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TECHNICAL NOTE

ANTHROPOLOGY

Kim-Diêp Dang-Tran,[1,2] *M.S.; Fabrice Dedouit,*[1,2,3] *M.D.; Francis Joffre,*[1] *M.D., Ph.D.; Daniel Rougé,*[2] *M.D., Ph.D.; Hervé Rousseau,*[1] *M.D., Ph.D.; and Norbert Telmon,*[2] *M.D., Ph.D.*

Thyroid Cartilage Ossification and Multislice Computed Tomography Examination: A Useful Tool for Age Assessment?

ABSTRACT: Many authors have studied the thyroid cartilage ossification process using radiographic features for age estimation of individuals. Their results were various: Sugiyama reported excellent correlation coefficients between ossification rates and civil age, unlike most other authors. We hypothesized that recent advances in imaging techniques would enable more precise evaluation of the ossification of the thyroid cartilage. We retrospectively analyzed the CT scans of 312 French patients using postprocessing software to examine the pattern of thyroid cartilage ossification, calculating the ossified volume of the cartilage on 3D reconstructions. Pearson's correlation coefficients for volume were 0.73 for men and 0.75 for women (standard error 18.02 and 17.06), indicating considerable inter-individual variability. Although a correlation between civil age and morphological changes was found, these methods based on thyroid cartilage ossification were not accurate enough for the assessment of individual age.

KEYWORDS: forensic science, forensic anthropology, age determination, thyroid cartilage ossification, imaging, multislice computed tomography

Age estimation is a major part of forensic medical work, and there is increasing demand for identification of cadavers and human remains (1). Age estimation is also frequently required for living individuals who have no valid birth certificate (refugees, illegal immigrants, cases of impersonation) (1–3). Several reliable methods, which can be used alone, are commonly employed for children and adolescents (1–3). Estimation of the age of adults is less accurate and a combination of several methods is required (1,3). Various anatomic areas may be used (1–7). Each method must be chosen according to its accuracy, simplicity, and advantages, assessed for each individual case (1–4).

Among these methods, study of thyroid cartilage ossification using radiographic features has been considered by many authors since the beginning of the last century. The first detailed X-ray study dated back to 1958, when Keen and Wainwright carried out postmortem examination of 133 adult and child larynges (8). These authors classified thyroid cartilage changes into five main stages and intermediate stages because of the many variations seen. They observed that cartilage ossification progressed at a much slower rate in women and that correlation between age and ossification was poor. A genetic factor in ossification was the main hypothesis discussed.

Vlcek (1980) (9) found a correlation between age and ossification and defined male ossification stages for each age decade from 0 to 70 years. In 1993, Turk radiographed 48 postmortem specimens (10). Based on the description of Keen and Wainwright, the author defined five stages for the male ossification process and four for women. Ossification varied widely between individuals and could not be linked to an age index.

Studies by Sugiyama et al. in 1995 (11,12) indicated that increasing thyroid cartilage ossification was a reliable method of age determination. The thyroid cartilages of 501 Japanese men and 513 Japanese women were radiographed at autopsy, and computerized measurement of ossification areas was performed to obtain an ossification rate for each specimen.

De la Grandmaison et al. in 2003 used Keen and Wainwright's classification to estimate age in 82 laryngeal radiographs of autopsy specimens. The correlation coefficient between laryngeal opacity and age was 0.74, but inter-individual variability was wide (standard error ± 12.7 years), preventing this method from being used alone for forensic purposes (13).

Cerny phases (14) were used for radiographic scoring of 104 laryngeal structures in a recent study (2008) by Garvin, who found this method inaccurate and not applicable (15).

The thyroid cartilage is one of the larynx cartilages and is known to go through an ossification process from the second or third decade, as shown by numerous radiographic findings (8,10–12,15–19). Ossification increases with advancing age (8,11,12,15,17–20). The radiographic features of the thyroid cartilage have been examined on postmortem dissection specimens and in living individuals (8,10–13,15–17,21). Male laryngeal cartilages tended to be ossified to a greater extent than female cartilages (8,10–13,15,17,19,21). For both genders, thyroid cartilage ossification appeared to pass through different phases until complete ossification, and each phase appeared to be correlated to a decade (8–10). However, this method using radiographic techniques has been contested (8,10,15,18,21).

