Radiographic evaluation of secondary dentin formation in lower premolars for forensic age diagnosis of 18 years in a sample of south Indian adolescents and young adults

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ABSTRACT

There has been an increase in the need for alternate methods of dental age assessment, especially for the forensic age diagnosis of the 18th year of life. This is due to the completion of the third molar development before 18 years or the agenesis or therapeutic extractions of the third molars. The present study aimed to verify whether the secondary dentin formation in lower premolars can be used to determine the completion of the 18th year of life in a sample of South Indian adolescents and young adults. For this purpose, 800 orthopantomograms of 400 male and 400 female South Indian subjects aged 14- 22 were evaluated. The characteristics of the secondary dentin formation were determined in all mandibular premolars using the stage classification according to Olze et al (Int J Legal Med 126(4):615-21). The results showed that when stage 3 of secondary dentin formation was reached in the first premolars, the probability of the subject completing the 18th year of life was very high. However, only a few individuals in the studied population were at stage 3. Therefore, proceeding cautiously with this degenerative change in lower premolars is advised due to the higher inter-examiner differences. It is also recommended to use this method in conjunction with other age estimation methods. Further research should investigate other degenerative characteristics in the studied population.

INTRODUCTION

Age estimation of the living and dead people is imperative in forensic and medicolegal practice. Research in this area, especially in living individuals, has become a priority in recent years due to increased refugee migration, juvenile delinquency, and competitive sports.[1- 3] In European countries, the age thresholds of legal relevance usually lie between 14 and 21 years.[1] In India, the legally relevant age thresholds are 14 years (child labour), 16 years (age of criminal responsibility), 18 years (age of majority), and 21 years (legal age of marriage). The validation of completing the 18th year of life is of particular importance. From this point of view, numerous researchers have focussed on third molar development to provide proof of completion of the 18th year with a forensically required certainty.[4- 7] However, the complete mineralization of third molars before the 18th year, their agenesis or therapeutic extraction in adolescents necessitated the need for alternate methods for age estimation.

Various methods were tested to predict the attainment of the

legal age of 18 years as an alternative to third molar development. In 2010, Olze et al. proposed two staging methods based on the radiographic visibility of the root pulp[8] and periodontal ligament[9] on lower third molars. Later the staging method of pulp visibility was tested in lower first and second molars for the prediction of 18 years in the absence of third molars.[10, 11] However, the shape and positional variations of third molars influencing the observations of root pulp or the periodontal ligament visibility has warranted for other dental age estimation methods.[12]

In 1947, Gustafson presented a scientific method for age estimation using degenerative dental changes based on extracted teeth.[13] He studied characteristics of secondary dentin formation, periodontal recession, attrition, dentinal translucency, cemental apposition, and external root resorption and confirmed their correlation with chronological age. Later in 1981, Matsikidis studied these changes and proved they could be evaluated using dental films.[14] In 2010, Olze et al. studied these characteristic features in orthopantomograms (OPGs) and proposed a formula for age estimation based on only four characteristics from Gustafson's criteria.[15] Furthermore, they advised proceeding with caution as the applicability of this method is limited by the quality of the X-ray images. In 2017, Timme et al. suggested that the study of degenerative dental characteristics can be used as evidence to predict the completion of the 18th year of life.[16] In 2019, Hou et al. studied these degenerative changes in Chinese juveniles and young adults. They concluded that their developmental stages could prove the completion of the 18th of life beyond a reasonable doubt.[17] To the best of our knowledge, no investigation of these criteria demonstrating the completion of the 18th year of life in the Indian population has been conducted.

The present study aimed to verify whether the degenerative changes can be used for age estimation in South Indian adolescents and young- adults. To this purpose, the secondary dentin formation in lower premolars was evaluated.

