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ORIGINAL ARTICLE

Radiological study of the ossification process of medial clavicular epiphysis: The influence of sex and laterality

Dominic Marera, Kapil Sewsaran Satyapal, The influence of sex and laterality in age estimation

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ABSTRACT

Background: Radiological examination of bones such viz, clavicle have been developed to estimate the age of an individual without valid identification, or when suspected of providing inaccurate age. However, there is a paucity of information on the influence of sex and laterality on age the estimation process. Therefore, the aim of this study was to evaluate the impact of sex and laterality in the ossification process of the medial clavicular epiphysis within the South African and Kenyan population.

Materials and methods: A retrospective study of 1605 digital radiographs selected from South African and Kenyan population aged between 14–30 years were evaluated. The ossification process of the medial clavicular epiphysis was scored in accordance with Schmeling's (2004) staging system. The difference between the chronological age and estimated age for each sex was recorded in the Bland-Altman chart. A log linear regression test was used to test for bilateral asymmetry in the ossification status of the medial epiphyses

Results: While statistically significant differences between males and females were observed in stages 1, 2, 3 and 4 of the ossification process, a paired sample t-test did not report any statistically significant difference in the asymmetry of the medial epiphysis (P = 0.89).

Conclusions: The present study concurs that the maturation of the clavicle starts earlier in the female population. However, no significant differences were recorded in the time-frame of maturation between the right and left clavicles.

Keywords: clavicle, sex, laterality, age estimation, medial epiphysis

INTRODUCTION

Forensic Age Estimation is a subset of forensic medicine which aims to define, in the most accurate way, the chronological age (CA) of a person of an unknown age involved in judicial or legal proceedings [21]. In the case of the living, age estimation plays an important role in solving problems regarding criminal aspects within different age groups, viz, juvenile status for criminal responsibility, sports categorization, voting rights, driving license, age of marriage and adoption, old age pension and asylum proceedings [9].

Several authors [1, 8, 14, 18, 19, 21, 24–27] have shown more interest in the development process of the clavicle when estimating the age of young subjects below 30 years old. This is due to the slow fusion of the medial epiphysis, which begins at the onset of puberty to until one reaches approximately 30 years, while other bones ossify completely by 20 years [23].

A review of several published studies has indicated considerable variations with regard to the onset and completion of the ossification of the medial clavicular epiphysis in different geographical areas [14]. These inconsistencies may be due to the influence of sex and laterality of the clavicle being assessed. Therefore, an important question of practical relevance is whether the results of previous studies may act as reference data to subjects of different sex or if the right and left clavicular epiphysis may be used interchangeably.

Sex dimorphism

Some authors have reported that the epiphyseal plates of the clavicle specifically fuse earlier in females than in males [11, 22, 27, 32]. However, Todd and D'Errico [30] and Flecker [5] have disputed the notion of sex difference in the maturation process of the clavicle.

Webb and Suchey [32] studied the fusion process of the medial clavicular epiphysis in the American population and reported non-union as late as ages 25 and 23 in men and women respectively, whilst complete fusion occurred between ages 17 to 30 in males and 16 to 23 years in females. They then concluded that the fusion of epiphyseal plates began approximately one year earlier in females.

However, Flecker [5] pointed out that while female standards may vary between 1 to 2 years from those of males, the epiphyseal ossification timing of both the sexes were similar. Flecker [5] studied the medial end of the clavicle radiologically in six hundred and fifty five (n = 655) Australian subjects under the age of 30 years. He observed that the maximum numbers of cases showing a center of ossification in this particular epiphysis was in the age group of 19 years in the case of males (63.6% cases) and 21 years in the case of females (50% of the cases). His findings were not in tandem with those of Webb and Suchey [32].

Some authors have also discussed the relationship of physical stress and bone fusion [7]. During a study of radiological changes in the upper limb, Garamendi et al. [7] concluded that ossification of the clavicle and first rib are usually more advanced in men than in women as they are more exposed to recreational and labour stress.

Sex dimorphism is not limited to studies using the clavicle Lakha [15] studied the knee, elbow and hip joints of subjects aged between 6 and 24 years in the South African population and demonstrated that maturation in female subjects was significantly more advanced than in male subjects by about 3 years.

Incidence of asymmetry

The variation or asymmetry in the onset and complete fusion of right and left clavicles is considered to be controversial, even though most researchers [1] noted insignificant differences between the two, others still maintain that these small differences must be taken into consideration in the case of legal proceedings [24]. To date there is still no agreement on which side of the clavicle to use, especially when one shows earlier ossification than the other.

