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Article

# Reliability of maxillary canine calcification stages and salivary alkaline phosphatase for pubertal growth prediction (cross-sectional study)

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**ABSTRACT**
Orthodontic growth estimation for growing individuals is essential for diagnosis and treatment planning, essentially in managing cases with skeletal growth defects. Research has revealed a substantial correlation between tooth calcification stages and skeletal maturity. Also, many biomarkers have been investigated as maturity indicators, and studies show promising results. Therefore, the present research assessed if salivary alkaline phosphatase (ALP) level and maxillary canine calcification stages with developmental stages of modified middle phalanx of the third finger (MP3) are reliable skeletal maturity indicators. No previous study conducted the reliability of both canine calcification stages and ALP level as skeletal maturity indicators. In this prospective observational study with a cross-sectional design, 80 subjects aged 8-16 years were selected. Unstimulated saliva to assess ALP, periapical radiographs for the maxillary canine to assess the dental calcification by relying on the Dermirjian's stages, and periapical radiographs for the middle phalanx of the third finger region were taken from 80 individuals (from eight to sixteen years old) to assess the skeletal maturation state. The data were analyzed using the statistical package of social science (SPSS version 26), using the Spearmen correlation test and the Kruskal-Wallis test. The maxillary canine's calcification stages and MP3 stages showed a significant correlation coefficient; however, the correlation coefficient of MP3 stages and maxillary canine calcification stages with salivary ALP was non-significant. The maxillary canine calcification stages can practically be utilized as an indicator for assessing skeletal maturity, but the salivary ALP level showed no correlation with skeletal maturity indicators.

**Keywords:** Skeletal maturity indicator; Canine calcification stages; Alkaline phosphatase; Middle phalanx of third finger.

## INTRODUCTION

Puberty growth is a dynamic stage of development accompanied by substantial changes and increases in body size, shape, and composition, all of which are sexually dimorphic. The commencement of puberty corresponds to a skeletal (biological) age of approximately 11 years in females and around 13 years in males.[1]

Growth estimation for orthodontic patients in growing phases is essential for Diagnosis and treatment planning, essentially in the treatment of malocclusions with skeletal defects.[2] Clinical decisions regarding many orthodontic issues, such as the use of extraoral and orthopedic appliances, myofunctional devices, non-extraction versus extraction therapy, or corrective surgery, and protocol of retention after treatment, depending on growth estimation.[3] Therefore, estimation of pubertal growth phases in growing patients with dentofacial problems is essential in the diagnostic and treatment plane.

Different methods have been reported by several studies, to estimate the most reliable indicator of skeletal maturation, like weight, height, chronological age, frontal sinus development methods, biological age, sexual maturation, hand-wrist maturity, cervical vertebrae maturation, dental eruption, and teeth calcification stages and, later on, different biomarkers were introduced, such as the insulin-like growth factor, growth hormone, creatinine, and alkaline phosphatase (ALP) in saliva and serum.[4]

The interpretation of the hand-wrist bone radiograph is a conventional and recommended method for skeletal maturity estimation, which numerous studies have proved as a valid and reliable method.[5, 6, 7] Fishman introduces a system of hand-wrist skeletal maturation indicators that rely on four phases of bone calcification at six anatomic areas on the hand and wrist radiograph.[8] However, these classical approaches to skeletal maturity investigation need a sophisticated device, higher radiation dose, longer exposure duration, and are expensive.[9]

The middle phalanx of the third finger (MP3) area can be assessed to detect the skeletal maturation of a human.[10] in which the anatomic changes can be recorded conveniently by using dental X-ray film and machine reducing the X-ray exposure to a minimum; therefore, it is an easy, reliable and relatively inexpensive, and fast procedure.[11]

Several studies have revealed a high correlation between skeletal maturation and dental calcification phases [2,7,12,13,14]. Furthermore, less invasive methods of biological mediators have been introduced to assess skeletal maturity.[15] Recently, different biomarkers have been investigated to show their reliability as maturity indicators, such as insulin-like growth factor 1, growth hormone, creatinine, and ALP. [4, 16, 17]

