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Forensic Anthropology Population Data

# Cameriere's third molar maturity index in assessing age of majority

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ABSTRACT

Estimation of chronological age of an individual is one of the main challenges in forensic science. Legally to be able to treat a person as a minor or an adult, it is necessary to determine whether their age of majority (if they are older or younger than 18, in most countries). Methods for estimating age are especially important when an individual in question lacks personal documents or other means of identification. As the dental age differs in various populations, the aim of this study was to evaluate applicability of third molar method for assessing age of majority in Croatia.

Cameriere's third molar maturity index ($I_{3M}$) value of 0.08, measured by the open apices of the teeth, was verified in sample of 1336 panoramic images aged between 14 and 23 years. Chronological age gradually decreased as $I_{3M}$ increased in both genders. Males showed statistically significant advanced maturation when $I_{3M}$ was between 0.0 and 0.3 value. The results indicate that the sensitivity of the test for 0.08 value was 84.3% (95%CI 80.6%, 87.5%) for females and 91.2% (95%CI 88.7%, 93.1) for males. Specificity was 95.4% (95%CI 92.5%, 97.5%) and 91.9% (95%CI 88.8%, 94.3%). The proportions of accurately classified males were 88.8% and that of females 91.5%. The estimated post-test probabilities, of individuals, in other word the probability that a Croatian individual with an $I_{3M} < 0.08$ is 18 years or older is 94.5% for females, and 96.5% for males.

With high accuracy, the third molar maturity index should be used as a determinant of the age of majority in Croatia.



## 1. Introduction

Estimation of the real age of individuals is one of the main challenges in forensic science. Most methods used today can be divided to either non-medical procedures that include personal check and identification documents, medical procedures that include physical and radiological examinations or those that are the combination of the two [1,2]. Study Group on Forensic Age Diagnostics (AGFAD) presented criteria for age estimation in living individuals and listed: physical examination and anthropometric measures, inspection of sexual maturation, radiological examination of left hand or computer tomography (CT) examination of the clavicle in cases when the hand skeletal development is completed, and dental examination with radiologic analyses of the dentition [3]. In line with AGFAD guidelines, an additional radiographic or CT examination of collar bones is recommended to evaluate the age of persons who are assumed to be at least 18 years old [2]. Estimation methods that involve hand and wrist bones are not suitable for adults because skeletal development of hand bones is completed by the age of 17 years in girls and 18 years in boys [4].

Croatian War for Independence, which lasted from 1991 to 1995, lead to a large number of persons still presumed missing, and

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caused particular challenges for experts in forensic odontology in identifying these victims [5–7]. Furthermore, many countries within the European Union and countries such as Norway or Switzerland, have been seeing a large influx of foreigners pursuing settlement or political asylum [8]. Croatia joined the European Union in 2013 and was identified as one of the preferred countries for immigration, transition and asylum seeking. One of the main questions in penal and criminal law and general legislation – in Croatia and other nations inside European Union – is whether the person is older or younger than 18 years, an adult or a minor. This is especially pertinent when an individual is without personal documents or other means of identification. Beside 18 years cut-off, there are several important ages cut-offs in Croatian legislation. Depending on the age, laws impose different misdemeanour and penalties. Persons younger than 14 years are considered children, persons aged 14–16 years are considered younger minors, persons aged 16–18 years are elder minors, persons aged 18–20 are consider younger adults, while those older than 21 years are considered adults [9]. The age limit of 15 years is important in criminal law with respect to offences of sexual abuse and exploitation of children and is a minimum required age for acquiring employment [9,10]. Dental examination methods on growing teeth are today either the evaluation of clinical emergence of teeth or the radiographic evaluation of the mineralization of crowns and roots of primary and permanent dentition [11,12]. Assessment of tooth mineralization can be done by: (1) applying methods which estimate the developmental stages of the target group of teeth, (2) measuring apices of dental roots with incomplete development, (3) using the atlas growth and teeth development index? [12–15]. Furthermore, assessment of regressive changes in primary and permanent teeth can also be used [16–22]. Age estimation by analysis of mineralization of permanent teeth, excluding third molars, is highly effective until the age of 13 years from the development of second molars [23,24]. Assessment of third molars is applied for those between 13 and 23 years of age [23,25–27]. Mincer et al. [28] studied third molars of American whites and blacks, aged 14–24, using Demirjian staging system (A–H), to estimate their chronological age. Cameriere at al. [29], for determining 18 years of age introduced a method based on the relationship between age and the third molar maturity index ($I_{3M}$), calculated by measuring the open apices of the third molar. Recent studies from Italy on 397 subjects aged between 13 and 22 years from Milan area another from Albania on 286 subjects aged between 15 and 22 years showed a very high percentage of correctly classified individuals and estimated post-test probability [30,31]. As no studies so far have evaluated the $I_{3M}$ method on individuals from the Republic of Croatia, it was our goal to do so on a young adult Croatian sample.

