Age estimation in the Thai male population using epiphyseal union of the medial clavicle

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Objectives
To study the relationship between the union of the epiphyseal plate of the medial clavicle and age in the Thai male population.

Methods
Age-at-death estimation was evaluated from clavicles obtained from 200 cadavers of Thai males aged between 11 and 35 years. After soft tissue removal, the fusion status of the medial clavicular epiphysis was classified using a morphological classification. Stages of the epiphyseal union were categorized as follows: 1) nonunion with no epiphyses, 2) nonunion with separate epiphyses, 3) partial union, and 4) complete union.

Results
The stage of development of the epiphyseal union increased with age. The nonunion stage was found in individuals up to 22 years old. Complete epiphyseal union was found as early as age 22. Partial union was found in individuals 19-26 years old.

Conclusions
The stage of the epiphyseal union of the medial clavicle in Thai males is related to age. We recommend using complete union (stage 4) of epiphyseal development to indicate age more than 22 years and nonunion (stage 1 and 2) to represent age less than 22 years in Thai males. Chiang Mai Medical Journal 2021;60(2):149-55. doi 10.12982/CMUMEDJ.2021.13

Keywords: age estimation, epiphyseal union, medial clavicle, Thai skeletal remains

Introduction
Estimation of the age of a deceased individual is one of the most important issues in crime investigations as well as following mass disasters. Various methods of age estimation from skeletal remains have been developed in recent years which depend in part on the skeletal materials as well as the methods used for the analysis (1-15). Methods using morphological traits of the human skeleton have focused on the pubic symphysis (4), cranial suture closure (5) as well as union of the epiphyseal plate (16).

Currently, forensic anthropology relies heavily on standard methods for aging juvenile remains based on the epiphyseal union (16). In endochondral ossification, a bone grows by deposition of skeletal matrix on epiphyseal cartilage. Over time, bone growth stops and the epiphyseal plate unites with the diaphysis, leaving a temporary line. Finally, this visible line become completely obliterated by the remodeling process as the individual ages (1,16). Thus the progress of the epiphyseal fusion of various bone elements provides valuable data for age-at-death estimation, particularly in teenagers and young adults.

Estimation of age-at-death is important for purposes of identification of individuals. Recent studies have investigated at the relationship between clavicular development and age (2,3,6,9-16). The
medial epiphysis of the clavicle is the last bone to fuse and thus is useful for estimating age in the post-pubertal period (2-15,16). Variations in the range of fusion time have been identified between the sexes and in different populations. Epiphyseal fusion data from the medial end of the clavicle has been obtained from samples of dry bones (2,3) as well as from radiological images (12-15). However, those two methods provide different results depending on the condition of the skeletal remains (1). The application of older standards can result in an inaccurate age estimation (7). Changes in secular trends in growth and maturation rates have occurred in all developed countries (7). Certain non-genetic conditions, e.g., better health practices and living conditions, can promote a faster growth rate during childhood and the teenage years. Thus, a forensic anthropologist should establish criteria based on more recent samples to accurately reflect the modern population. What is needed are population-specific standards for individual countries such as Thailand.

**Objectives**

The purpose of this study was to develop standards for age-at-death estimation using morphological analysis of medial clavicular epiphysis in the Thai male population.

**Methods**

This prospective study was conducted on 200 pairs of Thai male clavicles collected from autopsy cases and were examined in the Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Bangkok, Thailand. Ethical approval was obtained from the Human Research Protective Unit, Faculty of Medicine, Siriraj Hospital, Mahidol University (Code 630081). From 15 May 2020 to 30 December 2020, Thai male cadavers of known age between 11 and 35 years were selected for study. Clavicles exhibiting any morphological pathology e.g., a fracture, deformity or bone developmental anomaly, were excluded from the study.

After obtaining permission from relatives, both left and right medial clavicles were dissected from their surrounding muscles and carefully removed from the deceased with an electric saw. Subsequently, the clavicles were wrapped in gauze pads and simmered in a solution containing washing detergent for 6-8 hours or until the muscle coverings could be removed (17). Next, the bones were washed then wiped with a piece of gauze and dried at room temperature. Finally, the bone samples were examined to determine the stage of epiphyseal closure (Table 1 and Fig. 1). Right and left elements were scored separately.

