Evaluation of Skeletal Maturation Using Mandibular 1st Premolar and 2nd Molar Calcification Stages: A Cross-sectional Radiographic Study

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ABSTRACT

Aim and objective: The study aimed at, whether the calcification stages of mandibular 1st premolar and 2nd molar can be used to assess skeletal maturity.

Materials and methods: Lateral cephalogram and OPG of 72 growing subjects with good health (38 girls and 34 boys; age-group of 8–17 years) were selected for a cross-sectional study, in which the identity of patients was not revealed to the investigator. The dental calcification stage of mandibular 1st premolar and 2nd molar were estimated in accordance with the Demirjian system (DI) and skeletal maturation was estimated according to cervical vertebral maturation index (CVMI) given by Hassel and Farman and these two indices were compared.

Results: Chi-square test and Spearman rank-order correlation coefficient test showed that a significant relationship of mandibular 1st premolar and 2nd molar calcification stages with CVMI exists, for both males and females. In females, CVMI stage 2 showed maximum correlation to DI stage E of lower 2nd molar. In males, CVMI stage 2 showed maximum correlation to DI stage E of lower 1st premolar.

Conclusion: The result of this study revealed that each stage developed prior in female subjects compared to male subjects and the calcification stages of lower 1st premolar and 2nd molar can be used to assess the peak of growth.

Keywords: Cervical vertebral maturity index, Demirjian system, Peak velocity of growth, Skeletal age.

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INTRODUCTION

The growth potential of the patient plays an important role in orthodontic treatment planning. Human growth and development is not consistent. Different skeletal components shows peak as well as retardation in growth at various developing maturational stages. Some factors like genetics, environment, sex and nutrition influence normal growth and development excessively, and orthodontists should attempt to evaluate each patient in relation to these influences.

Dentofacial orthopedics as well as the functional appliance therapy can be used in patients in accordance to their maturational status. Although use of insulin like growth factor (IGF) and frontal sinus has also been reported as a skeletal maturity indicator.

The stability of the result of these therapies depends largely over the correct skeletal maturational age, that is more potential indicator than chronological age to check the status of maturity.

Maturational indices like hand wrist radiographs cervical vertebral maturity index (CVMI) have been used in knowing the peak of growth both in body height and mandibular size. Dental age assessment is another precise method to check skeletal maturity by using stages of tooth calcification on radiographs. Demirjian et al. gave a method (DI) to assess the stages of calcification in various teeth and classified it into various stages according to tooth mineralization; this can be done on periapical (IOPA) or panoramic radiographs (OPG). OPG is very common in the day-to-day clinical practice of orthodontics. Krallassiri et al. and Coutinho et al. in their study showed a strong relation in-between the Dental calcification stages and the skeletal maturation, which is helpful for the clinician to determine the stages of the skeletal growth from the OPG.

Some structures like the palate, maxillary sinus septum usually overlap the roots of the upper canine and molars in radiographs, so these teeth are not used as the parameter in this study, and mandibular 1st premolar and 2nd molar are more reliable teeth. Many previous studies used either lower 3rd molars or canines for dental age assessment. Complete root formation and apex closure of lower canines is achieved by 13 years of age but children show active growth till the age of 16–17 years. Apex closure of mandibular 2nd molar tooth generally continues and extends up to 16 years of age in healthy children, so is more precise to assess the peak of growth.

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3rd molars are the most commonly missing teeth, so they cannot be used as a part of skeletal maturity indicators. Therefore, the aim of the study was to examine if, whether the calcification stages of mandibular 1st premolar and 2nd molar can be used to reveal the skeletal maturity by comparing the Demirjian index with the cervical vertebral maturity index.

**Materials and Methods**

The data comprised of randomly selected pretreatment lateral cephalometric radiographs and panoramic radiographs of 72 subjects (34 boys and 38 girls) in the age range of 8–17 years, reporting to the Department of Orthodontics and Dentofacial Orthopedics, Mahatma Gandhi Dental College and Hospital, Jaipur, Rajasthan. Ethical clearance for the study was obtained from the Institutional Ethical Committee of Mahatma Gandhi Dental College and Hospital, Jaipur.

**Inclusion Criteria**

- Age between 8 and 17 years.
- Healthy patients with no medical problem.
- Normal healthy dentition without any impactions and transpositions.

**Exclusion Criteria**

- Radiographs of poor quality.
- Radiographs showing obvious dental pathology.

