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Radiographic assessment of third molar development in a Russian population to determine the age of majority

Raquel Porto Alegre Valente Franco^{a, e}, Ademir Franco^{b, f}, Anna Turkina^c, Marianna Arakelyan^c, Alina Arzukanyan^c, Pavel Velenko^d, Priscilla Belandrino Bortolami^b, Irina Makeeva^c, Ricardo Henrique Alves da Silva^{a, e, *}

^a Department of Forensic Medicine and Pathology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

^b Division of Forensic Dentistry, Faculdade São Leopoldo Mandic, Instituto de Pesquisas São Leopoldo Mandic, Campinas, Brazil

^c Department of Therapeutic Dentistry, Institute of Dentistry, Sechenov University, Moscow, Russia

^d Department of Forensic Medicine, Faculty of Medicine Sechenov University, Moscow, Russia

e Department of Stomatology, Public Health and Forensic Odontology, School of Dentistry of Ribeirão Preto, Ribeirão Preto, Brazil

^f University of Dundee, Centre of Forensic and Legal Medicine and Dentistry, Dundee, United Kingdom

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ABSTRACT

Objective: To test the applicability of "Gleiser and Hunt dental staging system modified by Kohler" (GHK) to assess third molar (3 M) development in a Russian population in order to determine the age of majority. *Design:* The sample consisted of 918 panoramic radiographs from Russian females (n = 551) and males (n = 367) within the age interval between 8 and 23 years. On each radiograph, 3 M development was classified based on the GHK technique. Statistics tested the data for normality. Mean age and standard deviation were described for each 3 M position. Ordinal logistic regression tested the performance of the technique to classify individuals below or above the 18-year threshold. Receiver Operating Characteristic (ROC) curves were used. *Results:* The mean ages and standard deviation (SD) for apex closure in females were 21,11 (SD = 1,47), 21,11 (SD = 1,43), 21,24 (SD = 1,39), and 21,29 (SD = 1,28) wears for the teeth #18, 28, 38, and 48, respectively.

(SD = 1,43), 21,24 (SD = 1,39), and 21,29 (SD = 1,28) years for the teeth #18, 28, 38, and 48, respectively. Among males, the same teeth showed mean closure ages of 20,57 (SD = 1,69), 20,64 (SD = 1,76), 20,68 (SD = 1,68), and 20,81 (SD = 1,62) years, respectively. Area under the curve (AUC) reached 0.904 and 0.915 for classifying females and males below or above the 18-year threshold.

Conclusion: The GHK technique was able to describe 3 M development in a Russian population. The statistic model was able to classify individuals below or above the 18-year threshold. However, the outcomes must be carefully interpreted, especially in borderline cases (17–19-year spectrum).

1. Introduction

Dental age estimation is one of the fields of forensic dentistry (Deitos et al., 2015). Didactically, the methods available in this field may be divided according to age groups of interest, namely children, adolescents and adults (Kumagai, Willems, Franco, & Thevissen, 2018; Pfau & Sciulli, 1994). In children and adolescents, dental development is one of the parameters of choice for age estimation. The transition between children and adolescents is usually marked by the progressive root formation of the permanent teeth (Franco, Thevissen, Fieuws, Souza, & Willems, 2013)

Human third molars develop from childhood to early adulthood (Gunst, Mesotten, Carbonez, & Willems, 2003; Preeti, Wadhwan, & Sharma, 2018). From a forensic perspective, third molars are useful to point out if an individual is below or above age thresholds of legal interest, such as the age of legal majority – 18 years in most countries (Franklin, Flavel, Noble, Swift, & Karkhanis, 2015). Techniques of dental age estimation via third molars exist and need testing (Franco, Vetter, Coimbra, Fernandes, & Thevissen, 2020).

