Original article

Age estimation using Hounsfield unit values from computed tomography of proximal femur trabecular: A validation study in the Thai population

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Abstract

Background: Age is one of the biological profiles that are important in human identification, not only of unknown remains but also forensic clinical patients who need their identification confirmed, such as marginal patients who needs to obtain a national identification (ID) card.

Objective: The present study aimed to validate the age estimation equation of Ford JM in the Thai population to test the accuracy of each method when applied to a Thai sample.

Methods: Computed tomography (CT) scans and the resultant 3D models of 48 male and 48 female patients aged 18-to 88-years-old were used to measure their trabecular bone density of the proximal femur in Hounsfield units (HU). Additionally, the actual age and age calculated by the age estimation equation were compared.

Results: The actual age and age calculated using the combined sex, male and female equations were significantly different ($P < 0.05$). The bias and inaccuracy range were 2.8 - 37.4, the combined sex age estimation equation provided an overall accuracy of 16.7% ± 5 years, with the highest accuracy of 83.3% found in females aged 60 - 69 years.

Conclusion: The equation of Ford JM cannot be in age prediction among the Thai population and this suggests that inter-population variation does exist. Further study with a larger sample, using cadavers, might give better results for age estimation among the Thai population.

Keywords: Age estimation, computed tomography, Hounsfield units, proximal femur, Thai population.

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To support the death investigation process, forensic anthropologists play an important role in the identification of human skeletal remains by gathering biological specific parameters such as age, sex, stature, and ancestry. Although there are more specific investigations, such as DNA analysis, the prediction of these parameters is still valuable as they can help narrow down the group to which victims belongs before searching for relatives and comparing antemortem and post-mortem data for positive identification. Especially in cases of mass disasters, decomposed bodies, dismemberment or unknown remains, the body is not always discovered in pristine condition. Age estimation can assist in other legal concerns, such as the identification of marginal individuals who need to obtain a nationality identification (ID) card, criminal perpetrators and victims. [1 - 3]

In the field of age estimation studies, various parts of the body are used, such as the skull, pelvis, teeth, ribs, vertebrae, and long bones including the femur. [1, 4 - 26] The femur is the longest, heaviest, and strongest bone in the body, and is most likely to be preserved in mass casualty incidents. It has a distinctive shape, and is therefore easy to recognize when discovered, while the skull and other parts are often destroyed or missing in such circumstances. [1, 24] In Thailand, studies of age estimation are limited, due to the lack of forensic anthropology expertise in the country, and most studies are morphologic observation. [4, 11, 20] The problem with age estimation from morphological examination is that it is subjective and depends on the experience of each observer. Thus, anthropologists are making efforts to search for other techniques for age estimation that are more objective in order to reduce errors, such as plain radiography, computed tomography (CT) and magnetic resonance imaging (MRI). [1, 2]

There are several benefits of using CT scanners for anthropological analysis. Firstly, a CT scanner has the ability to penetrate the body's soft tissue so that the thus anthropologists are able to view skeletal structures in a timely and efficient manner, regardless of the antiquity or preservation of the remains. Secondly, the documentation of the bones can be collected permanently, creating virtual copies that are always accessible even when the actual specimen is no longer available to be analysed for reasons such as cremation or reburial. Thirdly, CT is accessible and cost-effective in Thailand, making it reasonable to use a CT scanner, as the Disaster Victim Investigation Guidelines. Moreover, CT data allow evaluation of the macroscopic bone surface and structures below the surface, e.g., the trabecular bone, and can facilitate morphometric analysis. [3, 24, 27, 28]

The CT number, also known as Hounsfield units (HU), is a number that represents the X-ray attenuation. All other CT values are computed according to: HU = 1000 x (μ tissue - μ water)/ (μ water - μ air), where μ is the CT linear attenuation coefficient. Denser tissue, with greater X-ray beam absorption, has positive values and appears bright, while less dense tissue, with less X-ray beam absorption, has negative values and appears dark.[3]

Age estimation studies using CT machines have been performed in many countries, using various parts of bones such as the cranial suture, clavicle, pelvic bone, vertebrae, and femur. [6 - 9, 13, 15, 22 - 25, 29] To the best of our knowledge, the application of HU in the field of age estimation is limited to the study of Ford JM, *et al.* in 2020, which applies HU to estimate age and sex using femur CT data. Their study introduces new equations for age prediction using the HU value of the proximal femur trabecular as follows: 1) combined sex equation: Age = 118.021 - 0.248 x HU; 2) male age equation: Male Age = 111.946 - 0.227 x HU; and 3) female age equation: Female Age = 123.718 - 0.266 x HU. However, they suggest further study using larger sample sizes in various populations.[24] The purpose of the present study was to validate the estimation equation in the Thai population to test the accuracy of this method.

