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Original Article

Radiographic assessment of third molars development and it's relation to dental and chronological age in an Iranian population

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ABSTRACT

Background: The aim of the present study was to estimate chronological age based on third molar development and to determine the association between dental age and third molar calcification stages.
Materials and Methods: In this cross-sectional study, 505 digital panoramic radiographs of 223 males (44.2%) and 282 females (55.8%) between the age of 6 and 17 were selected from patients who were treated in Departments of Pediatrics and Orthodontics of Isfahan University of Medical Sciences between the years of 2009 and 2013. Correlation between chronological age and third molar development was analyzed with SPSS 21 using Spearman's Rank correlation coefficient, Chi-square test and multiple regression statistical tests ($P < 0.05$).
Results: All third molars demonstrated a highly significant correlation with dental age ($P < 0.001$). The teeth showing the highest relationship with dental age were mandibular left third molar in males and mandibular right third molar in females ($r_s = 0.072$). When multiple regression was used to predict dental age based on molar calcification stage, the only significant correlation was between maxillary left third molar in males ($P < 0.05$). There was no statistically significant correlation for any of third molars in females. Relationship between chronological age and molars development stage was significant in all age subgroups and in both gender ($P < 0.001$).
Conclusion: Strong correlation was observed between left third molars and dental age in males. Results showed that third molar calcification stage can be used as an age predictor and in general mandibular teeth seems to be more reliable for this purpose in both genders and in all ages.

Key Words: Chronological age, demirjian system, dental age

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INTRODUCTION

Human chronological or biological age doesn't directly show the real body maturation stage,hereditary, functional, environmental, nutritional, sexual, metabolic, social, emotional, cultural factors, affect the development.[1] Third molar radiographic examination which determines it's presence,[2] position and degree of calcification,[3] morphology and eruption,[4] effect on the dental arch development[5] is commonly used in oral surgery, orthodontics and pediatrics for diagnosis and treatment planning.[6]

As genes play a more important role in controlling the dental calcification and development compared to environmental factors, methods based on the stages of tooth formation revealed to be more appropriate for age assessment than those based on skeletal development.[7,8] Hormonal and pathological changes seem to have less effect on teeth especially in children; therefore, analyzing radiographic developmental stages of teeth can be used as a way to assess children age.[7,9]

Radiographic images are used as a mean to identify the stage of mineralization.[10] For this purpose,

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different radiographic images such as intraoral periapical radiographs, cephalometric radiographs, panoramic radiographs, digital imaging, lateral oblique radiographs and advanced imaging technologies can be used.[11,12] The radiographic images should include the area of interest and all stages of dental development can be rated according to chosen development standards.[13]

Teeth calcification stage can be used as a method for age estimation. Wisdom tooth root growth is one of the most important criteria in the dental examination.[14-16] Third molars can be used to estimate chronological age in a wide age range as they have a long period of development.[17] Several methods have been designed to assess dental calcification in the past.[18-19] Demirjian *et al*. 8-stage classification[19] has been widely accepted and being used because of its objectivity, simplicity and accuracy. Age estimation based on dental status has been also used in forensic dentistry. When a person with unknown age is being legally investigated, forensic expert should be able to estimate their age based on their biological signs of development.[20]

Recently, many of studies have investigated the influence of geographic and ethnic factors on chronology of third molar development in order to provide reference data for age estimation in different ethnic groups.[3,21-24] Since different populations indicate differences in third molar mineralization stage, it is essential to use specific reference data for each population.

Third molars can demonstrate different chronology related maturation. Hence, it is appropriate to investigate all third molars for each population and each gender.

In this study, the course of third molar development and its association to dental and chronological age and gender related differences was analyzed based on evidence obtained from digital panoramic radiographs of subjects. Based on these findings, the ability of third molars in chronological age estimation and their association with dental age will be assessed.