In Ghana, Brown [1] noted that 4.6% of X-rays showed a developmental gap between the left and right clavicles. However, there was no statistically significant difference when the mean values of the right and left clavicle were compared for each stage. Brown [1] concluded therefore that the results from either the left or right clavicle may be used during the forensic estimation of age. During an osteological study in Nigeria, Udoaka et al. [31] established that the right clavicle was not significantly longer than the left in adults. He attributed this to the frequent use of the right upper limb as compared to the left. When a part of the body is subjected to frequent stress, it develops to resist the stressor subjected to it [31].

In Germany, Kellinghaus [13] also noted the developmental differences between the left and right side of the clavicle in 17.3% of subjects when he used thin-slice CT scans. Howaver, He used the epiphysis that matures faster as a standard for his research. This led to criticism for not providing any scientific basis for choosing the most advanced side. The same has been done by other researchers like Kellinghaus [13].

The present study aimed to evaluate the influence of sex and laterality in the ossification process of the medial clavicular epiphysis among the Black African populations. This information is important in establishing whether there is a need for sex specific references in Africa, and to answer the question as to which clavicle to use (left or right) for age estimation when there is a developmental difference between the two.

MATERIALS AND METHODS

Postero-anterior (PA) views of three thousand (3000) digital chest X-rays were obtained from the Radiology Department at King Edward VIII Hospital, within the eThekwini Municipality, South Africa and West Kenya Diagnostics and Imaging Center, Kisumu, Kenya. The samples were derived from the Black South African and Kenyan populations aged between 14 and 30 years old. Ethical approval was obtained from both countries (BE 267/14).

The digital X-rays of the South African samples were developed using Toshiba X-ray diffraction (DRX) detector (DRX-Plus 3543 Detector with gadolinium (GOS) scintillator) while those of Kenyan samples were taken with Philips flat panel detector system (Digital-diagnostic Optimus-50). Any patient records with known skeletal growth problems, endocrine or metabolic disorders such as gigantism, acromegaly, and osteogenesis imperfecta were excluded as this study focused on the establishment of normal skeletal age parameters. Poorly taken radiographs showing overlapping of the ribs, vertebrae, and mediastinal shadows were also excluded. After the exclusion criteria, one thousand six hundred and five radiographs (n = 1605) met the inclusion criteria as the rest proved difficult to assess due to the over-lapping of ribs, vertebrae and mediastinal shadows. The sex and date at which the radiographs were taken were recorded for analysis. Each respondents' actual age (CA) was recorded after the staging process and the EA was established to avoid bias. The ossification stages of the medial clavicular epiphyses were defined in accordance with the scoring system of Schmeling et al. [24] as follows:

- Stage 1: no fusion of ossification taking place — stage 1 is observed when the medial end of the clavicle has a thin margin that is curved inwards. (Fig. 1).
- Stage 2: visible ossification center, but the epiphyseal plates are not fused (Fig. 2).
- Stage 3: metaphysis and epiphysis are partially fused (Fig. 3).
- Stage 4: complete fusion and union of metaphysis with epiphysis, but with a visible scar — this stage describes the complete union of the epiphysis and metaphysis of the medial clavicle (Fig. 4).
- Stage 5: total fusion of the ossification centers without any visible union scar (Fig. 5).

The study was subjected to analysis using Statistical Package for Social Sciences (Release 21.0.1, SPSS Inc. 1989–2001) for Windows 10. Descriptive (frequency-descriptive) and analytical statistical tests were applied. Results were expressed as minimum, maximum, mean ± standard deviation (SD) and median with lower and upper quartiles. The difference between CA and EA for each population group (South Africa and Kenya) was presented in Bland-Altman plots [4] at a confidence interval of 95%. The correlation of age with the ossification status of bilateral medial clavicular epiphyses was determined for both sexes.

The age intervals corresponding to different ossification stages were also compared between the sexes within the two population groups. A log linear regression test was used to test bilateral asymmetry in the ossification status for the medial epiphyses. Significance was assessed at $P < 0.05$ (exact, two-sided) whilst cross-tabulation was done between the EA and the 5 stages of ossification to determine the reliability and margin of error of this method of age estimation.