[1]Service de Radiologie Générale, Centre Hospitalier Universitaire Rangueil, 1 avenue du Professeur Jean Poulhès, 31403 Toulouse Cedex 4, France.
[2]Service de Médecine Légale, Centre Hospitalier Universitaire Rangueil, 1 avenue du Professeur Jean Poulhès, 31403 Toulouse Cedex 4, France.
[3]Imagerie de Synthèse en Anthropobiologie, Université Paul Sabatier, FRE 2960 CNRS, 37 allées Jules Guesde, 31000 Toulouse, France.

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Numerous nondestructive methods based on computed tomography (CT) have been developed during the past two decades, enabling major advances in forensic fields (5–7). The aim of our work was to study thyroid cartilage ossification using multislice computed tomography (MSCT) and to estimate its potential relationship to age. We hypothesized that the recent advances in imaging techniques would enable more accurate evaluation of thyroid cartilage ossification. We found only one published study that assessed bone age using CT (21). This study closely examined ossification localization on the thyroid cartilage and analyzed the link between ossification deficiency and invasion by laryngeal tumor. The authors found wide variability in the ossification process, no link between ossification deficiency and tumor invasion, and no correlation with the patients' age, but all subjects were 50 years or older.

Materials and Methods

Materials

We carried out a retrospective study of thyroid cartilages in patients undergoing CT examination at our institution. The patients were of various ethnic origins and were globally representative of the present population of southern France. Their CT examinations were mainly requested in a clinical context of stroke, aneurysm, suspicion of laryngeal infection, abscess, cyst or tumor, vertigo, cervicobrachial neuralgia, cervical arthritis, and minor cervical injury. Patients with a known history of laryngeal radiation therapy, invasive osseous laryngeal tumor, thyroid cartilage fracture, or calcium disorder were excluded. The scans were performed between April 2005 and April 2008. A total of 312 CT examinations of patients of known sex and age were included. Patient gender (156 men, 156 women) and age (range, 3 days to 98 years) were recorded anonymously at inclusion. Mean age was 46.51 years for men (standard deviation: 26.01) and 46.17 years for women (standard deviation: 25.97). The sample distribution is shown in Table 1.

MSCT was performed on a Sensation 16 scanner (Siemens, Erlangen, Germany), a Philips Brilliance 16-slice scanner (Best, The Netherlands) and a GE HiSpeed FX/I scanner (Milwaukee, WI), with 16×0.75 mm collimation, except for pediatric patients (up to 15 years old) where collimation was 1.5 mm. The image matrix was 512×512 pixels. A bone filter and a soft tissue filter were used. For adult individuals, depending on the purpose of the scan, axial 1 mm (or 0.75 mm) thick reconstructions were performed every 0.5 mm (or 0.3 mm). For pediatric patients, depending on the purpose of the scan, axial 3 mm (or 1 mm) thick reconstructions were performed every 1.5 mm (or 1 mm).

Postprocessing was performed using Amira 4.1.1 software (Mercury Computer System, Inc., Chelmsford, MA). For our study, we saved the 312 CT scans as DICOM (Digital Imaging and Communications in Medicine) files. The software excluded soft tissue, and cartilage ossification was visualized in three dimensions. The visual densities and Hounsfield unit densities of the cartilage were recorded, and according to density, the ossified part of the thyroid cartilage was differentiated from the cartilaginous part. Depending on the appearance of the ossification, manual or semi-automatic segmentation was performed. Segmentation provided accurate volume measurements of the ossified part and rotational 3D reconstructions. Volume was expressed in cm^3.

TABLE 1—*Sample distribution according to sex.*

Age (years)	Men n = 156	Women n = 156
0–9	14	15
10–19	16	16
20–29	19	18
30–39	17	18
40–49	18	18
50–59	18	18
60–69	17	17
70–79	18	17
80–89	17	17
90–99	2	2

Methods

Morphological 3D Analysis—The 3D reconstructions were studied and compared with Turk stages (Fig. 1), based on the description of Keen and Wainwright, and with Vlcek ossification stages (Fig. 2) (8–10). Each 3D reconstruction was classified into a precise stage: 5 and 4 stages for Turk male and female classification, 7 stages for Vlcek male classification. Undetermined stages were classified as "*U*." We studied the distribution of Turk and Vlcek stages according to gender and civil age.

Volume Analysis—After segmentation, we recorded each ossified volume measurement and related it to the patient's civil age and gender, seeking a correlation between ossified volume and civil age.