Detecting biomarkers in saliva and their use as a diagnostic tool have several advantages. It is much simpler to collect with no sophisticated laboratory devices, sufficient quantities can be easily obtained for analysis, and its collection is a noninvasive procedure compared to other body fluids. [18, 19.] Specifically, salivary ALP is used in the diagnosis and treatment plan as a biomarker for the pubertal growth phase identification.[16]

Hence, the present study aimed to assess the reliability of salivary ALP enzyme level and maxillary canine developmental stages as valuable methods to estimate skeletal maturity based on the modified MP3 analysis described by Rajagopal and Kansal.[20] The null hypothesis stated no correlation between canine developmental stages and salivary ALP with modified MP3 stages.

## MATERIALS AND METHOD

In this prospective observational study with a cross-sectional design, 100 subjects were recruited from the College of Dentistry, University of Baghdad, and different specialized dental centers and private clinics in Baghdad. Eighty Subjects fit the inclusion criteria (between chronological age of 8 to 16 years, healthy and free from any sign of serious illness). In contrast, the others were excluded according to the exclusion criteria (Subjects with a history of facial trauma, tooth malformation, periodontal disease, congenital or developmental anomalies, and previous orthodontic treatment).

The project was approved by the ethical committee at the College of Dentistry, University of Baghdad (ref. number: 340). With a thorough explanation of the research purpose and procedure, the subjects and their parents have signed the consent form. The patient's date of birth was registered with the exact day, month and year. Then on the same day, a periapical radiograph for maxillary canine and periapical radiograph for MP3 was taken. Additionally, the subjects were instructed to fast for at least 30 minutes,[21] then after mouth rinsing with distilled water, unstimulated saliva was collected into a pre-weighted tube for five minutes by drooling method and stored immediately in a cooling box; all samples were collected during the same period, from 9:00 AM to 12:00 PM, to overcome the circadian changes in saliva flowing and then centrifuged and kept in a freezer at −20°C until analysis.[22]

After completing the recruitment of subjects, they were allocated into eight groups of one-year intervals based on chronological age. For the assessment of MP3 stages, a periapical radiograph for the MP3 area was taken by guiding the subjects to place the left hand with the palm down on a flat table. The MP3 area was centered on the X-ray sensor (manufactured by Changzhou Sifary Medical, China) parallel with the long axis of the sensor, and the cone of the dental X-ray machine (Manufactured by Ningbo Runyes Instrument, China) was positioned in light contact with the middle finger of the left hand, perpendicular to the X-Ray sensor. The development of the MP3 region was evaluated using the modified MP3 method, including six stages: F, FG, G, H, HI, and I (Figure 1).[20]

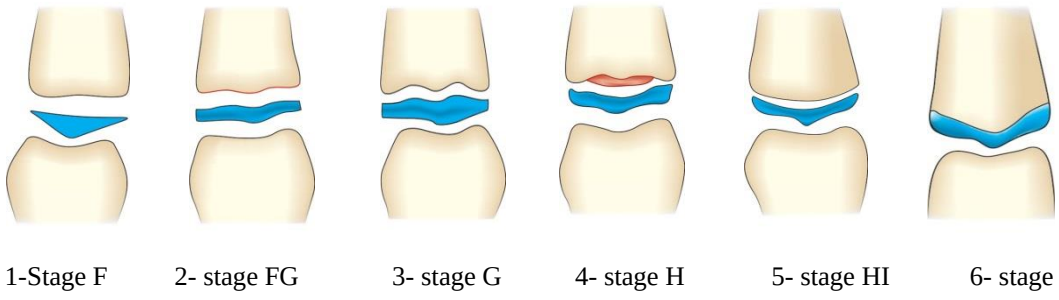


**Figure 1. Multiple graphs represent the stages of the modified MP3 method (adopted from Korde et al., 2015).[4]**

Then, after getting radiographs and saliva samples, the middle finger radiographs were analyzed according to the modified MP3 analysis described by Rajagopal and Kansal,[20]. Then, the subjects were allocated into six groups based on MP3 stages, to which maxillary canine developmental stage and salivary ALP were arranged accordingly.