Statistical analysis was conducted using the SPSS package (SPSS 27.0 for Windows; SPSS Inc.). Descriptive statistics were used to describe the basic characteristics. Differences between the left and right clavicles of each individual were explored using the Wilcoxon signed rank test. The distribution of the stage of epiphyseal union varied for ages between 11 and 35 years. The relationship between age and epiphyseal union stage was visually analyzed using Spearman’s Rank correlation coefficient. To test accuracy and reliability, a sample of 35 clavicles was randomized and re-evaluated for intra- and inter-observer error.

**Results**

Both the left and right clavicles of 200 Thai males aged between 11 and 35 met the inclusion criteria and were assessed for the stage of medial clavicular fusion. The mean age of the subjects was 24.37±5.95 years, the average height was 171.77±8.46 cm and the average weight was 68.14±19.19 kg. The left and right clavicles showed the same stage in 191 of the cases (95.5%), with 9 cases (4.5%) showing different stages. Table 2 illustrates the average age of both left and right clavicles. No statistically significant difference was observed between left and right sides in any

| Stage | Description                          |
|-------|--------------------------------------|
| 1     | Nonunion with no epiphyses           |
| 2     | Nonunion with separate epiphyses     |
| 3     | Partial union                         |
| 4     | Complete union                        |
Figure 1. Stages of the epiphyseal union of the medial clavicle were categorized as: Stage 1 = nonunion with no epiphyses; Stage 2 = nonunion with separate epiphyses; Stage 3 = partial union; and Stage 4 = complete union.

Table 2. Number of cases and an average age for each stage of epiphyseal union of the right and left medial clavicles.

| Stage | Right clavicle | | Left clavicle | |
|-------|----------------|----------------|----------------|----------------|
|       | No. | Mean age | SD. | No. | Mean age | SD. |
| 1     | 21  | 15.05    | 1.91 | 22  | 15.09    | 1.88 |
| 2     | 30  | 18.37    | 2.13 | 29  | 18.45    | 2.11 |
| 3     | 39  | 21.79    | 1.91 | 36  | 21.67    | 1.93 |
| 4     | 110 | 28.70    | 3.67 | 113 | 28.56    | 3.72 |

The stage of medial clavicular epiphysis showed a statistical difference at different ages. We found that the average age in each stage, analyzed by one-way ANOVA, was statistically significantly different ($p < 0.0001$). We also found a strong correlation between age and the fusion stage of the medial epiphysis of both the left clavicle ($r = 0.86, p < 0.001$) and right clavicle ($r = 0.87, p < 0.001$).

The reliability of this study, particularly inter- and intra-observer variation, was also studied. Using Cohen's kappa coefficient, the average intra-class correlation coefficient (ICC) was 1.0 for intra-observer reliability and 0.92 for inter-observer reliability. Based on the 95% confidence interval of the ICC estimate, values greater than 0.9 in this study are indicative of excellent reliability (21).
Discussion

Age estimation using the clavicle has been studied extensively in both forensic and archaeological settings because the clavicle is likely to survive inhumation more successfully than other long bones (2,6). In this study, the stage of the medial clavicular epiphyseal union was described using a version of the Webb and Suchey classification (3), which is an easy and well-defined system. Fusion time between primary and secondary ossification centers exhibits significant variation. Between the age of 11 and 35 years, the degree of the epiphyseal union of the medial clavicular epiphysis changes dynamically and increases with increasing age. In this study, we found no difference in the degree of left and right clavicle union, results which are in accord with previous studies (2,3,6,12-15).

During the early developmental stage, the small and unrecognizable piece of the epiphysis is isolated. Direct visualization of dry bone samples is not possible for purposes of age estimation in this period because of the absence of the epiphysis. The present study showed that a maximum age of 22 years can be inferred when the non-union stage is seen in a dry bone sample. If the complete union is observed, a minimum age of 22 years can be concluded. When the partial union stage is observed, the subject can be estimated to have been 19-26 years old at the time of death.