Inter-observer review

To validate the reproducibility of the study, an intra-observer precision test was run again using Fleiss' Kappa analysis [17]. The intra-observer results versus the expected observations of the primary researcher were expressed as zero (0) for no agreement and one (1) for a perfect agreement. All the parameters examined had a substantial (Kappa = 0.61–0.80) to almost perfect agreement (Kappa = 0.81–1.00) (Tab. 1).

RESULTS

Age distribution

Of the total sample, 53.5% (n = 1605) of the X-rays met the inclusion criteria and were selected for analysis. The sex distribution of the selected sample was 50.2% ($^{805}/_{1605}$) males and 49.8% ($^{800}/_{1605}$) females. The mean age of the sample was 22.70 ± 0.12 years with a median age of 23 years. In males (n = 805), the mean age was 21.31 ± 1.02 years, while in females (n = 800) was 21.9 ± 5.2 years. The least number (4.1%) of patients were 15 years old, while the majority of the patients (7.2%) were 28 years old (Tab. 2).

Sex dimorphism

Of the total samples evaluated (n = 1605), the beginning of fusion (stage 2) was observed at a range of 17–23 years while complete fusion (stage 4) was observed at a range of 24–27 years in male patients. In female patients, the age range was 14–23 years for the beginning of fusion and 23–27 years for complete fusion.

The mean age for the onset of fusion (stage 2) was observed at 20.02 ± 1.69 and 18.80 ± 2.11 years in males and females, respectively (P = 0.000). The onset of complete fusion (stage 4) was noted at 25.73 ± 0.83 years in males and 25.83 ± 1.04 years in females (P = 0.037). Statistically significant differences between males and females were observed in stages 1, 2, 3 and 4 (Tab. 3).

Incidence of asymmetry

The asymmetric growth of the right and left sides of the medial epiphyses was observed in 2.7% of the current population (n = 1605). Of the total reported cases of asymmetry, 0.8% were observed in males while 1.9% was observed in females. A paired sample *t*-test did not report any statistically significant difference in the asymmetry of the medial epiphysis (P = 0.89) (Tab. 4).

Comparison between CA and EA

The mean difference between CA and EA in each patient was plotted against the age frequency distribution to determine how wide the deviation was from the CA (mean standard error). In the current sample (n = 1605), the mean standard error of estimate was ± 1.49 years at 95% confidence interval (CI). Therefore, the CA that was estimated in the current population had a margin of error of ± 1.49 years.

The mean difference between the CA and EA was also plotted using the Bland-Altman plot. Since a negative deviation shows an underestimation of the CA and a positive deviation shows an overestimation. In the current sample population (n = 1605), the limit of agreement was 0.03 years. Therefore, the results of this study emonstrated that there is more likelihood of overestimating the chronological age by 0.03 years (Fig. 6).

DISCUSSION

Due to its longest growth related activity compared to other bones, the development of the medial clavicular epiphysis has been used for age estimation from adolescence to the third decade of life [3, 24]. In this study, the ossification stages of the epiphysis were graded and used for age estimation in the radiographs analyzed.

While comparing the results of authors from different countries, variable differences were observed in the onset of fusion of the secondary ossification center (stage 2). For instance, in the present study, the onset of fusion began at 17 years in males and 14 years in females (Tab. 3), whereas Kellinghaus et al. [13] reported the onset of fusion at 14 and 13 years in male and females, respectively. This was three years earlier in males and one year earlier in females than in the present population. Kaur [12] also observed an early onset of stage 2 at 15 and 14 years in males and females, respectively, while Schulz et al. [27] observed the onset of fusion at 15 years in both sexes using conventional X-rays.

On the contrary, a marked delay in the onset of fusion was observed by Webb and Suchey [32] at 16 years in both sexes. However, they employed a dry bone assessment technique. This reflected the problem of method-specific variations, thus the call for method specific references. Moreover, the results of Webb and Suchey are over 30 years old, this highlighted the relevance of secular trends in bone maturation. From the literature reviewed, it is apparent that the maturation process at stage 2 occurs much later in the current African population than that of previous reports [32].

In medico-legal practice, the earliest age of complete fusion is essential in confirming if one has reached 18 years, the age of legal responsibility. In the present population, the earliest onset of complete fusion (stage 4) was 24 years in males and 23 years in females (Tab. 3). The observed sex difference was statistically significant ($P = 0.037$). However, these results were approximately 2 to 4 years older than the reports of some authors who presented complete fusion at 19 years [6, 27]. Others, [1, 10, 24] reported the age of complete fusion at 20 years. The current study corroborates earlier reports that [6] complete fusion begins after 18 years. However, the onset of fusion has remarkably delayed in this cohort in comparison with the previous literature. Accordingly, there is a need to address the problem of population specific standards during bone age assessment, while taking into consideration the sex differences and technique specific variations.