Materials and methods

This study has been approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University (IRB no. 348/64).

Data from 96 patients, consisting of whole abdominal, lower abdominal, or pelvic CT scans, were retrospectively collected from living Thai patients who visited the Department of Emergency Medicine, King Chulalongkorn Memorial Hospital (KCMH) with abdominal pain, blunt abdominopelvic injury or suspected intraabdominal haemorrhage, from 2019 to 2021.

All the scans were acquired using a 256-slice multi-detector CT scanner, model GE Revolution, at 120 kV, using an automatic tube current (mA), variable with subject size, with a slice thickness of 1.25 mm,

calibrated each year with phantom and air calibration daily. All the images were reported officially by the radiologist and residents of the Department of Radiology. Any scans that had artificial implants of the hip or pelvis or osteopathology that prevented data collection, such as femoral neck fracture, avascular necrosis of femoral head, etc. were excluded. Patients with a body mass index (BMI) ≥ 30 kg/m^2, bedridden patients, patients with a diagnosis of cancer, osteoporosis, end-stage renal disease, parathyroid disease, vitamin D and calcium deficiency, chronic obstructive pulmonary disease (COPD), asthma, or any disease treated with steroids, were also excluded.

The sample of 48 males and 48 females were equally subdivided into 6 males and 6 females in each of 8 age groups, as shown in Table 1.

All the scans were imported into a medical software system, GE AW Server 3.2, for 3D reconstruction and analysis. The bones were initially 3D reconstructed and the femoral head was separated from the acetabulum via manual segmentation. Then, the lesser trochanter, a well-recognized anatomical landmark, was identified and the bone was cut at 90 degrees to the coronal view just inferior to this landmark in order to maintain reproducibility of the region of interest (ROI).[24]

Since HU in the equation is a representation of the trabecular density [24], a threshold of -100 to 500 HU was identified and kept as the ROI, subtracting all the cortical bone, which is > 500 HU in the Asian population [30], from the total bone density in order to detect all the trabecular area. The HU of the ROI were recorded. Figure 1 graphically shows the steps of the modelling process.

Table 1. Age and sex distribution of the study sample.

Age group	Male (n = 48)		Female (n = 48)	
Decade	N	Mean age (years)	N	Mean age (years)
10	6	18.6 ± 0.5	6	18.5 ± 0.5
20	6	25.0 ± 4.1	6	22.3 ± 1.7
30	6	33.8 ± 2.2	6	34.0 ± 3.5
40	6	43.8 ± 3.3	6	44.5 ± 4.0
50	6	54.6 ± 4.0	6	54.8 ± 3.1
60	6	64.8 ± 2.9	6	65.3 ± 2.2
70	6	74.0 ± 2.0	6	73.0 ± 3.2
80+	6	84.0 ± 2.0	6	83.6 ± 3.0

* Data were expressed as mean ± standard deviation

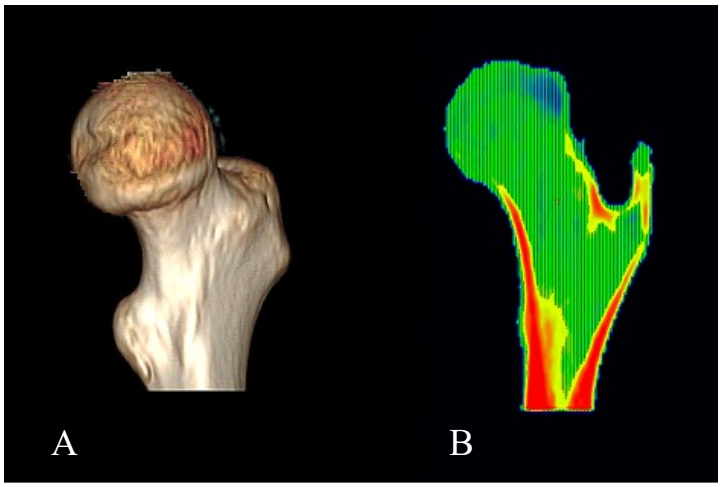


Figure 1. (A) Cut 3D femur, **(B)** 2D CT image showing (bright grey) cortical and (dark grey) trabecular bone delineation.

