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ORIGINAL ARTICLE

Forensic Age Estimation by Ossification of Medial Clavicular Epiphysis using Kellinghaus et al. Classification in an Indian Population

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Abstract:

Present study aimed to determine the chronology of medial clavicular union for an Indian population. A retrospective study was conducted by evaluating 556 (227 females and 329 males) Computed Tomographic (CT) images of chest and neck region. The evaluation was carried out based on five stages of maturation described by Schmeling et al. [2004] and sub-stages of stages 2 and 3 by Kellinghaus [2010]. In the results the range, mean age, median, upper quartile, lower quartile, standard deviation and student t test are presented for each stage of ossification. Comparison between males and females revealed statistically significant differences in mean age at maturation stage 1, 3b and 5 which was absent in remaining stages. Maturation stage 3a was first presented at 16 year of age for both sex, stage 3b was first presented at age 18 year in females and 16 year in males and stage 3c was first presented at 21 years for both sex. To conclude the likelihood of whether an Indian individual is at least 16, 18 and 21 years or not can be determined. It is a reliable indicator of chronological age and somatic maturity.

Keywords: Age estimation; Clavicle; Ossification; Skeletal age; Computed tomography; Identification.

Introduction:

Estimation of age is of prime importance in routine practice of law enforcement in case of identification, searching for unknown victims, estimating the age at death, classification of cluster victims in mass disaster, to know the age for availing social benefits and also aid in immigration services in processing of illegal immigrants.[1]

In majority of countries, the age limits of criminal responsibility lie between 14 to 21 year of life.[2,3] European nations like United Kingdom, Switzerland, Sweden, Norway, Italy use age 18 and 21 years as a threshold for assessment of an individual as juvenile or adult. If you look at the Indian subcontinent, criminal responsibility lies at 16, 18 and 21 years for both sexes. Indian judicial system will decide a male or a female as juvenile or an adult at 16 and 18 year respectively in heinous crimes.

Continuous research is going on worldwide to improve the accuracy of Forensic age estimation (FAE) to help in the administration of justice. Different population groups have variations in the attainment of the maturity indicator like eruption of teeth, ossification of bones based on the geographical, environmental and nutritional factors. Dentition and skeletal maturation are used either individually or in combination to estimate the age of an individual.[4] Ossification of the carpal bones and wrist is one of the easily available reliable methods of age estimation in children.[5] By 18 years of age majority of the skeleton will complete ossification except few centers like sacrum and clavicle among them clavicle is notable. Therefore many countries are using the clavicle as a gold standard in determining the age of an individual above 16 years.[4] If you look at the guidelines issued by the Study group on Forensic Age Diagnostics [Arbeitsgemeinschaft für forensische Altersdiagnostik (AGFAD)] from the German Association of Legal Medicine, X-ray/CT examination of the clavicle is mandatory to confirm the age over 18 and 21 years.[6] The study group further recommended that it should be followed by worldwide for better forensic age diagnostics.[7-9]

Development of the clavicle starts in the fetal life. The ossification begins from a primary center for central zone along with coalescent ossification centers appearing at both ends. The second phase of ossification begins in the adolescent age group. Secondary epiphyseal center for the medial end which is also called as medial clavicular epiphysis (MCE) fuses with the primary center.[10] Beginning of fusion of MCE (stage 3a) signifies > 16 years.[11] Complete ossification of MCE (stage 4) confirms age >21 years.[11-13]

Correlation with the chronological age of an individual with the maturation of clavicle has been studied in dry bones which revealed positive correlation but the age estimation was in wide range.[14] Recent research revealed the possibility of using imaging techniques in the field of age estimation. A study conducted in an European continent revealed the positive correlation between chronological age and age estimated at death of an individual based on the maturation of cranial sutures using CT scan.[15] The

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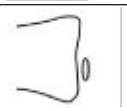
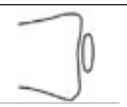
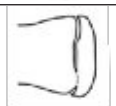
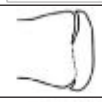
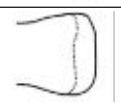
most common techniques used are X-Ray, ultrasound, CT, and magnetic resonance imaging (MRI).[16-18] When you compare them, CT has many advantages over other techniques. The notable ones are high image resolution, increased accuracy in detecting ossification centers, absence of overlapping soft tissue images.[2,11,19-22]

