The third molars for indicating legal adult age in Montenegro

Marija Antunovića, Ivan Galicb, Ksenija Zelicc,⁎, Nenad Nedeljkovicd, Emira Lazicd, Marija Djuricc, Roberto Camerieree

a University of Montenegro, Medical Faculty, Clinical Centre of Montenegro, Ljubljanska bb, 81000 Podgorica, Montenegro
b University of Split, School of Medicine, Spinčićeva 2, 21000 Split, Croatia
c University of Belgrade School of Medicine, Laboratory for Anthropology, Institute of Anatomy, Dr Subotica 4, 11000 Belgrade, Serbia
d University of Belgrade School of Dentistry, Clinic for Orthodontics, Gastona Gravijeva 2, 11000 Belgrade, Serbia
e AgEstimation Project, University of Macerata, Macerata, Italy

ARTICLE INFO

Keywords:
Montenegro
Age estimation
Third molar maturity index
Orthopantomogram
Legal adult age

ABSTRACT

Aim: From a medico-legal and penalty point of view, it is essential to conclude if an individual is a minor or an adult (18 years of age). Methods based on the third molar development have been used for this purpose. The present article aims to verify the Nolla's stages of mineralization of the third molars and a third molar maturity index (I3M) which is based on the measures of the projections of open apices normalized by their height in the sample of Montenegrins.

Method and sample: The sample consisted of 683 panoramic radiographs (324 males and 359 females) between 13 and 24 years of age. The specific mineralization stages of Nolla and the cut-off value of I3M < 0.08 was used to discriminate adults and minors.

Results: The best performance in discriminating adults and minors was for I3M < 0.08 followed by the Nolla stage 9. For I3M < 0.08 the results demonstrated high sensitivity of 0.92 and 0.82 and specificity of 0.94 and 0.96 in males and females, respectively. The proportions of correctly classified individuals were 0.93 in males and 0.89 in females. The Nolla stage 9 demonstrated the sensitivity of 0.95 and 0.85 and the specificity of 0.84 and 0.90 in males and females, respectively. The proportions of correctly classified individuals were 0.90 in males and 0.87 in females.

Conclusion: The suggested value of I3M < 0.08 followed by Nolla stage 9 can be used to discriminate adults from minors with high specificity.

1. Introduction

Age estimation of individuals in the legal medicine is necessary for both the dead and the living [1]. The age estimation of the dead is usually used to help in the identification and creation of the biological profile which can be compared with those of missing persons [2]. For the living, there is an increasing need for the forensic expertise in cases of continued escalation of the criminal activities [3]. The reality of the present time is the massive population migration. The most common reasons for migration are low economic status, political instability, and ongoing armed conflicts [4]. Migrations of the people put a burden on different state and governmental services, particularly in the target immigration countries. These days, the migration is still increasing, predominantly in European Union (EU) countries which are more economically developed and politically stable, as a destination for immigration and settlement [5–9]. Immigrants commonly request asylum in EU after organized passes through the transit countries, including countries on the Western Balkan route (Greece, Macedonia, Montenegro, Serbia, Croatia, and Slovenia). From a legal aspect, a particular concern is the growing number of illegal entries of migrants and those who are not registered [7]. A specific problem comes from a large number of minors or those who are aimed to be treated as minors when seeking asylum, so estimation of their age is of great importance [7,9]. To prevent mistreatment by the political system, in the country where the migrant granted asylum if the individual is a minor, an evidence-based approach and the procedure has to be performed for the assessment of the age in the cases of persons of unknown age [10]. The punishing of juveniles may be conducted in detention centers for adults due to the failure or inability to assess their age [11]. In listed cases, it is necessary to determine the age as accurately as possible. Many countries have introduced procedures for age estimation in cases where a given age is in question [7]. Approaches mainly used to determine the ages are:
a. The non-medical examination that includes the evaluation of all documents that a person possesses;
b. The medical approach, which involves determining the stage of bone mineralization, dental and physical development [12–16];
c. The combination and integration of medical and non-medical approaches [17].