## 2. Materials and methods

### 2.1. Sample

Panoramic radiographs (OPTs) of 1416 Croatian children and adolescents between 14 and 23 years of age, without recorded developmental abnormalities in either dental or medical records, were evaluated. The sample was randomly selected from the patients attending the Split-Dalmatia County Community Health Centre, and Dental Clinic and Radiology Department, School of Dental Medicine, University of Split (Croatia). Those individuals of unknown age or those with no third molars or showing badly rotated teeth, 80 (5.64%) were excluded from the evaluation sample. In total 1336 OPTs (758 females) were evaluated (Table 1).

Identification number of each patient, gender, dates of birth, and X-ray were recorded. In Croatia, each patient signs a general agreement with medical and dental institutions allowing dental record and radiographs to be used only for research and educational purposes, while preserving anonymity of the person. Agreement for usage of previously taken radiographs was approved by the Ethics Committee for Research Involving Human Subjects of the School of Dental Medicine, University of Zagreb, Croatia, and the study was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki (DoH).

**Table 1**
Sample of panoramic radiographs from Croatia according to sex and age categories.

| Age categories | Males | Females | Total |
|---|---|---|---|
| 14 | 63 | 48 | 111 |
| 15 | 70 | 96 | 166 |
| 16 | 63 | 79 | 142 |
| 17 | 63 | 84 | 147 |
| 18 | 60 | 54 | 114 |
| 19 | 58 | 65 | 123 |
| 20 | 50 | 84 | 134 |
| 21 | 50 | 74 | 124 |
| 22 | 49 | 95 | 144 |
| 23 | 52 | 79 | 131 |
| Total | 578 | 758 | 1336 |

### 2.2. Measurements

All OPTs were done and stored in digital form using Orthopantomograph OP200D (Instrumentarium Dental, Tuusula, Finland) and images were recorded as computer files in JPG format. The digital images were examined by using the Corel Draw software package (Corel Draw v.12.0, 2003, Corel Corporation, Ottawa, Canada). Only the left permanent third lower molars, tooth No. 38 according to classification by Federation Dentaire Internationale (F.D.I.), were evaluated. Dental age estimation was performed according to the Cameriere et al. [29] method. The apical ends of the roots of the left lower third molar of each individual were analyzed and the third molar maturity index, $I_{3M}$, was defined as follows: if the root development of the third molar is complete, i.e., the apical ends of the roots are completely closed, then $I_{3M} = 0$, otherwise $I_{3M}$ was calculated as the sum of the widths of the inner margins of the two open apices than divided by tooth length. Maturity index $I_{3M}$ is evaluated in a similar way to the ratio $A_i$ to $L_i$, when $i = 6$ or 7, as reported for the other two teeth with two roots in Cameriere et al. [32].

### 2.3. Statistical analysis and data management

SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL) and MS Excel 2003 (Microsoft Office 2003, Microsoft, Redmond, WA) were used for all statistical analysis and data management. The evaluation of maturity index $I_{3M}$ was done by the first author. Real age was calculated and recorded as difference between dates of OPT and dates of birth. A cut-off value of 0.08 was used for consideration if a person was a minor of 18 years of age or older, as described in DeLuca et al. [30]. Intra-class correlation coefficient (ICC) of $I_{3M}$ was used for intra-observer agreement and inter-observer agreement among three different observers. Cohen Kappa was used for intra-observer agreement and Fleiss Kappa was used for inter-observer agreement among three different observers for agreement in selection on adult ($I_{3M} < 0.08$) or minor ($I_{3M} \geq 0.08$). Fifty randomly selected OPTs were re-examined one month after examination by the first and two other observers. The sensitivity of the test, $p_1$ (i.e., the proportion of subjects older than or 18 years of age who have $I_{3M} < 0.08$) was evaluated, together with specificity $p_2$ (i.e., the proportion of individuals younger than 18 who have $I_{3M} \geq 0.08$). The third molar index has been shown to be efficacious in discriminating individuals below and above the age of 18 years

**Table 2**
Summary statistics of chronological age according to third molar maturity index ($I_{3M}$): number of individuals (*N*), Mean ± standard deviation (SD), minimum value (Min), 1st quartile (Q1), median, 3rd quartile (Q3) and maximum value (Max) of age distribution for each $I_{3M}$ class, for females and males.

