

Human age estimation combining third molar and skeletal development

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Abstract The wide prediction intervals obtained with age estimation methods based on third molar development could be reduced by combining these dental observations with age-related skeletal information. Therefore, on cephalometric radiographs, the most accurate age-estimating skeletal variable and related registration method were searched and added to a regression model, with age as response and third molar stages as explanatory variable. In a pilot set up on a dataset of 496 (283 M; 213 F) cephalometric radiographs, the techniques of Baccetti et al. (2005) (BA), Seedat et al. (2005) (SE), Caldas et al. (2007) and Rai et al. (2008) (RA) were verified. In the main study, data from 460 (208 F, 224 M) individuals in an age range between 3 and 26 years, for which at the same day an orthopantomogram and a cephalogram were taken, were collected. On the orthopantomograms, the left third molar development was registered using the scoring system described by Gleiser and Hunt (1955) and modified by Köhler (1994) (GH). On the cephalograms, cervical vertebrae development was registered according to the BA and SE techniques. A regression model, with age as response and the GH scores as explanatory variable, was fitted to the data. Next, information of BA, SE and BA + SE was, respectively, added to this model. From all obtained models, the determination coefficients and the root mean squared errors were calculated. Inclusion of information from cephalograms based on the BA, as well as the SE, technique improved the amount of explained variance in age acquired from panoramic radiographs using the GH technique with 48%. Inclusion of cephalometric BA + SE information marginally improved the previous result (+1%). The RMSE decreased

with 1.93, 1.85 and 2.03 years by adding, respectively, BA, SE and BA + SE information to the GH model. The SE technique allows clinically the fastest and easiest registration of the degree of development of the cervical vertebrae. Therefore, the choice of technique to classify cervical vertebrae development in addition to third molar development is preferably the SE technique.

Keywords Forensic science · Forensic odontology · Dental age determination · Skeletal age · Third molars · Cervical vertebrae

Introduction

Determination of the age of majority is of forensic importance during examinations considering the legal status of unaccompanied young asylum seekers. To evaluate chronological age of sub-adult individuals, dental age estimation methods regard foremost radiologically observed maturation stages of developing third molars [1]. Compared to all other developing teeth, timing of third molar development shows the highest variability [2]. Consequently, related age estimates have wide prediction intervals [3]. Worldwide, various forensic protocols for aging unaccompanied young asylum seekers combine dental and other age estimation methods [4–10]. The objective of pooling age estimation methods was to report the obtained age results and to interpret them in a conclusive age outcome with narrowed prediction intervals. For orthodontic treatment planning, correlations between skeletal and dental development were frequently investigated in an attempt to detect the maturity degree of the examined patient [11–19]. For sub-adult age estimation purposes, minor research was performed based on samples, including living individuals with known chronological age on

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which at the same moment dental and other age predictors were collected [4]. Therefore, the interpretations of pooled age estimation outcomes are not fully scientifically based and result to a certain extent in investigator-dependant conclusions.

Forensic age estimation protocols can combine methods based on third molar development and socio-psychological maturity [20], physical appearance [21, 22], secondary sexual development [23], clinical dental observations [24], radiologically observed secondary dentine apposition [25, 26], visibility on panoramic radiographs of the root pulp and the periodontal ligament in third molars [27] and skeletal variables. The latter group includes mainly noninvasive methods based on the degree of ossification of hand wrist bones [28–34], the medial part of the collar bone [35–41] and the costal cartilage of the first rib [42, 43].

