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PAPER

ANTHROPOLOGY

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The Reliability of Chronological Age Determination by Means of Mandibular Third Molar Development in Subjects in Croatia*

ABSTRACT: The aim of this study was to determine the development of the mandibular third molar and its relation to chronological age. The evaluated material consisted of 979 orthopantomograms of patients of Croatian Caucasian origin aged between 5.7 and 14.6 years. Third molar developmental stages were evaluated according to the stages proposed by Nolla. The frequencies of different stages of mineralization and the mean value of the mineralization of the mandibular third molars with regard to age, gender, and size of the mandible were determined and the coefficient of correlation determined between the age of the subject and the stage of development of the mandibular molars. Statistically significant correlation was determined between mineralization of the mandibular third molar and chronological age of the subjects (boys, mandibular left third molar $r = 0.779$, right third molar $r = 0.793$; girls, mandibular left third molar $r = 0.746$, right third molar $r = 0.725$). It can be concluded that the accuracy of age estimation based on Nolla's method is applicable for Croatian children.

KEYWORDS: forensic science, physical anthropology, third molar, tooth formation, dental age, Croatian population

Because of certain specifics of the third molar, such as its presence (1–4), commencement and end of mineralization (2,5–11), period of eruption (8,12), crown and root morphology (13,14), and its effect on the development of the mandibular dental arch (15–17), it has been the subject of numerous investigations of various dental disciplines. During recent years the commencement and end of mineralization of the third molar has been used in forensic dentistry to determine chronological age (2,4,7,8,10,11,18–27). Many methods can be used to determine mineralization of teeth, and thus of the third molar (7,8,28–34). The most frequently and commonly used method among Croatian orthodontists and pedodontists is the method according to Nolla (29). Many studies to date have indicated the influence of ethnic and geographic factors on the chronology of the development of the third molar (1,11,20,25,35–37). The aim of this study was to analyze the development of the mandibular third molar and its relation to chronological age in subjects aged 6–15 years in Istria (Croatia).

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Materials and Methods

Orthopantomograms of 1000 subjects (500 boys and 500 girls) were examined, which satisfied the following criteria:

- Adequate quality of the orthopantomogram, particularly in the region of the third molar.
- Normal growth, development and dental conditions (without impaction, congenital absence, transposition, and extraction of permanent teeth).
- No history of surgical disease that could affect the presence and development of permanent teeth.

All the orthopantomograms were performed during the period 2002–2005. With regard to age the subjects were divided into 10 age groups (Table 1) with difference between the groups of 1 year (e.g., 5.7–6.6 = 6 years). Twenty-one subjects (11 boys and 10 girls) with hypodontia in segment M2–M2 were excluded from the study. Thus the final number of subjects amounted to 979. There were 95–100 subjects in each age group in an almost identical number of boys and girls. The name and surname of each subject, sex, date of birth, and date of the X-ray was recorded on the orthopantomogram, together with data on the place of birth of the child and both parents. Chronological age of the subject was determined according to the date of birth of the subject and date the X-ray was taken.

TABLE 1—*Distribution of subjects according to age and sex.*

Sex			
Age	Boys *N*	Girls *N*	Total
6	50	48	98
7	49	49	98
8	47	48	95
9	49	47	96
10	49	49	98
11	49	50	99
12	47	50	97
13	50	49	99
14	49	50	99
15	50	50	100
Total	489	490	979

Method for Determination of Teeth Mineralization

The development of the mandibular third molar was assessed according to the method proposed by Nolla (29), who considers that the degree of calcification is based on 10 stages of maturity, from stage 1 (no sign of calcification) to stage 10 (apical end completed), besides 0 stage which indicates the absence of dental germs. The author suggests that the accuracy of the estimation is increased by using a fraction of the stage. Interstages were not used in the study. When the roots of the mandibular third molar were in different stages of mineralization, the mineralization of the root with a smaller degree of development was taken into account for evaluation. All the orthopantomograms were analyzed by the same orthodontist, with over 20 years' experience in the analysis of dental age in accordance with Nolla's method. Interobserver conformity was performed on 100 orthopantomograms selected at random, which were examined again after 1 month.