| $I_{3M}$ | Female | | | | | | | Male | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | Mean ± SD | Min | Q1 | Median | Q3 | Max | *N* | Mean ± SD | Min | Q1 | Median | Q3 | Max | *P*** |
| [0,0.04) | 330 (237)* | 21.66 ± 1.46 | 17.63 | 20.53 | 21.89 | 22.92 | 23.99 | 277 (207)* | 21.05 ± 1.83 | 16.79 | 19.51 | 21.13 | 22.60 | 23.94 | **<0.001** |
| [0.04,0.08) | 64 | 19.67 ± 1.84 | 16.16 | 18.26 | 19.62 | 20.69 | 23.53 | 35 | 18.95 ± 1.41 | 16.30 | 18.10 | 18.81 | 19.55 | 23.17 | **0.03** |
| [0.08,0.3) | 163 | 17.80 ± 1.82 | 14.22 | 16.65 | 17.33 | 16.64 | 23.07 | 99 | 17.15 ± 1.45 | 14.20 | 16.03 | 16.96 | 18.04 | 21.20 | **0.003** |
| [0.3,0.5) | 98 | 16.14 ± 1.40 | 14.02 | 15.13 | 15.90 | 16.85 | 21.51 | 82 | 15.78 ± 1.12 | 14.03 | 14.85 | 15.85 | 16.49 | 18.78 | 0.06 |
| [0.5,0.7) | 42 | 16.38 ± 1.45 | 14.18 | 15.27 | 15.91 | 17.45 | 19.40 | 28 | 15.85 ± 1.07 | 14.19 | 14.89 | 15.67 | 16.93 | 17.96 | 0.08 |
| [0.7,0.9) | 25 | 15.65 ± 1.30 | 14.13 | 14.72 | 15.58 | 16.07 | 19.21 | 25 | 15.38 ± 1.35 | 14.03 | 14.26 | 15.12 | 15.92 | 18.90 | 0.47 |
| [0.9,1.7) | 36 | 15.17 ± 0.90 | 14.05 | 14.44 | 15.05 | 15.85 | 17.63 | 32 | 15.05 ± 0.93 | 14.08 | 14.24 | 14.71 | 15.76 | 17.28 | 0.59 |

Bolded significance at the level of <0.05%.
* *n* – number of individuals with closed apex.
** Independent samples *t*-test.

by the post-test probability of being 18 years of age or more (i.e., the proportion of individuals with $I_{3M} < 0.08$ who are older than or equal to 18 years). According to Bayes' theorem, post-test probability may be written as:

$$p = \frac{p_1 p_0}{p_1 p_0 + (1 - p_2)(1 - p_0)} \quad (1)$$

where $p$ is post-test probability and $p_0$ is the probability that the individual in questioning is 18 or older, given that individual is aged between 14 and 23 years, which represents the target population. Probability $p_0$ was evaluated with data from the Croatian Bureau of Statistics [33] and considered to be 60.1% for females and 61.0% for males.

## 3. Results

The mean ages between genders varied across $I_{3M}$ classes (Table 2) and the differences were statistically significant for the first three age groups. In particular, the mean age of females was higher than that of males when $I_{3M}$ was between 0.0 and 0.04 ($p < 0.001$). ICC of intra-rater and inter-raters agreement of $I_{3M}$ were 0.95 (95%CI 0.92, 0.97) and 0.90 (95%CI 0.84, 0.94), respectively. Cohen Kappa for intra-rater agreement was 0.94 (95%CI 0.83, 1.05) and Fleiss Kappa for inter-rater agreement among three observers was 0.89 (95%CI 0.73, 1.05). Real age gradually decreased as $I_{3M}$ increased for both genders (Fig. 1). Males showed statistically significant advanced maturation when $I_{3M}$ was between 0.0 and 0.3 value. As a result, the efficiency of the cut-off value of 0.08, suggested by Cameriere et al. [29] and the validity of $I_{3M}$ with Croatian sample used for training purposes was analyzed separately for females and males. The results are presented in two 2 × 2 contingency tables (Tables 3 and 4), which list the numbers of individuals with $I_{3M} < 0.08$ who are older than 18 years, those with $I_{3M} < 0.08$ who are younger than 18 years, those with $I_{3M} \geq 0.08$ who are older than 18 years and those who have $I_{3M} \geq 0.08$ and are younger than 18 years. Close association was found between adult age and positive test that $I_{3M}$ is < 0.08 both in females (Table 3) and males (Table 4).