Some of the techniques available to assess third molar development focus on classifying crown-root formation in stages (Olze et al., 2005; Orhan, Ozer, Orhan, Dogan, & Paksoy, 2007; Thevissen, Fieuws, &

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^{*} Corresponding author at: Forensic Odontology Division, School of Dentistry of Ribeirão Preto, University of São Paulo, Av. do Café, s/n, Zip Code: 14040-904, Ribeirão Preto, SP, Brazil.

E-mail address: ricardohenrique@usp.br (R.H.A. da Silva).

Willems, 2013). Other techniques require metric analyses and the quantification of linear ratios (De Luca et al., 2014). Gleiser and Hunt (1955) staging system modified by Köhler, Schmelzle, Loitz, & Püschel (1994) (GHK) is an example of technique used in third molar investigations. The technique describes ten ordinal stages that predict third molar development from half-crown to complete apex formation (Altalie, Thevissen, & Willems, 2015). GHK was previously used in investigations with Belgian (Thevissen, Galiti, & Willems, 2012), Brazilian (Franco et al., 2013), Danish (Arge et al., 2018), Japanese (Ramanan, Thevissen, Fieuws, & Willems, 2012), Malaysian (Yusof, Thevissen, Fieuws, & Willems, 2014), and UAE (Altalie, Thevissen, Fieuws, & Willems, 2014) populations, but Russian individuals were never sampled and tested.

The influence of intrinsic and extrinsic variables over third molar development was the reason of previous studies in the scientific literature. While there is a trend suggesting that ancestry might not necessarily dictate how teeth will develop (Liversidge, 2008), there are authors that point out variables that may influence on the early maturation of specific dental developmental stages, namely high socioeconomic status and overweight (Carneiro, Caldas, Afonso, & Cardoso, 2017). The fact is that country-specific studies are encouraged to translate international techniques into more regional/local data and practice. The Russian population was never sampled before in a similar study, indicating a scientific gap worth of investigation. An additional gap relies on Russian asylum-seekers, and undocumented individuals traveling clandestine. According to the United Nations High Commissioner for Refugees (UNHCR - www.unhcr.org), clandestine migration via smuggling is a challenge to overcome. The UNHCR clarifies that an important number of the refugees and asylum candidates are investigated and arrested by the police. In this context, dental age estimation studies may be useful to establish Russian tools to deal with the eventual need of identification of the living - especially when it is necessary to report on legal majority.

The state-of the-art of dental age estimation studies applied to the living relies more often on low-dose bidimensional imaging. The choice behind these protocols is founded on the principle of justification and image optimization (Oenning et al., 2019) inherent to international recommendations for radiation exposure. For the same reason, cone beam computed tomography (CBCT) is not usually recommended for the living (despite the improved image visualization). Differently, magnetic resonance imaging recently emerged as an alternative for tooth visualization and dental stage classification in age estimation (De Tobel et al., 2017). The facilities, however, are not usually available in forensic units worldwide. Panoramic radiographs, on the other hand, are bidimensional records of the human teeth and maxillofacial region with "diagnostically acceptable" (ALADA principle) contribution to dental age estimation.

Based on the exposed, this study aimed to assess third molar development in a Russian population and to test the applicability of GHK technique to classify individuals below or above the 18-year threshold.

2. Materials and methods

2.1. Ethics and study design

This study was approved by the institutional committee of ethics in human research (protocol: 5-11, SU). All the ethical aspects followed the Helsinki declaration of 2013. The study was designed observational and retrospective. Accordingly, the study was structured following the STROBE (The Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (Von Elm et al., 2008).

2.2. Sample

The sample consisted of 918 panoramic radiographs of female (n = 551; 60 %) and male (n = 367; 40 %) Russians. The individuals

were from rural and metropolitan areas and their age ranged from 8 and 23 years (Table 1). The panoramic radiographs were exclusively obtained for diagnostic and therapeutic purposes, between 2017 and 2019, and all were retrospectively collected from an institutional image database. The inclusion criteria consisted of Russian individuals aged 23.99 years or younger, with good-quality panoramic radiographs, and available information about date of birth, date of radiographic acquisition and sex. The exclusion criteria consisted of systemic diseases (developmental, metabolic and genetic tooth-related disorders), visible bone lesion associated with any of the third molars, history of third molar extraction and any trace of therapeutic intervention related to third molars.

