RESEARCH REPORT

SEX ESTIMATION USING THE HUMAN VERTEBRA

: A SYSTEMATIC REVIEW

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LIST OF ABBREVIATIONS

BHa/BHp	: the ratio of anterior to posterior height of vertebral body
BHm⁄ BHp	: the ratio of the anterior to middle height of the vertebral body
C1	: first cervical vertebra
C2	: second cervical vertebra
C4	: 4^{th} cervical vertebra
C7	: 7^{th} cervical vertebra
CMA	: maximum length of the axis
CT	: computed tomography
DFA	: physical osteological examination discriminant function analysis
DMFS	: maximum distance between the superior articular facets
EPDl	: lower end plate depth
EPWu	: upper end plate width
iBDc	: coronal diameter of endplate on inferior plane
iBDcm	: maximum coronal diameter of endplate on inferior plane
iVL	: inferior length of the whole vertebrae
L1	: first lumbar vertebra
LMA	: maximum width of the axis
sAD	: distance between superior articular processes
sBDc	: superior coronal diameter of endplate of the vertebral body
sBDcm	: superior maximum coronal diameter of endplate of the vertebral body
sBDs	: sagittal diameter of endplate on superior plane
sBDsm	: superior maximum sagittal diameter of vertebral body endplate
sVL	: superior vertebral length
T12	: 12^{th} thoracic vertebra
WVF	: width of vertebral foramen

SEX ESTIMATION USING THE HUMAN VERTEBRA: A SYSTEMATIC REVIEW

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ABSTRACT

Background*: Sex estimation is one of the essential elements in an anthropological examination, as it may narrow down the possible match by half. The vertebral column is often used in archaeological skeletal and forensic studies due to its weight-bearing function and relative density. This systematic review explored the most sexually dimorphic vertebrae by using traditional morphometry*.

Main body*: An electronic comprehensive search was conducted using a database of Scopus and Ovid Medline for suitable studies between 2008 and 2020. The main inclusion criteria were studies in English, and studies on sex estimation by morphometric analysis in vertebrae by CT scan or dry bone specimen. Only studies related to human adult age and vertebrae were analysed. The literature search resulted in 57 potentially relevant articles whereby, 15 articles had fulfilled the inclusion criteria. These studies included cervical vertebra, thoracic, and lumbar in different populations.*

Conclusions*: All studies reported sexual dimorphism in the vertebrae with variable accuracies, hence they were useful for estimation of sex. Of all vertebrae, the second cervical (C2), the 12th thoracic (T12) and the first lumbar (L1) gave the highest accuracy in sex estimation. The vertebrae frequently used for sexual dimorphism were the second cervical (C2), the 12th thoracic (T12) and the first lumbar (L1), and the accuracy seems to increase when they were used in combination. The most sexually dimorphic part of the vertebra is the vertebral body, hence it is vital to be used for sex estimation.*

Keywords: Systematic review, sexual dimorphism, vertebrae, morphometric, forensic.

1. INTRODUCTION

Identification of unknown skeletal remains has been a challenge for forensic anthropologists, especially in cases of mass disaster, severe fragmentation, and advanced decomposition in human remains. Sex estimation is one of the most important elements in the anthropological examination, and its impact on facilitating the identification of other skeletal parameters such as estimation of age at death, race and stature, and its relevance to studies of compounding biological factors such as pathological conditions, environmental and dietary habits (Marlow 2016). Besides, by using sex estimation, a forensic scientist may be able to narrow down the possible match by half (Badr El Dine and El Shafei 2015; Ramadan et al. 2017a).

It has been reported that pelvic bone is the most accurate bone for estimation of sex (Marlow 2016; Hora and Sladek 2018). In the presence of a complete skeleton, sex could achieve nearly 100% accuracy in pelvis along with the skull (Badr El Dine and El Shafei 2015). However, both pelvis and skull are not always preserved due to fragmentation and decomposition. Hence, exploration of sex differences in non-pelvic bone is important to be explored and developed in future studies (Badr El Dine and El Shafei 2015, Ramadan et al. 2017a, Ramadan et al. 2017b).

In several cases, the vertebral column was considered to be the best-preserved bone among all skeletal elements (Padovan et al. 2019). Besides, the vertebral column is often used in archaeological and forensic investigations due to its comparative thickness and its capacity in resisting weight. It comprises a combination of outer dense cortical bone and an inner cancellous bone, that contribute to the weight-bearing function of the vertebrae (Tan et al. 2004; Gülek et al. 2007; Yu et al. 2008; Badr El Dine and El Shafei 2015). The vertebral bone investigation produces variable results for sex prediction, although most are significantly different between sexes. Morphometric studies on the vertebrae have demonstrated sexual dimorphism in the vertebral size and shape due to their later development in the vertebral stature and expanded development in the vertebral transverse diameter in males (Taylor and Twomey 1984; Marlow 2016; Torimitsu et al. 2016).

Several researchers have documented sexual dimorphism in variance vertebrae from the cervical (Marlow and Pastor 2011; Hou et al. 2012; Zheng et al. 2012; Amores et al. 2014; Torimitsu et al. 2016; Kaeswaren and Hackman 2019; Padovan et al. 2019), thoracic (Yu et al. 2008; Hou et al. 2012; Badr El Dine and El Shafei 2015) and lumbar regions of the spinal column (Bethard and Seet 2013; Badr El Dine and El Shafei 2015; Gama et al. 2015; Ostrofsky and Churchill 2015; Ramadan et al. 2017b; Azofra-Monge and Aguilera 2020).

2. OBJECTIVES

2.1. To determine the most sexually dimorphic bone among the vertebrae by morphometric method.

2.2. To summarize the morphometric parameters in different types of vertebrae by both radiography and dry bone specimen, which can be used for sex estimation.

3. METHODS

The systematic review search protocol PRISMA was adopted in this research (Fig. 1). The focus of this review was to determine relevant researches on sexual dimorphism in vertebrae by morphometric analysis. The Ovid Medline and Scopus were broadly used to search for articles from health science journals published between 2008 and 2020. The search strategy included a combination of three sets of words with truncation of an advanced search engine: (1) gender OR sex* (2) dimorphism OR estimation OR determination OR assessment OR identification OR characteristic (3) vertebra* OR spin* OR cervi* OR thora* OR lumba*.

