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Association between mesially displaced maxillary first premolars and early displaced maxillary canines

ABSTRACT

Aim To evaluate the association between the mesially displaced maxillary first premolar (MDP) and the early displacement of the adjacent permanent canine (EDC) before their eruption.

Materials and methods A sample of 1247 subjects in the intermediate mixed dentition stage was assessed for the presence of MDP and EDC. All subjects were divided into two groups: MDP group and noMDP group. For each subject two angular measurements (premolar-occlusal plane η and Π premolar-midline angles) were analysed on panoramic radiographs. The chi-square test with Yates correction was performed to compare the prevalence rate of EDC in MDP (MDP-EDC) and noMDP groups. The statistical comparisons for the values of η and Π angles between MDP vs noMDP, MDP vs MDP-EDC, and noMDP vs MDP-EDC groups were performed by means of ANOVA with Bonferroni correction.

Results The prevalence rate of EDC in the MDP group was significantly greater than in the noMDP group (66% vs. 12.1%). MDP-EDC group showed a significantly larger Π angle than in the MDP group

resulting in an increased mesial inclination of displaced premolars.

Conclusion MDP can be considered a dental anomaly associated to maxillary canine displacement.

Keywords Association of dental anomalies, Dental anomalies, Displaced maxillary permanent canine, Mesially displaced maxillary first premolars, Panoramic radiograph.

Introduction

The ectopic eruption and impaction of maxillary permanent canines can determine consequences for adjacent teeth, causing root resorption of the adjacent lateral incisors and/or cyst formation [Becker and Chaushu, 2005; Baccetti et al., 2011]. Careful supervision and early diagnosis of eruption disturbances have been considered important because management of impacted canines can lengthen treatment time, complicate orthodontic mechanics, and increase treatment costs [Ericson and Kurol, 1987; Barlow et al., 2009; Baccetti et al., 2011]. For early diagnosis of possible impaction of the maxillary canine both clinical and radiographic examinations should be used [Shapira and Kuftinek, 1998].

Several studies [Ericson and Kurol, 1987; Ericson and Kurol, 1988a; Ericson and Kurol, 1988b; Lindauer et al., 1992; Power and Short, 1993; Stivaros and Mandall, 2000; Warford et al., 2003] identified radiographic factors analysing the inclination and location of the cusp tip of the unerupted permanent canine (tooth germ position of the maxillary canine) relative to the erupted lateral incisor root as it appears on panoramic radiographs in the late mixed dentition. These radiographic factors, however, can be used for diagnostic and prognostic evaluation of intraosseous displacement only after 10 to 11 years of age. Only Sambataro et al. [2004] introduced a diagnostic and prognostic method to identify eruption disturbances of the upper canine at an early developmental stage evaluated on the posteroanterior radiograph.

The term "malposed" or "displaced" canine is referred generally to an anomalous position of the tooth recognised at an "early" stage of development. "Early" in biology is often considered as occurring before the usual or physiological time [Ricketts, 1998]. From a physiological point of view the maxillary canine between 5 and 9 years of age tends to move palatally with substantial movement in a buccal direction between 10 and 12 years [McSherry and Richardson, 1999]. Consequently in the early stage of development it is not possible to establish the exact side of the malposed tooth germ when the palatal-buccal localisation is considered.

Previous studies [Ericson and Kurol, 1987; Ericson and

Kurol, 1988a; Ericson and Kurol, 1988b; Lindauer et al., 1992; Power and Short, 1993; Stivaros and Mandall, 2000; Warford et al., 2003] focused their attention evaluating the localisation of the malposed permanent canine with respect to the roots of the mesial adjacent teeth (permanent lateral or central incisors). No study analysed the spatial relationship between the malposed maxillary permanent canine and the tooth germ of the distal permanent teeth (permanent first premolar). During their development and eruption, the buds of the maxillary first premolar and permanent canine have close intrabony relationships. The tooth germ of the developing maxillary first premolar is located closer to the occlusal plane and under the permanent canine; consequently the first premolar erupts before the canine following a vertical direction [Van der Linden, 1976]. Abnormal tooth germ position, deviated angular changes, and anomalous tooth eruption sequence can lead to an alteration between the tooth germs of the first premolar and permanent canine resulting into eruption disturbances. Several studies [Kettle, 1958; Jacobs, 1994; Leonardi et al., 2004] reported that the success rate of canine eruption was increased by combining the extraction of the deciduous canine with manipulation of the space conditions at the upper arch by distal movement of the buccal segments or by localised permanent tooth extractions. More recently, Baccetti et al. [2008] suggested that the use of the cervical pull-headgear associated to the extraction of the primary canine for the interceptive treatment of palatally displaced canines can restrain the distal segment of the upper dental arch from moving mesially, thus maintaining the space available for canine eruption.