On the first day, the subjects were explained about the investigation, and informed consent was obtained. On the same day, lateral cephalogram (lateral cep) and OPG of the subjects were taken. Both radiographs were taken with the subject in a standing position. For lateral cephal, the patients were asked to occlude the teeth and keep the lips in a relaxed position and for OPG the subjects were asked to place the tongue on the palate and bite the panoramic bite plane with the upper anteriors.

**Tools Used**

Kodak 8000/8000 C Digital Panoramic and Cephalometric System was used to take the lateral cephalogram and OPG radiographs. Lateral cephalogram and orthopantomogram were used for recording CVMI and DI stages.

**Interpretation of the Processed Radiograph**

**Assessment of Dental Calcification Stages of Mandibular 1st Premolar and 2nd Molar (DI)**

The calcification stages of the lower-left 2nd molar and 1st premolar were recorded according to the Demirjian et al.'s method. According to this method, the calcification stages were divided from A to H, but in this study we used the stages ranged from D to H.

**Assessment of Skeletal Age by Visual Analysis (CVMI)**

Visual inspection of anatomy of the three cervical vertebrae (C2, C3, C4) is done by using the method given by Hassel and Allan G Farman in 1995. They classified this into 6 stages from CVMI 1 to CVMI 6.

**Statistical Analysis**

All statistical analyses were performed using the Microsoft Office Excel 2007 and SPSS software package (SPSS for Window 07, Version 13.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated by determining the means and standard deviations of the chronological ages for the six phases of CVMI.

The same investigator re-evaluated the randomly selected OPG and Lateral Cephal. Radiographs were evaluated once again 2 weeks after the 1st evaluation for each group, to perform the test of the reproducibility of both dental and skeletal maturation stages, intra-examiner reproducibility differences were statistically tested and found to be statistically insignificant. No significant intra-observer difference (p > 0.05) was observed.

To check the relationship between DI and CVMI, the frequency and the percentage distribution of the stages of calcification of lower 1st premolar and 2nd molar were recorded and calculated, and was done both for male and female subjects. The Pearson Chi-square (χ²) test was used to study the relationship between the chronological age and gender with the stage of skeletal maturation and to determine the relationship between DI and CVMI.

To record the relationship between skeletal maturation indicators and dental calcification stages of each tooth individually, the Spearman rank-order correlation coefficients were used and the statistical significance of the correlation was examined.

**Results**

The data included in this study were 72 subjects with the age-group of 8–17 years. The CVMI stages were analyzed using lateral cephalogram by the method described by Brent Hassel and Farman (1995) and the DI of lower 1st premolar and 2nd molar were analyzed as per the radiographic appearance from OPG according to the method described by Demirjian et al.8

The statistical analyses of the data was performed for obtaining a relationship between CVMI and DI stages.

In Table 1, the result shows that for each category of CVMI, stage of DI for mandibular 2nd molar as well as mandibular 1st premolar appeared prior in female patients than in males for both mandibular first molar as well as first premolar.

The result shows that the value of the Chi-square test was highly significant at 41.787 with 15 degrees of freedom (p < 0.01). From Table 2, we can appreciate that the lower stage of DI is correlated to the lower stage of CVMI, similarly the higher stages of DI are associated with higher categories of CVMI in the female subject for a mandibular 2nd molar. Stage E included the highest percentage distribution (100%) at category 2 of CVMI, which shows that the highest correlation with category 2 of CVMI in female subjects for a mandibular 2nd molar. Stage F included 57.14% distribution at category 3 of CVMI. Stage G included 71.43% distribution at category 4 and 5 of CVMI. Stage H of DI displayed the highest percentage distribution with category 6 (100%). In mandibular 1st premolar stage, E and F were equally included at category 2 of CVMI which shows the highest correlation with category 2 of CVMI in female subjects.

In Table 3, associations between CVMI and DI for the male subject for mandibular 2nd molar is clearly appreciated that as the lower stage of DI is correlated to lower stage of CVMI, similarly the higher stages of DI is associated with higher categories of CVMI.