MATERIALS AND METHODS

The data for this retrospective cross-sectional study were derived from 505 digital panoramic radiographs collected randomly from patients who visited Departments of Pediatrics and Orthodontics, Faculty of Dentistry, Isfahan University of Medical Sciences from 2009 to 2013. Participants were aged between 6 and 17 years.

Initially, 1148 radiographs were examined by two observers and were selected if they demonstrated enough quality especially in third molars regions, normal dental development and no history of diseases that could affect development. This resulted in the final number of 505 digital panoramic radiographs of 223 males (44.2%) and 282 females (55.8%) between the age of 6 and 17. Subjects were divided into four age subgroups of 6-9, 9-12, 12-15 and 15-17 years old.

All radiographs were taken with PLANMECA PROLINE XC device (PLANMECA OY, Helsinki, Finland).

Subject's sex and date of birth were gathered from their records. The assessment of reproducibility was performed on a sub-sample of 50 randomly selected radiographs. For this purpose, the same investigator examined the radiographs twice in a period of 1 month and the intra-observer variability was calculated. The intra-observer agreement was found to be 97%.

As shown in Figures 1 and 2, teeth mineralization of the third molars and dental age were assessed based on the method proposed by Demirjian *et al*.[19] Demirjian *et al*. proposed 8 stages of maturity, from stage A (single occlusal points calcification, without fusion) to stage H (apical end completed). To increase the accuracy of the estimation, one code for "follicle with no calcification" was added and named stage 0. Demirjan classification stages were scored from "1" to "8" and "follicles with no calcification" was scored "0".[17] In order to make our research more appropriate for clinical use, we decided to classify these 9 stages into 5 categories which can be easily determined by a

Stage 0	Follicle without calcification
Stage A	Single occlusal points calcification, without fusion
Stage B	Fusion of calcified points form occlusal surface
Stage C	Enamel formation is complete, beginning of dentinal deposits
Stage D	Crown formation is complete up to cement enamel junction
Stage E	Initial formation of radicular bifurcation, root length shorter than crown height
Stage F	Root length is equal or longer than crown height
Stage G	Root formation finished but Apical foramen still open
Stage H	Apical foramen is closed

Figure 1: Developmental stages of the third molar (modified from Demirjian *et al*.)

clinician. These categories are based on the amount of crown and root formation as follows:

1. No calcification (stage O),
2. Beginning of crown formation until it's completion up to cement enamel junction (stage A, B, C),
3. Beginning of root formation until root length is equal to crown height (stage D, E),
4. Root length longer than crown height until completion of root formation, apical foramen still open (stage F, G),
5. Apical foramen is closed (stage H).

All panoramic radiographs were analyzed by the same researcher twice. Each radiograph was first seen in a negatoscop and then scanned and examined in a computer. This method provided a more accurate view of the selected region as the researcher can zoom in or magnify the image in computer.

The SPSS 21 (SPSS Inc., Chicago, IL, USA) was used for statistical calculations. Descriptive statistics were obtained by calculating the means and standard deviations (SD) of the chronological age. The Spearman rank order correlation coefficients and Chi-square test were applied to measure the relationship between the chronological age and the calcification stages of the subject's third molars teeth. Multiple regression statistical tests were used to analyze the possibility of dental age prediction from third molar calcification stages. The level of significance was $P < 0.05$.

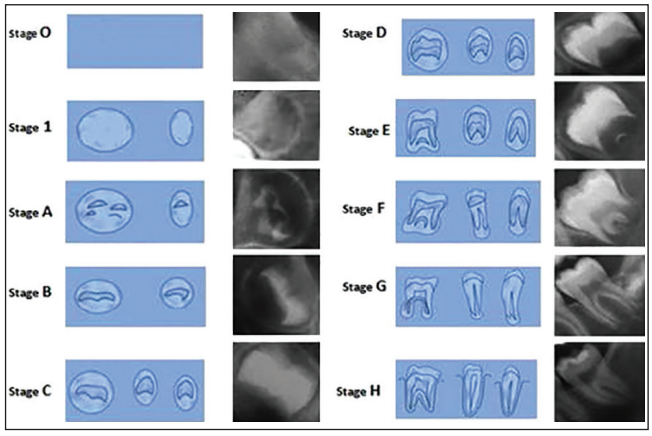

Figure 2: Classification stages of molar teeth according to Demirjian *et al*.