The difference in ossification of the medial epiphysis between sexes was statistically significant in the current population except in stage 5 (Tab. 3). In this stage, the epiphyseal plates had completely ossified and are unlikely to show any distinct variations between the sexes. It is therefore, apparent that the maturation process is more accelerated in females than males in the early stages of clavicular development. Developmental changes are accelerated in females than males during the pre-natal and post-natal periods [16]. The difference in the growth process is controlled by genes located both on the X and Y chromosomes [16]. The longer growth period experienced by males gives more time to gain additional height and body mass before their final cessation of growth.

From the literature reviewed, it is apparent that accelerated maturation is observed in the populations of the Northern Hemispheric countries as compared to results from the current African cohorts. The early maturation processes may underpredict an individual's chronological age. Consequently, application of references of a population with a faster bone maturation process to estimate the age of subjects with slow maturation process may lead to the underestimation of age. In legal proceedings, it may be detrimental for a child to be mistakenly evaluated as an adult when he/she should instead be afforded the rights of a child.

One of the objectives of the current study was to determine whether any significant difference in the ossification process between the right and the left clavicle exists and to correlate the current findings with the literature reviewed. This study also tried to answer the question of which clavicle (left or right) may be used for age estimation in cases where asymmetry is observed. This study demonstrated no statistically significant variation ($P = 0.89$) in asymmetry of the medial epiphyses in the present population (Tab. 4). Moreover, the incidence of asymmetry was higher in females (1.9%) than in males (0.8%). From the literature reviewed, the incidence of asymmetry varied from 0.6% [24] to as high as 21.6% [28] in different population groups.

Among the Indian population, Kaur [12] reported a 13% incidence in the developmental gap between the right and left clavicle. He noted a higher incidence in females than males. In addition, Kaur [12] observed that neither the left, nor the right clavicle could be identified to mature faster than the other as accelerated development was found on both sides. Against this background, it is apparent that application of reference values on either right or left clavicle may not influence the final results [24]. Table 5 shows the comparison of the incidence of bilateral asymmetry observed during evaluation of the medial epiphyses in the previous and current studies.

CONCLUSIONS

The present study demonstrated a more accelerated clavicular ossification process in females than males. However, the results did not demonstrate any significant difference in maturation process of either the right or left clavicle. All the cases of asymmetry in the current population differed only by one stage.

Limitations of the study

Finally, the radiographs collected in this study did not use the same projection and exposure parameters as noticed by the various exposure rates printed on the digital plates, the need for exposure and penetration re-adjustments depends on the patient's body weight, position and the type of X-ray machines being used. These variations may lead to poor bone penetration, thus interfering with the estimation of the chronological age.

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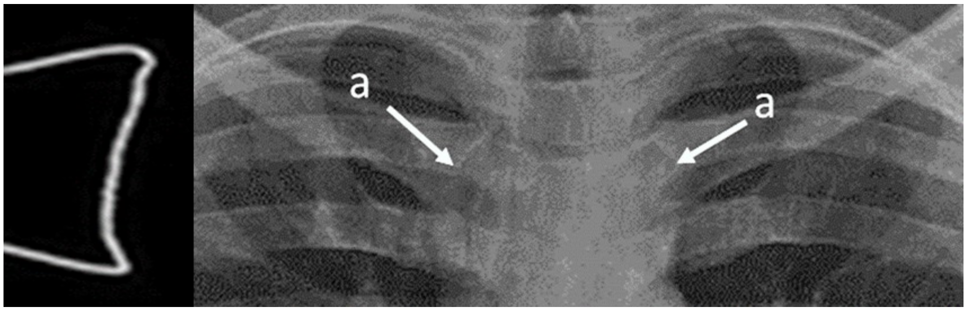

Figure 1. Radiograph of a 16-year-old patient showing stage 1 ossification: the estimated age = 15 years. a — curved medial epiphysis.

