

Age Prediction Using Pixel Value Sum from Radiographic Proximal Femur in a Thai Population

Predicción de la Edad Utilizando la Suma de Valores de Píxeles del Fémur Proximal Radiográfico en una Población Tailandesa

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SUMMARY: The present study aimed to investigate the utility of the proximal femur in the forensic age estimation by assessing changes in bone densities through radiographs. Using Otsu's threshold, bone density was quantified by counting all white pixel values within selected regions of interest, which include femoral head (FH), femoral neck (FN), Ward's triangle (WT), and greater trochanter (GT) from 354 left femora of Northern Thai descent. The pixel width of medullary cavity (MC) was also estimated. Furthermore, the study evaluated the performance of linear regression (LR) models for age estimation from radiographic images of proximal femora. Negative correlations were observed between FH, FN, WT, and GT pixel intensity with the age-at-death of the samples, with females exhibiting stronger correlations than males. Moreover, a positive correlation was found between age and MC width in female samples, while male MC widths did not show any relationship with increasing age. The results showed a slight difference between the LR model applied to both sexes, which integrated all variables, and the alternative configuration that only utilized relevant attributes. Both models exhibited similar performance, with a narrow range of root mean square error (RMSE) values, ranging from 12.67 to 12.71 years, and a correlation coefficient range of 0.51 to 0.52. For females, the LR model with FN and WT as selected attributes (RMSE = 11.85 years, correlation coefficient = 0.65) performed decently, while for males, the LR model with all variables showed RMSE of 12.52 years and correlation coefficient of 0.46. This study showcased the potential application of pixel intensity in predicting age.

KEY WORDS: Forensic anthropology; Proximal femur; Age estimation; Linear regression; Radiograph.

INTRODUCTION

Forensic anthropology involves the identification of unknown skeletal remains, which requires the determination of a biological profile, including age estimation. Age estimation is a complex process that involves the evaluation of various skeletal elements, with the skull and pelvis being commonly utilized. The former is assessed based on the obliteration of cranial sutures (Meindl *et al.*, 1985). However, age determination based on bone degeneration is deemed more reliable, with the pubis symphysis (Brooks & Suchey, 1990) and auricular surface (Lovejoy *et al.*, 1985; Buckberry & Chamberlain, 2002) of the pelvis being commonly

employed as age indicators due to their close association with age. Other skeletal elements, such as sternal rib ends (Iscan *et al.*, 1984, 1985), have been proposed as reliable age indicators due to their minimal mobility. In contrast, long bones, despite being suggested as age indicators, possess synovial joints that allow for constant movement, making them less reliable for age prediction than joints with limited mobility (Walker & Lovejoy, 1985). Nonetheless, the stage of ossification is typically used to estimate age from long bones, primarily to differentiate between adult and sub-adult bones. Recent studies, however, suggest that radiological

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techniques can be utilized to evaluate the proximal femur's potential as an age indicator (Curate *et al.*, 2013; Navega *et al.*, 2018; Ford *et al.*, 2020; Abdulai *et al.*, 2022; Curate *et al.*, 2022).

The proximal femur's composition, comprising both cortical and trabecular bone, renders age-related changes noticeable in the trabecular structure, which can be evaluated through X-ray imaging. Consequently, proximal femora have potential as a means of determining age, as distinct stages of trabecular degeneration are evident (Walker & Lovejoy, 1985). Walker & Lovejoy (1985) identified primary and secondary trabeculae within the trochanters, Ward's triangle, and medullary cavity as useful markers for age determination, with radiographs classified into eight age intervals based on radiolucency changes in these regions of interest. However, the high level of activity in the proximal femur leads to unreliable age estimation, as reported by the same study (Walker & Lovejoy, 1985). Furthermore, the method's subjective nature poses a challenge, as investigators must categorize each radiograph into various stages. A quantitative approach may be more appropriate for evaluating trabecular changes in the proximal femur than a qualitative approach. Additionally, the range of age intervals in this study is skewed towards individuals under 50 years old, with individuals over 60 years old clustered in one category. These limitations suggest that further research is necessary to validate the potential of proximal femora as a reliable indicator of advanced age.