The methods for age estimation on the orthopantomogram (OPT), based on the assessment of mineralization of crowns and roots of lower permanent teeth except for the third molars, are used for the age estimation up to 15 years of age [18–23]. In the age interval between 15 and 24 years, the third molars are the only teeth that are still developing [24–26]. The third molars potentially become a useful anatomical structure for the estimation of age [27–29]. However, there are some complicating factors in the assessment of the development of the third molars, including their angulation or rotation, high variability in size and number of roots and frequent absence [30,31]. Cameriere et al. [32,33] have presented a method for estimating the age and discriminate adults and minors based on the relationship between age and measurements on open third molars apices or the third molars maturity index \( \text{I}_{3M} \). This approach enables the determination of threshold or cut-off that separates individuals who are 18 years of age or older (adults) and minors [32]. The specific cut-off value of \( \text{I}_{3M} < 0.08 \) was used in studies on several populations for assessing the adults and minors [24,27,34–37]. Considering that the possible differences exist, even in the same geographical region, it is recommended to test the accuracy and applicability of any new method, including this one, on a new independent sample [38]. This approach was already compared with the method of Mincer et al. [29], which used Demirjian’s staging system on wisdom teeth to determine whether a person is minor or adult. It was shown that the third molar maturity index has better specificity and sensitivity [32,39]. However, for this purpose, Nolla’s stages have not been used up to now.

Montenegro is a small state in Southeastern Europe on the Adriatic Sea. Montenegrin population is wide spread in surrounding Balkan countries, as well as in other European countries. It is a state located on the transit route of massive emigrations from the different countries of Africa and the Middle East in recent years [8,9]. Therefore, the importance of this research is even more significant because it is the first study on the teeth of that kind in Montenegro. This study aimed to verify the Nolla’s stages of the mineralization and a cut-off value of \( \text{I}_{3M} < 0.08 \) that person is an adult or minor in Montenegrin population with the intention for applicability in the identification processes in the medicolegal and criminal proceedings when it is necessary.

2. Materials and methods

This study evaluated the development of lower left third molars and was done according to the Declaration of Helsinki ethical principles for the medical research [40]. An approval for the study was obtained by the Ethical Committee of the University of Montenegro, Faculty of Medicine (KCCG No. 03/01-13013/1). We evaluated OPTs of the Montenegrins, aged between 13 and 30 years, who obtained their OPT between 2007 and 2015 for different diagnostic and clinical purposes. Only individuals who were born and were living in Montenegro were included in this study. The sample was selected at random basis from patients at Medical Faculty, Clinical Centre of Montenegro and regional community dental clinics from Podgorica, Niksic, Pljevlja, Bijelo Polje, Cetinje, Bar, Herceg Novi, Berane and Budva, to accurately present the ethnic structure of the population of Montenegro which include Montenegrins, Serbs, Bosnians, Albanians and Croats. Dates of birth and radiography, as well as sex, were recorded. We found no single OPT with evidence of unfinished maturation of the third molars in individuals older than 24, and therefore we restricted this study to age range from 13 to 24-year-old participants. OPTs of participants without lower third molars, with gross pathologic processes or distortion that obstruct visibility of lower third molars and severely angulated, were excluded from the study. Chronological age of each participant was calculated and converted into decimal ages. In total, 683 OPTs aged 13–24 years (324 males and 359 females) were analyzed. All OPTs were recorded in uncompressed JPEG format and Image J software; version 1.49v was used for all measurements. The third molars were evaluated by using the system of ten stages of development of molars presented by Nolla [41] and by the third molar maturity index \( \text{I}_{3M} \) [32]. Nolla divided mineralization into stages: 0 – absence of crypt, 1 – presence of crypt, 2 – initial calcification, 3 – one-third of crown completed, 4 – two thirds of crown completed, 5 – crown almost completed, 6 – crown completed, 7 – one third of root completed, 8 – two third of root completed, 9 – root almost completed and 10 – apical end of root completed Fig. 1 [41].