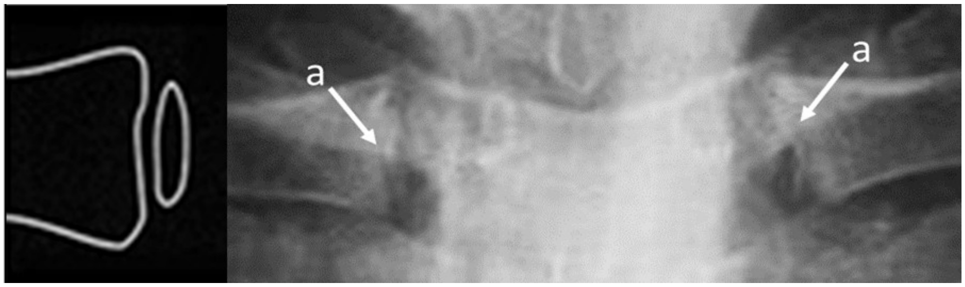

Figure 2. Radiograph of a 19-year-old patient showing stage 2 ossification: the estimated age =18 years. a — gap between epiphyses and diaphysis.

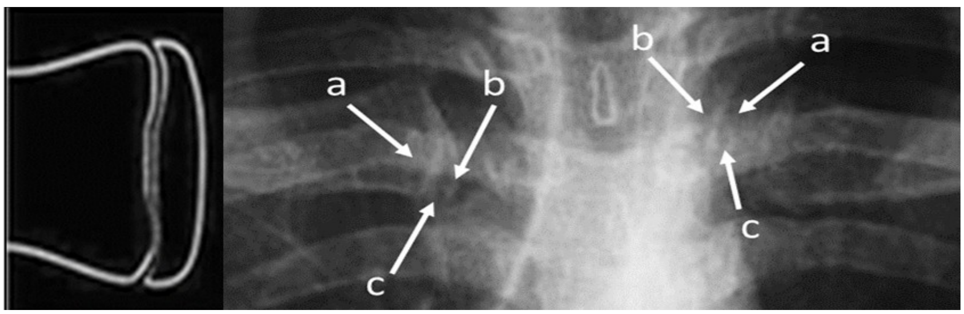

Figure 3. Radiograph of a 24-year-old patient shows stage 3 ossification: the estimated age = 24 years. a — epiphyses, b — bone tubercle closing the gap between epiphyses and metaphysis, c — metaphysis.

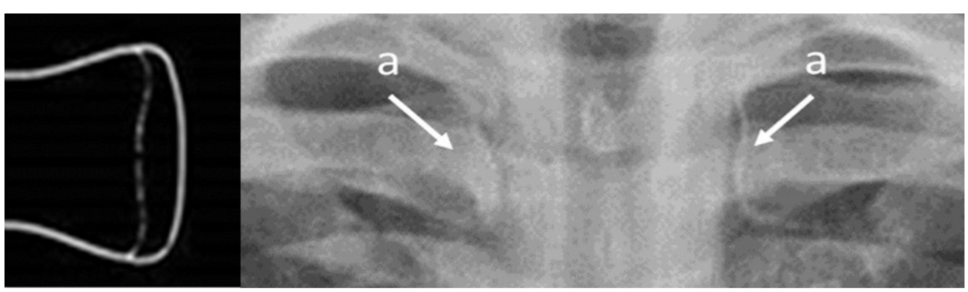

Figure 4. Radiograph of a 25-year-old patient showing stage 4 ossification: the estimated age = 27 years.

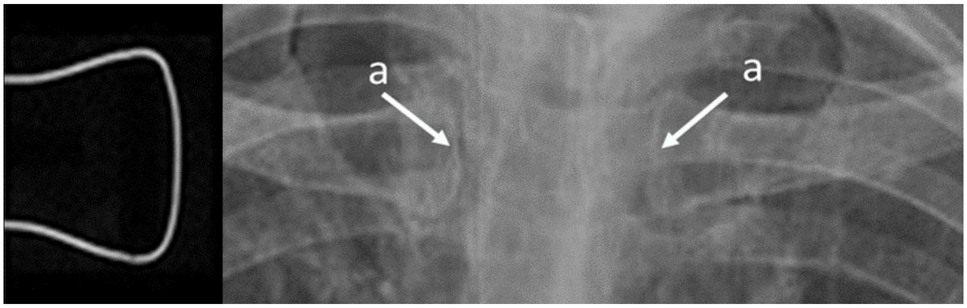

Figure 5. Radiograph of a 29-year-old patient showing stage 5 ossification: The estimated age = 27 years. a — convex edges without a visible scar.