Age grouping schemes are commonly utilized in forensic anthropology, with the most common categorization separating adults into young, middle, and older age groups (Falys & Lewis, 2011). The end of young adulthood is generally established at 25 years of age, as most late epiphyseal fusion will have occurred by this age. Middle adulthood is typically considered to be between 25 to 35 years old, leaving a broad age range for older adults. As age increases, the precision and accuracy of age estimation methods decrease due to the inconsistent relationship between chronological and biological age, and older adults tend to exhibit greater variation, resulting in less precise and accurate age estimates (Botha & Steyn, 2022). Issues with aging older adults have been identified in numerous studies (Listi, 2016; Cappella *et al.*, 2017), with even the most reliable aging methods yielding high errors in this population. Underestimating and overestimating the age of elderly individuals are the most commonly encountered challenges (Cappella *et al.*, 2017). These problems present a significant obstacle in identifying the skeletal remains of elderly individuals.

In comparison to other age estimation methods such as pubic symphysis (Brooks & Suchey, 1990), auricular

surface (Lovejoy *et al.*, 1985; Buckberry & Chamberlain, 2002), and sternal rib end (Iskan *et al.*, 1984, 1985), techniques involving phase analysis can prove challenging due to subtle changes between phases. Furthermore, the last phase of these methods often centers on individuals with a mean age of 60 years, resulting in a rather large age range and standard deviations for "older adult" samples. Cranial suture closure (Meindl *et al.*, 1985) and maxillary suture closure (Mann *et al.*, 1987) are other useful indicators of advanced age. Bone diseases, including osteoarthritis and osteoporosis, can also serve as possible indicators of more advanced age. In particular, osteoporosis, which results from bone reabsorption outpacing bone formation and causes an increase in bone porosity with increasing age, is palpable in the proximal femoral site, and also commonly used for diagnosis of hip fracture risk. Therefore, the proximal femur is often chosen as the skeletal element of interest for age estimation in cases where the skull and pelvis are absent during forensic recovery. In addition to its suitability as an age indicator, the proximal femur offers advantages such as greater bone density and better protection, resulting in better preservation compared to the pubic symphysis and sternal rib ends.

The need for age estimation methods that specifically cater to the aging population has become increasingly urgent in light of the recent rapid growth of the elderly demographic, particularly in cases where their deaths occur in isolation (Blatt *et al.*, 2020). Additionally, the definition of "older adult" age range varies considerably in the literature, with significant overlap, making it challenging to establish definitive age estimates. The root cause of this issue can be attributed to the underrepresentation of elderly samples in the osteological collections used in the development and validation of age estimation methods. According to Márquez-Grant (2015), the absence of adequate older adult samples in studies has hindered the development of anthropological age assessment methods for distinguishing between individuals aged 60, 65, 70, and 80 years old.

Hence, this study aims to capitalize on the abundance of older adult samples in our osteological collection to develop a novel age estimation technique tailored for older Thai individuals (50 years and above) using radiographs of proximal femora. The selection of X-ray as the imaging modality is motivated by its cost-effectiveness and widespread use in disaster victim identification. The quantification of bone densities in the proximal region of the femur using image analysis tools enables the detection of age-related bone degeneration. Linear regression analysis will then be employed to derive an age prediction model, which has been demonstrated to be effective in analogous studies. As noted by Ford *et al.* (2020), while an exact age

estimate would be ideal for age estimation, forensic anthropologists typically rely on estimated age ranges in practice. Therefore, the objective of this investigation is to identify the optimal configuration for age prediction that yields the narrowest age range, which would be more practical for estimating the age range of elderly adults with greater precision.

MATERIAL AND METHOD

Material. This study aimed to evaluate the utility of radiographic proximal femoral density in predicting age. A sample of 354 radiographs of documented left femora was acquired using AiRTouch Portable X-Ray Equipment (Aspenstate, USA) and derived from the skeletal collection located at the Osteology Research and Training Center (ORTC), Chiang Mai University. The radiographic procedure was taken between February – March 2022. The aforementioned sample consisted of 161 females and 193 males of Thai descent, with an age-at-death range of 19 to 94 years. The samples were born in Thailand between the years 1935 and 1998, and their deaths occurred between 2006 and 2019. Femora visually exhibiting signs of pathology and trauma were excluded from the study to ensure the accuracy of the results. The majority of samples in this osteological collection is skewed toward older adults, so this is an excellent opportunity for developing age prediction methods in older adults. The use of human specimens in this research was approved by the Research Ethics Committee of the Faculty of Medicine at Chiang Mai University, under ethical exemption number ANA-2564-08568.

