l'activité de la phosphatase alcaline (ALP) du fluide créviculaire gingival (GCF) s'est avérée être un biomarqueur formidable de la maturation squelettique chez des sujets en croissance. Dans cette étude, pour la première fois, les phases de denture et d'âge circumpubérales ont été étudiées pour chercher des corrélations avec l'activité ALP du GCF, utilisée comme biomarqueur de maturation squelettique.*
Matériels et méthode: *85 sujets en croissance (51 femmes, 34 hommes; âge moyen 11,7 ± 2,3 ans) ont été inclus dans cette étude transversale, prospective et en double aveugle. Les échantillons de GCF ont été collectés chez chaque sujet dans les parties mésiales et distales des deux incisives centrales, supérieures et inférieures. La phase de denture a été enregistrée comme mixte intermédiaire, mixte tardive et permanente. L'activité ALP du GCF a été déterminée d'un point de vue spectrophotométrique.*
Résultats: *Les phases de denture ont montré des médianes de l'activité ALP du GCF entre 42.0 et 67,5 mU/échantillon. Bien que l'activité ait été légèrement plus élevée pour la denture permanente, aucune différence significative n'a été enregistrée. Qui plus est, l'âge chronologique n'avait aucun lien significatif avec l'activité de l'ALP et aucune différence significative n'a été identifié entre les parties maxillaires et mandibulaires.*
Conclusions: *La détermination individuelle du timing pour le traitement des dysharmonies dento-faciales, chez des patients qui demandent le suivi de la maturation squelettique, ne devrait pas compter sur les phases de denture et d'âge chronologique circumpubérales.*

Resumen

Objetivo: *La identificación de la fase de maduración esquelética es de importancia fundamental en términos de reactividad a casi todos los tratamientos de ortopedia dentofacial. En este marco, aún se recurre con mucha frecuencia a la fase de dentición y edad cronológica para definir la temporización y la reactividad al tratamiento ortodóncico. Recientemente, la actividad de la fosfatasa alcalina (ALP) del fluido crevicular gingival (FCG) resultó ser un biomarcador efectivo de maduración esquelética en sujetos en crecimiento. En esta investigación fueron estudiadas, por primera vez, las fases de dentición y de edad circumpuberales, buscando correlaciones entre la actividad de ALP del FCG, utilizada como biomarcador de maduración esquelética.*
Materiales y métodos: *Ochenta y cinco sujetos en crecimiento (51 mujeres, 34 varones; edad promedio 11, 7 ± 2,3 años) fueron incluidos en este estudio transversal, prospectivo, doble ciego. Las muestras del FCG fueron recogidas por cada sujeto en las localizaciones mesiales y distales de ambos incisivos centrales, superiores e inferiores, La fase de la dentición fue indicada como mixta intermedia, mixta tardía y permanente. Determinación espectrofotométrica de la actividad de ALP del FCG Resultados: en las fases de la dentición destacaron medianas de actividad de ALP del GCF entre 42,0 y 67,5 mU/muestra. A pesar de que la actividad resultó ligeramente más alta para la dentición permanente, no se experimentaron diferencias significativas. Asimismo, la edad cronológica no resultó relacionada, de manera significativa, con la actividad de ALP, y ninguna diferencia de relieve fue identificada entre las localizaciones maxilares y mandibulares.*
Conclusiones: *La determinación individual de la temporización para tratar las disarmonías dentofaciales en pacientes que requieren del seguimiento de la maduración esquelética no debería confiar en las fase de dentición y de edad cronológica circumpuberales.*

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