ORIGINAL ARTICLE

Estimation of forensic age using substages of ossification of the medial clavicle in living individuals

Oguzhan Ekizoglu[1] · Elif Hocaoglu[2] · Ercan Inci[2] · Ismail Ozgur Can[3] · Sema Aksoy[2] · Ibrahim Sayin[4]

Abstract Forensic age estimation based on staging of ossification of the medial clavicular bone is one of the methods recommended by the Study Group on Forensic Age Diagnostics of the German Association of Forensic Medicine. In the present study, we analyzed the stages of ossification of the medial clavicular epiphyses on thin-sliced (1 mm) computed tomography (CT) images using the substages defined within stages 2 and 3. The retrospective CT analysis involved 193 subjects (129 males, 64 females) ranging in age from 13 to 28 years. Spearman's correlation analysis revealed a positive correlation between age and ossification stage in both male and female subjects. Stage 3c was first observed at 19 years of age in both sexes and may thus serve as a valuable forensic marker for determining an age of 18 years. Although further research is needed on the ossification stages of the medial clavicular epiphyses, the present findings could contribute to existing reports on observers' experiences using CT analysis of ossification combined with analysis of substages.

Keywords Age estimation · Schmeling method · Medial clavicular epiphyses · Computed tomography · Substages

✉ Oguzhan Ekizoglu
drekizoglu@gmail.com

1 Department of Forensic Medicine, Tepecik Training and Research Hospital, Güney mahallesi 1140/1 Yenisehir, Konak, Izmir, Turkey

2 Department of Radiology, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey

3 Department of Forensic Medicine, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

4 Department of Head and Neck Surgery, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey

Introduction

Forensic age estimation in living individuals has gained importance in civil and criminal law. Courts often require forensic scientists to determine the age of an individual (thus, adult or child); this is of legal importance in assessing criminal culpability. Also, refugees who lack age documentation, or who intentionally state they are younger than they are, require evaluation. In addition, the paucity of birth records of countries of low socioeconomic status is problematic [1]. Forensic age estimation of criminals is especially important, and various definitions and age thresholds are used among different countries' jurisprudences. In most countries, 18 or 21 years is the critical age in terms of imposition of criminal penalties. The hand–wrist or dental status is usually examined for forensic purposes [1, 2]. In particular, the Study Group on Forensic Age Diagnostics of the German Association of Forensic Medicine (AGFAD) recommends the combination of physical examination and radiographic examinations of the left hand, a dental examination, and an orthopantomographic examination. If ossification of the hand is complete, radiological examination of the degree of clavicular ossification with conventional radiography and/or computed tomography (CT) is recommended [2].

Work on age-staging ossification of the medial clavicular bone has attracted particular intention; assessments may be made by X-ray [3, 4], CT [5–20], ultrasonography [18, 21, 22], or magnetic resonance imaging [23–25]. During the last few years, CT has mainly been performed for research purposes in the forensic sciences; thus, it is recognized that the study of thin sections at high resolution may help eliminate mistakes by allowing more detailed assessments [6, 8, 10, 12]. Many researchers have also studied other noninvasive methods besides CT such as magnetic resonance imaging and ultrasonography, which also have some advantages (no

exposure to radiation) and are being used increasingly more often [21–25].

The Schmeling five-stage method is the most frequently preferred method for staging of ossification of the medial clavicle [7, 8, 10–12, 15–18]. Kellinghaus et al. [6] proposed the use of subclassifications as a supportive method for the Schmeling staging technique. However, few studies have focused on this subject [6, 8, 15, 17].

We previously presented data obtained using the Schmeling five-stage method; we evaluated a Turkish population using CT [16]. In the present study, we explored the utility of the subclassification of Kellinghaus et al. [6] in such a population and compared our data to those of previous studies, to contribute to efforts to enlarge the database supporting the recognized substages of medial clavicular ossification.

Materials and methods

This retrospective study was performed at Bakırköy Dr. Sadi Konuk Research and Training Hospital, Istanbul, Turkey, from January 2014 to August 2014. We evaluated CT images of patients who presented for trauma and other conditions. The local ethics board approved the study protocol.