Of particular interest is the comparison of the right with the left clavicle. Table 2 shows the timing of each fusion stage in each of the two sides for the medial clavicle. The finding of a strong

| Age (yrs) | Right clavicle | Left clavicle |
|-----------|----------------|--------------|
|           | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
| 11        | -      | -      | -      | -      | 1      | -      | -      | -      |
| 13        | 5      | -      | -      | -      | 5      | -      | -      | -      |
| 14        | 1      | -      | -      | -      | 1      | -      | -      | -      |
| 15        | 9      | 4      | -      | -      | 5      | 4      | -      | -      |
| 16        | 7      | 2      | -      | -      | 6      | 1      | -      | -      |
| 17        | 6      | 5      | -      | -      | 1      | 5      | -      | -      |
| 18        | 6      | 3      | 3      | -      | 3      | 3      | -      | -      |
| 19        | 12     | 8      | 4      | -      | -      | 8      | 4      | -      |
| 20        | 12     | 3      | 9      | -      | -      | 3      | 9      | -      |
| 21        | 6      | 2      | 4      | -      | -      | 2      | 4      | -      |
| 22        | 14     | -      | 3      | 8      | 3      | -      | 3      | 8      |
| 23        | 14     | -      | 7      | 7      | -      | -      | 5      | 9      |
| 24        | 10     | -      | 4      | 6      | -      | -      | 3      | 7      |
| 25        | 7      | -      | 1      | 6      | -      | -      | 1      | 6      |
| 26        | 12     | -      | 2      | 10     | -      | -      | 2      | 10     |
| 27        | 13     | -      | -      | 13     | -      | -      | -      | 13     |
| 28        | 13     | -      | -      | 13     | -      | -      | -      | 13     |
| 29        | 9      | -      | -      | 9      | -      | -      | -      | 9      |
| 30        | 6      | -      | -      | 6      | -      | -      | -      | 6      |
| 31        | 9      | -      | -      | 9      | -      | -      | -      | 9      |
| 32        | 7      | -      | -      | 7      | -      | -      | -      | 7      |
| 33        | 6      | -      | -      | 6      | -      | -      | -      | 6      |
| 34        | 6      | -      | -      | 6      | -      | -      | -      | 6      |
| 35        | 9      | -      | -      | 9      | -      | -      | -      | 9      |
| Total     | 200    | 21     | 30     | 39     | 110    | 22     | 29     | 36     | 113    |
correlation between age and fusion stage of the two medial clavicles confirms the reports of previous studies (14,15), thus either medial clavicle can be used interchangeably to conduct forensic age estimation. In cases where different stages of development in the left and right clavicle are found, a determination of skeletal maturity using alternative methods is recommended. The handedness of the subjects in this study is not known, but as the subjects were randomly selected, it can be concluded that the medial clavicle of the dominant hand appears to mature at the same rate as those of the non-dominant hand.

A matter of concern in conducting developmental status comparisons is the technique used to establish the stage of epiphyseal union. Most earlier studies were done using either morphological or radiological examination (Table 4). In comparing different techniques and bone preparation methods, the question arises whether the results obtained from computerized tomography (CT) scans can be compared with those from morphological examination. The present study used fresh bones obtained from autopsy cases and found some differences in the results from the two methods. In this study, the earliest age at which a partial union was detected was 19 years, which is in accordance with observations based on morphological examination in most of the published literature. However, earlier fusion was observed in studies which used CT scan (Table 4). For example, Pattamapaspong and colleagues (15) used CT scans to evaluate the development of the medial clavicular epiphysis in the Thai population. Their results showed that the partial union stage occurred in the age group 15-29 years and that the stage of complete epiphyseal union could be found as early as age 20 years. This indicates that fusion of the medial clavicular epiphysis can be identified earlier with CT scans than with morphological examination. That difference may be due to the greater sensitivity of CT scans in detecting the early stage of the union of the partial ossified epiphysis. In summary, the appearance of the medial clavicular epiphysis with radiological imaging is not necessarily the same as what appears in dry bone (11,14,20).