On cephalometric radiographs, the developmental changes of cervical vertebrae were described to evaluate the degree of physiological maturity of a growing individual and to calculate cervical vertebral bone age. Furthermore, on these radiographs, the development of the mandibular bone was registered and used as age predictor. The development of radiologically observed cervical vertebrae body(ies) was evaluated and registered using a staging or a measuring system. More specifically, Baccetti et al. (BA) classified the vertebral body growth of cervical vertebrae C2, C3 and C4 in six stages [44]. Seedat et al. (SE) considered a six-stage system on C3 [45],

discriminating the different stages as described by Hassel et al. [46]. Caldas et al. (CA) registered the ratio of the length and the height of the corpus of C3 and C4 to determine cervical vertebral bone age using multiple regression analysis [47, 48]. Rai et al. (RA) measured two mandibular lengths and a height on cephalometric radiographs and presented three related formulae to calculate age [49] (Fig. 1).

The aim of this study was, firstly, to compare existing skeletal development evaluation systems developed on cephalometric radiographs (BA, SE, CA and RA) in order to detect the most accurate age-predicting variable and related registration system and, secondly, to verify whether adding the detected most accurate age-predicting skeletal variable and related registration system to stages of third molar development obtained on panoramic radiographs resulted in an improved age estimation.

Materials and methods

In a pilot study, 496 cephalograms (283 M, 213 F) (Table 1) were collected to detect the most accurate age-predicting variable(s) and related registration system among the published BA, SE, CA and RA methods. All radiographs were taken from individuals of Central Indian origin. Their chronological age at the day of X-ray exposure was calculated based on an existing and valid birth certificate.

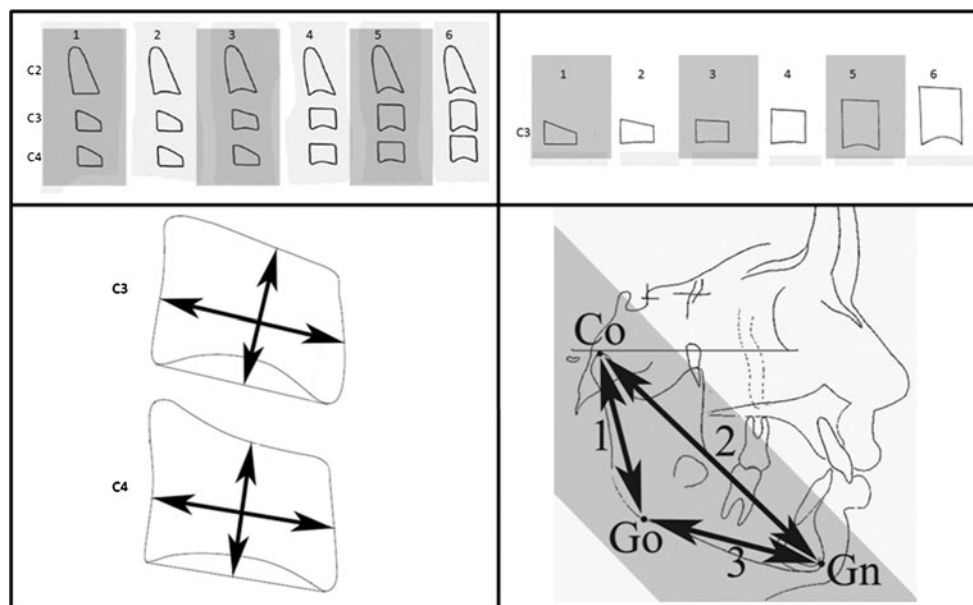


Fig. 1 Different skeletal development registration techniques applied on cephalometric radiographs. The *upper panels* illustrate six developmental stages of cervical vertebrae. At the left side, as described by Baccetti et al. and evaluated on the cervical vertebrae C2, C3 and C4. At the right, as described by Seedat et al. (SE) and evaluated on the cervical vertebra C3. The *lower panels* illustrate two measuring techniques. At the left side, the technique described by

Caldas et al. (CA) considers ratio of the shown height and width measures of the cervical vertebrae C3 and C4. At the right side, the technique described by Rai et al. (RA) considers three length measurements: #1 between Gonion (*Go*) and Condylion (*Co*), #2 between Condylion (*Co*) and Gnathion (*Gn*) and #3 between Gnathion (*Gn*) and Gonion (*Go*)