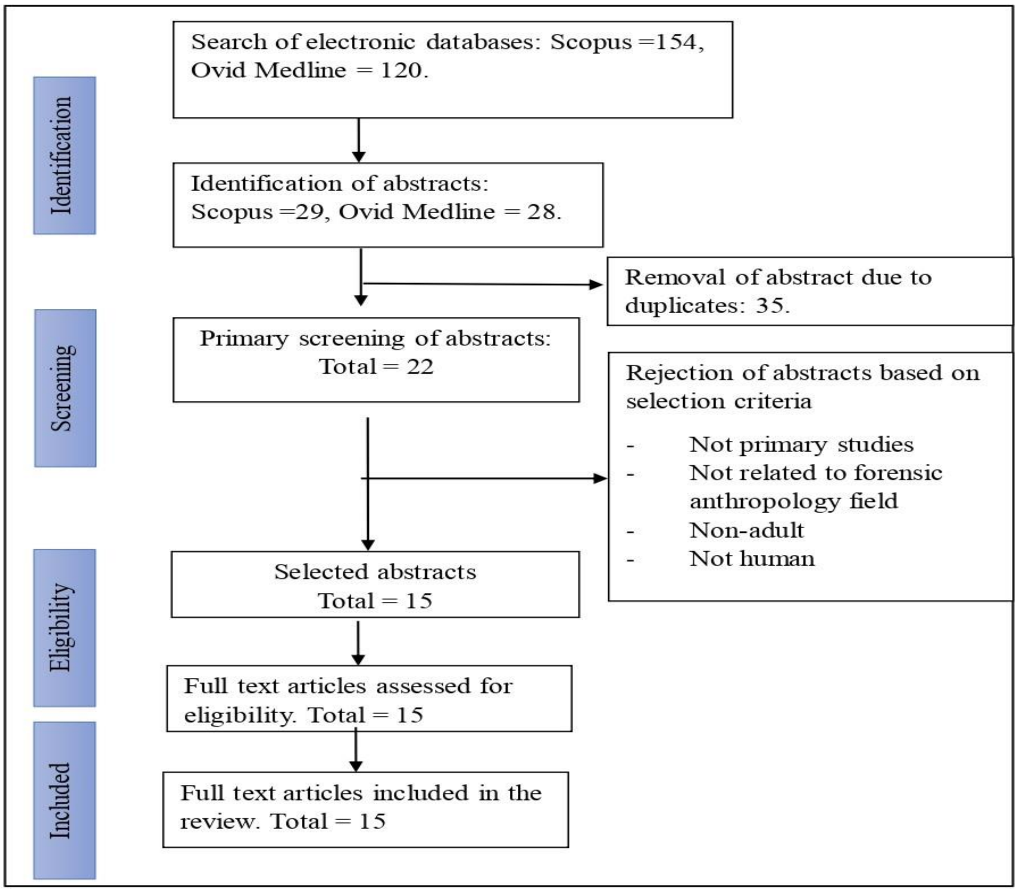

Fig 1. The flow chat of selection and processing systematic review

Selection Criteria

The results were selected from articles, that were published in English including the abstracts. The studies selected for review were studies that performed morphometric analysis of human vertebrae for sexual estimation by using CT scan or dried bones. Articles such as reviews, news, editorials, letters or case report were excluded from the review.

Data Extraction and Managing References

Prior to being included in the review, articles were screened in three stages. Firstly, title screening was done, and articles that were not suitable by the inclusion criteria were excluded. Secondly, abstracts of the remaining articles were screened, and those which did not meet the inclusion criteria were excluded. Finally, the remaining articles were screened to exclude papers which were not within the scope of the literature. Duplicates were taken out, and remaining papers were selected again by at least two reviewers.

Before the data extraction phase, the reviewers will approve the full papers, that matched the inclusion criteria, and any discrepancies in opinions were discussed by the reviewers. Data extraction was performed independently for data validation by using a data collection form. The following data were recorded from the studies i.e. title of the study along with the authors and year, type of vertebrae bone performed in the study, subject or sample, study methods, results, and remarks or conclusion of the study.

Inclusion and Exclusion Criteria

The inclusion criteria were primary studies, which included studies related to human adult age, studies related to vertebrae, studies by morphometric analysis, and studies using discriminant function analysis or regression analysis.

4. RESULTS

The literature search discovered 57 related articles. The reviewers had evaluated the inclusion and exclusion criteria of all articles based on the titles and abstracts. There were 22 papers chosen for evaluation and data extraction. Seven papers were removed due to nonrelational types neither to single bone vertebrae analysis, sexual dimorphism nor morphometric measurements, and the studies were not conducted within the field of forensic anthropology. The remaining 15 articles had matched with the inclusion and exclusion criteria, and hence were included in the review. The selection process of the systematic review was presented in a flow chart (Figure 1).

Study characteristics

The descriptions of the articles were presented (Table 1). Briefly, all of the articles were published from the year 2008 until 2020, which comprised studies of vertebrae by 3D radiography or dry bone specimens for estimation of sex. Based on the types of bone, six studies had focused on cervical vertebrae (Marlow and Pastor 2011; Hou et al. 2012; Zheng et al. 2012; Torimitsu et al. 2016; Kaeswaren and Hackman 2019; Padovan et al. 2019), two studies on thoracic vertebrae (Yu et al. 2008; Hou et al. 2012) and five studies on lumbar vertebrae (Zheng et al. 2012; Ostrofsky and Churchill 2015; Ramadan et al. 2017b; Decker et al. 2019; Azofra-Monge and Aguilera 2020). One study was done on both thoracic and lumbar (Badr El Dine and

El Shafei 2015), and another study was done on both cervical and thoracic vertebrae (Amores et al. 2014). According to review, seven studies used CT scan to assess the bone (Yu et al. 2008; Hou et al. 2012; Zheng et al. 2012; Badr El Dine and El Shafei 2015; Torimitsu et al. 2016; Ramadan et al. 2017b; Decker et al. 2019), while eight studies investigate on dry bones (Marlow and Pastor 2011; Bethard and Seet 2013; Amores et al. 2014; Gama et al. 2015; Ostrofsky and Churchill 2015; Kaeswaren and Hackman 2019; Padovan et al. 2019; Azofra-Monge and Aguilera 2020), and all of the studies had undergone conventional morphometric to analyse the data. All of the studies were conducted in various populations using different experimental designs and analysed by discriminant function analysis or regression analysis.

Table 1. The description studies on sexual dimorphism in vertebral bone.