The images were acquired in a digital device (Pan eXam Plus, Kavo Dental[™], Biberach, Germany) set with 66 Kv, 2.5 mA and 17 s. Next, the images were stored as. tiff files with 600 dpi. They were imported to a personal computer (Vaio PCG- 71911X, Sony Corp.[™], Minato, Tokyo, Japan) equipped with a 15.6" LCD screen and resolution of 1366 × 768. Adobe Photoshop CS6 (Adobe Inc.[™], San Jose, CA, USA) was used for image visualization and analysis. Image analysis was performed life size allowing maximum magnification of 200 %. All the analyses were performed in a dark room (Moshfeghi, Shahbazian, Sajadi, Sajadi, & Ansari, 2015).

2.3. Variables

The main examiner classified each third molar according to GHK technique (Gleiser & Hunt, 1955; Köhler et al., 1994) (Fig. 1). The technique describes ten ordinal stages, namely: i) $\frac{1}{2}$ crown formation, ii) $\frac{3}{4}$ crown formation, iii) complete crown formation, iv) initial root formation, v) $\frac{1}{2}$ root formation, vi) $\frac{1}{2}$ root formation, vii) $\frac{3}{4}$ root formation, vi) $\frac{1}{2}$ apex formation, and x) complete apex formation.

Third molar developmental stages were tested for correlation with chronological age and sex. The age threshold of legal interest related to majority (18 years) was considered. In this context, third molar stages were used to classify individuals below of above the selected threshold.

2.4. Minimizing the risk of bias

Intra-examiner agreement was tested by revisiting 100 panoramic radiographs of the total sample within a period of 30 days, while the inter-examiner agreement was tested by adding another examiner to revisit the same 100 panoramic radiographs. The analyses of the main and second examiners were supervised by a third examiner. Agreement tests were performed for the maxillary right (#18), maxillary left (#28), mandibular left (#38) and mandibular right (#48) third molars,

Table 1	
Sample distribution based on sex and ag	ge.

Age	F	М	$\mathbf{F} + \mathbf{M}$
8.00-8.99	14	22	36
9.00-9.99	8	18	26
10.00 - 10.99	29	26	55
11.00-11.99	36	26	62
12.00 - 12.99	32	32	64
13.00 - 13.99	36	38	74
14.00 - 14.99	46	30	76
15.00 - 15.99	42	20	62
16.00 - 16.99	44	32	76
17.00-17.99	38	22	60
18.00 - 18.99	36	18	54
19.00 - 19.99	32	13	45
20.00 - 20.99	31	24	55
21.00 - 21.99	54	19	73
22.00 - 22.99	51	17	68
23.00 - 23.99	22	10	32
Total	551	367	918

F: females; M: mal.

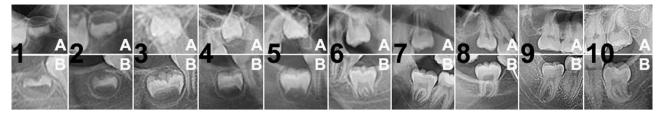


Fig. 1. Illustrative overview of dental maturation according to the staging system of Gleiser and Hunt (1955) modified by Köhler et al. (1994). Ten stages described crown, root and apex formation in maxillary (A) and mandibular (B) third molars as follows: 1) ½ crown formation, 2) ¾ crown formation, 3) complete crown formation, 4) initial root formation, 5) ¼ root formation, 6) ½ root formation, 7) ¾ root formation, 8) complete root formation, 9) ½ apex formation and 10) complete apex formation.

separately. Weighted Kappa was used to quantify intra- and interexaminer reproducibility.