Stage D included the highest percentage distribution (100%) at category 1 of CVMI, remaining stages showed a similar trend between DI and CVMI stages in male subjects. However, for 1st premolar, stage E included the highest percentage distribution...
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Table 1: Distribution of gender and chronological ages for all the subjects, grouped by CVMI categories, Demirjian index (DI) stages of the mandibular second molar and first premolar

| Categories/stages | Gender | No. of subjects | Chronological age (years) |
|-------------------|--------|----------------|--------------------------|
|                   |        |                | Mean | SD  |
| CVMI categories   |        |                |      |     |
| Category 1        | Males  | 1               | 8    | 0.00 |
|                   | Females| –               | –    | –   |
| Category 2        | Males  | 6               | 11.33| 1.36 |
|                   | Females| 2               | 9.5  | 0.707|
| Category 3        | Males  | 12              | 12.75| 0.754|
|                   | Females| 14              | 12.42| 0.852|
| Category 4        | Males  | 11              | 14.45| 0.5222|
|                   | Females| 14              | 14.21| 0.975|
| Category 5        | Males  | 3               | 15.86| 0.577|
|                   | Females| 7               | 15.67| 0.96 |
| Category 6        | Males  | 1               | 17   | 0.00 |
|                   | Females| 1               | 16   | 0.00 |
| Demirjian index (DI) stages of mandibular second molar | | | |
| D                  | Males  | 2               | 10.5 | 1.053 |
|                   | Females| –               | –    | –   |
| E                  | Males  | 4               | 11.17| 0.957|
|                   | Females| 6               | 10.75| 1.60 |
| F                  | Males  | 12              | 13    | 1.206|
|                   | Females| 12              | 12.83| 0.835|
| G                  | Males  | 15              | 14.56| 1.047|
|                   | Females| 16              | 14.33| 1.093|
| H                  | Males  | 1               | 17    | 0.00 |
|                   | Females| 4               | 16.25| 0.50 |
| Demirjian index (DI) stages of mandibular first premolar | | | |
| E                  | Males  | 6               | 10.33| 1.366|
|                   | Females| 1               | 9     | 0.000|
| F                  | Males  | 3               | 12    | 0.000|
|                   | Females| 6               | 11.50| 1.048|
| G                  | Males  | 13              | 13.38| 0.768|
|                   | Females| 7               | 13.29| 0.488|
| H                  | Males  | 12              | 15    | 0.953|
|                   | Females| 24              | 14.5  | 1.474|

Discussion

The identification of skeletal maturation level provides a useful method of identifying particular points along the progressive path of adolescent growth. This provides a new dimension for evaluating general and individual growth, including facial growth.

Dental, maturational, and chronological ages are not necessarily interrelated on a simple one-to-one basis, but they are nevertheless related. For example, an orthodontist to commonly see a patient who is tall in stature, advanced in age but with more retained deciduous teeth compared to normal patients of his age. A short child may be more advanced in skeletal maturation than a taller child because of genetic pattern that predestines a shorter adult.

Above mentioned variations make maturational age a more valid means of judging physiologic development than chronological age, which can provide misleading information.12

Dentofacial orthopedics as well as functional appliance therapy can be used in patients in accordance with their maturational status. For a long time, hand wrist radiographs and cervical vertebrae have been used to assess the mandibular growth spurt.10 While taking hand wrist radiographs, the radiation exposure and dose are very high, and according to ALARA principle, a limited radiation dose can be used, especially in small kids and pregnant ladies and so hand wrist radiographs were not used for our study.

Many methods have already been suggested for precise prediction of growth.13,14 Some studies15,16 showed maximum relationship dental calcification stages and skeletal maturity indicators.

Some studies17,18 showed high correlations between dental calcification stages and skeletal maturity indicators, which makes easy for clinicians to identify the growth stage from OPG.

Demirjian et al.8 gave a method to identify the stages of calcification in many teeth; the classification of this method is based on maturational stages of the teeth over the teeth size. This can be done on periapical or panoramic radiographs, which are used in the day-to-day clinical practice of orthodontic treatment planning.19

Hassel and Farman7 gave six stages of classification based on visual inspection in the change of shape of the 2nd, 3rd, and 4th cervical vertebrae on lateral cephalogram. The stale response of functional jaw orthopedics and myofunctional appliances occurs during the peak of puberty.

The aim of the study was to determine, whether the calcification stages of mandibular 1st premolar and 2nd molar can be used to assess the skeletal maturity by correlating the DI of mandibular 1st premolar and 2nd molar with the CVMI.

Some structures like the palate, maxillary sinus septum usually overlap the roots of the upper canine and molars in radiographs, so these teeth are not used as the parameter in the study, and mandibular 1st premolar and 2nd molar are considered more reliable teeth. Many previous studies19 used either lower 3rd molars or canines for dental age assessment. Till the age of 13 years, root formation completes lower canine apex closes, but the age of 16–17 years is most common up to which children shows active growth. Apex closer of mandibular 2nd molar generally continues and extends up to 16 years of age in healthy children, so is more precise to assess the peak of growth.9,17

Third molars are the most commonly missing teeth, hence it cannot be used as a part of skeletal maturity indicators.