Alessandri Bonetti et al. [2010, 2011] reported that by combining preventive extraction of both deciduous canine and deciduous first molar it is possible to accelerate eruption and promote uprighting of the first premolar. These events can stimulate the correct eruption of the permanent canine by providing more space for the physiologic uprighting movement of the canine crown in a distal direction into alveolar bone. It should be noted, however, that these studies [Alessandri Bonetti et al., 2010; Alessandri Bonetti et al., 2011] suggest a therapeutic approach and do not propose any diagnostic evaluation or a method for early detection or prevention of intraosseous malposition of the maxillary canine.

The purpose of this study, therefore, was to evaluate the association between the mesially displaced maxillary first premolar and the displacement of the adjacent permanent canine before their eruption.

Materials and methods

The initial sample for this study consisted of 1247 subjects from the Department of Orthodontics of the University of Rome Tor Vergata. All subjects were in the

intermediate mixed dentition stage (permanent incisors and first molars fully erupted and before the exfoliation of the posterior deciduous teeth) and at a prepubertal stage of skeletal maturity (CS1 or CS2) [Baccetti et al., 2005], and they were observed before any orthodontic treatment. For each subjects dental casts and radiographic records (panoramic radiographs and PA films) were examined. The application of exclusion criteria (presence of complex craniofacial malformations, cleft lip or palate, incomplete or inadequate records) reduced the initial sample to the final sample of 1180 subjects (622 females and 558 males; mean age 8 years 6 months \pm 1 year 4 months). The following parameters were recorded for each subject.

- Mesially displaced maxillary first premolar (MDP), defined as the mesial inclination or mesial position of the bud of the maxillary first premolar toward the erupting permanent canine. The stage of development of the unerupted maxillary first premolar was appraised according to the classification of Koch et al. [1991] and had to range from stage E to G: stage E is root length less than crown height, stage F is root length equal or greater than crown height, and stage G, the walls of the root canal are parallel and root apex is still partly open. The intraosseous position of the bud of the first maxillary premolar was evaluated on panoramic radiographs using sectors 1-5 according to Ericson and Kurol [1988a]. MDP occurred when the first premolar bud exceeded the tangent line to the distal border of the adjacent deciduous canine and the medial crown portion was in sector 1 (Fig. 1).
- Early displaced canine (EDC), defined as an anomalous position of the tooth germ of the maxillary permanent canine was evaluated on the basis of the panoramic radiograph and the PA film. The presence of early displaced canine was evaluated by the analysis of sector 1-5 [Ericson and Kurol, 1988a] and it was

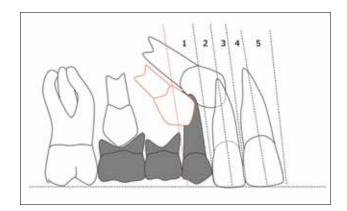
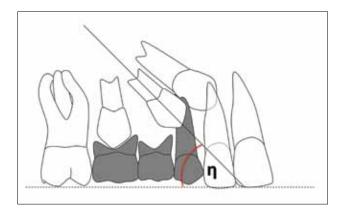


FIG. 1 MDP occurred when the premolar bud exceeded the tangent line to the distal border of the adjacent deciduous canine and the medial crown portion was in the sector 1 according to Ericson and Kurol [1988a]. EDC was identified when the crown of the permanent canine overlapped the root of the adjacent lateral incisor (sectors 2-5) [Ericson and Kurol, 1988a].