Statistical Analysis—Statistical analysis was performed using R 2.2.0 software (R Development Core Team [2008]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org). Our sample included 312 thyroid cartilages. Previous studies demonstrated significant differences between male and female ossification (8,10–13,15,17,19,21). As we had a large sample, we analyzed male and female data separately. The correlation between age and ossification was studied using Pearson's correlation coefficient. We performed ANOVA to compare age differences between Vlcek and Turk stages. Our analysis was performed with a significance level of $p < 0.05$ and with a 95% confidence interval.

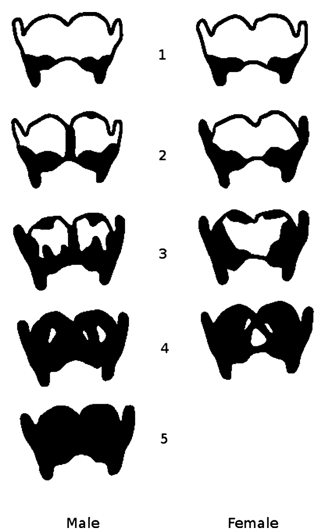

FIG. 1—*Turk stages of thyroid cartilage ossification.*

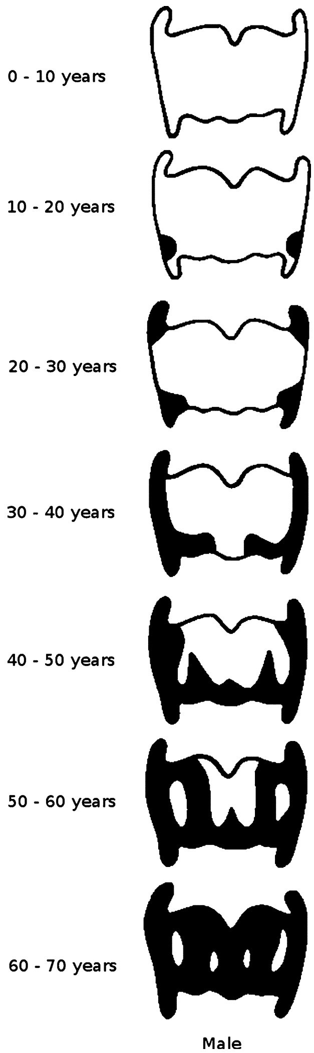

FIG. 2—*Vlcek stages of thyroid cartilage ossification, according to decade groups.*

Results

It is generally accepted that the onset of thyroid cartilage ossification occurs at a variable age, during or at the end of adolescence (8,10–12,15–19). In our sample, the youngest male individual with ossification foci was 15 years old, and the youngest woman was 13 years old. However, no precise age of onset could be determined as one 21-year-old man and five 15–19-year-old women had no sign of ossification.

Morphological 3D Analysis

Globally, ossification progressed with the age of the individual as previously described (8,11,12,15,17–20). In our study, male and female cartilage ossification generally began symmetrically at the base of the inferior horns (8–10,15,17–19). It spread along the posterior borders and to the inferior border of the laminae to form a low posterior triangle on each side. The ossification process then showed variability between men and women and in each age-related sample (8,10–13,15,17,19,21).

Male Ossification Process—We found that the ossification pattern usually began at the posterior border, near the base of the inferior horns (8–10,15,17–19). In some cases, there were two or more separate foci along the posterior borders (8). The ossification spread along the posterior borders, the inferior horns and along the inferior border of the laminae (8–10,15,18). The ossification process then extended to varying degrees and sometimes invaded the superior border of the laminae (10), or the lower or upper part of the anterior midline strip (18) that linked up with the bone along the lower border (8,10), or continued to extend along the inferior border of the laminae (8). In a second phase, it extended and invaded the laminae (8,18). Ossification was bilateral but was not strictly symmetrical in extent (21). The ossification process was extensive but seldom led to total ossification (8,11,17,18). Frequently, two symmetric rounded areas remained nonossified in the anterior part of the laminae, near the midline (8,17,18).

Female Ossification Process—We observed that the female process was similar to the male process in the early stages (10,15). It also spread along the posterior borders (10), the inferior horns and along the inferior border of the laminae (15,18). In most cases, the lower part of the anterior midline strip became ossified (18). The inferior and upper parts of the laminae were seldom totally ossified. We noted that the ossification process mainly showed right- and left-sided symmetry (8,17,19). It appeared less extensive in women than in men (8,10,15,17,19,21). In women, the ossified parts of the cartilage seldom showed complete ossification (10,12,15,17,19).