The mandibular premolar is also a precise indicator because the development of the mandibular 1st premolar begins at 21–24 years.
months and ends at the age of 12–13 years. Rozylo-Kalinowska et al.21 reported that the calcification stages of mandibular premolars and canine shows the highest correlation with CVMI.

Biomarkers, which include alkaline phosphates in serum, serum insulin-like growth factor-1,22 serum PTHrP,23 and DHEAS24 can be used to assess growth but these methods are invasive in nature.

The data included in this study were 72 subjects with the age-group of 8–17 years. The CVMI stages were analyzed using lateral cephalogram by the method described by Brent Hassel and Farman7 and the DI of mandibular 1st premolar and 2nd molar were analyzed as per the radiographic appearance from OPG according to the method described by Demirjian et al. 8

The indexing system of the Demirjian method used the ratio of root length and the crown height, so if any projection error is seen in the radiographs like foreshortening or elongation of developing teeth will not affect the reliability of assessment.17

The results of this study showed that in females, CVMI 2 showed the highest correlation with permanent mandibular 2nd molar stage E of DI, and CVMI 4 showed the highest correlation with stage G of permanent mandibular 2nd molar. In males, CVMI 2 showed maximum correlation with permanent mandibular 1st premolar stage E of DI, and CVMI 4 showed maximum correlation to stage H for permanent mandibular 1st premolar. This is in accordance with the study done by Priya Gupta et al.25

Fishman,26 Hagg and Taranger,27 and Bjork and Helm28 gave the report that just appearance of the adductor sesamoid of the thumb means it is the beginning of the pubertal growth spurt that is correlated to CVMI stage 2 and stage E of DI showed the maximum correlation with CVMI stage 2, for both males and females, which signifies the pre-peak of puberty. The same report was given by Vijayashree et al.20

A previous study by Kumar et al.17 showed that for males and females, DI stage F and G is maximally correlated with CVMI stage 3 and 4, but the present study shows a maximum correlation of stage F of DI with category 3 of CVMI. As it is already studied that CVMI stage 3 is the time when puberty is at its peak in both general body and mandibular growth29 and the present study shows, stage F of DI of lower 2nd molar shows a higher correlation to category 3 of CVMI which can be used to assess peak velocity of growth.

According to this study, correlation of mandibular 1st premolar and skeletal age is higher than the mandibular 2nd molar. This is the similar findings of Rozylo-Kalinowska et al.21 Valizadeh et al.30 and Krisztina et al.31 study.22

### Table 2: Association between CVMI and DI for a female subject for mandibular second molar and first premolar

| CVMI categories | Association B/W DI and CVMI | E (%) | F (%) | G (%) | H (%) | Total (%) | p value |
|-----------------|-----------------------------|-------|-------|-------|-------|-----------|---------|
| Mandibular second molar | Category 1 Frequency | 0 | 0 | 0 | 0 | 0 | – |
| | Percentage | – | – | – | – | – | – |
| | Category 2 Frequency | 2 | 0 | 0 | 0 | 2 | 0.001 |
| | Percentage | 100 | – | – | – | 100 | 0.002 |
| | Category 3 Frequency | 35.71 | 57.14 | 7.15 | – | 100 | 0.001 |
| | Percentage | – | 21.43 | 71.43 | 7.14 | 100 | 0.004 |
| | Category 4 Frequency | 5 | 2 | 7 | 71.43 | 28.57 | 100 |
| | Percentage | – | – | – | 100 | 100 | 0.001 |
| | Category 5 Frequency | 0 | 0 | 0 | 1 | 1 | 100 |
| | Percentage | – | – | – | 100 | 100 | 0.001 |
| | Category 6 Frequency | 0 | 0 | 0 | 1 | 1 | 0.001 |
| | Percentage | – | – | – | 100 | 100 | 0.001 |
| | Total Frequency | 7 | 11 | 16 | 4 | 38 | 100 |
| | Percentage | 18.42 | 28.95 | 42.11 | 10.52 | 100 |