If you look further into CT, a revolution in the multi detector CT scanners give you very thin slice equal to 0.6 mm. This high resolution enables clear vision of the ossification centers which are beginning as well as the thin growing epiphyseal growth plates. Therefore, presently CT imaging has been considered as a gold standard in assessing the ossification of MCE.[2,23]

Kreitner et al. introduced the use of CT in assessment of MCE ossification.[10] Later, Schmeling et al. designed the common staging system which divide the ossification of MCE into 5 stages.[24] Kellinghaus et al. brought forward sub-stage system which decrease the interval of assessed age thus enhancing estimation of accuracy in the chronological age of an individual.[25] That will help a forensic expert in FAE of an individual in turn helping the law enforcement authorities to enforce the law more accurately.

Table 1. Stage of maturation of the medial epiphysis of clavicle described by Schmeling et al. and the sub-stages of stage 2 and 3 by Kellinghaus et al.

Stage	Diagram	Description
1		Non-ossified epiphysis
2a		Ossified epiphysis, growth plate not fused. Ossified epiphysis is 1/3rd or less compared to the width of metaphysis
2b		Ossified epiphysis, growth plate not fused. Ossified epiphysis is over 1/3rd until 2/3rd compared to the width of metaphysis
2c		Ossified epiphysis, growth plate not fused. Ossified epiphysis is more than 2/3rd compared to the width of metaphysis
3a		Growth plate is partially fused. The epiphysis and metaphysis complete the fusion up to 1/3rd width
3b		Growth plate is partially fused. The epiphysis and metaphysis complete the fusion over 1/3rd until 2/3rd width
3c		Growth plate is partially fused. The epiphysis and metaphysis complete the fusion over 2/3rd width
4		Growth plate complete the union of epiphysis with metaphysis. Visible physeal scar
5		Complete union of the epiphysis and metaphysis. Invisible physeal scar

Presently most of the studies conducted on maturation of MCE with the help of CT were reported in European and Australian continents.[10,11,19,20,26,27] Although few studies were reported on Indian population which is based on either post-mortem examination of the clavicle or radiographic examination.[6] Authors reported similar study using CT scan over the Indian population with Schmeling et al. six stage classification of MCE union. The results revealed that the maturation of MCE stage 2 attained at 13 year, stage 3 at 16 year, stage 4 at 22 year and stage 5 at 25 year.[22] It help in estimation of age in wide range, which is less accurate.[22] If the Kellinghaus et al. sub stage classification is used, there may be a possibility of increased accuracy in age estimation because the ossification stage 2 and 3 are sub classified as 2a, 2b, 2c, 3a, 3b, 3c, which are attained at a very short interval of time. That will help us to assess the chronological age more accurately. There is paucity of such research in Asian continent using CT. It may be because of the unavailability of CT in the underdeveloped south Asian countries. Present study is aimed to assess the correlation between the ossification of the MCE and chronological age in an Indian individual using CT, to know whether the standard staging procedure of MCE ossification is applicable or not to the study demographic.

Materials and methods:

It is a retrospective study conducted in the Department of Radiology after approval from the institutional ethical committee. We collected the CT scans of the neck and chest of patients aged from 10 to 30 years taken between 2010 and 2016 for the period of six years. The age and place of birth of an individual were confirmed by the government issued

Table 2. Age by sex distribution of patients.

Age (Y)	Gender		Total
	Female	Male	
10	6	2	8
11	4	4	8
12	6	6	12
13	4	12	16
14	6	4	10
15	10	14	24
16	6	16	22
17	16	18	34
18	28	20	48
19	23	17	40
20	18	16	34
21	20	20	40
22	14	28	42
23	8	8	16
24	4	24	28
25	18	38	56
26	8	24	32
27	6	18	24
28	8	26	34
29	2	4	6
30	12	10	22
Total	227	329	556

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Table 3. Descriptive statistics for age by sex and ossification stage expressed in years.