The HU obtained from the left femur were used in the following equations: 1) combined sex Age = 118.021 - 0.248 x HU; 2) Male Age = 111.946 - 0.227x HU; and 3) Female Age = 123.718 - 0.266 x HU.

Both sides of the femurs were measured in the first 30 cases and analysed by the pair *t* - test. If there was no significant difference between the sides, only the left-side femurs were measured in the next 66 cases. The left side is non-dominant which means it is less physically active and therefore exposed to lower stress and lesser degeneration effects. [3]

Statistical analysis

The data collection process was performed by two collectors (a forensic anthropologist and a forensic resident) and tested for intra- and inter-rater agreement, calculated via intraclass correlation (ICC). Data were expressed as mean and standard deviation (SD) The Kolmogorov-Smirnov test was used to assess the normality of the distribution. An unpaired student *t* - test was used to compare male and female trabecular density. The pair *t* - test was used to test for symmetry of the left and right density and comparison between the actual and calculated age. The differences between the groups were considered significant if the *P* - values were less than 0.05. Bias (the mean error in predicting age, over and underestimation), and inaccuracy (the average of absolute error of age estimation) were also analysed to assess the reliability of the age estimation methods from the trabecular bone density of the patient. [31]

$$\text{Bias} = \frac{\sum(\text{estimated age} - \text{actual age})}{n}$$

$$\text{Inaccuracy} = \frac{\sum|\text{estimated age} - \text{actual age}|}{n}$$

Results

These findings show the normal distribution of HU and age of the samples. The intraclass correlation (ICC) shows an excellent degree of repeatability with the intra-rater agreement having an ICC = 0.973, and the inter-rater agreement having an ICC = 0.979. The descriptive statistics of HU for males and females in each age group are demonstrated in Table 2.

Further analysis shows no significant in HU between the left and right proximal femur ($P = 0.709$). There was no significant difference between male and female proximal femur trabecular density difference (male 225.67 ± 54.97 HU, female 226.25 ± 42.23 HU, $P = 0.954$), while there were significant differences in the combined sex, male, and female equations, as shown in Table 3. The correlation between actual and calculated age using the combined sex equation and male equation are 0.409 and 0.373, respectively, while the female equation shows a negative correlation of -1.0.

The bias and inaccuracy range for the combined sex equation is between 2.8 and 37.4, as shown in Table 4. The bias and inaccuracy for the male and female equations is shown in Table 5.

The combined sex equation has an overall accuracy of 16.7% ± 5 years. Focusing on female samples, the combined equation has an accuracy of 83.3% ± 5 years in the 60 decade age group with a bias of - 2.8 and an inaccuracy of 4.0. The male samples have an accuracy of 66.7% ± 5 years in the 70 decade age group, with a bias of 0.8 and an inaccuracy of 5.2, as shown in Table 6.

Table 2. Descriptive statistics of HU in each age group.

Age	Female			Male		
(Decade)	n	Mean HU	SD	n	Mean HU	SD
10	6	250.63	22.33	6	277.97	33.11
20	6	242.80	28.47	6	272.68	44.23
30	6	249.88	51.68	6	218.47	48.77
40	6	214.17	39.55	6	215.63	42.32
50	6	241.00	53.18	6	210.88	20.25
60	6	223.78	29.07	6	192.75	46.19
70	6	206.72	45.10	6	163.83	28.77
80	6	181.03	20.97	6	253.15	68.09
Total	48	226.25	42.23	48	225.67	54.97

Table 3. Comparison between actual and calculated age using the combined sex, male, and female equations.