MATERIALS AND METHODS

Materials

The design of this study was a retrospective cross-sectional study of OPGs obtained from the clinical practices of southern India, which comprised five states, i.e., Andhra Pradesh, Telangana, Karnataka, Tamilnadu, and Kerala. Eight hundred OPGs (400 males and 400 females) of South Indian juveniles and young adults aged between 14 and 22 were studied. All the OPGs were made in the period between 2018 and 2022. Total subjects were divided into eight age groups encompassing one year; e.g., 14 years was defined as 14.00 to 14.99 years. Equal distribution of male and female subjects (50 per sex) was ensured in each age group (Table 1). The exclusion criteria for the evaluated premolars followed the recommendations made by Matsikidis that include premolars with caries, filling, crowned tooth or bridged abutment, post and core restoration, root filling, retained root, or premolars undergoing apicoectomy.[14]

Table 1. Age and sex distribution of the total sample

Age group	Male	Female	Total
14-14.9 years	50	50	100
15-15.9 years	50	50	100
16-16.9 years	50	50	100
17-17.9 years	50	50	100
18-18.9 years	50	50	100
19-19.9 years	50	50	100
20-20.9 years	50	50	100
21-21.9 years	50	50	100
Total	**400**	**400**	**800**

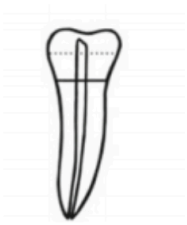
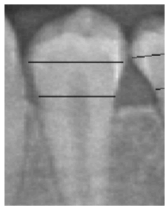
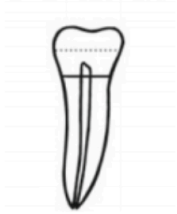
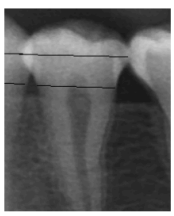
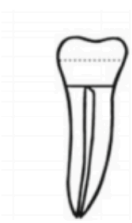
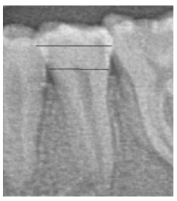
Following the collection, each OPG was provided with a unique identification number (UIN), and the details of the sex and chronological age were entered against each UIN. All the details were entered in a separate Microsoft Excel file. Examiners performing the radiographic analysis were blinded to the information about the sex and age of the subjects.

Method

The characteristics of the secondary dentin formation were determined in all mandibular premolars using the stage classification according to Olze et al.[15] In this classification, four stages were provided to assess the degree of secondary dentin, such as stage 0 (pulp horn reaches above to the above crown equator), stage 1 (pulp horn reaches at maximum to the crown equator), stage 2 (pulp horn exceeds enamel- cementum junction and falls short of the crown equator), and stage 3 (pulp horn reaches at maximum to enamel-cementum junction (Figure 1). In addition, non-evaluable teeth were distinguished between non-presence or due to other reasons such as developing premolars or lack of accessibility.

All the evaluations were performed randomised and blinded. A forensic odontologist with eight years of forensic age assessment experience did all the evaluations. A second examiner, a dentist with no experience in dental age assessment, analysed a few OPGs to study inter-examiner agreements.

Figure 1. Schematic representation of stage classification to determine the degree of secondary formation in mandibular premolars

Stage classification	Original Drawings	Example X- rays for different stages in study population
STAGE 0		
STAGE 1		
STAGE 2		
STAGE 3		

Statistical analysis

A Microsoft Excel file 2016 (Microsoft Office 2003, Microsoft, Redmond, WA) was used to enter the details of each individual, such as unique identification number, chronological age, sex, and stage classifications of all premolars. Statistical analysis was performed using SPSS 29.0 statistical software (SPSS Inc., Chicago, IL, USA). The significance threshold was set at 5% ($p< 0.05$). Side differences were tested using the Wilcoxon signed rank test. For each stage, the frequencies of the examined teeth, the mean, standard deviation, minimum, maximum, and median, were calculated in both sexes separately. Finally, two-by-two contingency tables were drawn to indicate the performance of the stages of secondary dentin formation for indicating the completion of the 18th year of life.