We retrospectively collected gender and medical information from the data-processing center of the hospital. Age, sex, and all other patient information were concealed from observers using a "hide data" feature of the software. Patients with clavicular fractures (28 cases), developmental abnormalities (1 case of clavicular aplasia), bilateral presence of anatomical shape variation (11 cases), and missing data on chronological age (14 cases) were excluded from the study. Patients with a history of any surgery or pathological bone conditions were also excluded. In total, the CT scans of 573 patients were evaluated. Finally, 193 cases aged between 13 and 28 years and classified as stage 2 (97 cases) and stage 3 (96 cases) according to Schmeling et al. [3] were included in the study.

Multidetector CT was performed using a 40-row multidetector CT scanner (Siemens Medical Solutions, Erlangen, Germany) from January 2014 to August 2014. All scans were obtained with the patients in the supine position. The following parameters were used: tube voltage, 120 kV; effective mAs, 120; and slice thickness, 1 mm. Row data were reconstructed using bone algorithms. All images were transferred to a commercially available Leonardo Workstation (Siemens AG, Munich, Germany). Axial views were obtained, and all slices were evaluated through the sternoclavicular joints.

Two radiologists (R1 and R2) evaluated all CT images. One radiologist (R1) had 10 years of experience with CT, and the other (R2) had 20 years of experience. Both radiologists had experience estimating age via CT by measuring the ossification stage of the medial clavicle [16].

The observers reevaluated all images after 2 weeks without knowledge of the results of previous clavicular ossification staging. We used the assessments of R1; if the left and right assessments differed, we recorded the ossification status of the more advanced side.

We analyzed the data using the substages 2a–c and 3a–c described by Kellinghaus et al. [6]:

- Stage 2a—the lengthwise epiphyseal measurement is a maximum of one third of the widthwise measurement of the metaphyseal ending.
- Stage 2b—the lengthwise epiphyseal measurement is more than one third to a maximum of two thirds of the widthwise measurement of the metaphyseal ending.
- Stage 2c—the lengthwise epiphyseal measurement is more than two thirds of the widthwise measurement of the metaphyseal ending.
- Stage 3a—the epiphyseal–metaphyseal fusion completes a maximum of one third of the former gap between the epiphysis and metaphysis.
- Stage 3b—the epiphyseal–metaphyseal fusion completes more than one third to a maximum of two thirds of the former gap between the epiphysis and metaphysis.
- Stage 3c—the epiphyseal–metaphyseal fusion completes more than two thirds of the former gap between the epiphysis and metaphysis.

Statistical analysis

Continuous variables regarding age estimation in both sexes according to stage are expressed as means, 95 % confidence intervals, and lower and upper quartiles, standard deviations, medians, minima, and maxima. Categorical variables are expressed as percentages. Evaluation of the relationship between age and stage was performed using Spearman correlation analysis. Comparisons between the two sexes were performed using the Mann–Whitney U test, and a P value of <0.05 was accepted as statistically significant. The Wilcoxon test was used to evaluate the differences between the ossification stages of the right and left medial clavicle. The Statistical Package for the Social Sciences (SPSS) software (version 17; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis.

Compatibility between the two radiologists and one forensic medicine expert was assessed with Cohen's κ statistical test. The κ value, weighted κ value, and agreement rate were calculated. The system proposed by Altman [26] was used to interpret the κ values:

- $\kappa<0.20$: poor agreement
- $\kappa=0.21–0.40$: fair agreement

- κ=0.41–0.60: moderate agreement
- κ=0.61–0.80: good agreement
- κ=0.81–1.00: very good agreement

Results

In total, 193 patients were evaluated (129 males, 64 females; age, 13–28 years). The average age of female patients was 18.55±3.05 years, and that of male patients was 18.75±2.67 years. Table 1 shows the distribution of the sample by number of cases and sex. No significant differences between the ossification stages of the right and left medial clavicle were observed between the two sexes.

Cohen's kappa test between the two observers was κ=0.868 and intraobserver variability was κ=0.916 for the medial clavicular ossification, respectively, indicating very good reliability.

A statistically significant positive correlation was found between age and determined stage based on Spearman's rank correlation values (two-tailed $P<0.001$). A statistically significant difference between the sexes was observed only for substage 2c ($P<0.05$).

We detected 11 instances of bilateral variation in anatomical shape and excluded all of those cases.