No.	Study	Types of vertebrae	Sample population, Number of subjects [male/female]	Methods	Results	Outcomes
1	Padovan L et al. (2019) [6]	C1 (atlas)	Brazil, 110/81	• Four measurements were made in each atlas from samples of skeletal remains in museum. • The unpaired t-test was used to test sexual dimorphism between the measurements. • The stepwise-forward Wald method was used to obtain a logistic regression.	• The unpaired t-test applied significant sex differences for all measurements (p<0.05) • Of four variables, only two were selected by establishing the best model equation i.e. anterior diameter of the atlas and maximum transverse diameter of the atlas.	The atlas showed 81.2% accuracy in sex estimation.
2	Marlow & Pastor (2011)[12]	C2	UK 153 known-sex individuals	• Using Wescott's eight proposed sexually dimorphic dimensions plus an additional dimension width of vertebral foramen (WVF), from samples of museum collection of skeletal remains. • The paired sample t-test was used to determine significant difference	• The discriminant function generated from C2 vertebra by stepwise procedure had the option to appraise sex with an accuracy of 83.3%, and had the option to arrange males and females with equivalent precision. • By stepwise selection, maximum breadth across superior facets (SFB), maximum sagittal	Sex determination method from the Wescott's measurements of the C2 vertebra displayed a significant discrimination between males and females with 83.3% accuracy.

				between sexes. • Utilize discriminant function analysis (DFA) to evaluate its efficacy in estimating sex. • The stepwise technique chooses a subset of factors based on squared partial correlation and the significance level from an analysis of covariance with the greatest number of separating capacity.	length (XSL), width of vertebral foramen (WVF), and dens sagittal diameter (DSD) form the discriminant functions. • The dimension SFB is the absolute best discriminator of sex.	
3	Bethard & Seet (2013) [20]	C2	American	• Following Wescott's measurements, five dimensions were measured from skeletal remains collection. • Wescott's five discriminant functions were utilized to estimate sex for each case.	• The five discriminant functions presented in Wescott utilized highly replicable measurements, that yielded an overall accuracy of 80% or greater.	The second cervical vertebra can provide a wealth of information in estimating sex.
4	Gama et al. (2015)[14]	C2	Portuguese First sample = 99/91 (as a training set	• Acquired 13 measurements from the skeletal collections by sliding calipers and registered with an approximation of 0.5 mm. • Performed a t-test (two tailed) to analyse	• The most dimorphic dimensions are the LMA (11.18%) and DSMC (10.6%). • The most predictive variables were LMA, DSMC, CMA and LMFS (right side).	• The second cervical vertebra was useful for sex estimation with accuracies, that ranged between 86.7-89.7%.

			sample). Second sample = 24/23 (testing the model constructed)	the differences in measurements between males and females. • Using logistic regression modelling to construct estimation models.	• The resulting model identified sex of individuals in the training set in 89.7% of cases. In the test sample, sex was accurately assessed in 86.7% of cases.	
5	Torimitsu et al. (2016) [10]	C2	Japanese 112/112	• Nine measurements were collected from cadavers by PMCT scanning and subsequent forensic autopsy was done. • Analysis ANOVA was utilized to look at mean differences between sex group. • Univariate discriminant function analysis (DFA) was performed for every variables to prescribe a formula for sex classification.	• Measurement of DMFS and LMA reached the most discriminant variable for the C2 vertebra, with expected cross-validated accuracies of 83.5% and 83.1%, respectively. • There were four variables (AS, DSD, DSMC, and DTMC), which revealed correct prediction rates by approximately 80%. • A five-variable model with an accuracy rate of 92.9%.	• The CT imaging of the C2 vertebra are able to estimate sex accurately on human skeletal remains.
6	Kaeswaren & Hackman (2019) [15]	C2-C7	White Scottish 13/12	• Using 25 human cadavers, three morphometric traits were measured for each cervical vertebra.	• Discriminant functions utilized all three vertebral measurements (CHT, CAP and CTR) with 77.3% - 100% accuracy for sex estimation	This study developed 25 multivariate discriminant functions, that successfully classified

				• A total of 150 individual cervical vertebrae were included in the analysis. • Performing an independent (two-sampled) t-test on the data to establish sexual differences for all three measurements. • Performing stepwise discriminant analysis to select the most dimorphic variables for sex determination.	in each vertebral level. • Sex was estimated with an accuracy of above 80% at every vertebral level with C2 giving the most accurate sex estimation of 100% in all four combinations of vertebral measurements. • Twenty-five functions from a total of 37 discriminant functions were significantly discriminating, which achieved sex predicting accuracy of more than 80%. • The CTR and CHT were found to contribute greatly towards biological sex variation.	individuals as male or female with an accuracy greater than 80%.
7	Yu et al. (2008)[7]	T12	Korean 52/50	• Using 33 linear measurements and two ratios by CT images. • Independent sample t-test was performed to evaluate differences between the means of the parameters. • For each measurement with significant sexual dimorphism, the respective discriminant equations were calculated for	• Twenty-three single variables with significant sex differences among 35 traits contributed to a correct classification of sex ranging from 62.7% till 85.3% accuracies. • Three measurements of vertebral body (sBDc, sBDcm, iBDcm, and iBDc) exhibited accuracies higher than 80%. • The coronal diameter of the superior endplate	The 12th thoracic vertebra was sexually dimorphic, with 90% accuracy in Korean individuals.

				sex determination using univariate, bivariate, and stepwise methods of discriminant function analysis.	of the vertebral body (sBDc), the ratio of the anterior to middle height of the body (Hm/Hp), and the length of left mammillary process and pedicle (lM&PL) predicted sex with 90% accuracy by DFA.	
8	Hou et al. (2012) [13]	T12	Chinese 78/63	• Using 30 measurements from CT scan samples. • The data were analysed by one-way analysis of variance (ANOVA). • Univariate discriminant function analysis and stepwise discriminant function analysis were performed, respectively. • A leave-one-out classification procedure was used to assess the validity of these functions.	• The accuracy of sex classification was between 56.4% and 90.1%. • Variables such as sBDs, sBDsm, sVL, sBDc, iBDs,iBDsm, iVL, iBDc, mBDs, mBDc and BHp displayed 80% accuracy. • The iVL had the highest accuracy of 90.1%. • By stepwise discriminant function analysis, an equation with four variables i.e. three linear measurements (superior maximum sagittal diameter of vertebral body endplate (sBDsm), inferior length of the whole vertebra (iVL), distance between superior articular processes (sAD) and one ratio (the ratio of anterior to	The 12^{th} thoracic vertebra in Northeasterners in China was sexually dimorphic with 94.2% accuracy.