To assess intra-examiner agreement, 100 OPGs were randomly selected and evaluated for the second time by the first examiner at three months. For the inter-examiner agreement, the same OPGs were reviewed by the second examiner. Cohen's kappa statistics were calculated for intra-and-inter-examiner agreements.

RESULTS

The Cohen's kappa coefficients for the intra-examiner agreement were 0.842 and 0.797 for the first and second premolars. The Cohen's kappa coefficients for the inter-examiner agreement were 0.597 and 0.583 for the first and second premolars, respectively. The output of the Wilcoxon signed-rank test showed that the stage classification did not elicit a statistically significant difference between the right and left sides for both for first premolars ($Z= -2.833$, $p= 0.07$) and second premolars ($Z=-2.167$, $p=0.09$), respectively. From here onwards, the results were presented for the lower left first (#34) and second (#35) premolars.

Table 2 shows the number and percentage of lower premolars which could not be evaluated and, therefore, could not be evaluated for statistical evaluation. The number of missing teeth was provided as well. Depending on the examined tooth, 77.25% to 82.5% of first premolars and 77% to 82.5% of second premolars were evaluable.

Tables 3 and 4 show the descriptive statistics results for each stage of secondary dentin formation for first and second premolars in both sexes. There were no significant differences in the mean age between males and females in different stages for both premolars. Tables 5 and 6 show the results of two-by-two contingency tables describing the discrimination performance of stage 3 of secondary dentin formation to indicate the completion of the 18th year of life.

Table 2. Number and percentage of teeth excluded or missing

Tooth	Sex	Number of cases	Missing teeth	Non-evaluable teeth	Evaluated teeth	Percentage evaluated
34	Male	400	14	77	309	77.25%
	Female	400	10	61	329	82.5%
35	Male	400	14	63	323	80.75%
	Female	400	11	63	326	81.5%
44	Male	400	14	74	312	78%
	Female	400	10	63	327	81.75%
45	Male	400	15	77	308	77%
	Female	400	11	59	330	82.5%

Table 3. Descriptive statistics on the age (in years) of the stages of secondary dentin formation of mandibular premolars in males

Tooth	Stage	N	Mean	SD	Minimum	Maximum	Median
34	0	6	18.73	2.08	16.42	21	18.88
	1	169	18.08	2.18	14.05	21.87	18.02
	2	119	17.95	2.41	14.02	21.65	18.12
	3	15	20.39	1.47	18.2	21.94	21.12
35	0	1	-	-	-	-	-
	1	60	18.57	2.16	14.02	21.63	19.05
	2	216	17.80	2.30	14.08	21.94	17.71
	3	46	19.39	1.82	15.50	21.94	19.44
44	0	6	19.49	1.41	17.61	21	20.01
	1	154	17.99	2.13	14.10	21.75	17.89
	2	129	17.87	2.29	14.02	21.94	18.24
	3	23	20.57	1.11	18.61	21.91	21.08
45	0	1	-	-	-	-	-
	1	41	18.21	2.39	14.02	21.63	19.14
	2	215	18.06	2.29	14.08	21.94	18.06
	3	51	18.50	2.05	15.24	21.94	18.63

Table 4. Descriptive statistics on the age (in years) of the stages of secondary dentin formation of mandibular premolars in females

Tooth	Stage	N	Mean	SD	Minimum	Maximum	Median
34	0	5	18.35	1.54	15.93	20.12	18.48
	1	175	18.26	2.21	14.10	21.94	18.38
	2	139	18.01	2.45	14.02	21.99	18.06
	3	10	20.55	1.01	18.95	21.71	20.96
35	0	0	-	-	-	-	-
	1	40	18.39	2.26	14.14	21.83	18.98
	2	243	17.84	2.23	14.02	21.94	17.84
	3	43	20.03	1.74	14.37	21.99	20.33
44	0	7	18.72	1.35	16.77	20.83	18.48
	1	162	18.15	2.12	14.14	21.99	18.27
	2	122	17.49	2.33	14.02	21.92	17.33
	3	37	20.38	1.21	18.22	21.98	20.45
45	0	0	-	-	-	-	-
	1	50	18.92	2.29	14.14	21.99	19.40
	2	220	17.84	2.24	14.02	21.92	17.7
	3	60	18.94	2.21	14.27	21.98	19.23