2.5. Statistical analysis

The obtained data were explored by mean of descriptive statistics, and bivariate/multivariate inferential analyses. First, third molar development was tested for normality based on the allocated GHK stages using Shapiro-Wilk test. Next, descriptive information of the distribution of individuals by age groups and the occurrence of third molars were obtained. Values of central tendency and dispersion (mean age and standard deviation), as well as absolute frequency were quantified per stage and for each third molar position. Pearson's Chi-square test was used to assess the association of age and sex within the age groups, as well as to assess the association between the quantity of available third molars between females and males. Ordinal logistic regression was used to verify the predictive power of third molar stage to classify individuals below the age threshold of 18 years. A logistic regression was designed with the universal polytomous model (PLUM), which incorporates the ordinal nature of the dependent variable. The applicability of this model is corroborated by the current scientific literature (Lopes et al., 2016). Receiver operating characteristic (ROC) curves and their inherent area under the curve (AUC) were calculated to compare individuals' age with the classification (below or above 18) predicted by the model. Sensitivity, specificity, and positive and negative predictive values were quantified. Statistical analyses were performed with SPSS 20.0 software (IBM Corp.™, Armonk, NY, USA) and MedCalc 19.1.3 (MedCalc Software Ltd.TM, Ostend, Belgium) considering statistical significance of 5% and confidence interval of 95 %.

3. Results

Intra- and inter-examiner agreement were excellent (>0.95) (Fleiss, Levin, & Paik, 2003). Shapiro-Wilk test revealed lack of normality for the distribution of GHK stages in the sample.

The mean ages at third molar closure were 21,11 (SD = 1,43) and

Table 2

Mean age (standard deviation) and number (n) of teeth detected for each developmental stage and third molar position among females and males.

	wear age (standard deviation) and number (ii) of teem detected for each developmental stage and time motal position among remains and mates.										
Sex #		Third molar developmental stages									
	#	1	2	3	4	5	6	7	8	9	10
28 F 38	28	10,04 (2,15) (n = 28)	11,77 (1,75) (n = 27)	12,07 (1,82) (n = 40)	13,68 (2,25) (n = 63)	14,85 (2,31) (n = 68)	16,46 (2,34) (n = 60)	17,35 (2,05) (n = 28)	18,68 (2,49) (n = 29)	20,07 (1,89) (n = 71)	21,12 (1,43) (n = 89)
	38	9,77 (1,47) (n = 43)	12,05 (1,68) (18)	12,48 (2,05) (n = 60)	14,23 (2,21) (n = 55)	15,11 (2,41) (n = 71)	16,25 (1,89) (n = 43)	17,48 (2,53) (n = 14)	18,68 (1,74) (n = 22)	20 (1,93) (n = 68)	21,24 (1,39) (n = 77)
М	28	9,19 (1,53) (n = 32)	11,31 (1,49) (n = 29)	11,48 (1,84) (n = 28)	12,92 (2,24) (n = 41)	14,07 (1,36) (n = 40)	15,14 (2,26) (n = 34)	16,17 (1,85) (n = 17)	17,28 (1,79) (n = 14)	19,26 (1,72) (n = 38)	20,64 (1,76) (n = 48)
M 38	38	9,6 (1,66) (n = 59)	11,47 (2,13) (n = 19)	12,29 (1,5) (n = 46)	13,07 (1,2) (n = 29)	14,9 (2,02) (n = 39)	15,52 (1,2) (n = 27)	16,25 (1,78) (n = 8)	18,1 (2,09) (n = 11)	19,08 (1,76) (n = 35)	20,68 (1,68) (n = 51)

Data presented as mean age (standard deviation) over the number of teeth (n) in each stage and third molar position; F: females; M: males; #: third molar position; 28 and 38: maxillary and mandibular left, respectively (International Dental Federation). 1–10: third molar developmental stages according to the staging system of Gleiser and Hunt (1955) modified by Köhler et al. (1994); Only left-side third molars was reported to avoid redundant outcomes.