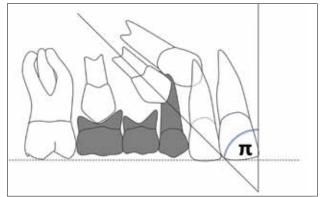


FIG. 2 Two angular measurements were analysed on the panoramic x-ray: A) the η angle between the long axis of the maxillary first premolar and B) the occlusal plane and the Π angle between the long axis of the premolar and the midline.

identified when the crown of the permanent canine overlapped the root of the adjacent lateral incisor (sectors 2-5). The PA film was used to predict eruption disturbance of upper canine according to the method by Sambataro et al. [2004] (score < -0.565). The radiographic variables used to identify the EDC become prognostic and indicative of diagnosis of intraosseous malposition at a later developmental stage, after 10 to 11 years of age [Ericson and Kurol, 1987; Ericson and Kurol, 1988a; Ericson and Kurol, 1988b; Stivaros and Mandall, 2000]. The lateral cephalograms could not be taken into consideration as in the early developmental stage, between 5 and 9 years, the unerupted canines physiologically tend to migrate in a palatal direction and to make a transition from the palatal side to the buccal side of the dental arch between 10 and 12 years [Coulter and Richardson, 1997; McSherry and Richardson, 1999].

All subjects were divided into two groups: MDP group and noMDP group according to the presence and absence of MDP, respectively. The prevalence rate of EDC was assessed in both groups.

The association MDP-EDC was recorded when the EDC was omolateral to the MDP.

For each subject two angular measurements were analysed on the panoramic X-ray (Fig. 2): the η angle between the long axis of the maxillary first premolar and the occlusal plane and the Π angle between the long axis of the premolar and the midline.

Statistical analysis

Reproducibility of the diagnosis was assessed by reexamining the records of 100 subjects 1 month after the first examination by the same operator (M.M.). Reproducibility was 100%.

The prevalence rates for MDP and EDC were recorded. The chi-square test with the Yates correction was performed to compare the prevalence rate of EDC in MDP and noMDP groups. For statistical purposes, individual subjects and not individual first premolars showing eruption disturbances were used as statistical units. This was accomplished in order to avoid inflation of statistical significance due to the greater number of teeth showing lack of eruption with respect to subjects. In case of bilateral MDP only one side was chosen randomly.

The values for η and Π angles were compared between the MDP group, the MDP-EDC (group with concurrent presence of MDP and EDC), and noMDP group. This statistical comparison was performed by means of ANOVA with Bonferroni correction.

The systematic error for the 2 angular variables was assessed with the paired t tests by repeating the measurements on 45 subjects. No systematic error was detected (p values of 0.486 and 0.096 for η and Π angles, respectively). The random error was evaluated with the method of moments' estimator (MME) and it was 0.30 and 0.32 degrees for η and Π angles, respectively.

All statistical computations were carried out with a

	MDP group (1)		MDP-ED	MDP-EDC group (2)		NoMDP group (3)		Statistical comparisons		
	Mean	SD	Mean	SD	Mean	SD	1 vs 2	2 vs 3	1 vs 3	
η angle	63.8	6.5	63.6	7.0	70.7	6.2	NS	*	*	
Л angle	17.6	5.3	18.9	4.8	9.2	2.0	*	*	*	
NS: not sig	nificant; * p	<0.001		·		•				

TAB. 1 Descriptive statistics and statistical comparisons for the angular measurements.

statistical software (Statistical Package for the Social Sciences, SPSS, Version 12, Chicago, USA).

Results

The prevalence rate of MDP in the total sample was 34.4% (406 out of 1180 subjects). The male-tofemale (M:F) ratio in MDP subjects was 152:254 which approximates a M:F ratio of 1:1.7. The unilateral-tobilateral ratio of MDP was 1:1 (204:202 subjects). The prevalence rate for EDC in the total sample was 31.0% (366 subjects), with a M:F ratio of 1:1.5 (146:220). The unilateral-to-bilateral ratio of EDC was 1:1.9 (242:124). The prevalence rate of EDC in the MDP group (268 subjects, 66.0%) was significantly greater than in the noMDP group (94 subjects, 12.1%) (chi-square =183.35, p=0.000). The unilateral association of EDC with MDP was more than 3 times greater than the bilateral one with a ratio of 3.3:1 (206:62). The M:F ratio in noMDP group was 1:0.8 (54:44 subjects), whereas the occurrence of the bilateral EDC was smaller than the unilateral one with a ratio of 1:2.6 (26:68 subjects). Descriptive statistics and between-group comparisons for the two angular measurements are reported in Table 1. The η angle was significantly smaller in both MDP and MDP-EDC groups when compared to the noMDP group (-6.9 and -7.1 degrees, respectively). The Π angle was significantly larger in both MDP and MDP-EDC groups with respect to the noMDP group (+8.4 and +9.7 degrees, respectively). The MDP-EDC showed a significantly larger Π angle than the MDP group (+1.3) degrees) while no significant differences were found between MDP and MDP-EDC groups for the η angle.