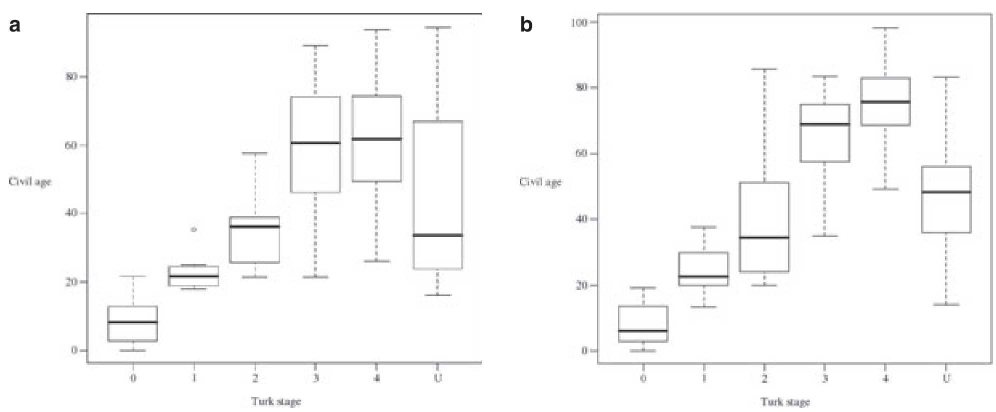

FIG. 3—*a: Box-and-whiskers plot for Turk male stages: box represents interquartile range, thick black horizontal line represents median, t-bars represent extremes. (°) represents outlier. b: Box-and-whiskers plot for Turk female stages: box represents interquartile range, thick black horizontal line represents median, t-bars represent extremes.*

We had difficulties classifying the 3D ossification reconstructions into Turk (Tables 2 and 3) or Vlcek stages (Table 4): 16.67% of men and 33.97% of women did not match any Turk stages, while 50.64% of men did not fit the Vlcek classification and were classified as undetermined. Defining new or intermediate stages in the Vlcek or Turk stages was impossible because of the considerable inter-individual variability. If we looked only at the cartilages whose Turk stage could be determined, the distribution showed a correlation between ossification pattern and age. But this relationship was debatable for male or female patients over 60 years old (Fig. 3*a,b*). The 3D views of the ossification process did not show any correlation with age according to Vlcek stages (Fig. 4).

TABLE 2—*Distribution of ossified thyroid cartilage in males, correlated with Turk stages.*

Male Turk Stages	Number of Subjects	Sample Percentages	Mean Age (Years)	Standard Deviation (Years)
0	25	16.02	8.06	6.05
1	8	5.13	22.82	5.68
2	7	4.49	34.93	12.46
3	26	16.67	59.59	17.30
4	64	41.02	61.42	16.70
Undetermined	26	16.67	44.13	25.73

TABLE 3—*Distribution of ossified thyroid cartilage in females, correlated with Turk stages.*

Female Turk Stages	Number of Subjects	Sample Percentages	Mean Age (Years)	Standard Deviation (Years)
0	22	14.10	45.21	26.45
1	15	9.62	38.20	24.58
2	20	12.82	65.83	31.05
3	9	5.77	45.04	21.88
4	37	23.72	47.13	26.47
Undetermined	53	33.97	43.02	26.88

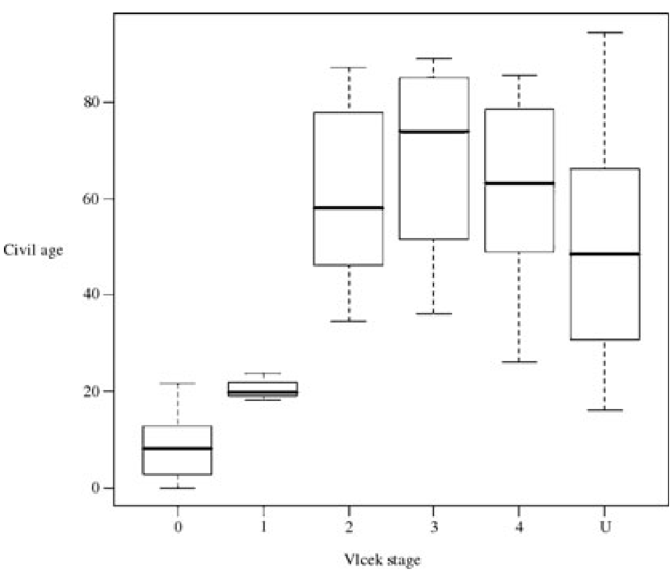

FIG. 4—*Box-and-whiskers plot for Vlcek male stages: box represents interquartile range, thick black horizontal line represents median, t-bars represent extremes.*