\[ \text{I}_{3M} \] is a ratio of the sum of projections of open apices in multi-rooted teeth or apex width in single-rooted teeth \((a + b)\) and a tooth length \(c\) [32] Fig. 2. If the third molar was with entirely closed roots than \( \text{I}_{3M}=0.0 \) was recorded. Finally, the cut-off value of \( \text{I}_{3M} < 0.08 \) was
tested to discriminate those who are 18 years and older (adults) and those under 18 years (minors) [32]. Cohen Kappa (Kappa) of Nolla’s stages and intra-class correlation coefficient (ICC) of I3M was used to test intra-observer and inter-observer agreement between the same and the second observer (IG), respectively. For this purpose, 60 randomly selected OPTs were reexamined one month after evaluation. The authors performed all measurements without knowledge of the date of birth of individuals to avoid bias. Scatter-plot and box-plot were used to show relationships between real age, Nolla’s stages and I3M indexes for males and females. Discrimination performance of the specific Nolla’s stages and I3M < 0.08 was evaluated by 2 × 2 contingency tables for males and females separately [42]. Briefly, the 2 × 2 contingency table shows the number of participants who have reached specific Nolla’s stage or I3M < 0.08 and are 18 years and older (true positives). Next, participants with specific Nolla’s stage or I3M ≥ 0.08 who are younger than 18 years (false positives), followed by those with specific Nolla’s stage or I3M < 0.08 who are 18 years and older (false negatives) and finally those with specific Nolla’s stage or I3M ≥ 0.08 who are younger than 18 years (true negatives). A performance of the cut-off value was measured by the accurate classified individuals (Acc); the sensitivity of the test (Se) or proportion of 18 years and older, selected as adults; the specificity of the test (Sp) or proportion of those who are younger than 18, selected as minors. The predictive values, positive (PPV) and negative (NPV), measure the probability that the specific cut-off will correctly classify adults from minors [43]. PPV is the proportion of participants with positive test results who are adequately classified while NPV is the proportion of participants with negative test results who are correctly classified [43]. The positive likelihood ratio (LR+) and negative likelihood ratio (LR−) summarize how many times more or less likely adults are to have specific Nolla’s stage or I3M < 0.08 than minors and minors are to have specific Nolla’s stage or I3M ≥ 0.08 than adults, respectively [43,44]. LR+ above 10 and LR− below 0.1 are considered to provide substantial evidence to accept or rule out an assessment in most situations [28]. Values of the test were presented with 95% confidence interval (95% CI).

The Bayes post-test probability (p) of being 18 years or older may help to discriminate between those who are or are not aged 18 years or more [32]. According to Bayes’ theorem, p may be written as:

\[
p = \frac{Se \times p_0}{Se \times p_0 + (1 - Sp)(1 - p_0)}
\]

In the post-test probability p, value p_0 is the probability that the participant in question is 18 years or older given that he or she is aged between 13 and 24 years, which represents the evaluated population. In this study, p_0 was calculated as the proportion of participants between 18 and 24 years of age who live in Montenegro and those between 13 and 24 years. This data was obtained from the 2011 census from the Statistical office of Montenegro-MONSTAT in Podgorica [45] and is considered to be 0.58 in males and females.

### 3. Results

The mean chronological age of 324 males and 395 females were 18.80 ± 3.47 years and 18.79 ± 3.46 years, respectively, without a statistically significant difference (p = 0.967). Age and sex distribution of OPTs are presented in Table 1. Intra-rater and inter-rater Kappa for discriminating those who were 18 years or older and those who were younger than 18 years were 0.93 (95%CI, 0.84–1.00) and 0.93 (95%CI, 0.84–1.00) respectively, which is almost perfect agreement according to Landis and Koch [46]. The intra-rater and inter-rater agreements of I3M were 0.96 (95%CI, 0.94–0.98) and 0.95 (95%CI, 0.92–0.97), respectively. The relationship between the real age of participants and I3M is presented in Fig. 3.