Stage	Gender	N	Age range (Min-Max)	Lower quartile	Median	Upper quartile	Mean (SD)	p-value
1	Female	24	10-15	10.75	12	13.25	12.08 (1.792)	0.009*
	Male	36	10-16	12	13	15	13.39 (1.886)	
2	Female	71	13-23	17	18	19	17.68 (2.020)	0.49
	Male	79	13-21	17	18	19	17.91 (2.039)	
2a	Female	21	15-19	16	17	18	17.19 (1.436)	0.608
	Male	31	13-20	17	18	19	17.45 (1.981)	
2b	Female	26	13-19	15	17	18	16.46 (1.860)	0.423
	Male	24	15-18	15.75	17.5	18	16.83 (1.308)	
2c	Female	24	18-23	18.75	19	20	19.42 (1.349)	0.719
	Male	24	15-21	19	20	21	19.58 (1.692)	
3	Female	86	16-26	19	21	22	20.93 (2.419)	0.009*
	Male	92	16-27	21	22	24	21.98 (2.855)	
3a	Female	34	16-22	18	20	21	19.41 (1.743)	0.796
	Male	22	16-22	17	20	21.75	19.27 (2.272)	
3b	Female	26	18-22	19	21	21	20.23 (1.451)	0.009*
	Male	36	16-24	21	22	23	21.61 (2.296)	
3c	Female	26	21-26	22	23	25	23.62 (1.627)	0.298
	Male	34	21-27	22	25	25	24.12 (1.966)	
4	Female	20	24-27	25	25	26	25.30 (0.801)	0.557
	Male	84	22-29	24	25	27	25.55 (1.852)	
5	Female	26	27-30	28	29	30	28.85 (1.190)	0.01*
	Male	38	25-30	27	28	29.75	27.84 (1.653)	

*** Significance was set at P-value < 0.05**

identification document. The date of performing the CT scan, date of birth and any medical illness were reviewed from the medical records section. Patients who are not of Indian origin and Indian nationals, patients treated with chemotherapeutic drugs, steroids, immunosuppressant drugs and patients suffering from chronic diseases which affect the bone development were excluded from the study. Data regarding the socioeconomic status and nutrition was unavailable.

All the axial and coronal CT images of 1 mm slice thickness were used in assessment. High resolution thin-slice imaging was obtained using 128 row multi detector scanner. Scan images were assessed on a standard picture archiving and communication system (PACS). The standard viewing tools were used. The thin-slice-axial images helped us in reconstructing superior quality images on coronal, sagittal, and oblique planes. If the staging remained doubtful on axial and coronal views, the multiplanar images in the long axis of clavicle were used.

The medial clavicular ossification was scored by two independent radiologists with more than 8 years of experience separately at two different time intervals to see any inter-observer variability. Both radiologists routinely assess skeletal ossification using clavicles and other bones on CT as a part of evaluation for head and neck as well as chest wall. The assessment of stages and scoring was done as described by Schmeling et al. [24] and sub-stages of stages 2 and 3 by Kellinghaus et al.[25] (Table 1).

The right and left clavicles were assessed separately as given in table 1. The images were scored by consensus. Both radiologists were blinded for patient's age. In cases where a different maturation stage appeared between right and left clavicle, the more advanced (stage) side was used for analysis.

The data was analyzed using SPSS software. Results are expressed as age range, mean, median and standard deviation for each stage. The differences of every maturation stage between both sexes were analyzed by using the two-sample t-test. The statistical significance was set at $P < 0.05$. The box-and-whiskers plot was drawn to explain the degree of maturation of MCE with chronological age and sex.

Table 4. Comparison of study results conducted on different population using maturation of medial clavicular epiphysis.

	Kellinghaus et al.[19]	Kellinghaus et al.[17]	Wittschieber et al.[7]	Pattamapaspong N et al.[20]	Present study
Study characteristics					
n	502	185	493	409	556
Age	10–35	13–26	10–40	11–29	10-30
Slice thickness (mm)	0.6–1.5	0.6–1.5	0.6	0.6–1	1
Minimum–maximum age by maturation stages (years)					
1	10–15	–	10–15	11–16	10-16
2	13–20	–	14–20	12–21	13-23
2a	–	13–20	14–18	12–20	13-20
2b	–	15–20	14–20	13–19	13-19
2c	–	15–20	17–20	15–21	15-23
3	16–26	–	15–36	15–27	16-27
3a	–	16–22	15–23	15–24	16-22
3b	–	17–25	16–36	15–24	16-24
3c	–	19–26	19–30	17–27	21-27
4	21–35	–	21–40	18–29	22-29
5	26–35	–	26–40	20–29	25-30