Table 1 Age and gender distribution of individuals included in the studied samples

| Age | Pilot sample | | | Main sample | | | Additional sample | | |
|-------|--------------|-----|-----|-------------|-----|-----|-------------------|-----|-----|
| | M + F | F | M | M + F | F | M | M + F | F | M |
| 3 | – | – | – | 2 | 2 | – | – | – | – |
| 4 | 2 | 1 | 1 | 7 | 2 | 5 | – | – | – |
| 5 | 3 | 1 | 2 | 7 | 5 | 2 | – | – | – |
| 6 | 3 | 1 | 2 | 14 | 7 | 7 | – | – | – |
| 7 | 7 | 6 | 1 | 18 | 5 | 13 | – | – | – |
| 8 | 12 | 6 | 6 | 16 | 5 | 11 | – | – | – |
| 9 | 25 | 12 | 13 | 3 | 2 | 1 | 3 | 2 | 1 |
| 10 | 18 | 6 | 12 | 19 | 7 | 12 | 19 | 7 | 12 |
| 11 | 37 | 17 | 20 | 19 | 9 | 10 | 19 | 9 | 10 |
| 12 | 53 | 27 | 26 | 40 | 26 | 14 | 40 | 26 | 14 |
| 13 | 51 | 24 | 27 | 65 | 30 | 35 | 65 | 30 | 35 |
| 14 | 46 | 24 | 22 | 58 | 30 | 28 | 58 | 30 | 28 |
| 15 | 33 | 22 | 11 | 57 | 29 | 28 | 57 | 29 | 28 |
| 16 | 38 | 28 | 10 | 42 | 26 | 16 | 42 | 26 | 16 |
| 17 | 31 | 19 | 12 | 25 | 11 | 14 | 25 | 11 | 14 |
| 18 | 25 | 14 | 11 | 10 | 3 | 7 | 10 | 3 | 7 |
| 19 | 16 | 13 | 3 | 11 | 5 | 6 | 11 | 5 | 6 |
| 20 | 14 | 9 | 5 | 10 | 5 | 5 | 10 | 5 | 5 |
| 21 | 12 | 9 | 3 | 3 | – | 3 | 3 | – | 3 |
| 22 | 20 | 10 | 10 | 4 | 2 | 2 | 4 | 2 | 2 |
| 23 | 14 | 9 | 5 | 11 | 8 | 3 | 11 | 8 | 3 |
| 24 | 14 | 7 | 7 | 8 | 7 | 1 | 8 | 7 | 1 |
| 25 | 6 | 6 | – | 11 | 8 | 3 | 11 | 8 | 3 |
| 26 | 6 | 3 | 2 | – | – | – | – | – | – |
| 27 | 5 | 5 | – | – | – | – | – | – | – |
| 28 | 5 | 3 | 2 | – | – | – | – | – | – |
| 33 | 1 | 1 | – | – | – | – | – | – | – |
| Total | 496 | 283 | 213 | 460 | 234 | 226 | 396 | 208 | 188 |

F female, *M* male

None of these individuals presented congenital or acquired malformations affecting their dental or skeletal development. The cephalograms were taken in an analog way using a Soredex unit (Soredex, Tuusula, Finland) and scanned digitally (Hewlett-Packard 8300, NY, USA). Care was taken so that the individuals were positioned correctly during cephalographic radiography and that all radiographs were of good image quality. The cephalograms were imported into Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, CA) and scored or measured following the techniques described by BA, SE, CA and RA. Scatter plots with a smoothed trend line were used to explore the shape of the relationship between age and the obtained stages or measurements (e.g. nonlinear). Regression models were derived, with age as response and the stages or measurements as explanatory variable. From each model, determination coefficients (R^2) and root mean square errors (RMSE) were analysed. R^2 indicates the proportion of the explained variability in the

response variable, age. The variable that leads to the highest R^2 contains the largest amount of information on age. Alternatively, RMSE denotes the magnitude of the error in age prediction. Interaction effects with gender and main gender effects were checked for all variables.