In females, 673 out of 758 individuals were accurately classified, 88.8% (95%CI 86.3%, 90.9%). These results show that the test for sensitivity ($p_1$) for females, the proportion of individuals being 18 years of age or older whose test was positive,

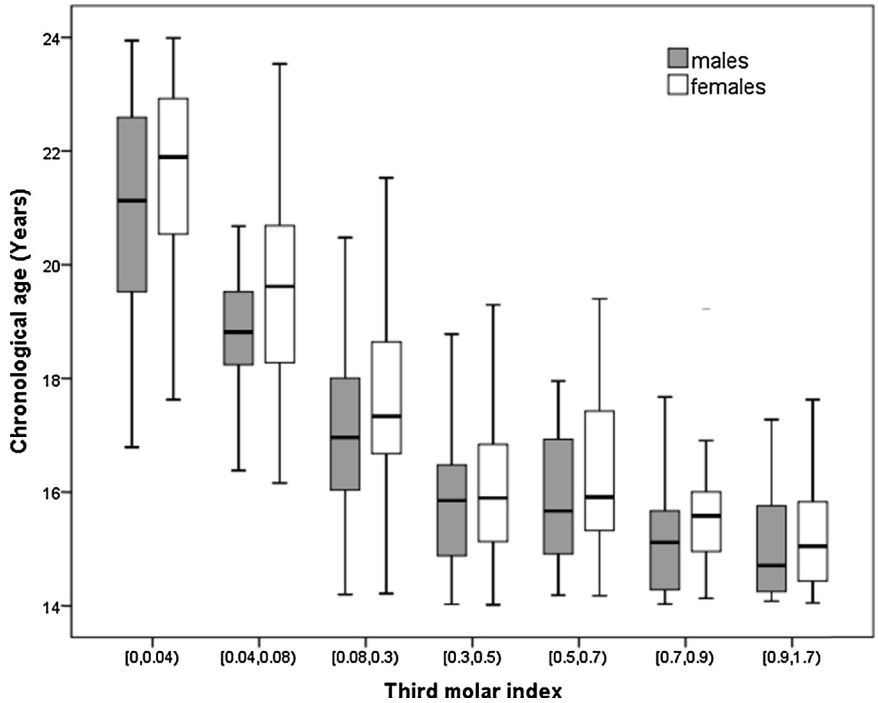


**Fig. 1.** Boxplot of relationship between chronological age and Cameriere's third molar index of open apices of mandibular left third molar, in females and males. Boxplot shows median and inter-quartile ranges while whiskers are lines extending from box to maximum and minimum ages, excluding outliers.

**Table 3**
Contingency table describing discrimination performance of the test for females.

| Test | Age | | Total |
|---|---|---|---|
| | ≥18 | <18 | |
| $I_{3M} < 0.08$ | 380 | 14 | 394 |
| $I_{3M} \geq 0.08$ | 71 | 293 | 364 |
| Total | 451 | 307 | 758 |

**Table 4**
Contingency table describing discrimination performance of the test for males.

| Test | Age | | Total |
|---|---|---|---|
| | ≥18 | <18 | |
| $I_{3M} < 0.08$ | 291 | 21 | 312 |
| $I_{3M} \geq 0.08$ | 28 | 238 | 266 |
| Total | 319 | 259 | 578 |

was 84.3% (95%CI 80.6%, 87.5%) and test for specificity ($p_2$), the proportion of individuals younger than 18 years whose test was negative, was 95.4% (95%CI 92.5%, 97.5%). The estimated post-test probability ($p$), that a subject positive on the test (i.e., $I_{3M} < 0.08$) was 18 years of age or older, was 96.5% (95CI 95.9%, 97.0%).

In males, 539 out of 578 individuals were accurately classified, 91.5% (95%CI 89.0%, 93.5%). These results show that the test for sensitivity ($p_1$) for males was 91.2% (95% CI 88.7%, 93.1%) and the test for specificity ($p_2$) was 91.9% (95%CI 88.8%, 94.3%). The estimated post-test probability ($p$) was 94.5% (95CI 94.3%, 94.7%).

## 4. Discussion

Our findings show earlier maturation of third molars in boys when compared to girls. The results of mean age within first three $I_{3M}$ groups in females were statistically significantly higher compared to males. Results of discrimination performance of the test, using 0.08 value of maturity index $I_{3M}$ for cut-off value, were good. Specificity was better in females (95.4%) compared to males (91.9%) while sensitivity was better in males (91.2%) compared to females (84.3%). These findings are valuable because the method was tested on a separate, larger, balanced sample of different nationality than that used for cut-off value modelling for $I_{3M}$ and the previously tested population samples from Italy [29,30]. Technically, the possibility of clear and unambiguous measurement of anatomical projections or estimates of developmental stages is a prerequisite of objective and accurate assessment of anthropological development.