The maxillary canine was chosen in this study to get a good visualization and estimation of the calcification stages. However, the maxillary molar roots overlap with anatomic structures such as the palate, the inferior border of the zygomatic arch or the maxillary sinus septum, which make it difficult to estimate the calcification of maxillary molars. [2, 14]

A periapical radiograph of the maxillary canine was taken with the periapical dental X-ray sensor size one (25×37 mm), using the parallel technique to assess the canine calcification stages.[23] The calcification of the maxillary canine was evaluated relying on Demirjian's stages of teeth calcification (Figure 2).[13]

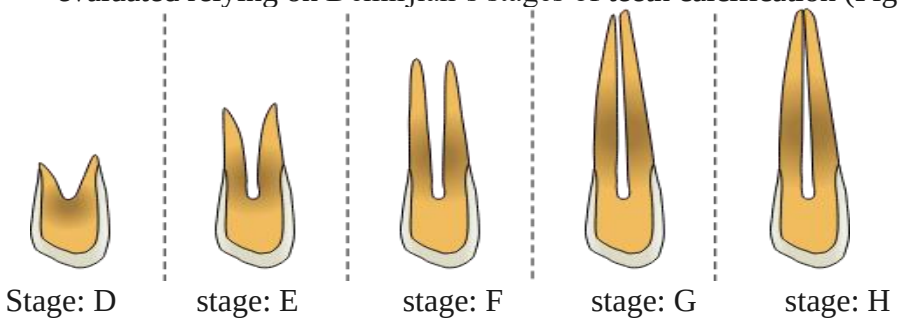


**Figure 2. Calcification stages of canine described by Demirgian et al.** [13]

For the assessment of ALP level, the salivary ALP assay was performed using a commercial kit (manufactured by BIOLABO SAS, maizy/France) by colorimetric method, thereafter saliva was analyzed by using a spectrophotometer (Cecil 1011, France).[16]

According to the modified MP3 method, the subjects were allocated into three growth phases i.e., pre-pubertal (stage 1 and stage 2), pubertal (stage 3 and stage 4), and post-pubertal (stage 5 and stage 6).

### Statistical analyses

The statistical package of social science (SPSS program version 26) was utilized to analyze data. The descriptive statistics include means, median, minimum, maximum standard deviations, and graphics. The normality of data distribution was checked using the Shapiro-Wilk test, and data was not normally distributed except for the chronological age variable. Inferential statistics include testing the variables' correlation (Spearman test), and the Kruskal-Wallis test is utilized to check the statistical significance. Levels of $P<0.05$ were considered statistically significant.

## RESULT

The descriptive statistic includes the minimum, maximum, mean and standard deviation of chronological age illustrated in Table 1. Spearman correlation test was utilized to evaluate the correlation degree among variables with a significance level of $p<0.05$. The correlation was significant between chronological age and canine calcification stages (r=0.888), chronological age and MP3 stage (r=0.867), and MP3 stages and canine calcification stages (r=0.930). However, the correlation was insignificant between chronological age and salivary ALP level, MP3 stages and ALP level, and canine calcification stages and ALP level (Table 2 and Figure 3). There is no effect of gender on correlation test results (Table 3).

| Group | Min. | Max. | Mean | SD |
|---|---|---|---|---|
| **1** | 8.02 | 8.66 | 8.21 | 0.18 |
| **2** | 9.07 | 9.61 | 9.27 | 0.15 |
| **3** | 10.00 | 10.49 | 10.20 | 0.17 |
| **4** | 11.13 | 11.64 | 11.37 | 0.14 |
| **5** | 12.00 | 12.71 | 12.21 | 0.20 |
| **6** | 13.00 | 13.28 | 13.12 | 0.32 |
| **7** | 14.00 | 14.75 | 14.36 | 0.27 |
| **8** | 15.00 | 15.94 | 15.40 | 0.32 |
| **Total** | 08.02 | 15.94 | 11.77 | 2.23 |