	Mean (years)	Mean differences (years)	*P* - value
Actual age(Combined)	49.73 ± 22.48	12.25 ± 2.11	< 0.001
Calculated age(Combined)	61.98 ± 12.09		
Female actual age	49.52 ± 22.65	61.03 ± 28.68	< 0.001
Female calculated age	110.55 ± 6.03		
Male actual age	49.94 ± 22.55	10.78 ± 21.32	0.001
Male calculated age	60.72 ± 12.48		

*Data were expressed as mean ± standard deviation

Table 4. Bias and inaccuracy of the combined sex equation; Age = 118.021 - 0.248 x HU.

Age	Female (n = 48)			Male (n = 48)		
(Decade)	n	Bias	Inaccuracy	n	Bias	Inaccuracy
10	6	37.4	37.4	6	30.4	30.4
20	6	35.5	35.5	6	25.4	25.4
30	6	22.0	22.0	6	30.0	30.0
40	6	20.4	20.4	6	20.7	20.7
50	6	3.4	3.4	6	11.1	11.2
60	6	- 2.8	4.0	6	5.4	11.8
70	6	- 6.2	11.3	6	3.4	5.8
80	6	- 10.5	10.5	6	- 29.4	29.4

Table 5. Bias and inaccuracy of the male age equation; Male Age = 111.946 - 0.227 x HU and female age equation; Female Age = 123.718 - 0.266 x HU.

Age	Female (n = 48)			Male (n = 48)		
(Decade)	n	Bias	Inaccuracy	n	Bias	Inaccuracy
10	6	100.3	100.3	6	30.2	30.2
20	6	95.4	95.4	6	25.0	25.0
30	6	80.7	80.7	6	28.5	28.5
40	6	67.4	67.4	6	19.2	19.2
50	6	54.3	54.3	6	9.9	9.9
60	6	41.0	41.0	6	3.4	10.4
70	6	31.3	31.3	6	0.8	5.2
80	6	17.8	17.8	6	-30.2	30.2

Table 6. Five-year accuracy rate of Ford JM, *et al.*'s equation applied to Thai samples.

Age group	Combined sex equation						Female equation		Male equation	
(Decade)	Female		Male		Total					
	n	%	n	%	n	%	n	%	n	%
10	0/6	0	0/6	0	0/12	0	0/6	0	0/6	0
20	0/6	0	0/6	0	0/12	0	0/6	0	0/6	0
30	1/6	16.7	0/6	0	1/12	8.3	0/6	0	0/6	0
40	0/6	0	1/6	16.7	1/12	8.3	0/6	0	1/6	16.7
50	2/6	33.3	1/6	16.7	3/12	25.0	0/6	0	2/6	33.3
60	5/6	83.3	1/6	16.7	6/12	50.0	0/6	0	1/6	16.7
70	0/6	0	4/6	66.7	4/12	33.3	0/6	0	3/6	50.0
80	0/6	0	1/6	16.7	1/12	8.3	0/6	0	1/6	16.7
Total	8/48	16.7	8/48	16.7	16/96	16.7	0/48	0	8/48	16.7

Linear regression analysis was performed to generate the age estimation equations for combined sex, male, and female for the Thai population, giving the following:

1) Combined Age = – 0.19HU + 92.33 (R = 0.409, standard error of estimation (SEE) = 20.63).

2) Male Age = – 0.15HU + 84.42 (R = 0.373, SEE = 21.15).

3) Female Age = – 0.25HU + 105.86 (R = 0.464, SEE = 20.28).

The scatterplots for these regression models are shown in Figure 2.

Discussion

The ICC shows an excellent degree of repeatability, which may be the result of using the lesser trochanter, a well-recognized anatomical landmark, as a cut point for the ROI. A potential source of error in this method is the manual segmentation process when the femur is separated from the acetabulum.

The study of Ford JM, *et al*. indicates the threshold of the femoral trabecular ROI is between 260 and 661 HU.[24] However, the present study in a Thai population is unable to use the same threshold as the cortical and trabecular bone of Thai population has a lower HU. Thus, in order to maintain the ROI of trabecular bone, the threshold in this study is reduced to - 100 to 500 HU. The difference between the mean HU in the population of Ford JM, *et al*. and this study cannot be analysed due to the lack of available data from the previous study.