| Mandibular first premolar | Category 1 Frequency | 0 | 0 | 0 | 0 | 0 | – |
| | Percentage | – | – | – | – | – | – |
| | Category 2 Frequency | 1 | 1 | 0 | 0 | 2 | 0.006 |
| | Percentage | 50.00 | 50.00 | – | – | 100 | 0.004 |
| | Category 3 Frequency | 0 | 5 | 2 | 7 | 14 | 0.005 |
| | Percentage | – | 35.71 | 14.29 | 50.0 | 100 | 0.005 |
| | Category 4 Frequency | 0 | 0 | 5 | 9 | 14 | 0.001 |
| | Percentage | – | – | 35.71 | 64.29 | 100 | 0.001 |
| | Category 5 Frequency | 0 | 7 | 7 | – | – | 100 |
| | Percentage | – | 100 | 100 | – | – | 100 |
| | Category 6 Frequency | 0 | 0 | 0 | 1 | 1 | 0.001 |
| | Percentage | – | – | – | 100 | 100 | 0.001 |
| | Total Frequency | 1 | 6 | 7 | 24 | 38 | 100 |
| | Percentage | 2.63 | 15.79 | 18.42 | 63.16 | 100 |

* p <0.05 = significant, p <0.01 = highly significant
The following conclusions can be drawn from the study:

- A statistically significant correlation exists between dental calcification and cervical vertebrae maturation, for both males and females.
- In females, Demirjian index (DI) stage E of mandibular 2nd molar corresponds to the CVMI category 2 (prepubertal phase).
- In males, Demirjian index (DI) stage E of mandibular 1st premolar corresponds to the CVMI category 2 (prepubertal phase).
- Demirjian index (DI) stage F of mandibular 2nd molar corresponds to the CVMI category 3, which signifies the peak pubertal growth for both males and females.
- Demirjian index (DI) stage G and H of mandibular 2nd molar correspond to CVMI category 4 and 5, which signifies the post-pubertal phase for both males and females.

**References**

1. Kuc-Michalska M, Baccetti T. Duration of the pubertal peak in skeletal class I and class III subjects. Angle Orthod 2010;80(1):54–57. DOI: 10.2319/020309-69.1.
2. Green LJ. The interrelationships among height, weight, and chronological, dental, and skeletal ages. Angle Orthod 1961;31(3):189–193.
3. Leite H, O’Reilly M, Close J. Skeletal age assessment with 1st, 2nd, and third fingers of the hand. Am J Orthod Dentofacial Orthop 1987;92(6):492–498. DOI: 10.1016/0889-5406(87)90231-9.
4. Masoud M, Marghalani H, Masoud I, et al. Prospective longitudinal evaluation of the relationship between changes in mandibular length and blood-spot IGF-1 measurements. Am J Orthod Dentofacial Orthop 2012;141(6):694–704. DOI: 10.1016/j.ajodo.2011.12.021.
5. Ruf S, Pancherz H. Frontal sinus development as an indicator for somatic maturity at puberty. Am J Orthod Dentofacial Orthop 1996;110(5):476–482. DOI: 10.1016/s0889-5406(96)70053-7.

6. Fishman LS. Radiographic evaluation of skeletal maturation. Angle Orthod 1982;52(2):88–112. DOI: 10.1043/0003-3219(1982)0522.0.CO;2.

7. Hassel B, Farman AG. Skeletal maturation evaluation using cervical vertebrae. Am J Orthod Dentofacial Orthop 1995;107(1):58–66. DOI: 10.1016/s0889-5406(95)70157-5.

8. Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. Hum Biol 1973;45(2):211–227.

9. Kraliassii S, Anuwongnukroh N, Dechkunakorn S. Relationships between dental calcification stages and skeletal maturity indicators in Thai individuals. Angle Orthod 2002;72(2):155–166. DOI: 10.1043/0003-3219(2002)0722.0.CO;2.

10. Coutinho S, Buschang PH, Miranda F. Relationships between mandibular canine calcification stages and skeletal maturity. Am J Orthodont Dentofacial Orthop 1993;104(3):262–268. DOI: 10.1016/s0889-5406(05)81728-7.

11. Engström C, Engström H, Sagne S. Lower third molar development in relation to skeletal maturity and chronological age. Angle Orthod 1983;53(2):97–106. DOI: 10.1043/0003-3219(1983)0532.0.CO;2.

12. Chertkow S. Tooth mineralization as indicator of pubertal growth spurt. Am J Orthodont Dentofacial Orthop 1980;77(1):79–91. DOI: 10.1016/0002-9416(80)90226-2.