Image Processing. The radiographic images were saved as DICOM (Digital Imaging and Communications in Medicine) and processed in Fiji software (Schindelin *et al.*, 2012). The images were converted into binary values (0 = black pixel, 255 = white pixel) using the "Otsu thresholding" filter [21]. The study focused on five regions of interest (ROIs): femoral head (FH), femoral neck (FN), Ward's triangle (WT), greater trochanter (GT), and medullary cavity (MC). The quantification of bone density within each ROI (FH, FN, WT, and GT) was performed by calculating the sum of white pixel values. This process involved utilizing the freehand selection tool and the "Measure" command. Each white pixel value, which corresponded to a value of 255, represented the bone density within the selected ROIs. The resulting sum of white pixel values for each ROI can be observed in the "RawIntDen" column within the "Results" window. Furthermore, the width of MC at the region adjacent to the lesser trochanter was measured using the straight-line tool and the "Measure" command (Fig. 1). All data were converted to megapixel units and recorded in a Microsoft Excel spreadsheet.

Statistics. Statistical analyses were conducted using Jamovi (The jamovi project, 2024), an open-source software for data analysis. Descriptive statistics were calculated for each ROI, including mean and standard deviation (SD), to examine trends across different age classes. Student's t-test for independent samples was employed to analyze sexual dimorphism in pixel sum values. A Pearson correlation coefficient analysis was used to determine the relationship between pixel sum values and age. In this study, a p-value less than 0.05 was considered statistically significant.

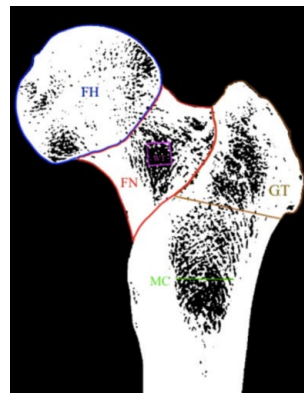


Fig. 1. Illustration of ROIs on radiographic proximal femur that was applied with Otsu thresholding filter. Abbreviations: FH: femoral head; FN: femoral neck; WT: Ward's triangle; GT: greater trochanter; MC: medullary cavity

Linear Regression Analysis. The data from Excel were exported in CSV format to formulate linear regression models for age estimation, using the Waikato Environment for Knowledge Analysis software (Weka; version 3.8.3) (Frank *et al.*, 2010). To develop the age prediction models, a dataset of 354 radiographic left femora was used, incorporating all variables. Linear regression (LR) is a simple and widely used statistical model for regression tasks. It assumes a linear relationship between the input features and the target variable and aims to find the best fitting line through the data points to make predictions on new data. However, the inclusion of irrelevant attributes can further diminish the performance of linear regression. To circumvent this issue, WEKA provides M5 method, an automatic feature selection process that only selects pertinent attributes. This process is enabled by default, but it can be disabled if necessary. In this study, we tested the performance of LR models with both approaches. The performance of each model was assessed using a 10-fold cross-validation method, which involves dividing the dataset into 10 equal segments and consecutively using each segment as a test set in 10 iterations, ensuring each segment is used as the test set once. The selection of an appropriate cross-validation technique is a critical component in evaluating the effectiveness of age prediction models. In this study, the 10-fold cross-validation approach was deemed optimal due to its ability to reduce both processing time and computational requirements, as compared to other commonly utilized techniques such as the leave-one-out method.

RESULTS

Table I presents a summary of the descriptive statistics of the pixel sum values for each ROI, categorized by age class and sex. All values are expressed in the unit of megapixels. Table I also includes the results of an independent sample t-test analysis, which shows statistically significant differences in bone densities between male and female proximal femora, as demonstrated by the p-values ($p < 0.05$). However, no sexual dimorphism was observed in MC pixel width (Table II). Furthermore, a Pearson's correlation analysis was conducted to investigate the relationship between pixel sum values and the age at death of the individuals. In Table I, the findings suggest that FH, FN, WT, and GT were negatively correlated with the age of the samples ($p < 0.001$), with stronger correlations observed in females (Pearson's r values ranged from -0.515 to -0.643) than males (Pearson's r values ranged from -0.318 to -0.505). Additionally, a positive correlation was found between age and MC pixel width in female samples ($p = 0.004$), but no association was observed between male MC pixel widths and increasing age (Table II).