In the main study, an orthopantomogram and a cephalogram taken on the same day were retrospectively selected of 460 Caucasian individuals (208 F, 224 M) out of the dental clinic files of the Katholieke Universiteit Leuven. The radiographs were taken during the intake of patients presenting for dental check-up. Their chronological age at the day of X-ray exposures was calculated based on their identity card records and ranged between 3 and 26 years (Table 1). None of these individuals presented congenital or acquired malformations affecting their dental or skeletal development. The radiographs were taken digitally: the orthopantomograms on a Cranex unit (Soredex, Tuusula, Finland) and the cephalograms on an Orthoceph OC100

unit (Instrumentarium Corp, Graven, Finland), using in both units phosphor plate technology (Siemens, Berlin, Germany).

On the orthopantomograms, third molar development was evaluated using a ten-point scoring system according to the method of Gleiser and Hunt and modified by Köhler (GH) [50, 51]. Pearson's correlation coefficients between the developmental scores of different wisdom tooth positions were calculated and induced multicollinearity in the regression model. Because of the highly correlated left and right third molar scores, this problem was reduced using only stages of the left third molars. The development of third molars cannot be measured before the onset of the calcification of third molars, neither when third molars are absent. This missing information may in itself contain some information about age. Therefore, four prediction models, including this information, were constructed based on the present third molars: firstly, upper and lower molars present; secondly, upper molar present; thirdly, lower molar present and, fourthly, no third molars present. To apply the maximal available information, for each subject, the predictions were used, which agree with the missingness pattern for this subject.

The cephalometric radiographs were scored by following the most (BA) and second most (SE) accurate age-predicting skeletal variables and related registration systems detected in the pilot study.

The interaction effects with gender and the main gender effects were checked at first for regression models with age as response and the third molar scores (GH) as explanatory variable. Secondly, the same models were fitted, including additional information of, respectively, BA, SE and BA + SE. From each model, determination coefficients (R^2) and root mean square errors (RMSE) were analysed.

Because no third molar development was observed for any subject younger than 9 years, in an additional study, a sample including all individuals of the main study older than 9 years was selected (Table 1). On this reduced sample, all analyses from the main study were repeated.

All analyses have been performed using PROC GLM in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

In the pilot study, the age-predicting variable(s) and related registration system providing the most information on age were BA (58%), immediately followed by SE (55%). Combining these two techniques provided an additional gain of 5% explained variability in age (63%). The two CA ratios jointly explained 26% of this variability. All RA measures clearly contained very little information on age, and their regression models explained maximally 3% of the

variability in age. The calculated RMSE values increased from 3.20 to 4.90 years, hereby classifying the magnitude of the error in age prediction of the age-predicting variable (s) and related registration systems in the same order of best performance as based on the detected R^2 values (BA > SE > CA > RA) (Table 2).

In the main study, a Pearson's correlation coefficient of 0.98 was detected between the left and right third molars from the upper, as well as the lower, jaw. Between the upper and lower third molars, this coefficient was 0.91 and 0.90 for, respectively, the left and right sides. Inclusion of information from cephalograms based on the BA, as well as the SE, technique improved the amount of explained variance in age acquired from panoramic radiographs using the GH technique with 48%. Inclusion of cephalometric BA + SE information marginally improved previous result (+1%). The RMSE decreased with 1.93, 1.85 and 2.03 years by adding, respectively, BA, SE and BA + SE information to the GH model (Table 3).

In the additional study, the amount of explained variance in age by adding BA, SE and BA + SE information to the GH model was reduced to, respectively, 19%, 17% and 21%. The RMSE ranged between 1.62 and 1.78 years for the models, including cephalometric information (Table 4).

In all study samples and for all variable(s) and related registration systems, age was better predicted for males than for females.