Primarily, the actual age increased as the Nolla’s stages increased and I3M decreased, Fig. 4. Males were ahead of females in the dental maturation of lower third molars. Mean age was lower in males than in females for most of Nolla’s stages except for the stage 4. The same was found in all I3M classes which are statistically significant for [1.6, 0.9], (0.3, 0.08) and (0.03, 0.00) I3M classes, Fig. 4 and Table 2. The first closure of the apices of third molars, Nolla’s stage 10 and I3M = 0.00

### Table 1

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>38</td>
<td>29</td>
<td>67</td>
</tr>
<tr>
<td>14</td>
<td>24</td>
<td>42</td>
<td>66</td>
</tr>
<tr>
<td>15</td>
<td>27</td>
<td>29</td>
<td>56</td>
</tr>
<tr>
<td>16</td>
<td>21</td>
<td>33</td>
<td>54</td>
</tr>
<tr>
<td>17</td>
<td>24 (3)</td>
<td>29 (1)</td>
<td>53 (4)</td>
</tr>
<tr>
<td>18</td>
<td>26 (9)</td>
<td>19 (4)</td>
<td>45 (13)</td>
</tr>
<tr>
<td>19</td>
<td>20 (15)</td>
<td>19 (3)</td>
<td>39 (18)</td>
</tr>
<tr>
<td>20</td>
<td>55 (48)</td>
<td>56 (36)</td>
<td>111 (84)</td>
</tr>
<tr>
<td>21</td>
<td>25 (19)</td>
<td>26 (20)</td>
<td>50 (39)</td>
</tr>
<tr>
<td>22</td>
<td>20 (14)</td>
<td>21 (14)</td>
<td>41 (28)</td>
</tr>
<tr>
<td>23</td>
<td>19 (19)</td>
<td>33 (29)</td>
<td>52 (48)</td>
</tr>
<tr>
<td>24</td>
<td>25 (25)</td>
<td>24 (24)</td>
<td>49 (49)</td>
</tr>
<tr>
<td>Total</td>
<td>324 (1 5 2)</td>
<td>359 (1 3 1)</td>
<td>683 (2 8 3)</td>
</tr>
</tbody>
</table>

Fig. 2. Third molar maturity index (I3M) is the proportion of the sum of open apices (a + b) and height (c) of the projection of the third molar on an ortho-pantomogram.

Fig. 3. Scatterplot of the relationship between third molar maturity index (I3M) and chronological age.
was found at the age of 17 years in both sexes (Table 1). Next, not all apices were closed at age 23 in females, which was not the case in males (Table 1).

Table 3 shows the contingency tables which describe discrimination performance of the cut-off value of I₃M < 0.08 in males and females. Regarding Nolla's stages, the stage 9 could be used for discrimination of adults. In other words, when the third molar reaches the stage 9 in maturation (almost completed root formation), it can be assumed that the person is 18 years or older. The accuracy is similar to males and females. However, in males, there were more incorrect negative results, meaning that more minors were assumed adults, which is an ethically unacceptable error. Regarding female, the results showed more technically unacceptable errors meaning that more adults was assumed to be minors.

In comparison with the results gained using the cut-off value of I₃M < 0.08, use of Nolla's staging system was found to be less accurate (Table 3). Table 4 shows the derived values (95% confidence interval) of 2-by-2 contingency tables to test the age of majority in Montenegro of different Nolla's stages and third molar maturity index (I₃M < 0.08). The values of the sensitivity, the specificity, PPV, NPV, LR+, LR− and the Bayes post-test probability for different Nolla's stages and I₃M < 0.08 were also presented in Table 4.