RESULTS

A total of 505 radiographs of 44.2% (n = 223) males and 55.8% (n = 282) females with the mean age of 11.6 (ranged from 6 to 17) were studied. Table 1 demonstrates the mean age (SD) for each calcification stage of the third molar based on location (maxillary/mandibular). Tables 2 and 3 show the distribution of calcification stages (our five stage classification) in different age subgroups based on third molar location (maxillary/mandibular and left/right) in males and females.

Results based on 8 stages of Demirjian method showed that mandibular left and right molars in both genders and maxillary right molar in females showed stage 0 of calcification in subjects with <15 years of age. For maxillary left molar in males and females and maxillary right molar in females, all the subjects in stage 0 of calcification were aged <12 years.

Early calcification, which is the stage A of the developmental level according to Demirjian method, for all third molars were seen in approximately 90% of male subjects and 85% of female subjects aged <12 years old.

Table 1: Means of age in stage 0 and in the Demirjian stages of calcification for each 3rd molar tooth (tooth no. LL8, LR8, UL8 and UR8)

Stage of calcification	3rd molar tooth			
	Mean ± SD (min, max)			
	LL8	LR8	UL8	UR8
0	10.0±1.8 (7,13)	10±2.05 (7,13)	9.6±0.6 (8.9,10)	11±1.5 (8.9,13)
A	10.7±1.7 (7,16)	10.4±1.5 (7,15)	9.7±1 (7,11)	9.9±1.2 (7,13)
B	11.0±1.6 (8,17)	11.2±2.1 (8,17)	10.7±1.9 (7,17)	10.6±1.9 (7,17)
C	11.4±1.7 (8,17)	11.3±1.5 (8,17)	11.6±1.7 (8,17)	11.7±1.8 (8,17)
D	12.2±2 (8,17)	12.7±2.2 (8,17)	14.1±2 (10,17)	13.7±2.1 (10,17)
E	14.9±1.6 (11,17)	14.5±1.9 (10,17)	15.2±1.7 (11,17)	15.1±1.7 (11,17)
F	16.3±0.8 (14,17)	15.9±1.2 (12.9,17)	16.6±0.5 (16,17)	16.6±0.5 (16,17)
G	16.4±2 (9,17)	16.5±1.8 (9,17)	16.2±2 (9,17)	16.2±1.9 (9,17)
H	16.7±0.8 (14,17)	16.7±0.8 (14,17)	16.9±0.2 (16,17)	16.9±0.2 (16,17)

SD: Standard deviation

Table 2: Percentage of each calcification stages in different subgroups for molars based on their location (maxillary/mandibular and left/right) in males

Age (years)	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
6-9					
LL8	23.30	67.70	0	0	0
LR8	38.70	58.10	3.20	0	0
UL8	10	90	0	0	0
UR8	6.10	93.90	0	0	0
9.1-12					
LL8	11.70	73.40	14.90	0	0
LR8	5.60	77.80	16.70	0	0
UL8	3.40	89.60	6.90	0	0
UR8	5.10	86	8.90	0	0
12.1-15					
LL8	2.90	55.80	41.20	0	0
LR8	3	51.60	45.40	0	0
UL8	0	60	40	0	0
UR8	7.40	63	29.60	0	0
15.1-17					
LL8	0	7.60	26.90	50	15.40
LR8	0	7.70	30.80	46.20	15.40
UL8	0	8	28	44	20
UR8	0	19.20	19.20	46.20	15.40

0: No calcification (stage 0); 1: Beginning of crown formation until it's complete up to cement enamel junction (stage A, B, C); 2: Beginning of root formation until root length is equal to crown height (stage D, E); 3: Root length longer than crown height until finishing of root formation, apical foramen still open (stage F, G); 4: Apical foramen is closed (stage H)