Discussion

It should be recommended not to use the RA technique for age estimations. The pilot study revealed that measures from the RA technique provide an extremely low maximal explained variability in age (3%) and high RMSE values

Table 2 Determination coefficient (R^2) and root mean squared error (RMSE) from the regression models with age as response and the indicated explanatory variable(s) developed on the pilot sample

| Explanatory variable | R^2 | | | RMSE | | |
|----------------------|-------|------|------|-------|------|------|
| | M + F | F | M | M + F | F | M |
| BA | 0.58 | 0.55 | 0.61 | 3.20 | 3.33 | 3.00 |
| SE | 0.55 | 0.50 | 0.59 | 3.29 | 3.43 | 3.10 |
| BA + SE | 0.63 | 0.60 | 0.66 | 3.01 | 3.13 | 2.84 |
| CA | 0.26 | 0.20 | 0.30 | 4.23 | 4.36 | 4.11 |
| RA(Go-Co) | 0.03 | 0.01 | 0.12 | 4.85 | 4.99 | 4.43 |
| RA(Gn-Co) | 0.02 | 0.01 | 0.05 | 4.88 | 4.98 | 4.60 |
| RA(Go-Gn) | 0.01 | 0.01 | 0.04 | 4.90 | 4.99 | 4.61 |

M male, F female, BA Baccetti, SE Seedat, CA Caldas, RA Rai, Go-Co length from Gonion to Condylion, Gn-Co length from Gnation to Condylion, Go-Gn length from Gonion to Gnation

Table 3 Determination coefficient (R^2) and root mean squared error (RMSE) obtained from the regression models with age as response and the indicated explanatory variable(s) developed on the main sample

| | R^2 | | | RMSE | | |
|--------------|-------|------|------|-------|------|------|
| | M + F | F | M | M + F | F | M |
| GH | 0.39 | 0.30 | 0.53 | 3.60 | 3.99 | 2.99 |
| GH + BA | 0.87 | 0.86 | 0.90 | 1.67 | 1.81 | 1.39 |
| GH + SE | 0.87 | 0.84 | 0.91 | 1.75 | 1.97 | 1.33 |
| GH + BA + SE | 0.88 | 0.87 | 0.92 | 1.57 | 1.75 | 1.22 |

M male, *F* female, *GH* Gleiser and Hunt, *BA* Baccetti, *SE* Seedat

(approximately 5 years). Moreover, Dibbets et al. [52] and Cohen [53] reported that the magnification inherent to the technique of radiographic projection should be taken into account when comparing linear dimensions on cephalometric data. The RA technique is not allowing us to correct for magnifications of data from different sources. In contrast, the CA technique is overcoming this correction using ratio of linear dimensions obtained on the same radiograph.

The best age-related age-predicting variable(s) and related registration system were the staging and corresponding scoring techniques of BA and SE. The measuring technique of CA was remarkably less performing. Thevissen et al. [54] ascertained that scorings of third molar stages (categorical data) were best related to age and provided the most accurate age predictions compared to tooth measurements and ratio of tooth measurements from third and second molars (continuous data). They stated that measures and related ratio used to register molar development incorporate the variance in tooth size between individuals. A similar reason for the minor performance of the measured observations of skeletal development of cervical vertebrae is that these measurements (and their ratios) incorporated the human variability in corpus vertebrae size. The used staging techniques ignored corpus vertebrae size differences between individuals, delivering higher percentages of explained variability in

Table 4 Determination coefficient (R^2) and root mean squared error (RMSE) obtained from the regression models with age as response and the indicated explanatory variable(s) developed on the additional sample

| | R^2 | | | RMSE | | |
|--------------|-------|------|------|-------|------|------|
| | M + F | F | M | M + F | F | M |
| GH | 0.59 | 0.60 | 0.62 | 2.29 | 2.47 | 1.97 |
| GH + BA | 0.78 | 0.79 | 0.81 | 1.69 | 1.80 | 1.41 |
| GH + SE | 0.76 | 0.74 | 0.79 | 1.78 | 1.99 | 1.33 |
| GH + BA + SE | 0.80 | 0.80 | 0.85 | 1.62 | 1.76 | 1.25 |