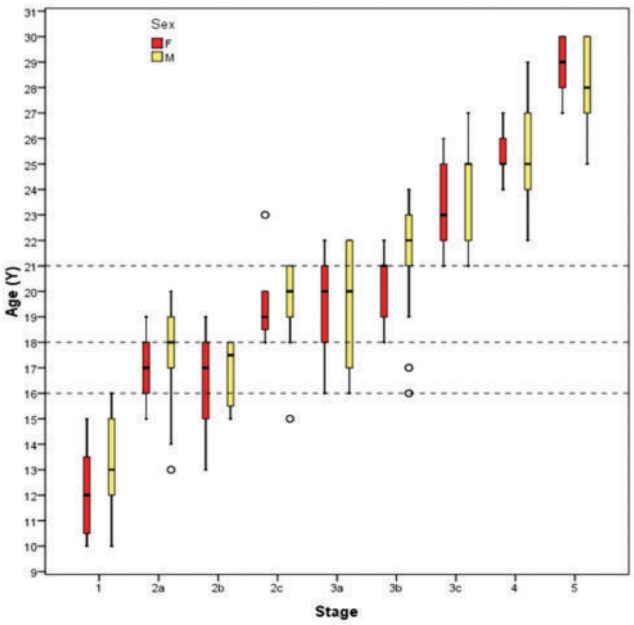

Figure 1. Box plots of the correlation between chronological age and stages for maturation of the medial epiphysis of clavicle with reference line at 16,18 & 21 years and outlier marked (O) (statistically).

Results:

The selection of 556 CT scans were done according to inclusion and exclusion criteria. Scans with blured/unclear/invisible image quality and unclassifiable clavicle union were excluded. The medial clavicle union was assessed and scored in 556 (227 females and 329 males) scans. The difference in the maturation of right and left clavicle was observed in 76 scans [total 13.6%, 32/227 females (14%) and 44/329 males (13%)]. The inter-observer variability was negligible [Kappa value of 0.851]. It reflect that there is good strength of agreement between two observers.

Table 2 describes the age and sex distribution of patients. Table 3 gives the descriptive statistics for age by sex which are divided into maturation stages. Comparison between males and females revealed statistically significant differences in mean age at maturation stage 1, 3b and 5. The maturation at stage 1 attained 16 months earlier in females (mean age 12.08 vs. 13.39 years), at stage 3b 17 months earlier in females (mean age 20.23 vs. 21.61 years) compared to their male counterparts whereas at stage 5, 12 months earlier in males (mean age 27.84 vs. 28.85 years) compared to females. The remaining maturation stages (2a, 2b, 2c, 3a, 3c and 4) did not show statistically significant difference between males and females at mean age of maturation. Figure 1 describe the box-and-whisker plot expressing the degree of maturation of MCE with chronological age and sex at various stages.

Table 3 describe the maturation stage 3a was first noted at 16 year of age for both sexes with mean age at 19.4 and 19.2 for females and males respectively. Maturation stage 3b was first noted at the age of 18 year in females and 16 years in males with mean age at 20.2 and 21.6 respectively. Maturation stage 3c was first noted at 21 years for both sexes with a mean age at 23.6 and 24.1 for females and males respectively.

Discussion:

As per the review of literature the maturation of clavicle in the form of MCE ossification stages were used in young adults of various populations to assess the chronological age which aid in the administration of justice. If you look at table 1, Schmeling A et al. described clavicular ossification into five stages, in which stage 2 and 3 are further sub-classified by Kellinghaus et al.[11,25,27,28]

The active phase of maturation (stage 2) started at <14 years in Indians which is similar to European and Thai populations.[25,27,28] Whereas study on German population by Wittschieber D et al. revealed it at 14 years[11] (Table 4).

The first appearance of MCE in the present study was noted at 13 years, a year earlier than German population. It may be due to an outlier in the statistical analysis or due to data collection process where completed age in years is considered. Similar finding of early stage 2 maturation in females was observed in another study conducted on Caucasians.[21]

Many European studies revealed maturation stage 3a represent 16 years or older for FAE.[25,27] This criteria was derived from European studies where maturation stage 3a did not occur before 16 years. Whereas few studies conducted on the same population revealed that stage 3a represented 15 years or older.[11] However, in the present study we found stage 3a at the age of 16 years. That is a significant benchmark in the Indian judicial system to classify an Indian male as juvenile or adult in heinous crimes.