**Table 1: Descriptive statistic of chronological age. Min, Minimum. Max, Maximum. SD, Stander deviation.**

| Sample no. | Variables | | r | *p* value |
|---|---|---|---|---|
| **80** | CA | CCS | **0.888** | **0.000*** |
| **80** | CA | MP3 | **0.867** | **0.000*** |
| **80** | CA | ALP | -0.115 | 0.309 |
| **80** | MP3 | CCS | **0.930** | **0.000*** |
| **80** | MP3 | ALP | -0.014 | 0.901 |
| **80** | CCS | ALP | -0.66 | 0.560 |

**Table 2: Correlation among variables. (*) significance (p≤0.05). CA, Chronological age. MP3, Middle phalanx of third finger. CCS, Canine calcification stages, ALP, Alkaline phosphates.**

The distribution of canine stages at pre-pubertal, pubertal and post-pubertal growth phases revealed that D and E stages are 100 percent distributed in the pre-pubertal phase. The F stage is 82 percent distributed in pre-pubertal and 18 percent in pubertal phases. The G stage is 90 percent distributed in pubertal and 10 percent in pre-pubertal phases, while the H canine stage is 29 percent distributed in post-pubertal and 71 percent in pubertal phase (Table 4).

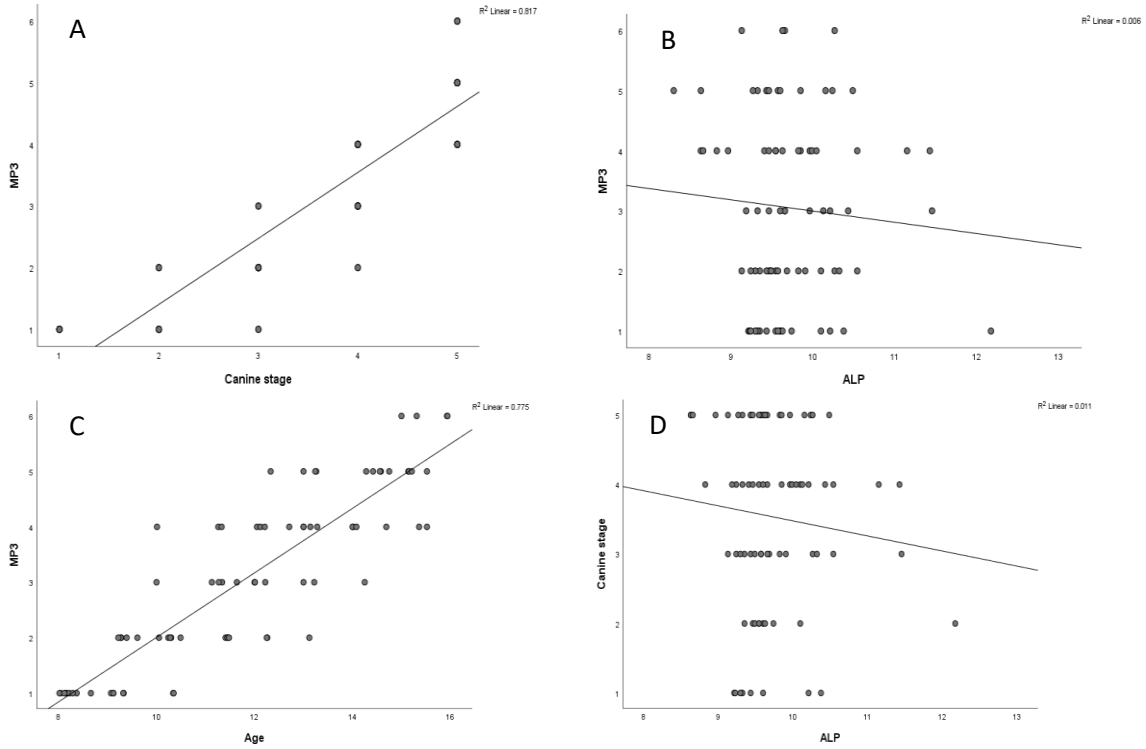


**Figure 3: Multiple scatterplots represent the correlation among variables. A, a strong correlation between MP3 stages and canine stages. B, no correlation between MP3 stages and ALP level. C, a strong correlation between MP3 and chronological age. D, no correlation between canine stages and ALP level**