					posterior height of vertebral body (BHa/BHp) were obtained with 94.2% accuracy.	
9	Amores et al. 2014 [16]	C7 and T12	Mediterranean 61/60	• Using eight measurements from samples of skeletal collections. • T-test analysis was used to compare the data between sexes, and evaluate the homogeneity of variance (F-test). • The effectiveness of the measurements was analysed by discriminant function analysis. • The discriminant capacity of the selected variables was then evaluated using a cross-validation procedure.	• By the t-test, the mean values of all variables were significantly higher in males than in females ($p<0.05$) with the exception of LSFc7, LIFc7, LVFt12, and WVFt12. • The discriminant analysis yielded five functions i.e. four for the 7th cervical and one for the 12th thoracic with 80% accuracy.	• The length of vertebral bodies of the 7th cervical and 12th thoracic vertebrae offered the highest discriminant power for sex estimation. • The percentage accuracy for sex estimation using C7 and T12 vertebrae was approximately 80%.
10	Badr el Dine & El Shafei 2015[2]	T12 and L1	Egyptian 54/66	• Using 24 linear measurements and four ratios from the images of multi-slice computed tomography (MSCT) • T-test analysis was used to establish differences between sexes. • Unstandardized coefficient. • Linear regression analysis was performed,	• About 14 of 24 linear measurements showed significant sex differences using T12 vertebrae (predictive accuracy ranged from 49% till 85.5%), with three variables i.e. lower end plate depth (EPDH), upper end plate width (EPWu) and superior vertebral length (VLs having more than 80% predictive accuracy).	The T12 vertebrae demonstrated a better sex estimation than L1 in the Egyptians. The accuracy increased when two vertebrae (T12 and L1) were used incombination as predictors for sex determination.

				in which individual variables of vertebral measurements were analysed for sex determination.	• By using L1 vertebra, only seven linear measurements and one ratio were sexually dimorphic (predictive accuracy ranged from 47% till 79%). Only the upper end plate depth (EPDu) showed an accuracy above 75% (79%). • The accuracy derived from T12 vertebra was 93.1%. • The accuracy derived from L1 was 68%. • With a combination of T12 and L1 vertebrae, only five variables were used in the equation, that predicted sex with a high level of accuracy (96.3%).	
11	Zheng et al. (2012) [14]	L1	China 113/97	• About 29 linear measurements were taken from 3D models of the CT, and five aspect ratios were calculated from the linear measurements. • All measurements were considered	• About 25 traits demonstrated significant sexual dimorphism ($p<0.01$), ranged from 57.1% till 86.6 %. • EPWu showed the highest predictive accuracy.	The L1 vertebra may be used for sex assessment by discriminant analysis with 88.6% accuracy.

				significant (p< 0.01), and stepwise discriminant analysis was applied. • The accuracy for the discriminant equations were obtained by cross-validated procedure.	• Discriminant functions were the upper end-plate width (EPWu), left pedicle height (PHI) and middle end-plate depth (EPDm) with predicted sex accuracy of 88.6%.	
12	Ostrofsky & Churchill (2015) [18]	L1-L5	47/51 South Africa	• Samples comprised skeletal collection, and 11 measurements were taken to the nearest 0.1 mm with digital calipers. • To compare male and female sample means, t-test was applied (for variables normally distributed) and nonparametric Wilcoxon ranks sum test was used (for data not normally distributed) • Each variable that showed significant sex differences (p<0.01) was subjected to univariate discriminant function analysis (DFA) to test its effectiveness for sex estimation.	• The highest accuracy was obtained predominantly by the measurements of vertebral body. • Four variables produced accuracies over 80% i.e. vertebral body superior and dorsoventral and transverse diameters of L1 and L2. • The discriminant functions predicted sex with accuracies over 80% for L1-L4, with the highest accuracy produced for L1 at 87.1%.	The lumbar vertebrae exhibited a large degree of sexual dimorphism, which may be used for sex estimation.

13	Ramadan et al. (2017) [5]	L1	Egyptian 61/62	• About 15 linear measurements of L1 were taken by MSCT. • Independent T-test was applied to compare between different sexes. • Correlation analysis was done followed by discriminant function analysis.	• Descriptive statistics showed significant difference between sex for all measurements except for length of the vertebral foramen (LVF). The upper-end plate width (EPWu) showed the highest accuracy. • Additionally, sex could be predicted from L1 by discriminant analysis at an accuracy of 84.6%.	Sex could be estimated from L1 at 84.6% accuracy.
14	Decker et al. (2019)[17]	L1-L5	US North American 76/78	• There were 36 measurements taken from the abdominal CT scans image data, comprising 30 linear measurements on the vertebral body wedging angle, and five aspect ratios for each vertebra. • A stepwise analysis method used the measurements to create discriminant function equations for L1 through to L5 individually as well as collectively, all accuracies were obtained from cross-validation.	• The L1 had 21 out of 29 measurements that were statistically significant, with a predictive accuracy ranged between 57.1–81.2%. • The L2 had 23 out of 29 measurements that were statistically significant, with a predictive accuracy ranged between 57.1–79.9%. • The L3 had 25 out of 29 measurements that were statistically significant, with a	• The study presented that the L1-L5 vertebrae can be used for sex estimation with an accuracy range of 81.2% - 85.1% • When all five vertebrae were used in combination, the accuracy was 92.2%.

					predictive accuracy range of 56.5–77.3%. • L4 had 24 out of 29 measurements that were statistically significant with a predictive accuracy range of 54.5–74.7%. • The L5 vertebra had 23 out of 29 measurements that were statistically significant, with a predictive accuracy range of 56.5–77.9%. • The discriminant function for the five lumbar vertebrae had an overall 81.2- 85.1% accuracy rate for sex estimation.	
15	Monge & Aguilera (2020) [19]	L1-L5	Spain 46/48	• Samples comprised identified adult individuals from the skeletal collections. • Thirty-three linear measurements were taken with digital calipers in millimeters. • Differences between means comparing sex were analysed by student's T-test and non-parametric Mann-Whitney U-test, and the	• All variables from L1 and L2 were higher in males than females, and were statistically significant ($p<0.05$) in 11 variables. • L1 and L2 were the most sexually dimorphic vertebrae among the lumbar vertebrae. • The total width (TW) which is the measurement of the maximum distance	• The discriminant equations for sex displayed accuracy of 90.1% till 94.5% for L1, 85.4% till 89.4% for L2, from 85.3% till 88.3% for L3, from 85.3% till 88.2% for L4, and from 80% till 85.3% for L5.

	equations were obtained by a binary logistic regression.	between the ends of the transverse processes, was the variable with higher deviation and variability among the sample population, and was mostly included in the equations for L1, L3 and L4.