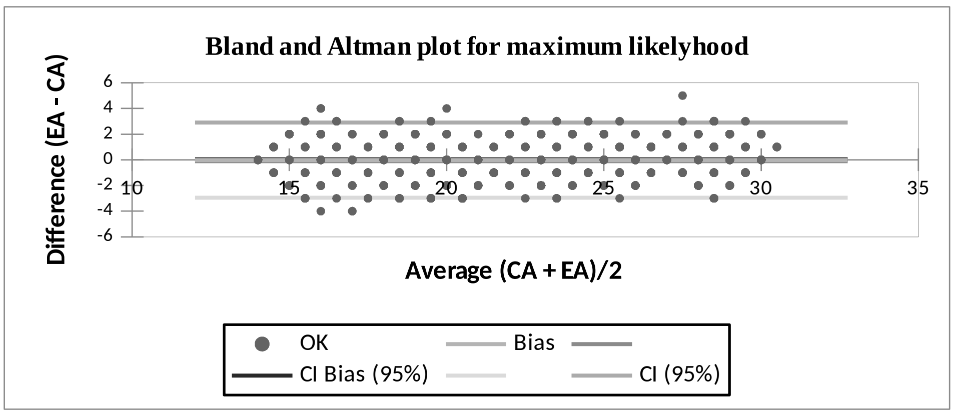

Figure 6. The Bland–Altman plot for comparison of the mean difference between chronological age and estimated age (the maximum overestimation is demonstrated by the thick blue line above the 0 mark).

Table 1. Probit regression and Kappa analysis.

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Attributes	Laterality		SE	95% CI	P-value	R
Medial epiphysis	Right	0.78	0.04	0.54	0.96	0.19
	Left	0.77	0.07	0.54	0.84	0.18

ϰ

Fleiss' Kappa analysis; SE = standard error; CI — confidence interval; R — regression coefficient.

Table 2. Age distribution in the sample population.

Chronological age	Male	Female	Total	Percentage
14	41	35	76	4.70%
15	26	40	66	**4.10%**
16	43	33	76	4.72%
17	48	37	85	5.30%
18	44	45	89	5.50%
19	40	39	79	4.90%
20	41	37	78	4.90%
21	44	40	84	5.30%
22	53	48	101	6.30%
23	53	58	111	6.98%
24	56	55	111	6.90%
25	51	58	109	6.80%
26	58	53	111	6.90%
27	40	58	98	6.10%
28	61	55	116	**7.20%**
29	53	55	108	6.70%
30	53	54	107	6.70%
Total	805	800	1605	100.0%

Table 3. The mean estimated age of the medial clavicular epiphysis of males and females.

Stages	Sex	n	Mean	Min-Max	P-value
Stage 1	Male	171	15.98 ± 1.55	14–19	**0.005**
	Female	94	15.37 ± 1.25	14–20	
Stage 2	Male	182	20.02 ± 1.69	17–23	**0.000**
	Female	206	18.80 ± 2.11	14–23	
Stage 3	Male	143	23.08 ± 1.46	19–26	**0.000**
	Female	184	22.90 ± 1.87	16–26	

Stage 4	Male	120	25.73 ± 0.83	24–27	**0.037**
	Female	127	25.83 ± 1.04	23–27	
Stage 5	Male	189	28.71 ± 1.03	26–30	0.927
	Female	189	28.70 ± 1.07	26–30	

Table 4. Paired sample t-test testing for significance in asymmetry of medial clavicular epiphyses.

Paired Samples t-test						
					95% confidence interval	
Test	**statistic**	**df**	**p**	**Cohen's d**	**Lower**	**Upper**
Student's	3.917	4	0.089	1.752	5.276	30.96
Wilcoxon	15.000	—	0.163	1.752	1.220	27.42

Table 5. Comparison of the incidence of bilateral asymmetry observed during evaluation of medial epiphyses.

Author and year	**Sample [n]**	**Incidence of asymmetry**	**Percentage**
Kaur. [12]	506	68	13.5%
Kreitner et al. [14]	380	6	1.6%
Schmeling et al. [25]	873	174	0.6%
Kellingaus et al. [13]	592	31	6.2%
Singh and Chavali [29]	343	None	None
Brown et al. [2]	1035	48	4.6%
Schulz et al. [28]	144	15	21.6%
Current study	**1605**	**43**	**2.7%**