Volume Analysis

In our analysis, ossified volumes showed a wide distribution and an average correlation with age (Figs. 5 and 6). We confirmed the marked inter-individual variability of the thyroid cartilage ossification process (8,10,13,15,21). Pearson's correlation coefficients were 0.73 for men, and 0.75 for women (18.02 and 17.06 standard errors). This distribution did not enable us to determine the age of individuals. As found in other studies, female cartilages were less ossified than men (8,10,15,17,19,21). The ossified parts of female cartilages seldom covered all the cartilage (10,12,15,17,19). We could not conclude on any

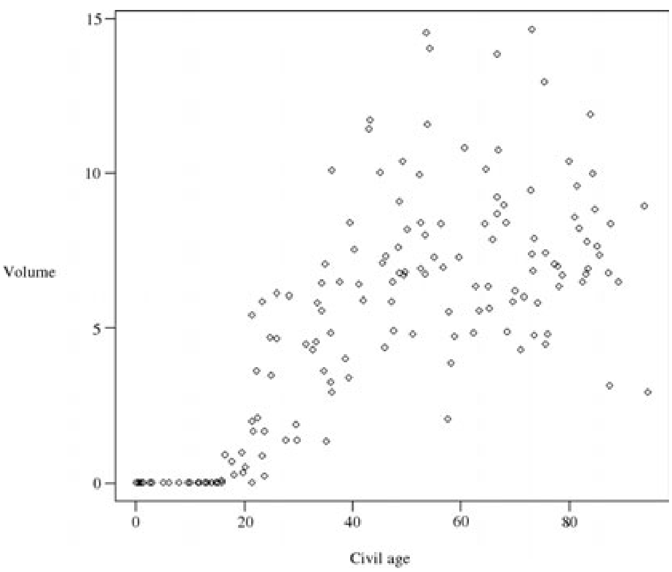

FIG. 5—*Distribution of the volume of ossified thyroid cartilage (cm^3) in males, correlated with civil age.*

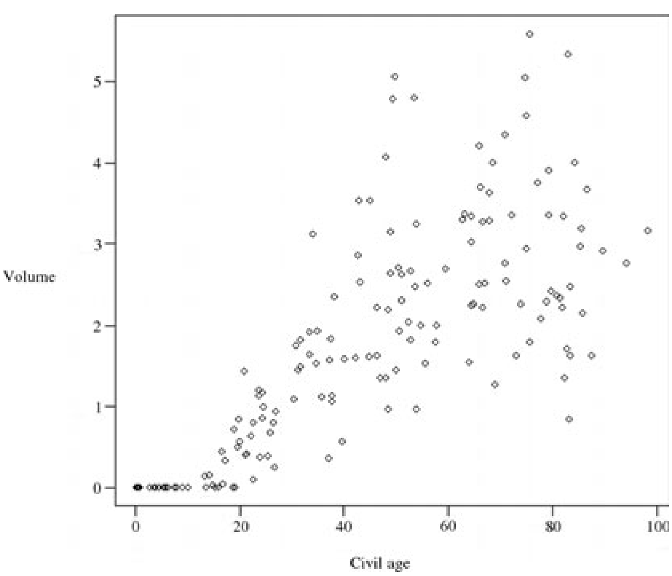

FIG. 6—*Distribution of the volume of ossified thyroid cartilage (cm^3) in females, correlated with civil age.*