Table 5. 2×2 Contingency tables describing discrimination performance of the test on being adult (≥18) or minor (<18) for "stage 3" of secondary dentin formation in lower left first premolars (#34)

Males			
	Age status		**Total**
	<18 years	**≥18 years**	
≤ Stage 2	145^{TP} (100)	149^{FN} (90.8)	294 (95.1)
Stage 3	0^{FP} (0)	15^{TN} (9.2)	15 (4.9)
Total	145 (100)	164 (100)	309 (100)
Females			
	<18 years	**≥18 years**	**Total**
≤ Stage 2	147^{TP} (100)	172^{FN} (94.5)	319 (96.9)
Stage 3	0^{FP} (0)	10^{TN} (5.5)	10 (3.1)
Total	147 (100)	182 (100)	329 (100)

Table 6. 2×2 Contingency tables describing discrimination performance of the test on being adult (≥18) or minor (<18) for "stage 3" of secondary dentin formation in lower left second premolars (#35)

Males			
	Age status		**Total**
	<18 years	**≥18 years**	
≤ Stage 2	141^{TP} (94.6)	136^{FN} (78.1)	277 (85.7)
Stage 3	8^{FP} (5.4)	38^{TN} (21.9)	46 (14.3)
Total	149 (100)	174 (100)	323 (100)
Females			
	<18 years	**≥18 years**	**Total**
≤ Stage 2	145^{TP} (97.9)	138^{FN} (77.5)	283 (86.8)
Stage 3	3^{FP} (2.1)	40^{TN} (22.5)	43 (13.2)
Total	148 (100)	178 (100)	326 (100)

DISCUSSION

Regressive alterations or degenerative changes generally begin immediately after the eruption and continue throughout life.[18-21] Lately, the study of these features has become a subject of interest, especially in the absence of third molars or due to their completed development in subjects younger than 18.[22] In this regard, the authors, in their previous investigations, studied the root pulp visibility in the fully mineralised lower first and second molars for indicating the completion of the 18th year of life in south Indian adolescents and young adults.[10, 11] The accuracy of these methods was reported to be moderate to high, thus warranting the need for other methods or the study of alternate teeth.

The reaction of the pulp dentinal complex in response to various physiological and pathological stimuli results in the formation of secondary dentin. It leads to the reduction of the size of the

pulp cavity.[23] This regressive tooth change attracted interest in the forensic literature, and its correlation with age was extensively studied. In 2012, Olze et al.[15] developed a staging system using the conventional OPGs using mandibular premolars as they are predominantly single-rooted teeth. In this study, the authors developed regressive equations using degenerative changes to estimate the living individuals aged between 15 and 40. They recommended using their method for age estimation with the restriction that the quality of OPGs limits the application of the method. In 2017, Timme et al.[16] investigated the validity of Olze et al. stages of regressive changes in 2346 German subjects aged 15 to 70 years. They performed regression analysis and derived regression equations for age estimation. They concluded that this method is inaccurate in older age groups and is applicable and reliable for dental age diagnostics up to 40 years. For the first time in 2019, Hou et al.[17] studied these regressive changes in lower premolars to demonstrate the completion of the 18th year of life. However, no investigations have been reported in the literature studying these regressive changes in the south Indian population. The current study aimed to test whether the degenerative change, i.e., secondary dentin formation in lower premolars, can be used to exclude south Indian individuals under 18 years.