Overall, the bias reflects an overestimation in younger samples and an underestimation in older samples, using the combined sex equation.

Among the female sample, an overestimation is found in all age groups when using the female age estimation equation, but this reduces progressively with age. This may result from the lower trabecular density of Thai females than Caucasian samples of the same age, as the bone mass density (BMD) of Asian women is lower [32, 33], and the bone size is smaller than Caucasian women. [34] Peak BMD of Thai women's femurs occurs between 30 and 40 years old [35 - 37], corresponding with that of Chinese women which occurs at the age of 30 to 34 years.[38] Meanwhile Caucasian women's peak BMD occurs at age 16 to 19 years [39], which makes the great inaccuracy in the younger age group reduce progressively after the 30 or 40 decade for both the combined equation and female equation, as the peak BMD for Thai women comes after the peak BMD for Caucasian women. When menopause occurs at around age 50, hormonal changes in the female have an obvious impact on trabecular density, and the inaccuracy of the combined equation is reduced more than in the male sample.[32] The age of osteoporosis in Asian women is around 60 – 85 years, close to the 70 - 85 years in Caucasians, suggesting there is less inaccuracy in the older age group.

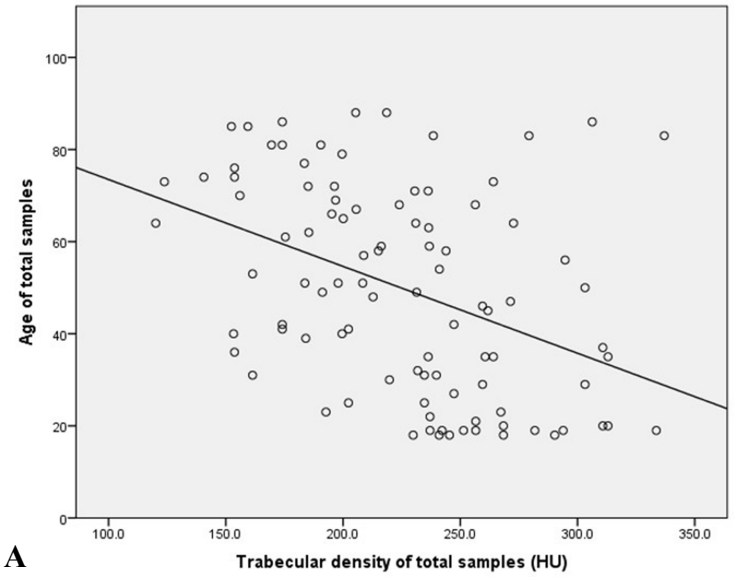
In the male group, overestimation is found from the 10 decade to the 70 decade. This may result from the lower trabecular density of Thai males than those of Caucasian males and the age of peak BMD of Thai males being 20 - 29 years [37], later than the 19 - 21 years for Caucasians. [39] This closer age of peak BMD among males may be the reason that the inaccuracy is lower than for females. There is a degenerative process that has an impact on the bone, without the effect of menopause, at around age 50 years in females only, which may cause the lower bias and inaccuracy for males in this age group.

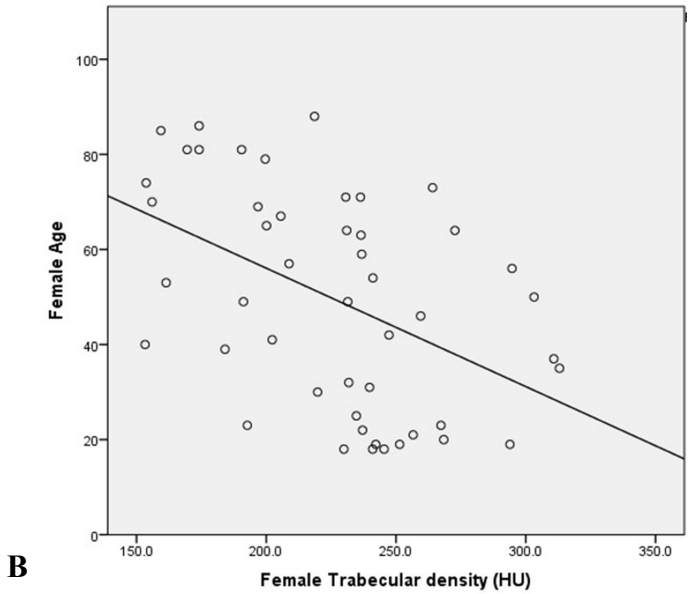
Underestimation of age is found for 60 - 89 year-old men. At age 50 years, Caucasian males start to have osteoporosis [39], which is earlier than Thai males at the age of ≥ 70 years. [37] The highest underestimation of male age is found in the 80 decades, which may be due to an outlier with more trabecular density than others. Such outliers may occur due to differences in socioeconomic status, nutrition, unknown medical conditions, or daily activity.[24]