This study presents an evaluation of LR models for estimating age based on radiographic images of the proximal femora. The summarized findings are presented in Table III. When considering both sexes, the LR model that included FH, FN, and WT as selected attributes for age prediction yielded a RMSE of 12.67 years and a correlation coefficient of 0.52. However, its performance exhibited a marginal improvement over the LR model that encompassed all variables, resulting in RMSE of 12.71 years and a correlation coefficient of 0.51. Among females, the LR model incorporating FN and WT as selected attributes demonstrated the highest efficacy in age prediction (RMSE = 11.85 years), exhibiting a correlation coefficient of 0.65, the strongest among all tested LR models. In the case of males, the LR model that incorporated all attributes exhibited the RMSE of 12.52 years, accompanied by a correlation coefficient of 0.46. Interestingly, an alternative LR model that solely utilized FN as the selected variable for age prediction displayed inferior performance, characterized by RMSE of 12.56 years and a correlation coefficient of 0.46. The LR equations for age-at-death estimation proposed in this study are provided in Table IV.

Table II. Summary of statistical analysis for pixel widths in the MC.

| | Age class | MC pixel width | | | | N (354) | |
|-----------------|-----------|----------------|--------|---------|--------|---------|---------|
| | | F (161) | | M (193) | | F (161) | M (193) |
| Mean (SD) | >18-30 | 14.3 | (2.07) | 16.0 | (2.19) | 7 | 2 |
| | 31-40 | 13.2 | (1.16) | 13.8 | (1.93) | 4 | 8 |
| | 41-50 | 13.6 | (1.93) | 14.7 | (2.28) | 15 | 26 |
| | 51-60 | 13.9 | (2.32) | 14.5 | (1.83) | 31 | 37 |
| | 61-70 | 13.5 | (1.56) | 14.5 | (1.44) | 41 | 51 |
| | 71-80 | 14.7 | (1.82) | 14.6 | (2.26) | 33 | 42 |
| | >80 | 15.5 | (2.43) | 14.6 | (2.13) | 30 | 27 |
| Mean difference | | | | -0.318 | | | |
| p-value | | | | 0.140 | | | |
| Pearson's r | | 0.224 | | 0.024 | | | |
| p-value | | 0.004 | | 0.736 | | | |

Abbreviations: MC: medullary cavity; SD: standard deviation; F: female; M: male

Table I. Summary of statistical analysis for pixel sum values in the ROIs (values are expressed in megapixels).

| Age class | FH pixel sum values | | | | FN pixel sum values | | | | WT pixel sum values | | | | GT pixel sum values | | | | N (354) | | | |
|-----------------|---------------------|--------|-----------|--------|---------------------|--------|-----------|--------|---------------------|--------|-----------|--------|---------------------|--------|-----------|--------|---------|---------|--------|--|
| | F (161) | | M (193) | | F (161) | | M (193) | | F (161) | | M (193) | | F (161) | | M (193) | | F (161) | M (193) | | |
| | Mean (SD) | | Mean (SD) | | Mean (SD) | | Mean (SD) | | Mean (SD) | | Mean (SD) | | Mean (SD) | | Mean (SD) | | | | | |
| >18-30 | 12.9 | (1.47) | 18.2 | (1.24) | 3.86 | (0.73) | 4.52 | (0.09) | 1.18 | (0.41) | 1.20 | (0.45) | 8.49 | (2.63) | 11.2 | (0.8) | 7 | 2 | | |
| 31-40 | 13.7 | (2.08) | 18.3 | (3.32) | 3.84 | (0.53) | 4.63 | (0.95) | 1.06 | (0.5) | 1.07 | (0.57) | 9.23 | (1.23) | 11.4 | (1.91) | 4 | 8 | | |
| 41-50 | 12.4 | (3.70) | 17.7 | (1.89) | 3.37 | (1.01) | 4.09 | (0.54) | 0.84 | (0.5) | 0.79 | (0.28) | 7.87 | (2.39) | 11.3 | (1.89) | 15 | 26 | | |
| 51-60 | 11.6 | (2.67) | 17.7 | (2.43) | 2.95 | (0.78) | 4.00 | (0.59) | 0.59 | (0.45) | 0.71 | (0.33) | 7.23 | (2.24) | 11.6 | (1.74) | 31 | 37 | | |
| 61-70 | 9.75 | (2.61) | 16.9 | (2.44) | 2.43 | (0.66) | 3.58 | (0.63) | 0.35 | (0.25) | 0.51 | (0.31) | 6.07 | (2.18) | 11.0 | (1.92) | 41 | 51 | | |
| 71-80 | 9.48 | (2.51) | 15.8 | (3.29) | 2.23 | (0.55) | 3.35 | (0.73) | 0.27 | (0.2) | 0.49 | (0.33) | 5.34 | (2.05) | 10.0 | (2.45) | 33 | 42 | | |
| >80 | 7.88 | (2.68) | 14.6 | (3.33) | 1.94 | (0.52) | 3.15 | (0.89) | 0.2 | (0.17) | 0.51 | (0.43) | 4.21 | (1.67) | 9.13 | (3.01) | 30 | 27 | | |
| Mean difference | | | -6.48 | | | | -1.1 | | | | -0.162 | | | | -4.55 | | | | | |
| p-value | | | < .001 | | | | < .001 | | | | < .001 | | | | < .001 | | | | | |
| Pearson's r | | | -0.515 | | | | -0.639 | | | | -0.643 | | | | -0.387 | | | | -0.318 | |
| p-value | | | < .001 | | | | < .001 | | | | < .001 | | | | < .001 | | | | < .001 | |