In the evaluation of the stage 2 subclassification, stage 2a was first seen at 14 years of age among male patients and at 13 years among female patients. The earliest appearance of ossification for stage 2b was at 16 years in both male and female patients. The earliest appearance of ossification for stage 2c was 17 years in male patients and 16 years in female patients.

In male patients, the latest appearance of stages 2a, 2b, and 2c was 20, 21, and 21 years, respectively. In female patients, the latest appearance of stages 2a, 2b, and 2c was 19, 19, and 21 years, respectively.

In male patients, the earliest appearance of stages 3a, 3b, and 3c was 17, 18, and 19 years, respectively; the latest appearance of these stages was 22, 25, and 25 years, respectively. In female patients, the earliest appearance of stages 3a, 3b, and 3c was 16, 17, and 19 years, respectively; the latest appearance of these stages was 21, 28, and 24 years, respectively.

Table 2 shows the minimum and maximum ages and the means±standard deviations and lower and upper quartiles of all parameters.

Discussion

In clavicular medial ossification assessment, the five-stage classification system described by Schmeling et al. [3] and CT age estimation analyses have become major research tools [7, 8, 10–12, 15–18]. Slice thickness is one of the key factors in CT-based research. It has been emphasized that the slice thickness should be at least 1.0 mm; in recent studies, slice thicknesses of 0.6 to 1.0 were used [6, 8, 10–12, 15, 16]. A thickness of 1 mm was used in the present retrospective study. Greater thickness may reportedly cause evaluation errors because of partial volume effects, anatomic shape variants, and overlooking of epiphyseal scar factors [8, 10, 11, 15]. Observer experience is also important to the evaluation. Wittschieber et al. [15] reported that observer experience had a strong effect on the results of medial ossification grading results; anatomic shape variants and overlooking the epiphyseal scars were the most common errors. Another important factor is the need to analyze all of the slice sections. Both the inner and outer parts of the clavicle end in all slice sections must be searched for the epiphyseal scar [15, 16, 23]. One of the limitations of our study is observer experience. Although our observers had experience with rating ossification of the medial clavicle and our kappa values seemed good, the observers had no previous experience determining subclassifications. Wittschieber et al. [15] emphasized that the kappa values for intraobserver and interobserver agreement should not be accepted uncritically as objective and commented that professional competence, experience, and knowledge were required. In the present study, shape variants were observed in 11 patients, who were thus excluded from the analysis. Subject numbers were very low, as is also true of previous studies [8, 15]; the lack of observer experience with slices thinner than 1 mm may render it difficult to identify variants of anatomical shape.

Table 1 Ages of male and female subjects

Age	Male (n)	Female (n)
13	–	3
14	7	1
15	2	8
16	20	8
17	19	4
18	17	5
19	19	13
20	10	5
21	9	4
22	15	8
23	5	3
24	3	1
25	3	–
26	–	–
27	–	–
28	–	1

Table 2 Minimum and maximum ages and the mean±SD and median and lower and upper quartiles at all ossification stages

Stage	Sex	No.	Mean age (95 % CI)	Min–max	SD	Median	LQ, UQ
2a	Male	30	16.03 (15.44–16.63)	14–20	1.586	16	14.75, 17
	Female	10	15.10 (13.69–16.51)	13–19	1.96	15	13, 16.25
2b	Male	25	17.44 (16.88–18.0)	16–21	1.35	17	16, 18.5
	Female	10	16.7 (15.05–18.35)	15–21	2.31	15	15, 19
2c	Male	13	18.46 (17.62–19.30)	17–21	1.38	18	17, 20
	Female	9	17 (16.14–17.86)	16–19	1.18	17	16, 18
3a	Male	8	18.5 (16.66–20.34)	17–22	2.2	17.5	17, 21
	Female	6	18.5 (16.32–20.68)	16–21	2.07	19	16, 20.25
3b	Male	20	20.45 (19.46–21.44)	18–25	2.11	20.5	18.25, 22
	Female	9	20 (17.48–22.52)	17–28	3.27	19	18, 21
3c	Male	33	21.36 (20.71–22.01)	19–25	1.83	22	19.5, 22.5
	Female	20	21.25 (20.51–21.99)	19–24	1.58	22	20, 22

SD standard deviation, *CI* confidence interval, *LQ* lower quartiles, *UQ* upper quartiles