Tables 5 and 6 show accuracy of Nolla's stage 9 and a cut-off value
When I3M < 0.08 was applied, this type of error was noticed in 15 out of 134 male minors in adult group and 6 out of 162 female minors. A s stage 9 gives slightly better results at ages 18, 19 and 20 in males. In both methods, the significant error rate was for 18 years old although the highest error rate for Nolla's stage 9 was at the age of 17.

Of I3M < 0.08 in each age category. Nolla's stage 9 gives slightly better results at ages 18, 19 and 20 in males. In both methods, the significant error rate was for 18 years old although the highest error rate for Nolla's stage 9 was at the age of 17.

4. Discussion

The possibility to indicate an adult age in the population of Montenegro by use of the procedures based on radiography of developing teeth has not been reported in the contemporary forensic literature. The current study aimed to test the methods based on the Nolla's stages and the third molar maturity index for this purpose on the sample of OPTs from Montenegro [32,41]. We compared both methods on all available OPTs, despite uneven distribution of the sample among age groups, because the sample of OPTs represents the distribution of individuals of specific age range with various dental clinical indications for obtaining OPT.

Previous reports showed the good results of I3M on other populations while Nolla's staging used for this purposes was not reported at all [39,47–49]. The fraction of accurately classified males for I3M were 0.93 (95% CI, 0.90–0.96) and 0.89 (95% CI, 0.85–0.91) females in this research. Our results were within the range of similar studies of I3M < 0.08 in discriminating adults and minors [9,24,27,35–37,50,51]. According to literature, the best accuracy in males was reported in Turkish (97.6%) and Peruvian (96%) studies, in females in Libyan (94.5%) and Colombian (94.4%) studies, while the lowest accuracy was reported in Saudi Arabian study-only 75.6% males and 72.4% females were accurately selected [37,39,52–54]. The accuracy in our study was somewhat better than in Croatian [27], Albanian [36] or Italian [35] studies. In the Serbian study on I3M < 0.08, the overall performance showed similar results with somewhat better accuracy [24]. However, it should be taken into account when comparing our results with other studies that this research, as well as the one on Serbian sample, covered different age range (13–24 years).

From the forensic point of view, it is ethnically unacceptable if a minor is missclassified as an adult. Therefore, we need the method with better specificity. In our study, I3M < 0.08 incorrectly classified 8 out of 134 male minors in adult group and 6 out of 162 female minors. A method based on Nolla's showed a higher rate of this kind of error for the Nolla's stage 9–21 out of 134 male minors and 17 out of 162 female minors were incorrectly classified. When an adult is incorrectly classified as minor, this is considered as a technically unacceptable error. When I3M < 0.08 was applied, this type of error was noticed in 15 out of 190 male and 35 out of 197 female adults. The results with the use of
Nolla’s stage 9 were 10 out of 190 male and 29 out of 197 female incorrectly classified adults. Thus, Nolla’s stage 9 performed better in classifying adults but I3M < 0.08 was better in classifying minors. Both methods performed better in males than in females.

The best specificity reported Turkish (100%) or Libyan (100%) studies in males while female Indian study reported the lowest, 83.6% and 79.6% for males and females, respectively [48,53,54]. The best sensitivity was reported in Serbian study for males (96%) and Colombian for females (95.1%), while the smallest sensitivity was found in a study from Saudi Arabia-only 52.3% in males and 51.3% in females [24,37,52]. In our study, the values of the specificity, PPV, LR +, LR – and the Bayes post-test probability were better for I3M < 0.08, while the sensitivity and NPV were better for Nolla’s stage 9.

Our study also provides the results for the error rate in each age group for both methods. In Nolla’s stage 9, most of the errors occurred in the age group of 17 years where only 38% males and 59% females were correctly classified as minors. In the age group of 18 error rate was also high but worse in females (42% of correctly classified). The inaccuracies are present from the age 15 until the age 22. Regarding I3M < 0.08, most of the errors occurred at the age of 18 years for both sexes, 69% of males and 42% of females have been classified as minor. The inaccuracies are also present in males between 16 and 20 years and in females between 17 and 22 years.