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21,24 (SD = 1,39) years for female maxillary and mandibular teeth, respectively (only the left side reported to avoid redundant outcomes). In males, the maxillary and mandibular third molars had mean closure age of 20,64 (SD = 1,76) and 20,68 (SD = 1,68) years, respectively (Table 2).

The mean age of the sample was 15,69 (SD = 4,24 years; median: 16 years; IIQ: 12–19 years). Age assessment showed that 591 (64.4 %) individuals were classified below the 18-year threshold, while 327 (35.6 %) individuals were classified above the threshold (Table 3). Statistically significant association between individuals' sex and age within the age group of legal interest of 18 years was detected (p < 0.001). The distribution of the number of available third molars (i.e.: four, three, two and one) was similar between females and males (p = 0.865).

The AUC outcomes related to the performance of the model to classify individuals below or above 18 (based on the predictions of chronological age using all GHK stages) reached 0.908. The AUC outcome maintained high when separately assessed for females (0.904) and males (0.915). Sensitivity, specificity, positive predictive, and negative predictive values were reported in Table 4.

4. Discussion

The scientific literature dedicated to the forensic sciences registered several dental age estimation studies over the last decade (Alsaffar et al.,

Table 3

Distribution of individuals based on sex and age group, and distribution of quantity and arch position of the available third molars.

Variables	n	%
Sex (n = 918)		
Female	551	60
Male	367	40
Age threshold of 18 $(n = 918)$		
<	591	64.4
\geq	327	35.6

N = absolute number of occurrences; % relative number of occurrences.

Table 4

Outcomes of predictive accuracy for the use of third molar staging according to GHK technique.

Sex	Age	AUC (SE)	Sensitivity	Specificity	PPV	NPV
Females	$<$ 18 / \geq 18	0.904 (0.018)	91.18 %	90.21 %	93.94 %	86.00 %
Males	$<$ 18 / \geq 18	0.915 (0.024)	96.13 %	85.71 %	92.55 %	92.31 %

AUC = area under the curve; SE = standard error; PPV = positive predictive value; NPV = negative predictive value. Outcomes calculated by pooling all the GHK stages detected in the present study. AUC values over 0.9, sensitivity/ specificity over 85 %, and PPV/NPV over 86 % reveal optimistic application of the model to distinguish minors and adults (females and males). However, it must be noted that the sample interval is large (8–23 years) making it easier to distinguish age based on third molar stage (e.g. children with third molar stage 4 are more easily classified as minors than an adolescent with third molar stage 7). Sensitivity rates show how adequate was the classification of minors as minors and adults as adults. Specificity rates deserve attention since they can depict minors that were classified as adults. In this case, a decrease of specificity rate on all positive predictions, while the NPV measures the true negative predictions based on the total negative predictions.

2017; Anastácio, Serras, Santos, & Palmela, 2018; Jayaraman, Roberts, Wong, & King, 2018). More recently, studies focused on testing the validity of available techniques (Jayaraman, Wong, King, & Roberts, 2016). Most of the studies tested the accuracy of methods by comparing estimated and chronological ages (Deitos et al., 2015; Franco et al., 2013). Other studies investigated the reproducibility of the staging techniques in different types of image exams (Franco et al., 2020). The present study investigated the applicability of GHK staging technique in a Russian population and took as reference the age threshold of legal interest of 18 years for inferential analyses.