| Variables | N | Gender | r | p |
|---|---|---|---|---|
| **MP3 VS CA** | 43 | M | **0.902** | **0.000*** |
| | 37 | F | **0.870** | **0.000*** |
| **MP3 VS CCS** | 43 | M | **0.932** | **0.000*** |
| | 37 | F | **0.857** | **0.000*** |
| **MP3 VS ALP** | 43 | M | 0.012 | 0.944 |
| | 37 | F | -0.032 | 0.845 |
| **CCS VS CA** | 43 | M | **0.938** | **0.000*** |
| | 37 | F | **0.730** | **0.000*** |
| **CCS VS ALP** | 43 | M | -0.047 | 0.777 |
| | 37 | F | -0.053 | 0.744 |
| **CA VS ALP** | 43 | M | -0.001 | 0.997 |
| | 37 | F | -0.187 | 0.242 |

**Table 3: correlation test among variables for male and female groups. (*) significance (p≤0.05). CA, Chronological age. MP3, Middle phalanx of third finger. CCS, Canine calcification stages, ALP, Alkaline phosphates.**

| Growth phases | D Stage | E Stage | F Stage | G Stage | H Stage |
|---|---|---|---|---|---|
| **Pre-pubertal** | 100% | 100% | 82% | 10% | |
| **Pubertal** | | | 18% | 90% | 29% |
| **Post-pubertal** | | | | | 71% |

**Table 4: Distribution of canine stages at each growth phase (pre-pubertal (1), pubertal (2), and post-pubertal (3) growth phases. (D, E, F, G, H stages) are Canine calcification stages**

Kruskal-Willis test was used to compare the level of ALP among pre-pubertal, pubertal and post-pubertal phases; the highest level of ALP was in the pubertal growth phase (Table 5); however, the differences were non-significant (Figure 4).

| Growth phases | Median | Min. | Max. |
|---|---|---|---|
| **Pre-pubertal** | 67.66 | 65 | 75 |
| **pubertal** | 69.62 | 64 | 81 |
| **Post-pubertal** | 67.86 | 59 | 74 |
| **Total** | 68.05 | 59 | 81 |

**Table 5. Descriptive statistics of salivary ALP level IU/L. (Min) Minimum. (Max) Maximum.**

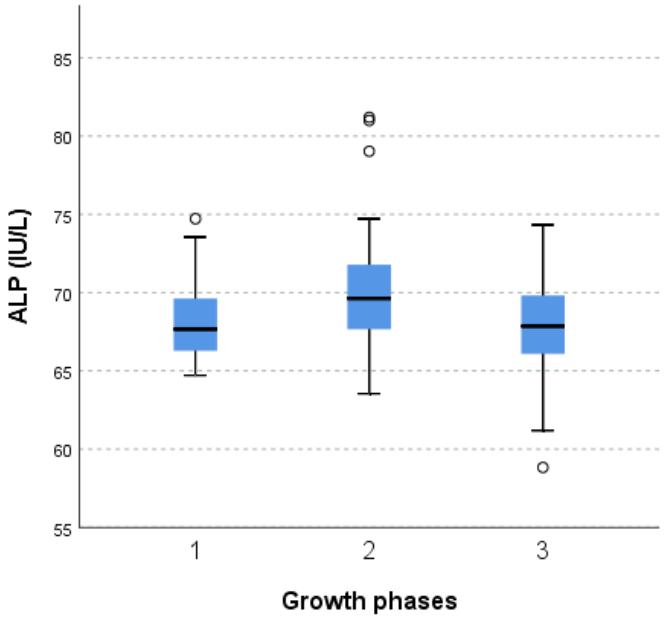


**Figure 4: Boxplot represents the Comparison among pre-pubertal (1), pubertal (2), and post-pubertal (3) growth phases according to the level of ALP.**