Abbreviations: FH: femoral head; FN: femoral neck; WT: Ward's triangle; GT: greater trochanter; SD: standard deviation; F: female; M: male

Table III. The performance of linear regression models for age estimation.

| Models | Correlation coefficient | | | MSE | | | RMSE | | | RAE | | | RRAE | | |
|-------------------------------|-------------------------|---------------|-----------|-------------------|---------------|------------|--------------------|----------------|------------|----------------------|------------------|--------------|----------------------|------------------|--------------|
| | All | F | M | All | F | M | All | F | M | All | F | M | All | F | M |
| LR | 0.51 | 0.62 | 0.46 | 10.0 | 9.64 | 9.97 | 12.71 | 12.18 | 12.52 | 84.22 % | 78.89 % | 85.58 % | 85.83 % | 78.12 % | 88.37 % |
| LR (with selected attributes) | 0.52 (FH, FN, WT) | 0.65 (FN, WT) | 0.46 (FN) | 10.0 (FH, FN, WT) | 9.45 (FN, WT) | 10.05 (FN) | 12.67 (FH, FN, WT) | 11.85 (FN, WT) | 12.56 (FN) | 84.05 % (FH, FN, WT) | 77.35 % (FN, WT) | 86.18 % (FN) | 85.57 % (FH, FN, WT) | 76.05 % (FN, WT) | 88.65 % (FN) |

Abbreviations: LR: linear regression; MSE: mean square error; RMSE: root mean square error; RAE: relative root absolute error; F: female; M: male; FH: femoral head; FN: femoral neck; WT: Ward's triangle

Table IV. The linear regression models for age estimation generated in this study.

| Models | Formula | | RMSE | |
|-----------------------------------|---------|---|------------------|--------|
| All | Age = | (0.92) FH + (-10.22) FN + (-5.98) WT + (0.4) GT + (-0.04) MC | FN + (-5.98) WT | ±12.71 |
| All (with selected attributes) | Age = | (1.11) FH + (-9.67) FN + (-6.31) WT | FN + (-6.31) WT | ±12.67 |
| Female | Age = | (-0.18) FH + (-4.54) FN + (-14.14) WT + (-0.1) GT + (0.27) MC | FN + (-14.14) WT | ±12.18 |
| Female (with selected attributes) | Age = | (-5.78) FN + (-13.49) WT | FN + (-13.49) WT | ±11.85 |
| Male | Age = | (0.35) FH + (-13.29) FN + (4.19) WT + (0.6) GT + (-0.5) MC | FN + (-13.29) WT | ±12.52 |
| Male (with selected attributes) | Age = | (-9.13) FN | FN | ±12.56 |

Abbreviations: FH: femoral head pixel sum value; FN: femoral neck pixel sum value; WT: Ward's triangle pixel sum value; GT: greater trochanter pixel sum value; MC: medullary cavity pixel width.

DISCUSSION

The current investigation explores the association between proximal femoral pixel sum values in radiographs and age, achieved by utilizing Otsu's threshold to binarize white pixel values (255) from black pixel values (0). Results from statistical analyses indicate that all regions of interest (ROIs) with the exception of MC demonstrate potential for age prediction. The assessment of LR models for age estimation indicates that the LR model is most effective at predicting age in females, with RMSE of 11.85 years, utilizing FN and WT as selected attributes. The best model for male age prediction was the LR model that incorporates all variables, with RMSE of 12.52 years. The LR model with FH, FN, and WT as selected attributes demonstrated RMSE of 12.67 in estimating age for all samples. This study underscores the efficacy of the linear regression analysis in developing robust age prediction models.

The rationale for generating sex-specific age prediction models stems from the well-established sexual dimorphism in bone densities in the proximal femora, as reported in various studies (Wheatley, 2005; Castillo *et al.*, 2011; Paschall *et al.*, 2018). Moreover, this study reveals that age-related changes in bone densities are more prominent in females compared to males, as a result of differential hormonal regulations and higher susceptibility of females to age-related bone diseases such as osteoporosis. Notably, the femoral neck, a site associated with a higher risk of hip fracture in elderly women, shows the greatest potential for age prediction in females, with FN and WT demonstrating the strongest association with age in this region.