Statistical Analysis of Results

The results obtained were analyzed by means of the program for statistical analysis of data Statistica 7.1 (StatSoft, Inc., Tulsa, OK). Kappa coefficient was used to determine the degree of conformity. The values of Kappa coefficient ranged from 0.89 to 0.95. Kolmogor-Smirnov test was used to test normality of distribution of the developmental stages of both mandibular third molars in each sex and age group. As normality of distribution of the developmental stages of both mandibular third molars was determined they were shown by arithmetic mean (mean) and standard deviation (SD). In order to determine differences in the values of the degree of mineralization between the boys and girls and between some age groups, multivariate analysis of variance (MANOVA) was performed, and analysis of variance (one-way ANOVA). Correlation analysis of the degree of mineralization between the left and right third molar and age of the subjects was carried out according to Pearson's coefficient of correlation. In the examined age groups significance of the frequency of the occurrence of a certain degree of mineralization was determined by χ^2 test. All levels of statistical significance were determined at the level $p < 0.05$.

Results

Distribution of the degree of mineralization of the mandibular right third molar in boys in different age groups is presented in Table 2, and of the mandibular left third molar in Table 3. Statistically significant frequency of the occurrence of a certain degree of mineralization of the mandibular right third molar was determined (Table 2: $\chi^2 = 255.0$; $p < 0.001$) and of the mandibular left third molar (Table 3: $\chi^2 = 251.0$; $p < 0.001$) in boys in different age groups. Mineralization of the mandibular third molar commenced after the sixth year when it was established that 14% of boys (Tables 2 and 3) had stage one mineralization. With 9 years 59.2% of the boys (Tables 2 and 3) had stages 1–6 mineralization. In 12-year-old boys 55.3% (Tables 2 and 3) had mineralized crown of the third molar (stage 6) and the beginning of root mineralization (stage 7). In 15-year-old boys mineralization of the roots of the third molar was present in various stages in 27% of the boys (Tables 2 and 3). From 11 to 15 years mineralization of the roots of the third molar was present in various stages in 16.5% of boys.

TABLE 2—*Distribution of the frequency of the occurrence of a certain degree of mineralization of the mandibular right third molar in boys in different age groups.*

		Degree of Mineralization										
Age		0	1	2	3	4	5	6	7	8	9	10
6	*N*	42	8									
	%	84	16									
7	*N*	27	19	3								
	%	55	39	6								
8	*N*	19	23	5								
	%	40	49	11								
9	*N*	21	20	2	1	4		1				
	%	43	41	4	2	8		2				
10	*N*	14	9	8	4	7	6	1				
	%	29	19	16	8	14	12	2				
11	*N*	10	6	6	2	1	7	14	3			
	%	20	12	12	4	2	14	30	6			
12	*N*	8	2	4	1	1	4	19	8			
	%	17	4	9	2	2	9	40	17			
13	*N*	7				5	13	17	7	1		
	%	14				10	26	34	14	2		
14	*N*	6					9	25	3	6		
	%	12					19	51	6	12		
15	*N*	7				1	9	209	5	7	1	
	%	14				2	18	40	10	14	2	

TABLE 3—*Distribution of the frequency of the occurrence of a certain degree of mineralization of the mandibular left third molar in boys in different age groups.*

		Degree of Mineralization									
Age		0	1	2	3	4	5	6	7	8	9
6	*N*	44	6								
	%	88	12								
7	*N*	30	16	3							
	%	61	33	6							
8	*N*	23	20	4							
	%	49	43	8							
9	*N*	19	19	5	2	3		1			
	%	39	39	10	4	6		2			
10	*N*	14	10	7	5	6	6	1			
	%	29	21	14	10	12	12	2			
11	*N*	7	4	8	5	1	6	15	3		
	%	14	8	16	10	2	13	31	6		
12	*N*	7	4	4		2	5	19	6		
	%	15	9	9		4	10	40	13		
13	*N*	7				6	11	18	7	1	
	%	14				12	22	36	14	2	
14	*N*	6				1	9	24	4	5	
	%	12				2	18	50	8	10	
15	*N*	6				1	10	19	6	7	1
	%	12				2	20	38	12	14	2