In our study, the third molars maturate faster in males than in females, and this difference was statistically significant in Nolla’s stage 7 and some I3M classes (i.e. [0.00, 0.03]; [0.08, 0.3]; [0.9, 1.6]). As mentioned earlier, this sexual dimorphism was also reported in other studies [4,24,49,55]. In our study, the first apical closure is registered at the age of 17, which indicates that individual, with a closed apex of the third molar, has to be older than 16 years in both sexes. The number of closed apices follows the increase of age but faster in males than in females [24]. All males finished maturation at the age of 23 years while all females mature at the age of 24. Very similar results were reported for the black population of Botswana where the first apical closure was also reported at the age of 17, the third molar develops faster in males than in females [9]. However, at age 23 some third molars are still not fully developed in male, which was not the case in the sample from Montenegro [9]. On the other hand in the study by Olze et al. [56] on Japanese population, it was reported that the first apical closure could be seen at age 16 in both male and female. Also, open apices are notices after the age 24 up to the age 30 in both sexes.

One of the essential responsibilities in age assessment for different medico-legal and the forensic question is reliable discrimination of adults from minors [7,57]. Among the various anatomical regions which are studied to evaluate whether a person reached legal adulthood, the third molars may still maturate after the age of 18 in most of the individuals and are available for study on OPTs [1,29,58]. It has been shown that differences between dental maturation can be found between races and populations, but also in the same population between sexes and different socioeconomic groups. In a study based on Portuguese population, the results showed the earlier development of third molars in children belonging to the higher socioeconomic group [59]. As differences may be possible in the populations living in the same region, there is a rational need for studying a new specific sample, especially to discriminate adults from minors. Thus, further investigation is needed even in a small population like Montenegro [30,59].

We used Nolla’s stages while most of the studies have used the Demirjian’s stages (DS) of mineralization, in which the development of the permanent tooth was divided into eight stages of mineralization, from initial calcification to complete development of apex closure. Demirjian uses only three stages of root development before apical closure [29,60–62]. Many studies showed a low accuracy of DS when selecting adults and minors [26,28,29,63]. In this study, a method based on Nolla’s stages was tested as it was consisted out of 10 stages. It was presumed that this method could be more accurate than methods using DS. Our results showed that stage 9 of Nolla’s classification could be used for discriminating adults from minors. However, in analyzing Table 6 it can be assumed that a significant number of minor could be incorrectly classified as adults (especially at age 17). This kind of error is ethically unacceptable [64]. The same can be found by analyzing contingency tables. Cameriere et al. [32] showed that a small number of mineralization stages of DS affected both the sensitivity and specificity of the test. Nolla’s staging system is very similar to DS. From stage 6 (crown completed) mineralization of the root was also divided into only three stages before final apex closure [41,60]. Additional stages in Nolla’s system refer to the mineralization of the crown [41]. Thus, it can be concluded that lower specificity of this method (Table 4) is also a consequence of a small number of root development stages.

5. Conclusion

Our study showed the usefulness of both methods in discriminating adults and minors. Nevertheless, I3M < 0.08 showed better accuracy. Next, I3M < 0.08 showed high sensitivity and even better specificity in dividing adults from minors. The mean results for sensitivity were better for males while specificity was better for females. The best discrimination performance among Nolla’s stages showed stage 9. However, its specificity was lower than I3M < 0.08 while the sensitivity was a bit higher. The results from this study from Montenegro as well as from other recent studies showed that some differences in the performance of the test might be expected among samples from a geographically closed region as well from entirely different continents. This study contributes to the application of the Nolla’s stages and a specific cut-off value of I3M to discriminate adults from minors. With high specificity, the third molar maturity index can be used as a useful tool for discriminating of the individuals as adult or minors in Montenegrin population.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgment

This study was supported by the Ministry of Education and Science of the Republic of Serbia Grand no. 45005.

References


C. Nolla, The development of the permanent teeth, ASDC J. Child. 27 (14) (1960) 254–266.