Mincer et al. [28] pointed to the low accuracy of approach using only eight developmental stages on third molars for the target age span of 11 years of age. The first three stages (A–C) are rare, and the remaining small number of stages of development, only four (D–G), are not evenly distributed in the age span. The terminal eighth stage (H) cannot be associated with a specific chronological age. For answering the question of whether or not an individual is an adult or a minor, Mincer et al. [28] found that terminal stage H of lower molars indicate empirical probability of 92.2% in females and 90.1% in males, respectively. Stage G indicates empirical probability of 69.8% and 56.0%, respectively that the person is ≥18 years of age. Stages F and lower indicate empirical probability under 50%. Study also showed that mean ages were over 18 years for stage G and under 18 years of age for stage F. Comparable findings for stages H and G were presented by Cameriere et al. [29]. Cameriere et al. [29] also showed that when H stage was exchanged with G stage for cut-off of adult age, sensitivity of the model increased, but specificity and post-test probability significantly decreased. Another study, comparing Demirjian (DT) and Modified Scoring System (MST) techniques [28] was performed among Brazilian young adults [34]. The findings have shown that DT presented better reproducibility than MST, and both underestimated the ages in about 6 months.

For the medical and legal purposes, it is important to minimize the proportion of false negative results – i.e. individuals who are ≥18 years of age but identified as minors. Improvement in better classification using staging of development of third molar could be reached by using a system with a greater number of stages – in particular during the period of development of the third molar [25]. Thevissen et al. [25] also showed that between eight procedures based on the developmental stages, Gleiser and Hunt [35] and Mooress et al. [36] were the best techniques. Selection of method should not compromise feasibility of correctly registering all stages where many stages can lead to errors in the classification [25].

In this manner, specific cut-off value of the tested continuous variable $I_{3M}$ in the evaluated sample and classification of individuals ≥ 18 years and <18 years, could be determined by receiver operating characteristic (ROC) curve. Value of 0.08 for $I_{3M}$,sugested by Cameriere et al. [29], were found in the Croatian sample thus making these results comparable with those in previous studies. When compared with results of study by De Luca et al. [30], sensitivity in both genders was slightly better in Croatian sample, while specificity and accurate classification were slightly higher in the Italian sample. Estimated post-test probability was equal. Study on Albanian sample also showed substantial success of suggested value for $I_{3M}$, with the 87.4% and 92.5% correctly classified females and males, respectively[31].

It is important to emphasize that in assessment of each individual case, ethnicity, hereditary, climate, and other considerations should also be taken into consideration. Studies on diet and extreme nutritional condition found no important influence in average timing of teeth development and mineralization [37,38]. Several studies have discussed the importance of population and ethnicity, with miscellaneous results [39–41]. Lewis and Senn [41] concluded in their review of a third molar age estimation techniques in different American population groups that population specific studies should be used when estimating dental age from third molars because of displayed varying rates of development and sexual dimorphism of third molars. Others, recent studies by Thevissen et al. [25,42] reported that no important differences was found in the degree of third molar development among the 14 examined countries which suggest that ethnical or national differences in third molar development are of clinically minor effect.

Possible gender difference in $I_{3M}$ value should be statistically evaluated, and sample composition and ethnicity should be weighted judiciously before any decision about changing the cut-off value be made. Inclusion of a larger sample from different nations may determine the new reference values [43,44]. In our study, eighty individuals (5.64%) were not assessed because the third molar was missing or it was impossible to measure their apices and lengths. De Luca et al. [30] pointed that some individuals could intentionally remove their third molars to prevent their assessment of age. It should also be noted that there are other useful indicators for assessing skeletal age with applicable confidence intervals. Medial clavicle epiphysis was most commonly studied, and a recent study of epiphyseal fusion of knee opened up new possibilities in forensic and legal applications, however the methods validity should be checked in other population samples, and if necessary, adjusted. Special attention should also be given to vulnerable group who needs enhanced protection and special provisions, as well as unaccompanied minors who enter the EU for harassment and seeking protection,

family unification, economic and aspirational reasons, transit to other member states, victims of trafficking and smuggling, health reasons and other [1]. When assessing age, it is especially important that minors are not classified as adults because it may lead to violations of their legal rights, including their right to asylum, exploitation by adults or health care services [30].

Finally, our study is the first to highlight the efficacy of $I_{3M}$ in estimating 18 years cut-off age in Croatian population, and showed consistent success over previously tested samples. Other skeletal system methods should be evaluated in this population including medial clavicle epiphysis and epiphyseal fusion of knee with the intention to develop more accurate methods for establishing the real age of examined individual of target age groups.

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