TABLE 4—*Distribution of the frequency of the occurrence of a certain degree of mineralization of the mandibular right third molar in girls in different age groups.*

		Degree of Mineralization								
Age		0	1	2	3	4	5	6	7	8
6	*N*	36	12							
	%	75	25							
7	*N*	31	17	1						
	%	63	35	2						
8	*N*	23	13	7	5					
	%	48	27	15	10					
9	*N*	11	16	5	8	2	3	1	1	
	%	23	35	11	17	4	6	2	2	
10	*N*	7	5	6	10	3	11	7		
	%	14	10	12	20	6	22	14		
11	*N*	4	1	6	4	9	10	14	2	
	%	8	2	12	8	18	20	28	4	
12	*N*	10	3	3	1	6	5	18	4	
	%	20	6	6	2	12	10	36	8	
13	*N*	8				7	14	15	5	
	%	16				14	29	31	10	
14	*N*	7				2	17	21		3
	%	14				4	34	42		6
15	*N*	8					26	4	6	6
	%	16					52	8	12	12

Table 4 (mandibular right third molar) and Table 5 (mandibular left third molar) show distribution of the stage of mineralization of the mandibular third molar in girls in different age groups. Statistically significant difference was determined in the frequency of the occurrence of a certain degree of mineralization of both mandibular third molars in girls in different age groups ($\chi^2 = 250.1$; $p < 0.001$ mandibular right third molar; $\chi^2 = 262.3$; $p < 0.001$ mandibular left third molar). Mineralization of the mandibular third molar commenced after the sixth year when it was determined that 24.5% of the girls had stage 1 mineralization (Tables 4 and 5). After 9 years 72.3% of the girls (Tables 4 and 5) had stage 1–7 mineralization. In 9-year-old girls 43% (Tables 4 and 5) had mineralized crown of the third molar (stage 6) and commencement of mineralization of the roots (stage 7). With the exception of one case (Table 5) the first stage of root mineralization of the third molar occurred after the age of 11. From 11 to 15 years root mineralization of the third molar was present in various stages in 10.6% of the girls. After the age of 13 none of the subjects of either sex had mineralization stages 1, 2, and 3, and it can therefore be concluded that hypodontia of the third molar in the examined population can be decided after the age of 13 years. Hypodontia of mandibular third molars was determined in 13.0% of the boys and 15.4% of the girls.

TABLE 5—*Distribution of the frequency of the occurrence of a certain degree of mineralization of the mandibular left third molar in girls in different age groups.*

		Degree of Mineralization								
Age		0	1	2	3	4	5	6	7	8
6	*N*	36	12							
	%	75	25							
7	*N*	35	13		1					
	%	71	27		2					
8	*N*	21	13	9	5					
	%	44	27	20	10					
9	*N*	15	13	3	9	3	2	1	1	
	%	32	28	6	20	6	4	2	2	
10	*N*	4	4	11	11	2	10	7		
	%	8	8	22	22	4	21	15		
11	*N*	4	1	6	5	10	9	13	2	
	%	8	2	12	10	20	18	26	4	
12	*N*	11	3	1	4	5	5	18	3	
	%	22	6	2	8	10	10	36	6	
13	*N*	8				8	13	14	6	
	%	16				16	27	29	12	
14	*N*	8				4	15	18	4	1
	%	16				8	30	36	8	2
15	*N*	7				2	25	5	6	5
	%	14				4	50	10	12	10

TABLE 6—*Mean values of mineralization of the mandibular right third molar in boys and girls in the examined age groups according to gradation method used by Nolla.*