M male, *F* female, *GH* Gleiser and Hunt, *BA* Baccetti, *SE* Seedat

age and smaller magnitudes of error in the age estimates. Caldas et al. [48] reported that a computerized CA technique was used in their study because it allowed skeletal age to be measured and calculated in an objective manner. Their decision was based on the findings of Özer et al. [55], namely, that in the Lamparski technique [56], which was modified into the SE technique [46], the first and last stages were most accurate because, in other stages, the borderline cases blended into each other. The current pilot study denoted that, for age estimation purposes, the staging techniques were outperforming the measuring technique. In addition, no agreement exists on the reproducibility of cervical vertebrae staging techniques. On one hand, Gabriel et al. [57] detected interobserver agreement levels below 50% and slightly better intraobserver agreement, and on the other hand, Jaqueira et al. [58] reported a good interobserver agreement for the staging techniques of BA, SE and Hassel et al. [46]. Further on, Baccetti et al. [44], SanRomán et al. [59] and Chen et al. [60] observed that the concavity of the lower board of the vertebral bodies is higher with greater maturity. This finding should be considered in an attempt to diminish the borderline cases. Moreover, during forensic age estimations on living individuals, the advantage of the doubt has to be given to the examined individual. In most cases, this advantage has to be accorded to the youngest age outcomes. In this context, the detected borderline cases during the staging of cervical vertebrae development have to be classified in the earliest of the questioned stages.

Clinically, the SE technique allows a faster and easier registration of the degree of development of the cervical vertebrae compared to the BA technique. Both techniques classify the observed cephalometric radiographs into six stages, but against the more complex combined examination of three vertebrae (C2, C3 and C4) considered in the BA technique, the SE technique simplifies the evaluations to the developmental examination of one vertebral corpus (C3). Since statistically the performances of SE or BA added to GH are likely for the main, as well as the additional, sample, based on clinical conveniences, the choice of technique to classify the added cervical vertebrae development is SE. Further on, optimal accuracy in age predictions is obtained using gender-specific regression models (Table 5). In particular, for males, all obtained RMSE values are reduced compared to the gender-independent values. In forensic context, the use of gender-specific models is not a constraint because the sex of examined unaccompanied young asylum seekers is always known.

The main study indicated that registrations of cervical vertebrae development added to stages of third molar development improved drastically the age predictions. This improvement was largely ascribed to the fact that the main sample contained subjects with ages between 3 and 25 years, while no dental stages and related scores were

Table 5 Gender -specific regression formulae for GH and added SE, fitted on the additional sample

| Present third molar(s) | Gender | Regression formula |
|------------------------|--------|---|
| ul + ll | F | Age=7,38+1,27UL-0,25UL ² +0,02UL ³ +0,03LL+0,84SE |
| | M | Age=8,19+0,32UL-0,19UL ² +0,02UL ³ +0,37LL+0,01SE |
| ul | F | Age=7,49+1,17UL-0,23UL ² +0,02UL ³ +0,86+0,84SE |
| | M | Age=8,16+0,48UL-0,14UL ² +0,02UL ³ +0,35+1,07SE |
| ll | F | Age=7,24+1,40LL-0,31LL ² +0,28LL ³ +0,39+0,92SE |
| | M | Age=8,38+0,20LL-0,08LL ² +0,01LL ³ +2,19+1,10SE |
| | F | Age=5,61+3,66+1,96SE |
| | M | Age=4,78+0,14+2,21SE |