It is unsurprising that femoral neck (FN) was included in various configurations of the LR models, as it demonstrated the strongest correlation to age compared to other ROIs (Table I). Our study employed the same age markers that were analyzed in Walker & Lovejoy (1985), which include femoral head, femoral neck, Ward's triangle, greater trochanter, and medullary cavity. However, our study differed in approach as we employed a quantitative method using image analysis tools. The results demonstrate that counting the sum of white pixels in the ROIs of radiographs has potential for generating data for age prediction models. However, as revealed in this study, the radiographic proximal femur can exhibit relatively high variability, which makes it challenging as a reliable age indicator. The high degree of physical activity in the proximal femur may contribute to the inaccuracy of determining an individual's age based on this region. Given the continuous movement and weight-bearing stresses on the proximal femur, methods that rely on examining the inner structures of this region may be less reliable compared to those that utilize joints with limited mobility, such as the pubic symphysis, which have been found to be more reliable for age prediction (Rattanachet, 2022).

The issue of accurately estimating age is further compounded by the limited number of samples from individuals over 90 years old. The 80-year-old demographic also presents a challenge as it is difficult to distinguish from the 90-year-old demographic, and to some extent, the 70-year-old demographic, which can hinder the precision of age assessment (Cunha *et al.*, 2009). In the present study, the number of individuals over 80 years old is significantly lower than the number of individuals within the age range of 40-60 years old. Although the impact of this on the efficacy of the generated age prediction model is unknown, it is crucial to have a more diverse age demographic composition of skeletal collections to improve the accuracy of age estimates, especially for elderly individuals. Imaizumi *et al.* (2021) suggested that increasing the sample size used to derive the model can minimize mean absolute errors in aging older adults. Therefore, a larger sample size, especially from individuals over 60 years old, is needed to improve the reliability of age prediction methods for elderly-presenting human remains.

The research conducted by Curate *et al.* (2013), explored the relationship between age-at-death and bone mineral content within the proximal femur, which led to the generation of a linear regression equation. Interestingly, the study categorized the samples into three age subgroups: Young, middle-aged, and older adults, and reported an increase in accuracy for the older adult category (Curate *et al.*, 2013). Botha *et al.* (2019), conducted a similar study that formulated linear regression equations for age estimation using bone density of the proximal femur in White and Black South Africans. When separated by ancestry, the error estimates of age prediction decreased significantly. Abdulai *et al.* (2022) utilized the osteometric technique in radiographs for age estimation using hip axis length, although their method was less precise. The study by Navega *et al.* (2018), investigated the potential of utilizing artificial neural networks for age estimation in proximal femoral bone mineral density (BMD), resulting in promising outcomes. These findings formed the basis for the development of a web application (DXAGE) incorporating the proposed model. However, the study was limited by a sample size that was confined to a specific demographic (females of European descent), thus highlighting the need for expanding the sample to include a more diverse population that includes a greater representation of males and individuals from various ethnic backgrounds (Navega *et al.*, 2018).

Bethard *et al.* (2019), validated Navega's DXAGE application (Navega *et al.*, 2018) by testing it against BMD data from females in the National Health and Nutrition Examination Survey, and compared its performance to linear regression equations formulated by Paschall & Ross (2018)

for age estimation using femoral neck BMD. Both DXAGE and LR models tended to overestimate the age of younger samples and underestimate the age of older samples (Bethard *et al.*, 2019). Although Bethard *et al.* (2019) appreciated the quantitative approach used in Navega's DXAGE, they were skeptical about using the application for individuals in the age range of 20-40, as peak BMD would not have been attained yet until the fourth decade of life (Henry *et al.*, 2004). The subsequent investigation by Curate *et al.* (2022), sought to enhance Navega's DXAGE by incorporating male samples in the training process. However, the revised version of DXAGE, DXAGE 2.0, is still encumbered by certain limitations. For instance, the study's scope is restricted to individuals of South European descent, and there is a dearth of samples from older males, which may hinder its applicability to other populations.