## DISCUSSION

This prospective observational study with a cross-sectional design was conducted on subjects with the age range to match the age of pubertal periods around eight to 16 years.[1] This study reveals that maxillary canine calcification stages strongly correlated to skeletal maturation (MP3 stages) and consider reliable skeletal maturity indicators, and the correlation between MP3 stages and canine calcification stages with ALP level was not significant; the study also revealed a strong correlation between MP3 and chronological age. Skeletal maturation of subjects was detected based on modified MP3 methods that were confirmed as a simple and reliable method to detect skeletal maturation, for which the skeletal maturity of the subjects was classified into six stages as described by Rajagobal and Kansel .[20] (modified MP3), these six stages were considered as the control of skeletal maturity with which the canine calcification, ALP, and chronological age were correlated.

The result revealed a statistically strong correlation between MP3 and maxillary canine calcification with chronological age in agreement with previous studies [23,24] However, a significant statistical correlation does not mean clinically significant, as most studies revealed there was a statistically significant correlation but do not reach the strong level of correlation to consider chronological age as a reliable method for maturity indicator. In more detail, chronological age was considered the first line of maturity indicators used clinically to decide whether the patient was in growing phases. From this point, we used chronological age to know if a person was growing. However, studies revealed that chronological age was unreliable for detecting the exact phase of pubertal growth phases. Therefore, in clinical practice, we cannot consider chronological age as a reliable skeletal maturity indicator to detect the exact phase of puberty.

The correlation between the modified MP3 stages and maxillary canine calcification stages was very strong (r =0.930). The findings of several studies confirm this [25, 26, 27, 28]. Therefore, we can consider maxillary canine calcification stages a reliable maturity indicator. Distribution of canine stages at pre-pubertal, pubertal, and post-pubertal growth phases: results revealed that canine stages (D and E) are just found in the pre-pubertal phase, and the G stage is 90 percent distributed in the pubertal phase. This means that D and E canine stages represent the pre-pubertal phase, whereas stage G represents the pubertal growth phase. The F canine stage is 82 percent distributed in pre-pubertal and 18 percent distributed in pubertal; therefore, the F canine stage could be considered the end of pre-pubertal and the beginning of pubertal. The H canine stage is 71 percent distributed in post-pubertal and 29 percent distributed in pubertal (Table 5); thus, the H canine stage can be used as an indicator of the post-pubertal phase and the end of the pubertal phase, which comes in line with the findings of previous studies.[26, 27, 28,29]Additionally, there was no correlation between salivary ALP with MP3 stages and canine calcification stages. The highest level of ALP was in the pubertal growth phase, these are in agreement with the results of previous studies [19, 21, 22, 30]. However, the differences were non-significant in salivary ALP levels among growth phases.

This agreement between skeletal maturity and teeth calcification stages could allow practitioners to utilize maxillary canine calcification stages as reliable skeletal maturity indicators to assess the skeletal maturation stage in growing individuals from only periapical X-rays. However, individual variations of tooth formation should be deliberated. The result of the research about the correlation

between skeletal maturity with chronological age and ALP level was not crucial. Further studies are to investigate the role of ALP level in skeletal maturation. For that, in clinical practice, we cannot use chronological age and ALP level as reliable skeletal maturity indicators to detect the exact phase of puberty.
Although the study shows a strong correlation between maxillary canine calcification and MP3 stages, more studies suggested confirming this result with a larger sample size and on different ethnicities. The result of the study about ALP level correlation with MP3 stages was not significant; however, investigation of the ALP level and its correlation with skeletal maturation to detect the exact role of ALP in skeletal maturation.
The limitation of the research includes a restricted sample size and the fact that the research primarily focuses on a specific community living in Baghdad, Iraq. Hence, further studies are needed on a different population with an extended sample size.