GH Tooth scoring according to Gleiser et al., SE Cervical vertebrae score according to Seedat et al., ul Upper left, ll Lower left, F Female, M male, UL Score upper left third molar, LL Score lower left third molar

available for any subject younger than 9 years. For these young subjects, the cervical vertebrae development was the only age-related information available, apart from the fact that the absence of third molar development, especially in this young age category, is age related. As expected, in the additional sample, adding skeletal information (BA) to dental information (GH) for age prediction reduced the explained variability in age from 87% in the main sample to 78%. The explained variability for the model with only dental information (GH) increased from 39% in the main sample to 59% for the additional sample. Subtracting both previous results reduced the gain of explained variability in age from 48% in the main sample to 19% in the additional sample. By combining GH + BA techniques, the RMSE diminished with 0.6 years in the additional sample compared to the single GH technique. These findings indicate a considerable gain in accuracy of age prediction by combining third molar and cervical vertebrae information. However, the period of vertebral development is not completely overlapping the span of third molar development. The older the considered individual gets, the minor the amount of overlap is. This was reflected in a related decrease of the added R^2 values as well as the gain in RMSE. Indeed, when calculating these values from the models (GH and GH+BA) based on all individuals from the main sample older than 14 years ($n=250$) and those older than 16 years ($n=135$), the added R^2 for both groups was reduced to 3%, and the gain in RMSE was, respectively, 0.12 and 0.09 years. Further on, the last BA stage with potential of cervical vertebrae development (BA, stage 5) ranged between 11.51 and 19.47 years, while the last stage with potential third molar development (GH, stage 9) ranged between 17.27 and 25.7 years. Consequently, during the period of late third molar development, no or a neglectable gain in accuracy of age prediction is obtained after adding cervical vertebrae information to third molar information. In summary, it should be recommended to take additional

cephalometric radiographs when aging individuals with third molar development lower than GH stage 7 (root 3/4 developed).

The effective radiation dose needed for a cephalometric exposure varies between 2 and 3 microsievert (μSv). In forensic age estimation investigations, additional skeletal age information is most frequently obtained from data observed on hand wrist and chest radiographs [61], with respective effective radiation doses around 5 and 30 μSv . The relatively low cephalometric dose is usually less than 1 day of natural background radiation. This has to be considered as an advantage of the proposed age estimation technique, especially because the examined individuals are maturing children.

Gabriel et al. [62] analysed facial proportions of developing juveniles for age estimation purposes. The authors will assemble frontal and lateral anthropometric data in age-related reference samples. Since cephalometric radiographs visualize soft tissue contours, some of the aforementioned lateral measurements can be obtained from these radiographs. In this way, cephalometric radiographs could be considered as a source of soft tissue and skeletal age-related information. In future research, the accuracy of age predictions by adding these nonskeletal lateral measurements to the dental and skeletal information described in this study could be searched. The major problem in establishing current research was to collect retrospectively individuals on which, at the same day, panoramic and cephalometric radiographs were taken. In future research, the current main sample will be extended to obtain a sample that includes both genders of individuals homogeneously distributed in age categories of maximally 1 year, allowing for optimal statistical analysis [63–65]. Further on, under the same inclusion conditions, a test sample will be collected to verify the established regression models. Age estimation methods based on tooth development for the age categories till 16 years consider all developing teeth, except the third molars, and for age categories of young individuals above 16 years, the developing third molars. Since cervical vertebrae development is not equally overlapping both age categories, in future research, the skeletal information will be added to the dental information obtained using two techniques. For the age group below 16 years, the Willems technique [66] will be applied on all lower left permanent teeth, and for the group above 16 years, the third molar development will be staged as described in this study

Conclusions

On cephalometric radiographs, the skeletal age-predicting variable(s) and related registration systems providing the most information on age were the cervical vertebrae scoring system of BA and SE. Adding the BA or the SE

information to the third molar model developed on GH scores improved the age predictions drastically, especially in the period of early third molar development. Because the SE technique allowed clinically the easiest and fastest registration of the degree of development of the cervical vertebrae, the choice of technique to classify the added cervical vertebrae development is SE.

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