Our study aimed to improve upon previous research by including both male and female samples with a similar age range to the study conducted by Navega *et al.* (2018), (19-94 in our study compared to 21-95 in their study). However, our results showed that age estimates for females are more reliable than those for males due to a stronger correlation to age. As a result, males exhibited larger RMSEs than females. Table V provides a comparison of error estimations for our study and previous research that utilized proximal femur for age prediction. RMSE was chosen as a suitable metric for regression analyses, as it indicates the applicability of the models to samples that were not part of the studied group. Though, other metrics, such as mean absolute error (MAE) and standard error of estimation (SEE), were also used in previous research to assess the accuracy of age estimation models. Regardless of the chosen metric, researchers should strive to minimize these values as much as possible. Our results were comparable to previous studies that used bone mineral density (BMD) of proximal femur for age estimation, with the exception of studies by Chansingthong *et al.* (2022) and Ford *et al.* (2020), that used Hounsfield units of the proximal femur to predict age. Table V suggests that females are more suitable for providing reliable age estimates due to the strong correlation of bone density in this region with age. Artificial neural networks (ANNs) have shown effectiveness in predicting age using proximal femur density, as demonstrated by the studies conducted by Curate *et al.* (2022) and Navega *et al.* (2018). Nonetheless, the lack of interpretability associated with ANNs presents a significant concern, impeding the identification of relevant predictors and their relationships to the modeled property (Zhang *et al.*, 2018). This limitation is commonly known as the "black box" issue, as ANNs only provide approximations without generating an identifiable model due to unclear relationship between network weights and the property being modeled. In contrast, "non-black box"

Table V. Meta-analysis of the error estimations in previous research using proximal femur for age estimation.

| First author | Population (Age range) | Model | All | | | Female | | | Male | | | | | |
|------------------------------------|------------------------------|-------|-------|-------|-------|--------|-------|---|------|-----|------|-------|-------|------|
| | | | RMSE | MAE | R | RMSE | MAE | R | RMSE | MAE | R | | | |
| Abdulai <i>et al.</i> (2022) | Ghanaian (31-82) | LR | | | | | | | | | | | | |
| Botha <i>et al.</i> (2019) | White South Africans (21-91) | LR | 14 | | 0.387 | | 10.23 | | | | 0.24 | | 10.71 | 0.43 |
| Chansingthong <i>et al.</i> (2022) | Central Thai (18-86) | LR | 20.63 | | 0.409 | | 20.28 | | | | 0.45 | | 13 | 0.44 |
| Curate <i>et al.</i> (2013) | Portugese (20-95) | LR | | 11.1 | | | | | | | 0.73 | 12.9 | 21.15 | 0.37 |
| Curate <i>et al.</i> (2022) | Portugese (20-96) | ANN | | 8.44 | | | | | | | 0.77 | 10.78 | 13.24 | 0.57 |
| Ford <i>et al.</i> (2020) | USA 29-72.6 | LR | 12 | | 0.56 | | 12.66 | | | | 0.72 | | 12 | 0.72 |
| Navega <i>et al.</i> 10 | Portugese (21-95) | ANN | | 10.12 | | | 12 | | | | 0.76 | | 12 | 0.64 |
| Paschall & Ross (2018) | USA 23-73 | LR | 13 | | | | 12.54 | | | | | | | |
| Present study | Northern Thai (19-96) | LR | 12.67 | | 0.52 | | 11.85 | | | | 0.65 | | 12.52 | 0.46 |

Abbreviations: LR: linear regression; ANN: artificial neural network; MAE: mean absolute error; RMSE: root mean square error; SEE: standard error of estimation; R: correlation coefficient

models, such as LR models, yield interpretable models with reproducible regression coefficients that elucidate the relationship between predictors and outcomes (Zhang *et al.*, 2018). Therefore, LR models are more preferable over ANNs due to this issue. Furthermore, the utilization of ANNs for age prediction in legal contexts cannot be recommended (Rattanachet *et al.*, 2023).

This study offers an innovative method for assessing bone density in radiographs for age estimation, which is an alternative to the traditional approach of obtaining BMD from dual-energy X-ray absorptiometry equipment (DEXA), or by using computed tomography (CT) scanner to measure bone densities in Hounsfield units. Ford *et al.* (2020), investigated the association between Hounsfield units of the proximal femur and age-at-death, and developed linear regression models for age estimation with a standard error of estimation of 12 years and accuracy rates ranging from 86-92 %. A validation study was conducted by Chansingthong *et al.* (2022), in a Thai population, wherein the authors reported that the age estimation models developed by Ford *et al.* (2020), were not applicable for estimating the age of Thai individuals. As a result, the authors formulated Thai-population-specific linear regression models for age estimation (Chansingthong *et al.*, 2022). The radiographs used in our study were obtained using a mobile X-ray scanner, which offers the advantage of rapid on-site analysis by forensic anthropologists. This approach can be particularly useful when remains are partially decomposed or burned, where the maceration of soft tissues can be invasive, and routine postmortem imaging can be used for biological profiling estimation. Additionally, the use of a mobile X-ray scanner is more cost-effective than a mobile CT scanner, a factor that is particularly important in forensic settings in developing countries where access to high-quality equipment may be limited. Finally, the implementation of a statistical model for biological profile estimation in this study has the potential to improve the objectivity of the analysis based on postmortem radiographs.