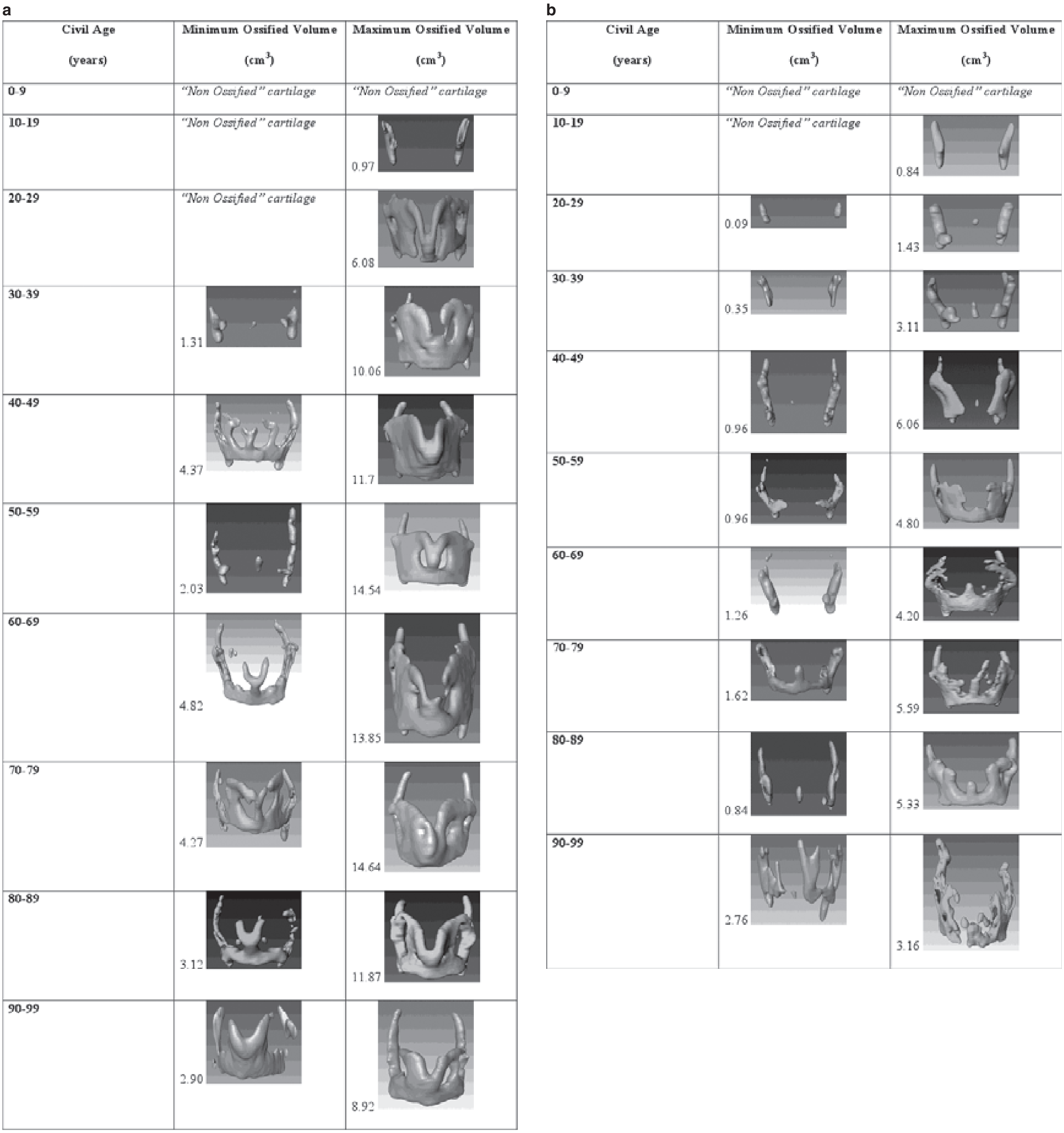

FIG. 7—*a: Ossified volumes of male thyroid cartilages showing inter-individual and intra-gender variabilities. NO = nonossified cartilage. 3D reconstructions of the ossified part of male cartilages. Anterior view, Amira 4.1.1 postprocessing. b: Ossified volumes of female thyroid cartilages showing inter-individual and intra-gender variabilities. NO = nonossified cartilage. 3D reconstructions of the ossified part of male cartilages. Anterior view, Amira 4.1.1 postprocessing.*

significant correlation between ossified volume and civil age for either men or women (Fig. 7*a,b*).