Cervical vertebrae for sex determination

There were seven articles, that utilised cervical vertebrae (Marlow and Pastor 2011; Bethard and Seet 2013; Amores et al. 2014; Gama et al. 2015; Torimitsu et al. 2016; Kaeswaren and Hackman 2019; Padovan et al. 2019). Most studies utilized only one vertebra i.e. the second cervical vertebra (C2), except for Padovan et al. (2019), who used the atlas first cervical (C1) (Padovan et al. 2019), while Amores et al. (2015) had used C7 and T12 (Amores et al. 2014), and Kaeswaren & Hackman (2019) used the cervical vertebrae from C2 till C7 (Kaeswaren and Hackman 2019). The C2, known as the axis, was commonly employed for sexing, and it has few morphological characteristics, that were easily recognizable. Besides, the literature demonstrated that cervical vertebrae were well-preserved bones (Gama et al. 2015). Studies have reported that C2 was significant for sex estimation, as was documented by Kaeswaren & Hackman (2019), who performed analysis on all of the cervical vertebrae (except the C1) (Kaeswaren and Hackman 2019). It was exhibited that C7 was sexually dimorphic in a study on C7 and T12 in combination (Amores et al. 2014). In another study, it was displayed that the first cervical was also sexually dimorphic (Padovan et al. 2019).

The Wescott's method was re-evaluated on the second cervical vertebra for sex estimation (Wescott 2000; Marlow and Pastor 2011; Bethard and Seet 2013). However, Wescott’s study (2000) was excluded from the review as it was an old publication, which has already been re-evaluated by several researchers. Samples from the Spitalfields’ anatomical collection that were held at the Museum in London were used to measure the Wescott's eight projected measurements on the second cervical and adding the width of vertebral foramen (WVF) (Marlow and Pastor 2011). This study had correctly classified male and female samples in 83.3% of cases, which was higher than that by Wescott's (2000). Bethrad & Seet (2013) reiterated the previous conclusions by Wescott (2000) and Marlow & Pastor (2011) by using samples of modern Americans from the skeletal collections in Tennessee, and the second vertebra was found to be good sex predictor, which was 86.7% correctly classified. (Bethard and Seet 2013).

Gama et al. (2015) updated the sexual dimorphism grade of the C2 vertebra in the Portuguese population, and created a logistic regression model, which allowed to estimate sex with an accuracy ranging from 86.7% till 89.7% (Gama et al. 2015). In this study, some measurements were adopted by Wescott (2000) with a total of 13 measurements. This study displayed measurements with the highest discriminant power i.e. sagittal maximum body diameter (DSMC), maximum width of the axis (LMA), maximum width of the right superior facet (LMFSD), and maximum length of the axis (CMA) (Wescott 2000). Torimitsu et al. (2016) was also utilising the C2 with nine parameters, and the most discriminating variable was the maximum distance between the superior articular facets (DMFS), followed by LMA with accuracies of 83.5% and 83.1%, respectively (Torimitsu et al. 2016). While the maximum distance between the superior facets (SFB) was exhibited as the best discriminating indicator, the maximum length of the axis (XSL), the maximum width of the vertebral foramen (WVF) and odontoid process sagittal diameter (DSD) were also found to be significant (Marlow and Pastor 2011; Gama et al. 2015; Torimitsu et al. 2016).

The discriminant functions were highly replicable with an accuracy rate of 80% or greater, a well-known benchmark for determining sex. According to the literature, C2 measurements that had high discriminating power with an accuracy rate of more than 80% comprised the length of C2 (Marlow and Pastor 2011; Bethard and Seet 2013; Gama et al. 2015), the width of C2 (Gama et al. 2015; Torimitsu et al. 2016), the sagittal diameter of the vertebral body (Gama et al. 2015; Torimitsu et al. 2016), and the distance between the superior articular facets (Marlow and Pastor 2011; Torimitsu et al. 2016). The sex estimation methods with correct classification rates of 80% or greater were found to be useful (Torimitsu et al. 2016). However, an important consideration here is that bone fragmentation may occur due to decomposition, where the spinous process and transverse process along with the superior articular facets are prone to damage. The bones or bone parts with the highest discriminant capability, that are exposed to fragmentation may be rendered unmeasurable. Hence, a fragmented specimen may be badly preserved for sex estimation, and limit the effective use of morphometric interventions in both forensic anthropology casework and archaeological studies.

Amores et al. (2014) conducted studies on the 7th cervical (C7) and 12th thoracic vertebrae (T12) in adult skeletal samples of the Southern Spain laboratory collection, and discriminant function analysis showed mean reliability of 80%. The C7 offered the greatest discriminant power based on the length of the vertebral foramen (LVF), the width and the length of the inferior vertebral body (LIVB and WIVB). (Amores et al. 2014).

Kaeswaren & Hackman (2019) used wet disarticulated cervical vertebrae (C2-C7) from the white Scottish human cadavers (Kaeswaren and Hackman 2019), and research on sex estimation using the cervical vertebrae was shown to be accurate. By using three morphometric traits on each cervical vertebra, 25 multivariate discriminant functions had an accuracy of more than 80%. This study presented that the second cervical (C2) displayed the greatest sexual variance among the four sets of vertebral dimensions. By stepwise discriminant analysis, two variables were established to be good sex indicators i.e. vertebral body height (CHT) of the C4 and transverse diameter of the vertebral foramen (CTR) of the C2 (Kaeswaren and Hackman 2019). The measurements of the second cervical vertebra are summarized (Figure 2).