Maturation stage 3b represents 17 years or older in the present study but when you look at the box plot, stage 3b represents 18 years or older except few outliers (Fig.1), while studies conducted on European continent represented 16 and 17 years.[11,24] Another study conducted on Thai population revealed stage 3b at the age of 15 year.[28]

Present study revealed maturation stage 3c was attained at the age of 21 years or older, while studies on Thai and European populations represented at 17 and 19 years or older respectively.[11,25,28] It highlights the fact that maturation of the MCE occurs at dissimilar age in different populations.

Therefore for the purpose of FAE in an Indian individual, the maturation stage 3a, 3b and 3c will represent the chronological age ≥ 16, ≥ 18 and ≥ 21 years respectively. This alteration is vital for Indian judiciary because the age thresholds for criminal responsibility of an Indian individual lies at 16, 18 and 21 years.

To increase the precision of sub-stage classification it is necessary to assess the CT scan images in multiple planes. If the assessment of particular stage is doubtful with axial plane, we discovered that images reformatted in multiplanes perpendicular to the growth plate have resolved the problem. There are numerous possible difficulties in exact staging of the epiphysis, notable once are variants of epiphysis, overlooking epiphyseal scars and misinterpretation within stage 1 and 4. Inappropriate grading of maturation is possible due to various forms of epiphyseal configuration.[29] If these variants are present it is better to avoid the staging as per the opinion of Wittschieber et al.[30] Stage 1 and 4 may counterfeit each other because both do not show separate epiphyseal centers. Such difficulty can be easily overcome by substitution of hand radiographs as well as dental radiographs. Stage 4 of maturation will be seen at 18 years or above which will be confirmed by skeletal maturation of hand radiographs or dental radiographs with the evidence of third molar.[31,32]

Radiological examination is widely available method worldwide including the suburban and remote rural areas. It is safe, inexpensive and easy to use without much expertise. But the assessment of ossification of MCE is difficult due to superimposed image of bones (rib and vertebrae) and mediastinal structures.[33] Other available imaging tools in living objects are ultrasonography and MRI, both of which have a great advantage of non-ionizing rays.[34] Ultrasonography is widely available compared to MRI. However its accuracy is limited because of limited viewing angle leading to incomplete visualization of the ossification. MRI is available in bigger centers and tertiary care centers which show the clear growth plate. But its reliability in age determination need to be established when compared to CT scan. When it comes to the CT, of course it is a better tool when compared to any other method described above in age determination of individuals. But a long standing debate is going on in India as well as in other parts of the world regarding the

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ethical issues in using the CT scan for criminal investigation because of its ionizing nature.[35,36]

Present study has several limitations. CT scans used for the analysis of study group were collected from the pool of hospital case records. In such records/scans artifacts are very common. The frequently encountered artifacts are contrast dyes and intubation tubes etc, which produced various artifacts overlapping the sternal end of the clavicle comparable to the growth plate. The considerable experience of the radiologist in differentiation of the artifacts from the ossifying epiphysis helped to score the stages accurately by excluding such cases. Linear streak artifacts usually appear dark compared to the growing end of the clavicle and also extend beyond the range of clavicle. Various techniques are used while exposing the patient to CT scan for example variable patient position, variable arm, neck and head position, variable radiation dose etc, which may affect the sharpness of images. By the observations of the present study, the sharpness of image did not affect interpretation because of thin slice of the images.

Conclusion:

The study found that the accuracy of age estimation can be improved in stage 3 with the help of Kellinghaus et al. sub-stage classification. Which represent stage 3a for age ≥ 16 years, stage 3b for age ≥ 18 years and stage 3c for age ≥ 21 years. CT scan is a helpful tool for FAE. We recommend to extend the study to larger set of population to create a reference data for study population.

List of abbreviations:

CT: Computed Tomography.

FAE: Forensic Age Estimation.

AGFAD: Arbeitsgemeinschaft für forensische Altersdiagnostik. (Study group on Forensic Age Diagnostics from the German Association of Legal Medicine).

MCE: Medial Clavicular Epiphysis.

MRI: Magnetic Resonance Imaging.

PACS: Picture Archiving and Communication System.

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