In summary, the quantification of white pixels in proximal femoral radiographs appears to be a promising approach for age estimation in elderly individuals. This method offers greater objectivity than visual assessments of trabecular loss, which are susceptible to inter-observer errors. Moreover, the method developed by Walker & Lovejoy (1985) was originally designed for individuals under 50 years old, whereas our approach is tailored to older samples, with the aid of statistical models. Our study supports the use of a LR model for age prediction based on pixel intensity, although the addition of highly age-correlated attributes slightly decreases the RMSE of age prediction. It is important to note that the age prediction models in this study are specific to forensic cases in Thailand. Limitations of the study include the relatively small number of samples to formulate LR equations and limited representation of samples from the 80s and 90s age range. Further research should involve more diverse samples to minimize RMSE in aging older adults.

CONCLUSION

The current study employs Otsu's threshold to quantify bone density by counting white pixels within a selected region of interest in proximal femoral radiographs. In addition to examining the differences in bone densities and medullary cavity widths between male and female proximal femora, the study explores the relationship between pixel sum values and age at death. Our findings demonstrate that significant differences exist in bone densities between males and females, with females exhibiting stronger correlations between FH, FN, WT, and GT and the age of the individuals, thereby resulting in more reliable age estimates for females. Evaluation of LR model performance for age prediction reveals that the LR model performs the best when the

most age-related features are selected for regression analyses, with female age estimation models achieving an RMSE of 11.85 years. Nonetheless, further research is warranted to minimize the RMSE in age prediction.

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RATTANACHET, P.; WANTANAJITTIKUL, K.; PANYARAK, W.; PALEE, P. & MAHAKKANUKRAUH, P. Predicción de la edad utilizando la suma de valores de píxeles del fémur proximal radiográfico en una población tailandesa. *Int. J. Morphol.*, 42(4):1011-1019,2024.

RESUMEN: El presente estudio tuvo como objetivo investigar la utilidad del fémur proximal en la estimación forense de la edad mediante la evaluación de cambios en las densidades óseas a través de radiografías. Utilizando el umbral de Otsu, la densidad ósea se cuantificó contando todos los valores de píxeles blancos dentro de regiones de interés seleccionadas, que incluyen la cabeza femoral (CF), el cuello femoral (CF), el triángulo de Ward (WT) y el trocánter mayor (TM) de 354 fémures izquierdos de ascendencia del norte de Tailandia. También se estimó el ancho de píxeles de la cavidad medular (CM). Además, el estudio evaluó el rendimiento de modelos de regresión lineal (RL) para la estimación de la edad a partir de imágenes radiográficas de fémur proximal. Se observaron correlaciones negativas entre la intensidad de los píxeles CF, CF, WT y TM con la edad de muerte, y las mujeres exhibieron correlaciones más fuertes que los hombres. Además, se encontró una correlación positiva entre la edad y el ancho del CM en muestras de mujeres, mientras que el ancho del CM del hombre no mostró ninguna relación con el aumento de la edad. Los resultados mostraron una ligera diferencia entre el modelo RL aplicado a ambos sexos, que integraba todas las variables, y la configuración alternativa que sólo utilizaba atributos relevantes. Ambos modelos mostraron un rendimiento similar, con un rango estrecho de valores del error cuadrático medio (RMSE), que oscilaba entre 12,67 y 12,71 años, y un rango de coeficiente de correlación de 0,51 a 0,52. Para las mujeres, el modelo RL con CF y WT como atributos seleccionados (RMSE = 11,85 años, coeficiente de correlación = 0,65) tuvo un desempeño satisfactorio, mientras que para los hombres, el modelo RL con todas las variables mostró un RMSE de 12,52 años y un coeficiente de correlación de 0,46. Este estudio mostró la posible aplicación de la intensidad de los píxeles en la predicción de la edad.

PALABRAS CLAVE: Antropología forense; Fémur proximal; Estimación de edad; Regresión lineal; Radiografía.

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