Discussion

This study was conducted to investigate the possibility of significant associations between the intraosseous position of the developing mesially displaced maxillary first premolar (MDP) and the eruption disturbance of the adjacent permanent canine (EDC) in a large sample of subjects during the early developmental phases, thus contributing to identify an early tooth position anomaly associated to canine displacement. The prevalence rate of MDP in the examined sample was 34.4%.

In this study the localisation of the upper premolars was analysed by using five sectors as described by Ericson and Kurol [1988a] for the palatally displaced erupting permanent canines on panoramic films. Root development of the first premolar had to range from stage E through G; stage D was not included in this investigation for the difficulty to trace the long axis of the tooth bud at such an early stage.

The prevalence rate of EDC in the initial examined sample was 31.0%. This prevalence rate was greater

than that reported for palatally displaced canines (2.43 - 5.2%) [Baccetti 1998; Sacerdoti and Baccetti, 2004] and that found for buccally displaced canines (3.06%) [Mucedero et al., 2013]. This evident difference might be explained by the fact that in our study the mean age of the examined sample ranged from 7 years 2 months to 9 years 10 months. The abnormal position of the ectopic canines is already present at a very early stage as 5-6 years and continues throughout the growth period. In fact, between 5 and 9 years the movement of the permanent canine is oriented in a palatal direction and then it tends to move buccally after 10 years of age [McSherry and Richardson, 1999]. Consequently in the early stage of development, as in our sample, it is not possible to establish the exact side of the malposed germ when the palatal-buccal localisation is considered. Moreover, this prevalence rate reflects the occurrence of the dental anomaly in an orthodontic population and does not indicate the absolute prevalence rate of EDC in the general population.

The prevalence rate of EDC in the MDP group was more than 5 times greater than in noMDP group (66.0% vs 12.1%). During root formation the maxillary first premolar is oriented in the alveolar bone almost perpendicular to the occlusal plane and almost parallel to the midline, as assessed on the panoramic X-ray [Van der Linden, 1976]. In presence of an intraosseous malposed canine, an excessive mesial inclination or mesial position (overlapping the root of the adjacent deciduous canine) of the first premolar can contribute to an incorrect eruption pathway of the permanent canine thus increasing its risk of impaction. The current investigation showed that MDP is an anomalous developmental position significantly associated to displaced canines in the intermediate mixed dentition.

Despite extensive analysis on the position of displaced canines, no study analyzed the intraosseous localisation of the adjacent unerupted maxillary first premolars. Nevertheless, studies on the interceptive treatment of the palatally displaced canines report that a successful outcome can be achieved by the extraction of the primary canine associated with a cervical pull headgear [Baccetti et al., 2008]. The addition of the cervical pull headgear seems to control the distal segment of the upper dental arch from moving mesially. It can be assumed that this therapeutic protocol helps canine eruption as the alveolar bone results to be cleared by the joining presence of the distal segments [Baccetti et al., 2008; Baccetti et al., 2011].

The two angular measurements assessed in this study, η and Π indicate the inclination of the long axis of the developing bud of the maxillary first premolar as well as of its position with respect to two different reference planes: the occlusal plane and the midline. In the noMDP group, the inclination of the long axis of the developing bud of the maxillary first premolar was about 71 degrees with respect to the occlusal plane and

about 9 degrees with respect to the midline as traced on the panoramic X-ray. During root formation, therefore, the maxillary first premolar has an orientation in the alveolar bone of the maxilla that is almost perpendicular to the occlusal plane and almost parallel to the midline. In the MDP group the inclination of the long axis of the developing bud of maxillary first premolar was 63.8 degrees with respect to the occlusal plane and 17.6 degrees with respect to the midline. Slightly different values for the mesial inclination of the tooth bud were found in MDP group with EDC (63.6 and 18.9 degrees, respectively). Both MDP and MDP-EDC groups showed a significantly greater mesial inclination of the first premolar than the noMDP group when measured at both the occlusal plane and the midline. This condition resulted in a η angle significantly smaller and a Π angle significantly larger with respect to the noMDP group.