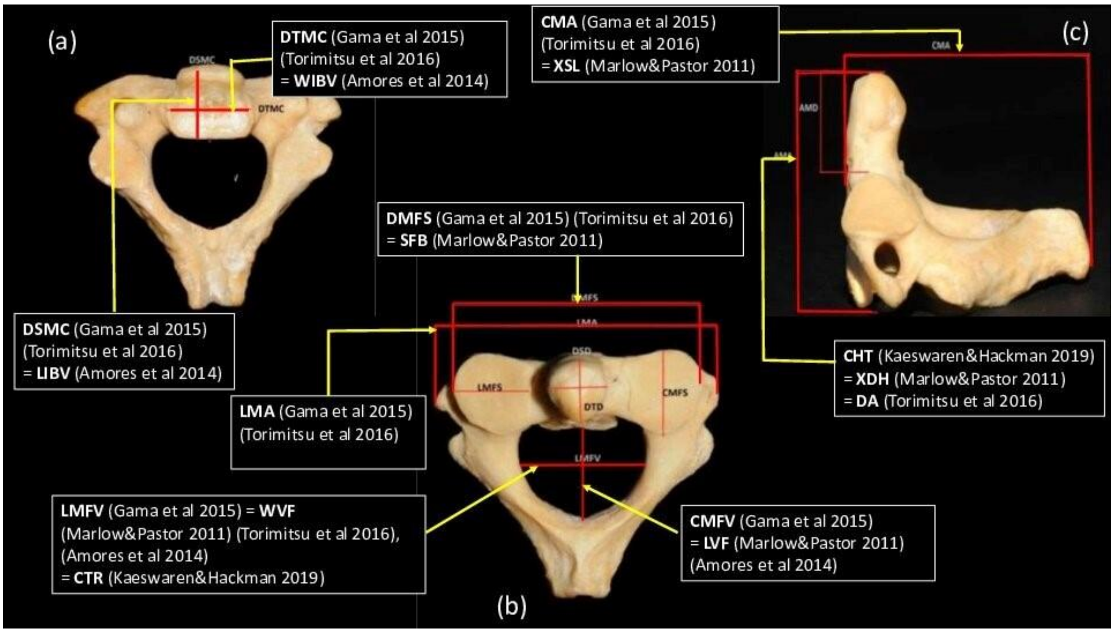

Fig 2. Measurements on the second cervical (C2). (a) Inferior view, (b) Superior view, (c) Lateral view. (Adapted by permissions from the Springer Nature : Springer Nature, International Journal of Legal Medicine, Sex Estimation using the second cervical vertebra a Morphometrics Analysis in a documented Portuguese skeletal sample, Gama et al, copyright, 2014)

Thoracic vertebrae for sex estimation

In this review, four articles were selected under the subject heading of thoracic vertebrae (Yu et al. 2008; Hou et al. 2012; Amores et al. 2014; Badr El Dine and El Shafei 2015). All of the articles used the 12th thoracic vertebrae (T12), as it was easily identified in the disarticulated skeleton, and being a transitional vertebra, it has distinct morphological characteristics (Ramadan et al. 2017a). Two articles were studies on the combination of vertebrae such as T12 with C7 and L1 (Amores et al. 2014; Badr El Dine and El Shafei 2015). All of the articles that used 12th thoracic vertebra were shown to be sexually dimorphic with an accuracy of more than 88% in different populations.

Yu et al. (2008) had performed 33 linear measurements and two ratios by CT scan on T12 in the Korean population (Yu et al. 2008). displayed that coronal diameter of the superior endplate of the vertebral body (sBDc), ratio of the anterior to middle height of the vertebral body (BHm⁄BHp), and length of left mamillary process and pedicle (lM&PL) had formed good predictors of sex with 90% accuracy.

Hou et al. (2012) exhibited that 30 measurements on CT scans in the Chinese population had obtained 94.2% accuracy for sexual dimorphism, which was based on three linear measurements i.e. superior maximum sagittal diameter of vertebral body endplate (sBDsm), inferior length of the whole vertebrae (iVL), distance between superior articular processes (sAD) and one ratio (the ratio of anterior to posterior height of vertebral body (BHa/BHp). (Hou et al. 2012).

Amores et al. (2014) used eight measurements on the 12th thoracic vertebra with the length of inferior surface of the vertebral body (LIVB) exhibiting the highest discriminant capacity with mean reliability of 80% (Amores et al. 2014). Badr El Dine and El Shafei (2015) had performed a study using a method by Yu et al. (2008), in which 24 linear measurements and four ratios of the 12th thoracic were utilized in the Egyptian population by multi-slice computed tomography (MSCT) (Yu et al. 2008; Badr El Dine and El Shafei 2015). From the analysis, 14 measurements had exhibited significant sex differences, with three variables i.e. the lower end plate depth (EPDl), upper end plate width (EPWu) and superior vertebral length (VLs) with more than 80% predictive accuracy. This study had generated 93.1% accuracy by regression analysis, which was comparable with Yu et al. (2008), [7] with predicted sex accuracy of 90% (Yu et al. 2008; Badr El Dine and El Shafei 2015). Measurements of the 12th thoracic are summarized (Figure 3).

Measurements on the 12th thoracic vertebra that were sexually dimorphic were mostly related to the vertebral body endplate and sagittal length of the vertebra. Yu et al. (2008) exhibited that the most reliable measurements were sBDc, sBDcm, iBDc, or iBDcm, which provided strong discriminant ability with accuracies over 80%. This study produced discriminant function for sex based on the vertebral body endplate measurements (sBDc, sBDcm, iBDc) with an accuracy of over 80%. This study produced discriminant functions based on the vertebral body measurements i.e. the coronal diameter of the superior endplate vertebral body (sBDc), and the ratio of the anterior to middle height of the body (BHm/BHp), and the non-vertebral body measurements, which is the length of the mammilary process and pedicle with 90% accuracy (IM&PL) (Yu et al. 2008).

Hou et al. (2012) also displayed measurements on the vertebral body (sBDs, sBDsm, iBDc, etc) by producing accuracies over 80%, with the sagittal length of the vertebra (iVL) displaying the highest accuracy of 90%. By DFA, this study had produced discriminant equation based on the superior sagittal diameter of vertebral body endplate (sBDsm) and the ratio of anterior to posterior height of vertebral body(BHa/BHp), and the non-vertebral body measurements i.e. the distance between superior articular process (sAD) and iVL with 94.2% correct classification for sex estimation. Besides, the vertebra sagittal length (iVL and sVL), which measured the distance from the anterior edge of vertebra body to the posterior edge of vertebral spinous process at the superior and inferior planes were believed to be sufficiently accurate, with the iVL displaying the highest accuracy (90%) among all measurements (Hou et al. 2012).

It concurred with the study by Badr el Dine et al. and El Shafei (2015), which produced discriminant functions for sex estimation in the Egyptians by using both 12th thoracic (T12) vertebra and first lumbar (L1) vertebra in combination (Badr El Dine and El Shafei 2015). In this study, the measurements of vertebra sagittal length of the 12th thoracic vertebra (sVLand iVL) were included in the multiple regression formulae for sex estimation with 93.1% accuracy. In accordance, three variables i.e. the lower endplate depth (EPDl) and upper endplate width (EPWu) and the superior sagittal length vertebral (sVL) were produced with greater than 80% accuracy rate.

Amores et al. (2014) studied sex estimation in the Mediterranean population using both 7th cervical vertebra and 12th thoracic vertebra, and by cross-validation analysis, mean reliability of 80% was shown by the length of the inferior endplate of the vertebral body (abbreviately LiBV). (Amores et al. 2014).

Hence, it can be concluded that the vertebral body and the sagittal length of the 12th thoracic played an important role in estimating sex. The accuracy of estimating sex may be achieved when the bone is complete and intact, including the vertebral body and the spinous process, which form the sagittal length of the vertebra.