	Degree of Mineralization			
	Boys		Girls	
Age	*N*	Mean ± SD	*N*	Mean ± SD
6	50	0.16 ± 0.37	48	0.29 ± 0.5
7	49	0.51 ± 0.62	49	0.41 ± 0.61
8	47	0.7 ± 0.66	48	0.87 ± 1.24
9	49	1.0 ± 1.35	47	1.91 ± 2.08
10	49	2.06 ± 1.86	49	3.18 ± 2.03
11	49	3.43 ± 2.56	50	4.18 ± 1.93
12	47	4.4 ± 2.58	50	3.94 ± 2.53
13	50	4.88 ± 1.98	49	4.55 ± 2.04
14	49	5.39 ± 1.73	50	4.86 ± 1.95
15	50	5.38 ± 1.83	50	4.88 ± 1.8

Correlation of the degree of mineralization of the left mandibular third molars and age was demonstrated by coefficient of correlation: $r = 0.779$; $p < 0.001$ for boys, and $r = 0.746$; $p < 0.001$ for girls. Correlation of the degree of mineralization of the right mandibular third molars and age was also demonstrated by significant coefficient correlation: $r = 0.793$; $p < 0.001$ for boys and $r = 0.725$; $p < 0.004$ for girls.

No significant difference was determined between mineralization of the right and left third molars: $r = 0.959$; p 0.001.

Table 6 shows mean values (mean ± SD) of the degree of mineralization of the mandibular right third molar in boys and girls in the examined age groups. Table 7 shows mineralization of the mandibular left third molar in boys and girls in the examined age groups. Comparison of the degree of mineralization of the mandibular right and left third molar between the boys and girls in all age groups showed significant differences (all $p < 0.05$). Mineralization of the mandibular third molar on both sides, with the exception of the seventh year (Table 6) was more advanced from the sixth to the 11th year in girls, and from the 12th to the 15th year in boys

TABLE 7—*Mean values of mineralization of the mandibular left third molar in boys and girls in the examined age groups according to gradation method used by Nolla.*

	Degree of Mineralization			
	Boys		Girls	
Age	*N*	Mean ± SD	*N*	Mean ± SD
6	50	0.12 ± 0.33	48	0.25 ± 0.44
7	49	0.45 ± 0.61	49	0.35 ± 0.59
8	47	0.6 ± 0.65	48	0.96 ± 1.03
9	49	1.08 ± 1.32	47	1.8 ± 2.13
10	49	2.02 ± 1.86	49	3.25 ± 1.85
11	49	3.67 ± 2.39	50	4.1 ± 1.92
12	47	4.37 ± 2.53	50	3.82 ± 2.53
13	50	4.5 ± 2.03	49	4.5 ± 1.86
14	49	5.3 ± 1.79	50	4.7 ± 1.8
15	50	5.5 ± 1.90	50	4.9 ± 1.8

(Tables 6 and 7). Significant difference was also determined of the degree of mineralization in both mandibular third molars, with regard to the age of the subject, regardless of the sex ($F = 89.2$; $p < 0.001$ right third molar; $F = 94.88$; $p < 0.001$ left third molar).

Discussion

In this study, the possibility of determining chronological age on the basis of mineralization of the mandibular third molar was examined. The method according to Nolla was used for the determination of mineralization of the third molar (29). Although this method was not used by many authors, it was applied in this study because of several reasons: (i) the results obtained by this method are no less reliable than those obtained by other methods (3,23), (ii) it offers two more degrees of mineralization of the crown than the more frequently used method according to Demirijan et al. (33), (iii) the reliability of the method greatly depends on the experience of its application, and the author who performed valorization of the mineralization of the third molars in this study has more than 20 years of experience using the Nolla method, (iv) intraobserver agreement in its application is well over 90% (38). Mineralization of the mandibular third molars in the subjects of this study began after the age of six.