The statistical comparison between MDP and MDP-EDC groups showed a significantly larger Π angle in the MDP-EDC group resulting in an increased mesial inclination of displaced premolars. In contrast, the statistical comparison was not significant for the η angle between the two groups. This outcome can be explained by the position of the occlusal plane. In fact, the latter is not necessarily perpendicular to the midline, therefore the η and Π angles can show independent values.

Only Alessandri Bonetti et al. [2010] in a recent study analysed the positional changes of the permanent maxillary canine and of the adjacent premolar in two groups of patients who received two different extraction protocols by evaluating the mesial position of the canine with α angle and sector (s) and of the maxillary first premolar by measuring its inclination with respect to the midline (Π angle). In their study no values of the Π angle are reported but only the changes of the radiographic variables in the two groups analysed between the initial observation (T0) and after an average period of 18 months (T1). The treatment approach consisted of the concomitant extractions of the deciduous first molar and the primary canine so that, by providing more space into the alveolar bone, it was possible to determine the uprighting of the first premolar and to stimulate the correction of the eruption path of the malposed canine in a more distal direction. The authors reported statistically significant changes in the sectors (or in the mesiodistal canine crown position) and in the α -angle variable (or in the inclination of the canine) in the double extraction group when compared with both the single extraction of the deciduous canine and the control groups. The advantage of the double extraction protocol consists in being no more technically difficult, biologically expensive or traumatic with respect to the single extraction approach and therefore it can be performed in the early mixed dentition stage without wearing any orthodontic appliance [Alessandri Bonetti et al, 2010;

Alessandri Bonetti et al, 2011]. It could be reasonably hypothesised that this clinical approach could increase its effectiveness as a prevention procedure at an early stage of dentition (between 8 and 10 years of age) when it is associated to a space maintainer (such as a transpalatal arch) to prevent the mesial movement of maxillary first molars during the transition to the permanent dentition as described by McNamara et al. [2003]. The use of a cervical pull headgear could be recommended when it is necessary to help an uprighting of premolars during their intraosseous pathway of eruption [Armi et al., 2011].

The clinical relevance of the current investigation is that early diagnosis of mesial displacement of the upper first premolar may reveal a potential risk of subsequent malposition and eruption anomaly of the adjacent permanent canine. The results of this study also add evidence to the clinical protocols proposed by Baccetti et al. [2009] and Armi et al. [2011], who found that the maintenance or improvement of the perimeter of the arch in the early mixed dentition (by using RME combined with the cervical pull headgear), as a measure to intercept the intraosseous displacement of maxillary canine, is effective in preventing canine impaction. Early detection of a mesially displaced premolar associated to a malposed maxillary canine provides to the clinician the possibility to perform the treatment of choice to prevent canine impaction and root resorption of the adjacent teeth.

Conclusion

Mesial intraosseous displacement of the maxillary first premolar is a developmental tooth malposition that is significantly associated with the displacement of the permanent canine in the intermediate mixed dentition.

References

- > Alessandri Bonetti G, Incerti Parenti S, Zanarini M, Marini I. Double vs single primary teeth extraction approach as prevention of permanent maxillary canines ectopic eruption. Pediatr Dent 2010; 32: 407-412.
- Alessandri Bonetti G, Zanarini M, Incerti Parenti S, Marini I, Gatto MR. Preventive treatment of ectopically erupting maxillary permanent canines by extraction of deciduous canines and first molars: A randomized clinical trial. Am J Orthod Dentofacial Orthop 2011; 139: 316-323.
- Armi P, Cozza P, Baccetti T. Effect of RME and headgear treatment on the eruption of palatally displaced canines. A randomized clinical study. Angle Orthod 2011; 81: 370–374.
- Baccetti T, Franchi L, McNamara JA Jr. The cervical vertebral maturation (CVM) method for the assessment of optimal treatment timing in dentofacial orthopedics. Semin Orthod 2005; 11: 119-129.
- Baccetti T, Leonardi M, Armi P. A randomized clinical study of two interceptive approaches to palatally displaced canines. Eur J Orthod 2008; 30: 381-385.
- Baccetti T, Mucedero M, Leonardi M, Cozza P. Interceptive treatment of palatal impaction of maxillary canines with rapid maxillary expansion: A randomized clinical trial. Am J Orthod Dentofacial Orthop 2009; 136:

657-661.