Ethnicity remains an unresolved factor, although it has been thought to have no influence on medial clavicular development (20). Compared with the results of previously published studies which were mostly conducted in Caucasian populations from the U.S. and Europe, variations in the time frame were observed in the developmental process of the ossification of the medial clavicle in the Thai population (Table 4). Despite an East Asian ethnic group e.g., Koreans, the period of the development of the medial clavicular

| Study                        | Nationality | Assessment | Sample size | Maximum age of nonunion | Partial union | Complete union |
|------------------------------|-------------|------------|-------------|-------------------------|---------------|----------------|
| McKern and Stewart (1957) (8) | American    | Gross      | 374         | 18                      | 18-30+        | ≥ 23           |
| Jit and Kulkarni (1976) (12)  | Indian      | Radiograph | 684         | 21                      | 18-24         | ≥ 22           |
| Webb and Suchey (1985) (3)    | American    | Gross      | 859         | 24                      | 17-30         | ≥ 21           |
| Kreitner et al. (1998) (19)   | German      | CT         | 380         | 22                      | 16-26         | ≥ 22           |
| Schaefer and Black (2007) (9) | Bosnian     | Gross      | 258         | 23                      | 17-29         | ≥ 21           |
| Langley-Shirley (2010) (11)   | American    | Gross      | 1,289       | 24                      | 17-30         | ≥ 19           |
| Singh and Chavali (2011) (2)  | Indian      | Gross      | 360         | 21                      | 18-31         | ≥ 22           |
| Wittschieber et al. (2014) (14)| German    | CT         | 493         | 20                      | 15-30         | ≥ 21           |
| Pattamapaspong et al. (2015) (15)| Thai     | CT         | 409         | 21                      | 15-29         | ≥ 20           |
| Yoon et al. (2016) (13)       | Korean      | Radiograph | 1,151       | 24                      | 16-30         | ≥ 18           |
| The present study (2020)      | Thai        | Gross      | 200         | 22                      | 19-26         | ≥ 22           |
epiphysis has been shown to be different (13). The reasons for the difference, however, is unclear (3,15,18). We hypothesized that these differences may be the result of differences in demographic origins and ethnicities (3,18). For that reason, this study emphasizes that reference values for one population may not be applicable to subjects from different demographic settings and ethnicities just as differences can result from using different evaluation techniques.

There are several limitations to this study. First, the effect of socio-economic and nutritional status on medial clavicular development was not assessed (20). The application of the data from this study to other ethnicities with different socio-economic situations should be made with caution. Second, only males were included in the experimental group, a result of the lack of female cadavers in our department. Future studies should also include females.

Conclusions

It appears that the stage of the epiphyseal union of the medial clavicle can be used to estimate age-at-death in Thai males. This age assessment technique is useful for evaluating age at death up to about 30 years of age. The findings of this study can be applied to dry bone examination of the Thai male population. Based on the results of this study, we recommend using the complete fusion of developmental stage to indicate ages greater than 22 years and the nonunion stage as representing ages less than 22 years in forensic anthropological examinations.

Ethics approval and consent to participate

Ethical approval was obtained from the Human Research Protective Unit, Faculty of Medicine, Siriraj Hospital, Mahidol University (Code 630081).

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Conflicts of interest

The authors declare they have no conflicts of interest.