Table 3: Percentage of each calcification stages in different subgroups for molars based on their location (maxillary/mandibular and left/right) in females

Age (years)	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)
6-9					
LL8	30.60	41.10	5.60	2.80	0
LR8	32.40	59.80	5.40	2.70	0
UL8	4.30	91.40	0	4.30	0
UR8	4.30	91.30	0	4.30	0
9.1-12					
LL8	9.60	77.90	12.50	0	0
LR8	13.60	67.90	18.50	0	0
UL8	1.10	94.50	4.40	0	0
UR8	3.50	87.20	9.30	0	0
12.1-15					
LL8	4.10	34.60	55.10	4.10	2
LR8	4.20	31.20	52.10	10.40	2.10
UL8	0	58.40	39.60	2.10	0
UR8	0	58.60	39.10	2.20	0
15.1-17					
LL8	0	13.10	23.70	39.50	23.70
LR8	0	13.50	21.60	45.90	18.90
UL8	2.60	13.20	26.30	34.30	23.70
UR8	2.70	13.50	27	35.10	21.60

0: No calcification (stage O); 1: Beginning of crown formation until it's complete up to cement enamel junction (stage A, B, C); 2: Beginning of root formation until root length is equal to crown height (stage D, E); 3: Root length longer than crown height until finishing of root formation, apical foramen still open (stage F, G); 4-Apical foramen is closed (stage H)

All the subjects demonstrated stage F, G and H of calcification for mandibular left and right molars in both genders and maxillary right and left molars in females were in 15-17 age subgroup. Of all study samples under age 12, 85.5% were in stage A Demirjian stage of the third molar calcification. Differences between all 8 stages of calcification and stage 0 for each molar in age subgroups were significant ($P < 0.001$).

All third molars demonstrated a highly significant correlation with dental age ($P < 0.001$). The teeth showing the highest relationship with dental age were mandibular left third molar in males and mandibular right third molar in females($r_s = 0.72$). When comparison between different age groups was conducted, mandibular third molars demonstrated a significant correlation with dental age in all age subgroups in both males and females ($P < 0.05$) and maxillary left molars showed a significant correlation except for the 6-9 subgroup. Maxillary right molar was not correlated in 15-17 female and 6-9 male subgroups.

When multiple regression was used to predict dental age based on molar calcification stage the only significant correlation was between maxillary left third molar in males ($P < 0.05$). There was no statistically significant correlation for any of third molars in females.

The linear regression coefficients were provided to assess the correlation of third molar development and chronologic age. Statistical analysis showed a strong correlation between age and third molar development for males ($r = 0.68$) and for females ($r = 0.66$).

DISCUSSION

Age estimation is important for both clinical and medico-legal (age at death, criminal cases, etc.) purposes and different methods have been used for age estimation. Dental developments are more associated to chronological age than somatic, skeletal or sexual maturity indicators.[25] In order to assess dental maturation, tooth calcification due to its progressive and continuous process and the fact that radiographs can be used to follow it, are more often used than tooth eruption. The panoramic radiographs due to their ability to enhance a wide view of the tooth and facial bones[26] are one of the best instruments to assess dental calcification.[17,24] Several factors such as genetics, climate, hormonal and environmental

differences and nutrition can affect individuals development.[25,27] In the present study, we evaluated the relation between third molar calcification with dental and chronological age.

Various methods have been used for age determination over the past decades; the radiological method due to its advantages such as not requiring either extraction or preparation of microscopic sections from each individual is often preferred over biochemical and histological methods. Therefore, in living individuals and where extraction of teeth for religious or scientific reasons are not acceptable, other methods are less applicable than radiological methods.[28,29] Furthermore, it should be noted that on the contrary to biochemical and histological methods which are expensive and require sophisticated laboratory equipment,[28] the radiographic method can be used for identifying the age in dead as well as living persons with a simple, economical, quick, non-mutilating and non-invasive method.[7]