The GHK technique is uncommon in dental age estimation studies possibly because of the variety of other techniques available with population-specific validation worldwide. Nevertheless, the staging technique was refined by Köhler et al. (1994) after the initial report by Gleiser and Hunt (1955). In particular, GHK reports on third molar maturity by predicting the remaining amount of crown/root formation (i.e. stage 6: 1/2 root formation). This technique differs from other anatomically descriptive staging systems, such as the one proposed by Solari and Abramovitch (2002) - that modified the original scheme of Demirjian, Goldstein, & Tanner (1973). Despite the popularity of Demirjian's technique and its use in international populations, such as Croatian (Sasso et al., 2015) and Chinese (Qing, Qiu, Gao, & Bhandari, 2014), the original system was not designed for adolescents, but for the classification of the seven mandibular left teeth of children. Adaptations were definitely an improvement, but in the present study, GHK figured as technique of choice. One of the contributions of GHK to the process of dental age estimation is the detailed description of the late phase of third molar formation (i.e. stage 7: 3/4 of root formation; stage 8: complete root formation, stage 9: 1/2 apex formation, and stage 10: complete apex formation) (Köhler et al., 1994). These stages are mainly important when investigations of legal majority are carried out. Previous population-specific studies in the field with GHK technique were performed by Mohd Yusof, Cauwels, & Martens (2015) in a Malay sample. Considering the mean age of 18, Malay females reached it within third molar stage 7, while 18-year-old males were already in stage 8. In Russians, stage 8 appeared in the age of 18 for both females and males. The difference between studies may rely on the methodological set up for censoring or not the last stage of third molar formation.

As the GHK stages were tested based on their applicability to classify individuals below or above ages of legal interest, the AUC outcomes for model performance reached 0.904 and 0.915 for females and males, respectively. It must be noted, however, that the present outcomes should be carefully interpreted because, at first sight, high AUC values

(>0.9) could suggest optimal application of third molar for the allocation of adolescents as minors or not. In practice, age estimation may be more challenging when individuals, such as asylum seekers, have third molars in late stages of root formation (not necessarily apex formation). In a recent study (Correia et al., 2020), the allocation of adolescents below or above 18 was hampered when the individuals had chronological age narrowed from 17 to 19 years. In the present study, the sample covered a large age interval that could give the impression of easier age allocation of subjects. Forensic experts must know that, in practice, these outcomes might not appear in such an optimal way justifying the use of third molar staging as an alternative/supporting tool to make inferences on legal majority. Especial attention is recommended to GHK stage 10, which has strong value to point out majority, but it is unbounded as well. In order words, panoramic radiographs with all third molars in stage 10 do not indicate how long that person reached full dental development.

This study did not aim to establish cut-off values from ordinal variables. This approach could be applicable and more realistic with continuous data. Stages 8–10 (late root and apex formation) are definitely predominant in individuals aged >18. However, these stages are not determinant to enable inferences about majority. In practice, evidence of these stages only provide clues of legal majority. Strong statement on majority based on the late stages are tricky since third molar development has a broad variability. The outcomes of the present study also may be influenced by the sample that was not equally distributed based on sex and age. Future studies in the field should strive for an optimal sampling process. Other techniques based on third molar staging should be studied and tested for their performance to distinguish minors and adults.

The outcomes presented in this study were the first obtained from a Russian population. Third molar development was staged and the mean age estimates per stage were quantified and tabulated. Forensic experts may benefit from these outcomes in casework practice that involve dental age estimation of Russian asylum seekers and judicial requests for the investigation of imputability. Experts must understand that dental age estimation through third molars have inherent limitations – making of this age estimation tool a small part of a larger armamentarium dedicated to justice.

CRediT authorship contribution statement

Raquel Porto Alegre Valente Franco: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - original draft. Ademir Franco: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing. Anna Turkina: Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Software, Writing - original draft, Writing - review & editing. Marianna Arakelyan: Conceptualization, Data curation, Investigation, Resources, Software, Writing - original draft, Writing - review & editing. Alina Arzukanyan: Conceptualization, Data curation, Investigation, Resources, Writing - original draft, Writing - review & editing. Pavel Velenko: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Software, Validation, Writing - original draft. Priscilla Belandrino Bortolami: Conceptualization, Data curation, Formal analysis, Resources, Software, Validation, Writing - original draft. Irina Makeeva: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Writing - review & editing. Ricardo Henrique Alves da Silva: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest related to

this study.

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