13. Różyło-Kalinowska I, Kolasa-Rączka A, Kalinowski P. Relationship between dental age according to Demirjian and cervical vertebrae maturity in Polish children. Eur J Orthod 2011;33(1):75–83. DOI: 10.1093/ejo/cjq031.

14. Kumar S, Singla A, Sharma R, et al. Skeletal maturation evaluation using mandibular 2nd molar calcification stages. Angle Orthod 2012;82(3):501–506. DOI: 10.2319/051611-334.1.

15. Rowland S. Series of collotype illustrations with descriptive text, illustrating applications of the new photography to medicine and surgery. London, England: Reibmans Publishing Ltd.; 1896.

16. Robison R. The possible significance of Hexosephosphoric esters in ossification. Biochem J 1923;17(2):286. DOI: 10.1042/bj0170286.

17. Greulich W, Pyle S. Radiographic atlas of skeletal development of the hand and wrist. 2nd ed., California: Palo, Alto, Stanford University Press; 1950.

18. Fishman LS. Maturational patterns and prediction during adolescence. Angle Orthod 1987;57(3):178–193. DOI: 10.1043/0003-3219(1987)0572.0.CO;2.

19. Baccetti T, Franchi L, McNamara JA. An improved version of the cervical vertebral maturation (CVM) method for the assessment of mandibular growth. Angle Orthod 2002;72(4):316–323. DOI: 10.1043/0003-3219(2002)0722.0.CO;2.

20. Priya Gupta K, Garg S, Grewal PS. Establishing a diagnostic tool for assessing optimal treatment timing in Indian children with developing malocclusion. J Clin Exp Dent 2011;3(1):18–24. DOI: 10.4317/jced.3.e18.

21. Krisztina MI, Ogodescu A, Reka G, et al. Evaluation of skeletal maturation using lower 1st premolar mineralization. Acta Med Marisien 2013;59(6):289–292. DOI: 10.2478/amma-2013-0066.

22. Vijayashree UH, Vikram P, Naik VR. 2nd molar calcification stages to evaluate skeletal maturation: a cross sectional radiographic study. APOS Trends Orthod. 2014;4:156–161.

23. Goyal S, Goyal S, Gugnani N. Assessment of skeletal maturity using the permanent mandibular canine calcification stages. J Orthod Res 2014;2(1):11–16. DOI: 10.5812/iranjradiol.9993.

24. Sierra AM. Assessment of skeletal and dental maturity: a new approach. Angle Orthod 1987(3):194–208. DOI: 10.1043/0003-3219(1987)0572.0.CO;2.

25. Chertkow S, Fatti P. The relationship between tooth mineralization and early radiographic evidence of ulnar sesamoid. Angle Orthod 1979;49(4):282–288. DOI: 10.1043/0003-3219(1979)0492.0.CO;2.

26. Fishman LS. Chronological versus skeletal age, an evaluation approach. Angle Orthod 1987(3):194–208. DOI: 10.1043/0003-3219(1987)0572.0.CO;2.

27. Lagg U, Taranger J. Maturation indicators and the pubertal growth spurt. Am J Orthodontic Dentofacial Orthop 1982;84(2):299–309. DOI: 10.1016/0002-9416(82)90464-x.

28. Bjork A, Helm S. Prediction of the age of maximum pubertal growth in body height. Angle Orthod 1967;37(2):134–143. DOI: 10.1043/0003-3219(1967)0372.0.CO;2.

29. Valizadeh S, Eil N, Ehsani S, et al. Correlation between dental and cervical vertebral maturation in Iranian females. Iran J Radiol 2013;10(1):1–7. DOI: 10.5812/iranradiol9993.

30. Gupta S, Jain S, Gupta P, et al. Determining skeletal maturation using insulin-like growth factor I (IGF-I) test. Prog Orthod 2012;13(3):288–295. DOI: 10.1016/j.pio.2011.09.006.

31. Hussain MA, Talapaneni AK, Prasad M, et al. Serum PTHrP level as a biomarker in assessing skeletal maturation during circumpubertal development. Am J Orthod Dentofacial Orthop 2013;143(4):515–521. DOI: 10.1016/j.ajodo.2012.11.022.

32. Srinivasan B, Premkumar S. Assessment of serum dehydroepiandrosterone sulphate in subjects during the prepubertal, pubertal, and adult stages of skeletal maturation. Eur J Orthod 2012;34(4):447–451. DOI: 10.1093/ejo/cjr041.