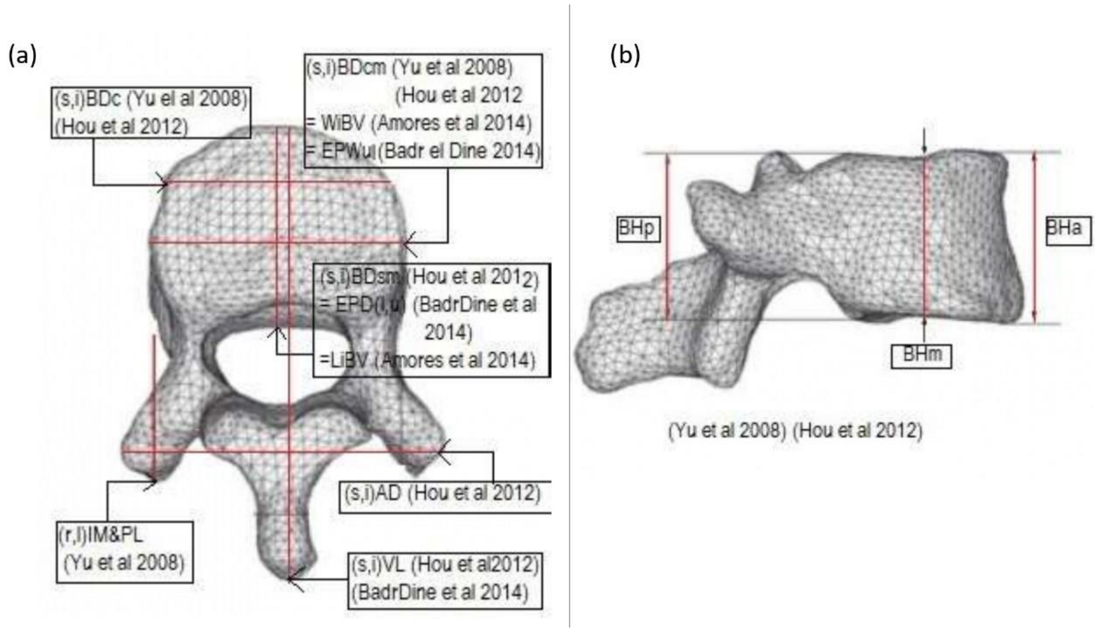

Figure 3**.** Measurements from the 12thhoracic vertebra (a) Superior /inferior view, (b) Lateral view. (Adapted by permissions from the John Wiley and Sons: John Wiley and Sons, Journal of forensic Science, Determination of Sex for the 12th Thoracic Vertebra by Morphometry of Three-dimensional Reconstructed Vertebral Models, Yu et al, copyright, 2008)

Lumbar vertebrae for sex estimation

Three articles had focused on the first lumbar vertebrae (L1), and documented that L1 was sexually dimorphic with varied accuracies by discriminant function analysis (Zheng et al. 2012; Badr El Dine and El Shafei 2015; Ramadan et al. 2017b). Three studies on lumbar vertebrae (L1 till L5) displayed that L1 had the highest accuracy among all of the lumbar vertebrae (Ostrofsky and Churchill 2015; Decker et al. 2019; Azofra-Monge and Aguilera 2020).

In a study by Zheng et al. (2012) in the Chinese population, they utilized 29 linear measurements and 5 aspect ratios from 25 traits, which demonstrated accuracies ranged from 57.1% to 86.6% (Zheng et al. 2012). About five measurements (EPWu 86.6%, EPDm 86.2%, EPWl 85.2%, EPDl 84.3% and EPDu 83.3%) associated to vertebral body, gave accuracies greater than 80%, with EPWu showing the highest predictive accuracy. The discriminant function produced an accuracy of 88.6% based on the upper end-plate width (EPWu), the middle end-plate depth (EPDm), and the left pedicle height (PHl) (Zheng et al. 2012).

Badr El Dine and Shafei (2015) discovered that the first lumbar vertebrae and the 12^{th} thoracic in the Egyptians, using the method by Zheng et al. (2012) i.e. 24 linear measurements and four ratios of the first lumbar, only seven measurements and one ratio were found to be sexually dimorphic with predictive accuracy ranging from 47% to 79% (Badr El Dine and El Shafei 2015), in which the upper endplate depth (EPDu) had 79% accuracy, lower than that by Zheng et al. (2012) (57.1–86.6%). Also, the level of accuracy obtained from L1 was 68%, lower than that by Zheng et al. (2012) (88.6%). With L1 in combination with the 12^{th} thoracic vertebra, the equation produced a higher accuracy (96.3%) for sex prediction (Badr El Dine and El Shafei 2015).

Ramadan et al. (2017) employed 15 linear measurements by CT scan in the Egyptians, adopting the methods by Zheng et al. (2012) and Badr el Dine et al. (2015), and showed an accuracy of 84.6% based on EPWu by discriminant function analysis (Ramadan et al. 2017b). The results were comparable with that by Zheng et al. (2012), in which nearly all measurements were significantly greater in males than females, and EPWu was highly accurate. (Zheng et al. 2012).

Ostrofsky & Churchill (2015) performed physical osteological examination (POE) on all lumbar vertebrae (L1- L5) in the South Africans (Ostrofsky and Churchill 2015). By discriminant function analysis, sex was predicted with an accuracy above 80% for L1 till L4, with the highest accuracy of 87.1% in L1. The vertebral body superior dorsoventral diameter (BSDVD) and body superior transverse diameter (BSTD) gave the highest accuracy of over 80%, similar to EPDm and EPWu/l (Zheng et al. 2011, Ramadan et al. 2017). The measurements of the first lumbar are summarized (Figure 4).

Discriminant function analysis on the body of the vertebra showed that it was sexually dimorphic (Zheng et al. 2012; Badr El Dine and El Shafei 2015; Ostrofsky and Churchill 2015; Ramadan et al. 2017b). The upper end-plate depth (EPDU) and upper endplate width (EPWU) were found to be sexually dimorphic in several studies (Badr el Dine et al. 2015, Ramadan et al. 2017, Zheng et al. 2015). By physical osteological examination (POE) of the first lumbar, the EPWu was shown to be the most accurate measurement with 87.1% accuracy rate in the South Africans (Ostrofsky et al. 2015).