- Baccetti T, Sigler LM, McNamara AJ Jr. An RCT on treatment of palatally displaced canines with RME and/or a transpalatal arch. Eur J Orthod 2011; 33: 601-607.
- > Baccetti T. A controlled study of associated dental anomalies. Angle Orthod 1998; 68: 267-274.
- > Barlow ST, Moore MB, Sheriff M, Irekand AJ, Sandy JR. Palatally impacted canines and the modified index of orthodontic treatment need. Eur J Orthod 2009; 31: 362-366.
- Becker A, Chaushu S. Long-term follow-up of severely resorbed maxillary incisors after resolution of an etiologically associated impacted canine. Am J Orthod Dentofacial Orthop 2005; 127: 650-654.
- Coulter J, Richardson A. Normal eruption of the maxillary canine quantified in three dimensions. Eur J Orthod 1997; 19: 171-183.
- Ericson S, Kurol J. Early treatment of palatally erupting maxillary canines by extraction of the primary canines. Eur J Orthod 1988; 10: 283-295.
- Ericson S, Kurol J. Radiographic examination of ectopically erupting maxillary canines. Am J Orthod Dentofacial Orthop 1987; 91: 483-492.
- Ericson S, Kurol J. Resorption of maxillary lateral incisors caused by ectopic eruption of the canines. A clinical and radiographic analysis of predisposing factors. Am J Orthod Dentofacial Orthop 1988; 94: 503-513.
- › Jacobs SG. Palatally impacted canines: aetiology of impaction and scope for impaction. Report of cases outside the guidelines of interception. Aust Dent J 1994; 39: 206-211.
- > Kettle MA. Treatment of the unerupted maxillary canine. Dent Pract 1958; 8: 245-255.
- > Koch G, Modeer T, Poulsen S, Rasmussen P. Pedodontics: a clinical approach. Copenhagen, Denmark: Munksgaard; 1991. p. 60.
- Leonardi M, Armi P, Franchi L, Baccetti T. Two interceptive approaches to palatally displaced canines: a prospective longitudinal study. Angle Orthod 2004; 74: 581-586.
- > Lindauer SJ, Rubenstein LK, Hang WM, Andersen WC, Isaacson RJ.

- Canine impaction identified early with panoramic radiographs. J Am Dent Assoc 1992; 123: 91-97.
- McNamara J Jr, Baccetti T, Franchi L, Herberger TA. Rapid maxillary expansion followed by fixed appliances: a long-term evaluation of changes in arch dimensions. Angle Orthod 2003; 73: 344-353.
- McSherry P, Richardson A. Ectopic eruption of the maxillary canine quantified in three dimensions on cephalometric radiographs between the ages of 5 and 15 years. Eur J Orthod 1999; 21: 41-48.
- Mucedero M, Ricchiuti MR, Cozza P, Baccetti T. Prevalence rate and dentoskeletal features associated with buccally displaced maxillary canines. Eur J Orthod 2013; 35: 305-309.
- > Power SM, Short MB. An investigation into the response of palatally displaced canines to the removal of deciduous canines and an assessment of factors contributing to favourable eruption. Br J Orthod 1993; 20: 215-223.
- Ricketts RM. Orthodontic treatment in the growing patient. Vol. I.
 Scottsdale, Ariz: American Institute for Bioprogressive Education; 1998.
 p. 16-21.
- Sacerdoti R, Baccetti T. Dentoskeletal features associated with unilateral or bilateral palatal displacement of maxillary canines. Angle Orthod 2004; 74: 725-732.
- Sambataro S, Baccetti T, Franchi L, Antonini F. Early predictive variables for upper canine impaction as derived from posteroanterior cephalograms. Angle Orthod 2004; 75: 28-34.
- Shapira Y, Kuftinek MM. Early diagnosis and interception of potential maxillary canine impaction. J Am Dent Assoc 1998; 129: 1450-1454.
- Stivaros N, Mandall NA. Radiographic factors affecting the management of impacted upper permanent canines. J Orthod 2000; 27: 169-173.
- Van der Linden FPGM, Duterloo HS. Development of the human dentition: an atlas. New York: Harper & Row; 1976.
- Warford JH Jr, Grandhi RK, Tira DE. Prediction of maxillary canine impaction using sectors and angular measurement. Am J Orthod Dentofacial Orthop 2003; 124: 651-655.