Compared to the results of Kellinghaus et al. [6], our data on females differed. The previous study considered females to be in stage 2c at 16 years of age, where we considered stage 2c at 17 years of age. Compared to Wittschieber et al. [8], our ages for stages 2b, 2c, and 3c in males and stage 3c in females were the same, whereas all later ages differed by ±1 year. We compare the data of those two previous studies to our data in Table 3. While Kellinghaus et al. [6] used a slice thickness of 0.6 mm for 2 cases and ≥1.0 mm for the remaining 183 cases, Wittschieber et al. [8] used a thickness of 0.6 mm for all cases. In the present study, all cases were analyzed at a thickness of 1.0 mm. The differences in the maximum age of the subclassification states between the present study and Wittschieber et al. [8] may have been related to partial volume effects associated with slice thickness. Stage 3c was first seen at the age

of 19 years in both sexes; thus, we consider that stage 3c contributes significantly to forensic evaluation of the age of 18 years. In the present study, stages 2c and 3a were first seen at the same age in both sexes, and the mean values were close to each other. This finding is the same as that in previous studies of these subclassifications [6, 8, 17]. This result might also be due to the earlier appearance of stage 3a. As already suggested [8], it may also be hypothesized that stage 2c does not always develop before arriving at stage 3a.

We believe that these variances may be due to the differences in the socioeconomic status of the countries in which the studies were performed, the limited experience of observers in the present study, and the differences in the slice thicknesses between the studies. Future studies performed in countries with an expanded socioeconomic status will be important to more accurately evaluate these differences. We believe that the discussion could be improved by considering studies such as Pattamapaspong et al. [18]. The age determinations that Pattamapaspong et al. [18] presented (stage 3c; 18.0 years for males and 17.4 years for females) are not entirely consistent with those of more recent studies. On one hand, the same authors stated that ethnicity is not effective for staging of ossification of the medial clavicle. Also, any effect of socioeconomic status remains unclear, although it was emphasized that supportive data were lacking. However, Ontell et al. [27] described advanced skeletal maturation in Asians during adolescence. Pattamapaspong et al. [18] suggested that their studies could be used as a reference in Thai society. However, there are two potential problems with these suggestions. First, the experience levels of the observers and the intraobserver–interobserver error analysis results were not presented. We believe that the possible observer-related assessment errors emphasized by Wittschieber et al. [15] should be assessed for this study. Second, in terms of the socioeconomic status, to the data of United Nations Human Development Index

Table 3 Comparison table between the present study and study of Kellinghaus et al. [6] and Wittschieber et al. [8]

Stage	Sex	Present study (min–max)	Kellinghaus et al. [6] (min–max)	Wittschieber et al. [8] (min–max)
2a	Male	14–20	14.4–20.0	15.0–16.5
	Female	13–19	13.1–18.2	14.8–18.4
2b	Male	16–21	16.4–20.1	16.1–20.4
	Female	15–21	15.4–19.3	14.1–15.8
2c	Male	17–21	17.1–20.2	17.4–20.2
	Female	16–19	15.6–18.2	17.9
3a	Male	17–22	17.5–20.7	16.4–22.3
	Female	16–21	16.8–22.1	15.5–23.3
3b	Male	18–25	18.3–25.4	17.6–36.5
	Female	17–28	17.8–24.4	16.4–23.3
3c	Male	19–25	19.7–26.2	19.0–30.0
	Female	19–24	19.5–26.2	19.4–26.5

[28], Thailand is in position 89. Climate differences, hormonal factors, and puberty-related factors have both developmental and socioeconomic effects [1, 29–34]. Because these major factors were not evaluated in their study, we believe that acceptance of their results as a reference for Thai society is controversial.

Conclusion

Forensic age estimation based on the ossification stage of the medial clavicle can provide important data, especially for individuals older than 18 years. We believe that the use of the subclassification described by Kellinghaus et al. [6] could help to achieve more accurate assessments, especially with respect to the earliest appearance of stage 3c at the age of 19 years. We believe that assessment of medial clavicular ossification based on thin-slice CT analysis will provide more accurate age estimation when applied to assess larger and more diverse multicenter cohorts with greater observer experience.

Conflict of interest The authors declare that they have no competing interests.

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