A considerable increase in the number of subjects with initial mineralization occurred only after the eighth and ninth year. The results of this study agree with those of numerous other authors (2,5,7,9,24). Mineralization of the crown of the third molar in different stages occurred up until the 15th year. However, already after the 12th year there was a significant increase in the number of subjects in whom the mandibular third molar crown was mineralized. Bolanos et al. (3) applied the Nolla method and determined mineralization of the mandibular third molars. They assessed that the crown of the mandibular third molars was mineralized on average by the age of 13.5 years. Comparing the findings of this study with those by Bolanos et al. (3) with regard to the average age of different stages from 1 to 6, there is no difference between the Spanish and Croatian population for the first and second stage. The third degree of mineralization was detected earlier in the Croatian population, while in the Spanish population it was the fifth and the sixth stage. With regard to the results of the end of the mineralization of the crowns of the mandibular third molars determined by other methods, mineralization of the crowns of the mandibular third molars occurred earlier in subjects in Israel (39), South Africa (40), Japan (2), Sweden (18), and Turkey (24), compared to the Croatian subjects. The end of mineralization of the crowns of the mandibular third molars in Croatian subjects corresponded to the age of Austrian (37) and German (41) subjects. According to all the above-mentioned authors, the period of time when the end of mineralization of the crown of mandibular third molar can be expected differ in more than 2 years. In our subjects, mineralization of the roots of the third molar commenced after the age of 11 years. According to Kullman et al. (7,33) it occurs after 15 years. According to different authors, mineralization of mandibular third molar roots extends up to the age of 18.5 years (24), 20 years (8,42), 21 years (10), and 22.8 years (7). According to Robetti et al. (43) the end of mineralization of the mandibular third molar in the Italian population can be prolonged from 18.1 to 25 years in girls, and from 18.4 to 25.6 in boys. In this study, it was found that after the age of 13 years there were no cases of the occurrence of the first stage of mineralization of the mandibular third molar. This would be the upper limit when hypodontia of the third molar could be considered. Gonzales and Del Rosario (1) and Rozkovcov et al. (4) agree with this age limit, while Tijanić (5) and Orhan et al. (24) raise this limit to 14 years and Bolanos et al. (3) to 14.8 years.

In this study, differences in mineralization of the mandibular third molar were determined with regard to sex. Up until 12 years mineralization was more advanced in girls, and later in boys. The results of this study confirm those of Prieto et al. (11) and Stella Martin et al. (26) who found that the stages connected with mineralization of the roots of the mandibular third molar were more advanced in boys. More advanced mineralization of this tooth in boys was also found in subjects investigated by Willershausen et al. (10), Harris (21), Sisman et al. (25), Meinl et al. (37), and Gunst et al. (42). However, Daito et al. (2) found that it was more advanced in girls. Kullman et al. (7), Friedrich et al. (22), and Olze et al. (35) did not find differences in the rhythm of mineralization with regard to sex. In this study, no differences were found between the rhythm of mineralization of the mandibular left and right third molar. Similar data have been recorded by many other authors (2,10,20,24,29,37,39). Tijanić (5) found differences in the degree of mineralization of the right and left mandibular third molar in 21% of subjects. In this study, the average stage of mineralization of the mandibular third molar in both sexes and for both third molars increased in like manner from the sixth to the 15th year. If we exclude cases with 0 stage mineralization (cases with hypodontia of the mandibular third molar) from the age groups after 13 years, the average stage in such age groups would be significantly higher. In this study, significant correlation was determined between the degree of mineralization of the mandibular third molar and chronological age of the subjects of both sexes. The results of the study were obtained by conforming to the conditions set up by Staaf et al. (44) as crucial for the accuracy of determination of dental age, prior experience in determination of dental age by the Nolla method, and exclusion from the study of those subjects who had one or more hypodontia of the third molars, which according Rozkovcov et al. (4) significantly influences the rhythm of mineralization of other teeth including the third molars.

The reliability of determination of the age of subjects by means of the mineralization of the mandibular third molar has also been affirmed by other authors (7,10,18,27,37). Many of the authors mentioned in this study consider that in determination of the chronological age of subjects by means of mineralization of teeth the specific rhythm of development of teeth of every population has to be observed.

Conclusions

- Mineralization of the crown of the mandibular third molar is completed by the 15th year and mineralization of the roots commences at the earliest after the 10th year.
- Significant differences exist between mineralization of the mandibular wisdom tooth with regard to sex.
- Accuracy of age estimation based on Nolla's method is applicable for Croatian children.

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