References

1. Byer SN. Introduction to forensic anthropology. 5th ed. London: CRC Press; 2017.
2. Singh J, Chavali KH. Age estimation from clavicular epiphyseal union sequencing in a Northwest Indian population of the Chandigarh region. J Forensic Leg Med. 2011;18:82-7.
3. Webb PA, Suchey JM. Epiphyseal union of the anterior iliac crest and medial clavicle in a modern multiracial sample of American males and females. Am J Phys Anthropol. 1985;68:457-66.
4. Suchey JM, Katz D. Applications of pubic age determination in a forensic setting. In: Reichs KJ, editor. Forensic osteology: Advances in the identification of human remains. Springfield: Charles C Thomas Publisher; 1998. p. 33-67.
5. Meindl RS, Lovejoy CO. Ectocranial suture closure: A revised method for the determination of skeletal age at death based on the lateral-anterior sutures. Am J Phys Anthropol. 1985;68:57-66.
6. Black, SM, Scheuer L. Age changes in the clavicle: From the early neonatal period to skeletal maturity. Int J Osteoarchaeol. 1996;6:425-34.
7. Roche AF. Secular trends in human growth, matura-
tion, and development. Monogr Soc Res Child. 1979; 44:1-120.
8. McKern TW, Stewart TD. Skeletal age changes in young American males: Analysis from the standpoint of age identification. (Technical report). Headquarters Quartermaster Research and Development Command. 1957; EP-45.
9. Schaefer MC, Black SM. Epiphyseal union sequencing: aiding in the recognition and sorting of commingled remains. J Forensic Sci. 2007;52:277-85.
10. Schaefer MC. A summary of epiphyseal union timings in Bosnian males. Int J Osteoarchaeol. 2008;18:536-45.
11. Langley-Shirley N, Jantz R. A Bayesian approach to age estimation in modern Americans from the clavicle. J Forensic Sci. 2010;55:571-83.
12. Jit I, Kulkarni M. Times of appearance and fusion of
การประมาณอายุจากการปิดของส่วนปลายด้านในของกระดูกไหปลาร้าในกลุ่มประชากรไทยเพศชาย

บทวิจารณ์ เพื่อศึกษาความสัมพันธ์ระหว่างการปิดของส่วนปลายด้านในของกระดูกไหปลาร้าและอายุในกลุ่มประชากรไทยเพศชาย

วิธีการ ทำการศึกษาโดยใช้ส่วนปลายด้านในของกระดูกไหปลาร้าจำนวน 200 ตัวอย่างที่เก็บจากศพเพศชายที่มีช่วงอายุระหว่าง 11-35 ปี โดยทำการเตรียมชิ้นส่วนกระดูกและทำการประเมินระยะการปิดของส่วนปลายกระดูกด้านในโดยใช้การประเมินด้วยตาเปล่า โดยแบ่งเป็น 4 ระยะได้แก่ ระยะที่ 1: ไม่พบการเจริญของส่วนปลายกระดูกด้านใน; ระยะที่ 2: พบการเจริญของส่วนปลายกระดูกด้านในแต่ยังไม่มีการเชื่อมต่อกับส่วนกระดูกไหปลาร้า; ระยะที่ 3: พบการเชื่อมต่อบางส่วนกับส่วนกระดูกไหปลาร้า; ระยะที่ 4: พบการเชื่อมต่ออย่างสมบูรณ์กับส่วนกระดูกไหปลาร้า

ผลการศึกษา พบว่าระยะการปิดของส่วนปลายด้านในของกระดูกไหปลาร้าในกลุ่มตัวอย่างเพศชายไทยมีความสัมพันธ์กับอายุที่มากขึ้น โดยระยะที่ 1 ไม่มีการเจริญของส่วนปลายกระดูกด้านในพบได้จนถึงอายุ 22 ปี กลุ่มที่มีการเจริญต่อโดยสมบูรณ์พบได้เร็วที่สุดที่อายุ 22 ปี กลุ่มที่มีการเชื่อมต่อบางส่วนพบที่อายุ 19-26 ปี

สรุป การเชื่อมต่อของส่วนปลายด้านในของกระดูกไหปลาร้าในกลุ่มตัวอย่างเพศชายไทยมีความสัมพันธ์กับอายุ โดยสามารถใช้ข้อมูลจากการเชื่อมต่อของสมรรถนะของส่วนปลายด้านในของกระดูกไหปลาร้าแสดงว่าผู้ตายมีอายุมากกว่า 22 ปี และการตรวจพบว่าไม่มีการเชื่อมต่อของส่วนปลายด้านในของกระดูกไหปลาร้าแสดงว่าผู้ตายมีอายุน้อยกว่า 22 ปี เชิงสำนวน

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คำสำคัญ: การประมาณอายุ การปิดของส่วนปลายกระดูก ส่วนปลายด้านในของกระดูกไหปลาร้า โครงกระดูกคนไทย