## CONCLUSION

In this study**,** the calcification stages of maxillary canine could be considered a reliable indicator for skeletal maturity; however, salivary ALP cannot be recommended as a maturity indicator.

### Author's contributions

OFN conceived and designed the study, conducted research, provided research materials, collected, organized, analyzed, and interpreted data, and was responsible for resources, visualization, and writing of the original draft of the article. HFS contributed to study design and logistic support and was responsible for project administration, validation, Data curation and manuscript language revision. All authors have a major contribution to the work and are responsible for its content.

### Ethical Policy and Institutional Review Board statement

This study was performed at dental clinics of the College of Dentistry, Baghdad University, in agreement with the ethical rules laid down by the Helsinki Declaration of 1975 (Version, 2013). A written consent form was taken from each patient before enrollment into the research after explaining the procedure in the patient's language, along with the potential risks and benefits involved. The project received ethical approval from the College of Dentistry, Baghdad University, in September 2021 with an ethical approval number (340).

### Patient declaration of consent

The authors declare that they have all the necessary patient consent forms. The patient(s) has/have granted permission in the form for his/her photos and other clinical data to be published in the journal. The patients know that while every attempt will be made to keep their identities hidden and their names and initials kept confidential, anonymity cannot be guaranteed.

### Data availability statement

Data will be provided from the corresponding author's mail upon reasonable request.

**List of Abbreviations**

ALP=Alkaline phosphatase

MP3=middle phalanx of the third finger

**Reference**


1. Rogol AD, Clark PA, Roemmich JN. Growth and pubertal development in children and adolescents: effects of diet and physical activity. *Am J Clin Nutr* **2000***;72(2):*521S-8S.
2. Hegde G, Hegde N, Anil Kumar K. A new system for assessment of growth using mandibular canine calcification stages and its correlation with modified MP3 stages. *J Pharm Bioallied Sci* **2014***;(Suppl 1):*S58.
3. Proffit WR, Fields HW, Msd DM, Larso B and Sarver DM. Contemporary orthodontics. 6th ed. Philadelphia, PA: Elsevier Health Sciences; 2019.
4. Korde SJ, Daigavane P, Shrivastav S. Skeletal Maturity Indicators-Review Article. *Int J Sci Res* **2015***; 6*:361-70.
5. Houston WJ, Miller JC, Tanner JM. Prediction of the timing of the adolescent growth spurt from ossification events in hand—wrist films. Br J Orthod 1979;6(3):145-52.
6. Flores-Mir C, Burgess CA, Champney M, Jensen RJ, Pitcher MR, Major PW. Correlation of skeletal maturation stages determined by cervical vertebrae and hand-wrist evaluations. Angle Orthod **2006**;76(1):1-5.
7. Reddy VV, Prasad GD, Hirey RK, Kumar SS, Krishnan KS. A comparison of skeletal maturation assessed from MP3 and its correlation with dental maturation. *J Indian Orthod Soc* **2014***;48(4_suppl1):*325-8.
8. Fishman LS. Radiographic evaluation of skeletal maturation: a clinically oriented method based on hand-wrist films. Angle Orthod 1982;52(2):88-112.
9. Joshi M, Rao D, AB S, Panwar S, Franklin S. A radiographic assessment of the correlation between the calcification stages of the mandibular second molar and the middle phalanx of the third finger of 9–16 years old children. *Egypt J Forensic Sci* **2018***;8(1):*1-7.
10. Hägg U, Taranger J. Maturation indicators and the pubertal growth spurt. *Am J Orthod* **1982***;82(4):*299-309.
11. Hegde DY, Baliga S, Yeluri R, Munshi AK. Digital radiograph of the middle phalanx of the third finger (MP3) region as a tool for skeletal maturity assessment. *Indian J Dent Res* **2012***;23(4):*447.
12. Nolla CM. The development of permanent teeth (Doctoral dissertation, University of Michigan).
13. Demirjian A, Goldstein H, Tanner JM. A new system of dental age
14. assessment. Hum Biol. **1973**; 45:211–27.
15. Vijayashree U, Pai V, Naik V. Second molar calcification stages to evaluate skeletal maturation: A cross-sectional radiographic study. APOS Trends Orthod **2014**;4(6):156-.
16. Perinetti G, Baccetti T, Di Leonardo B, Di Lenarda R, Contardo L. Dentition phase and chronological age in relation to gingival crevicular fluid alkaline phosphatase activity in growing subjects. Prog Orthod **2011**;12(2):100-6.
17. Irham F, Bahirrah S, Nazruddin D. The level of alkaline phosphatase in saliva as biomarker for pubertal growth phase. Adv Health Sci Res **2018**; 8:102-5.
18. Dhiman S, Maheshwari S, Verma SK. Assessment of maturity in orthodontics: A review. J adv clin res insights 2015;2(2):100-3.
19. Chiappin S, Antonelli G, Gatti R, Elio F. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clinica chimica acta **2007**;383(1-2):30-40. .
20. Tarvade SM, Ramkrishna S, Sarode S. Salivary alkaline phosphatase-a biochemical marker for growth prediction. Indian J Basic Appl Med Res **2015**;4(3):17-22.
21. Rajagopal R, Kansal S. A comparison of modified MP3 stages and the cervical vertebrae as growth indicators. J Clin Orthod: JCO **2002**;36(7):398-406.
22. Wijaya H, Kusdhany LS, Redjeki S, Soegiharto BM. The salivary bone spesific alkaline phosphatase in relation to pubertal growth phase in Indonesian children. *Asian J Pharm Clin Res* **2017***;10(5)*:389.