Moreover, the under- or overestimation of age might be explained by patient selection bias. Since KCMH is a tertiary healthcare centre, most patients are unhealthy, and therefore the sample may exclude some of the normal population with greater bone density. This study is unaffected by the age mimicry phenomenon due to there being an equivalent sample size in each age group.[31]

Even though the combined sex equation can be used for age prediction in 60 - 69 year-old females within 5 years with 83.3% accuracy and a bias of - 2.8, and 70 - 79 year-old males within 5 years with an accuracy of 66.7% and a bias of 0.8, this age range is too narrow to use for age estimation in real situations. This study proves that the age estimation equation used for people of other ancestry cannot be used for prediction of all populations.

Because of the exclusion criteria and limitations of the CT database, the samples in this study may not be sufficient. Thus, the linear regression equations have quite a low correlation, as shown in Figure 2.





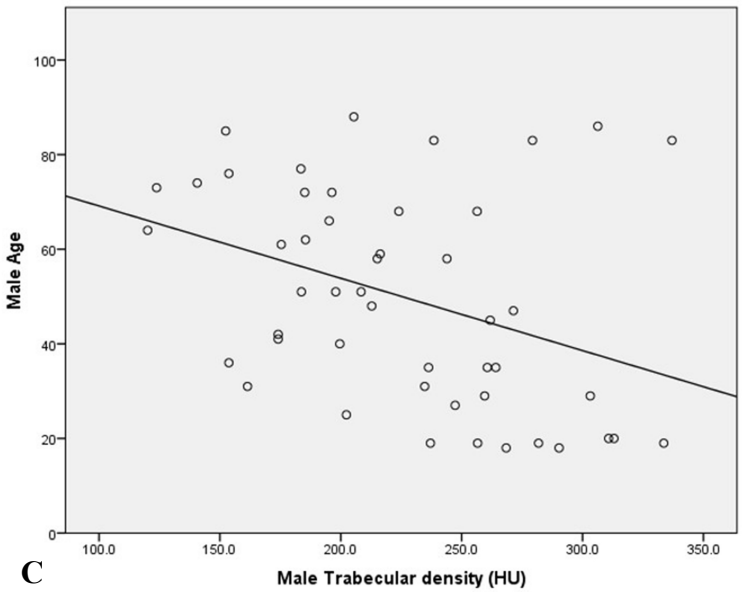


Figure 2. (A) Combined linear regression model, **(B)** female linear regression model, **(C)** male linear regression model. The X axis represents HU of left trabecular density and the Y axis represents actual age.

Since the results show no difference between left and right femoral trabecular density, the right femur measurements can be used in the equations. This result corresponds with the study of van Santen JA PC*,et al*. in 2019, that there are no clinical differences between the dominant and non-dominant hip BMD. [40]

In this study, the samples are living person data, and thus our equations can only be safely used on living people, due to the differences between fresh and dry bones, especially in respect of presented soft tissue.[41] When bone is burned or even frozen and thawed, specimens have differences in BMD.[42] Further study on forensic investigation of remains, larger samples of Thai cadavers and dry bones are needed.

Conclusion

This study found that the best accuracy resulted from the combined sex equation tested in female sample is 83.3%. Since the age parameter is population specific data, age estimation using the method of Ford JM*, et al.* should be applied in the Thai population with caution.

Conflicts of interest

All authors have no conflict of interest.

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