Pre-natal and neonatal age estimation are more difficult compared with the post-natal phase. However, for both pre-natal and post-natal phases, radiological examination of the developing tooth germs in the jaw bones can be used for age assessment.[28-30] No clinical manifestation can be used between the ages of 2.5 and 6 years up to the first permanent tooth eruption to determine the age; therefore, radiographs play a major role in assessing chronological age and permanent dentition development in this period.[30] When many under developed teeth and rapid growth are observed, the radiographic age estimation method has its best accuracy.[31] Since most of the dentition is completely developed after the age of 14 years, radiographic age estimation tend to become harder and less accurate.[28]

The validity of several radiographic methods for assessing third molar development such as Gleiser and Hunt,[18] Demirjian *et al*.,[19] Gustafson and Koch,[32] Harris[33] and Kullman *et al*.[27] methods were investigated by Olze *et al*.[34] They reported that of the methods tested, the Demirjian method was found to be the most accurate one for evaluation the third molar mineralization stages for age estimation.

Furthermore, in a study done by Dhanjal *et al*.,[35] the reproducibility of different methods to assess calcification stages of third molars was investigated and Demirjian *et al*. method was shown to be the best for both intra- and inter-examiner agreement and estimated and true age correlation. In the present study, in order to show the crypt stag (follicle with no calcification stage), which isn't primarily one of the original Demirjian stages, a stage 0 was added.

Third molar development (presence, formation, mineralization and position) has great variation and can't be accurately predicted.[26] However, Demirjian *et al*.[19] classification method seems to make it possible to use third molar calcification stage to determine the subjects age category in populations with various ethnic groups such as ours.[6,23,24,36]

Calcification stages should be used as an auxiliary tool to estimate chronological age and to investigate human characteristics within the ethical standards and professional good practice. Since physical and developmental characteristics vary greatly among the population groups in many regions of Iran and the world,[1] it is important to adopt a reliable and validated tool to study the chronological age by the mineralization of teeth in different populations.

In this study, a strong correlation was found between chronological age and molar calcification stages which are in accordance with findings of Costacurta *et al*.[37] The mean age of the individuals in stage D (crown completion) was 13.62 for the present population which is comparable to what has been observed in the Turkish population (12.90),[17] but much less than those of Japanese (18.2 for males, 18.0 for females)[4] or German (16.3 for males, 15.5 for females) populations[38] which may explain the differences between ethnicities and the dissimilarity between observers. In the present study, no significant differences related to gender existed in the calcification stage of the third molar which is in accordance with Araújo *et al*. study[39] and is in contrast with the Legovic *et al*. study.[40] While some of the previous studies have not found significant differences in calcification stages of third molars between genders,[40,41] some others have.[22,37,42,43]

In the present study, there was a significant correlation between the calcification stage of all four third molars with the chronological and dental age ($P < 0.001$). Costacurta *et al*.[37] in their study have also reported the same relation between chronological age and third molar calcification. Araújo *et al*.[39] found a similar correlation between third molar's calcification stages and chronological age in their Brazilian population. In their Caucasian study sample, Legović *et al*.[40] reported that crown calcification of mandibular third molars is completed by age 15. In the present study,

the mean age of completion of the calcification of the third molar crowns (D stage) was 13.62.

In this study, when multiple regression statistical test was used to determine dental age based on the third molar calcification stage, only the left maxillary third molar showed a significant ability in predicting the dental age.

CONCLUSION

It was concluded that:

1. There is a significant correlation between the calcification stage of third molars with dental and chronological age.
2. The teeth showing the highest relationship with dental age were mandibular left third molars in males and mandibular right third molars in females.
3. When comparison between different age groups was conducted, mandibular third molars demonstrated a significant correlation with dental age in all groups in both males and females. Maxillary left molars showed a significant correlation in all age groups except for 6-9 years old. Maxillary right molar was not correlated in 15-17 years female and 6-9 years male groups.
4. To predict dental age based on molar classification stage (using multiple regressions), only maxillary left third molars in males showed a significant correlation.

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