23. Alhazmi N, Trotman CA, Finkelman M, Hawley D, Zoukhri D, Papathanasiou E. Salivary alkaline phosphatase activity and chronological age as indicators for skeletal maturity. Angle Orthod **2019**;89(4):637-42.
24. Macha M, Lamba B, Avula JSS, Muthineni S, Margana PGJS, Chitoori P. Estimation of Correlation between Chronological Age, Skeletal Age and Dental Age in Children- A Cross-sectional Study. *J Clin Diagn Res.* **2017** *Sep;11(9)*: ZC01-ZC04.
25. Mohan R, Jain RK, Balakrishnan N. Correlation between chronological age and skeletal age using cvmi and modified MP3 methods. Bioinformation. **2020** Dec 31;16(12):1045-1050.
26. Nayak R, Nayak UK, Hegde G. Assessment of growth using mandibular canine calcification stages and its correlation with modified MP3 stages. *Int J Clin Pediatr Dent* **2010***; 3(1),* p.27.
27. Kumar S, Roy AS, Garg A, Hamid SB, Tyagi S, Kumar A. Correlation between maxillary canine calcification and skeletal maturation. J Clin Diagn Res: JCDR 2017;11(5): ZC13.
28. Trakinienė G, Smailienė D, Kučiauskienė A. Evaluation of skeletal maturity using maxillary canine, mandibular second and third molar calcification stages. *Eur J Orthod* **2016***;38(4):*398-403.
29. Kotecha T, Goel S, Gupta N, Soni G. Skeletal maturity assessment by comparing CVMI stages with calcification of maxillary canine, mandibular second molar and mandibular third molar-A RADIOGRAPHIC CEPHALOMETRIC STUDY. Eur j mol clin med **2020**;1(11).
30. Oncan E, Akan S. Assessment of the Relationship between Skeletal Maturity and the Calcifications Stages of Permanent Canines and Second Premolars. Turk J Orthod. 2021 Feb 23;34(1):31-38.
31. Hegde S, Revankar A, Patil A. Identification of bone-specific alkaline phosphatase in saliva and its correlation with skeletal age. *Indian J Dent Res* **2018***;29(6)*:721–5.

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