Discussion

To the best of our knowledge, MSCT had never been previously used to analyze thyroid cartilage ossification for age determination. It gave us more precise results than radiography (lateral cephalometric and cervical spine radiographs), especially for living individuals. Analyzing the extent of thyroid cartilage ossification was a difficult task even with quality radiographs. Perfect knowledge of anatomy and its variations is required. Even so, thyroid cartilage ossification could be confused with ossification of other laryngeal cartilages (cricoid, arytenoid, triticeal cartilages), foreign bodies,

TABLE 4—*Distribution of ossified thyroid cartilage in males, correlated with Vlcek stages.*

Male Vlcek Stages	Number of Subjects	Sample Percentages	Mean Age (Years)	Standard Deviation (Years)
0	25	16.02	8.06	6.05
1	3	1.92	20.55	2.86
4	9	5.77	61.07	19.38
5	4	2.56	68.36	23.41
6	36	23.08	62.81	17.03
Undetermined	79	50.64	49.48	21.51

metastatic calcifications, calcified lymph nodes, or calcified atherosclerotic plaques (17–19,21). For living individuals, generally only lateral radiographs were instructive because of superposition of the cervical spine on frontal radiographs (17).

Moreover, the accuracy of our results was improved as we examined a large sample of 312 individuals. Previous radiographic studies of thyroid cartilages did not examine such large populations because of inclusion and preparation difficulties (<150 specimens), except for Sugiyama et al. who studied 501 male and 513 female cartilages (8,10–13,16,21). For example, Turk and Hogg studied 21 men and 27 women, de la Grandmaison et al. 56 and 26, Keen and Wainwright 81 and 52 (8,10,13).

Our results agreed with those of most authors. None found a significant correlation between age and the appearance of radio-opaque ossified cartilage except de la Grandmaison et al. and Sugiyama et al. (8,10–13,15,18,21). De la Grandmaison et al. found a 0.74 correlation coefficient but too great an inter-individual variability (12.7 standard error) for their method to be used alone without combining it with other more accurate methods (13). Moreover, the authors took into account the total laryngeal opacity score and did not analyze the thyroid cartilage separately. Sugiyama et al. (11,12) found high correlation coefficients between ossification rates and age (0.96 for men and 0.99 for women). But this method did not take inter-individual variability into account, as statistical analysis considered only the mean ossification rates for each age sample.

In our study, the 3D reconstructions of the ossified part of the thyroid cartilage and measurement of the ossified volume both showed that ossification progressed overall with the age of individuals (8,11,12,15,17–20). We observed that female ossification was a less extensive process than male ossification, in agreement with many other authors (8,10,15,17,19,21).

Morphological 3D Analysis

The considerable inter-individual variability made it difficult to sort the 3D reconstructions into stages in order to estimate age. Compared with the Vlcek classification, Turk stages seemed to be the closest estimation. However, the rate of undetermined stages (16.67% for men and 33.97% for women) made it difficult to use this method as a reference method.

Volume Analysis

We observed a correlation between ossified volume and age in each gender-related sample. The correlation coefficient was higher in the female sample (0.73 for men, 0.75 for women). However, the correlation rates were not significant enough to enable us to use these methods for forensic purposes because of the low accuracy (18.02 and 17.06 standard errors). This was mainly because of inter-individual variability.

Inter-individual variability could have been reduced by sampling individuals of the same ethnic origin (11,12). But this method could not be used for a present-day population, because of population diversity, and was beyond the scope of this study. However, some authors believe that no racial differences exist (8). Other unknown factors may influence the ossification rate: genetics, hormonal factors, chronic local processes, muscular contraction strength and frequency, economic environment, or osteoporosis (8,10,13,15,18,21). Hormonal factors are known to underlie differences in male and female development and aging. These fundamental influences also affect bone synthesis and loss and so may well also affect the thyroid cartilage ossification process. However, no previous published data are available concerning this complex multifactorial process. Alcoholism, however, does not seem to have any impact on the ossification process (13).

Conclusion

The use of MSCT in forensic medicine has proved to be an efficient, simple, fast, and nondestructive technique (5–7). It provides accurate results and offers many precise postprocessing possibilities. Our study of thyroid cartilages for age assessment using MSCT was based on morphological 3D reconstructions and ossified volume measurement. Unfortunately, these methods based on thyroid cartilage ossification were not accurate enough for individual age assessment.

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Additional information and reprint requests:
Kim-Diêp Dang-Tran, M.S.
Service de Radiologie Générale
Centre Hospitalier Universitaire Rangueil
1 avenue du Professeur Jean Poulhès
TSA 50032
31059 Toulouse Cedex 9
France
E-mail: dtkimdiep@hotmail.com