Only one study has explored whether secondary dentin formation in the lower premolars can be used to indicate the completion of 18 years.[17] Hou et al.[17] stated that the respective mean ages for males and females were stage 0: 20.27 and 20.17 years; stage 1: 26.25 and 25.85 years; stage 2: 32.56 and 33 years; and stage 3: 39.19 and 35.55 years, for lower first premolars. For lower second premolars, the minimum age for stage 0 were 19.44 and 18.51 years; stage 1: 24.30 and 24.22 years; stage 2: 31.61 and 15.09 years; and stage 3: 39.28 and 40.05 years, respectively. As in our study, these mean age values are lower than those of the findings of Hou et al.[17], which could be due to the difference in the age range studied, with the upper end of the age extending to 40 years.

In forensic age estimation, determining the proof of being over or under the legally defined age limit is essential. This is due to the errors associated with the method and how these errors impact the fate of the assessed person. In this context, the "minimum-age concept" could be applied, which is designed to prevent the erroneous classification of minors as legal adults.[24] The minimum age is derived from the characteristic value, representing the age of the youngest person in the reference population with the ascertained characteristic value. Our study findings showed that stage 3 of secondary dentin formation in the lower first and second premolars could help indicate the completion of the 18th year of life. The minimum age corresponding to the determined stage 3 of secondary dentin formation was 18.2 years in males and 18.95 in females for the first premolar. Since the minimum age for stage 3 in lower first premolars in the population tested is above 18 years, a majority status, i.e., age over 18 years, seems possible. In this scenario, applying the minimum-age concept ensures that the forensic age of the assessed person is never underestimated.

We found that the repeatability was almost perfect while the reproducibility was moderate. Similar findings were reported by Hou et al.[17], where the reproducibility was reported between 0.285 and 0.652 for various regressive changes. The authors in the original study[15] mentioned that the quality of the radiographic images plays a significant role, and OPGs do not display fine anatomical details. It further leads to increased subjectivity and, therefore, observer error.[25] Therefore, observer training and calibration are needed.

To date, there has been limited research addressing the impact of patients' age and gender on the quality of panoramic radiographs. Gelbrich et al. investigated to explore how the image quality of panoramic radiographs is influenced by the age and gender of the patients.[26] Their findings revealed a consistent trend where image quality tended to decrease with the advancing age of patients. Importantly, this decline in image quality was observed regardless of the patient's gender or the specific imaging device used. Furthermore, Gelbrich and colleagues highlighted the significance of radiation levels emitted by the OPG device in relation to image quality. They noted that when radiation levels are reduced, the image quality becomes more susceptible to age-related changes. Additionally, the research by Dannewitz et al.[27] suggested that it is possible to reduce the tube current by approximately 50% without significantly affecting diagnostic accuracy, despite a potential decrease in subjective image quality. It

is worth noting that in our present study, we lacked specific information about the OPG devices used. This limitation was due to the retrospective nature of our research. Consequently, it is essential for future investigations to delve into the influence of OPG quality on the methodology employed in our study. One of the limitations of this research is its generalizability. It refers to the extent to which the present study's findings can be applied to a broader context, for example, to other populations. It was observed that the grading was not possible in approximately 20% of the first premolars and 20% second premolars of the total sample. Variations in the premolar position were the main reason while developing teeth was the least for the inability to grade premolars. Another important finding is stage 3 of secondary dentin grading in a very small percentage of individuals. This could be due to the smaller age range (14 to 22 years) selected for this study. Therefore, we recommend extending the upper boundary of the age range up to 40 years to verify whether the stage 3 secondary dentin grading can prove the completion of the 18th year of life beyond a reasonable doubt. Further studies are warranted to investigate other characteristics, i.e., attrition, periodontal recession and cemental apposition in lower premolars for indicating the completion of the 18th year of life.

CONCLUSIONS

Based on the findings, it can be concluded that the grading of second dentin formation in lower premolars is of limited value. Even though the presence of stage 3 in the lower first premolar was helpful in indicating the completion of the 18th year of life, the generalizability of this finding remains a question for multiple reasons. Further studies are warranted to investigate other characteristics, i.e., attrition, periodontal recession and cemental apposition in lower premolars for indicating the completion of the 18th year of life in south Indian adolescents and young adults.

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