There were two most recent studies on sex estimation, that used all five lumbar vertebrae (L1-L5). Decker et al. (2019) studied living patients in a modern adult population by abdominal CT scan. (Decker et al. 2019), while Azofra-Monge and Aguilera (2020) had used specimen collected from the laboratory in Spain (Azofra-Monge and Aguilera 2020). Both studies reported similar findings as that by Ostrofsky and Churchill (2015) with a similar trend of accuracy rate by discriminant analysis (Ostrofsky and Churchill 2015). Azofra-Monge and Aguilera (2020) reported the highest accuracy rate ranged from 90.1% to 94.5%. (Azofra-Monge and Aguilera 2020).

Decker et al. (2019) employed 36 measurements comprising 30 linear measurements, wedging angle and five aspects ratios by analysing all five lumbar vertebrae (L1 - L5) for sex estimation. The discriminant equation for all five vertebrae had an overall accuracy of 81.2% till 85.1% for sex estimation, with the highest percentage achieved by L3 (85.1%). By multilevel measurements, a discriminant equation had reached a higher accuracy of 92.2% (Decker et al. 2019).

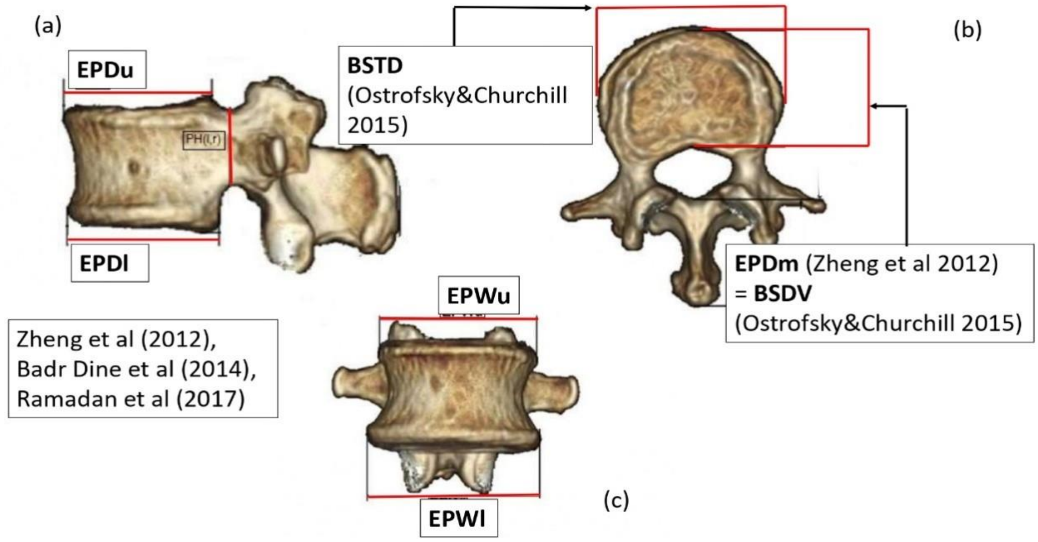

Fig 4. Measurements on the first lumbar vertebra (L1). (a) Lateral view, (b) Supero/Inferior view, (c) Anterior view. (Adapted from Forensic Science International, Vol 219, Zheng et al, Sex assessment using measurements of the first lumbar vertebra, page 215.e1-215.e5, 2012, with permission from Elsevier)

Discussion

The established search selected 15 studies for inclusion after reviewing the abstracts and full-texts (Figure 1). This review had revealed the benefits of using vertebrae for determining sex in the forensic anthropology casework. Of 24 vertebrae (excluding the sacrum and coccygeal), it can be concluded that the second cervical, 12th thoracic and first lumbar were sexually dimorphic in different populations. The significance of sexual dimorphism was apparent in combinations of several vertebrae rather than a single vertebra. As in Badr el Dine (2015), by using only L1 as a single bone, the accuracy in sexual dimorphism was 68%, but when combined with the 12th thoracic vertebra, the accuracy increased to 93.3% (Badr El Dine and El Shafei 2015). Also, by using all of the cervical vertebrae with three measurements in each vertebra, 25 multivariate discriminant functions were generated with 80% accuracy, but with C4 and C2 in combination, an accuracy of 100% was obtained for sex estimation

(Kaeswaren and Hackman 2019). Decker et al. (2019) documented that the lumbar vertebrae (L1-L5) were sexually dimorphic with an accuracy ranged from 81.2% to 85.1%, but when all of the vertebrae were applied in combination, the accuracy rose to 92.2% (Decker et al. 2019).

From the studies, it can be concluded that the vertebrae were sexually dimorphic based on their sizes, with the male being larger than the female, since this review was based on linear measurements focusing mainly on bone size. Studies on the ratios of linear measurements exhibited that the accuracies of the ratios were statistically lower than those by linear measurements (Yu et al. 2008; Hou et al. 2012; Zheng et al. 2012; Badr El Dine and El Shafei 2015). While the linear measurements are representing bone size, the ratios are representing bone shape, which is a combination of several linear measurements (Hou et al. 2012). However, further analysis may be done by geometric morphometric to evaluate bone shape for sexual dimorphism.

The vertebrae can be used as an effective tool for sex determination by discriminant function analysis. The discriminant function is created by multiplying the coefficients with variables of vertebral measurements. The discriminant score is obtained by having a value, that will act as the cut-off point between male and female, also known as the sectioning point. If the scores are greater than the sectioning point, it will be predicted as male, while scores smaller than the sectioning point, will be predicted as female (Omar et al. 2019). Gama et al. (2015) and Azofra-Monge & Aguilera (2020) used logistic regression analysis, instead of DFA (Badr El Dine and El Shafei 2015; Gama et al. 2015). They stated that the logistic regression analysis was more robust and flexible in terms of data assumptions. However, the equations produced by both methods established the fact that male vertebrae were generally larger than in female.

There were significant results on differences between sexes in the vertebral regions, but sexual dimorphism was mostly displayed from the vertebral body. The sexual variance in the vertebral body may be due to different growth spurts in males and females during puberty. Females seem to be having a vertical growth spurt and in comparison to males, they have a horizontal growth spurt (Taylor and Twomey 1984; Azofra-Monge and Aguilera 2020). Additionally, several factors may have contributed to the size and shape of the vertebrae, which may be influenced by mechanical and dietary interventions. While environmental factors and genes may have some effect on growth hormone function and control on bone development, dietary pattern, daily physical activity and mechanical loading may have some influence on bone density, shape and size, and hence the sexual variance (Torimitsu et al. 2016; Gilsanz et al. 2018; Munoz-Hernandez et al. 2018).

There are two types of measurement techniques on bones i.e. radiological technique mainly by CT scan, and metric measurements on dry bones by calipers. The traditional method in dry bones requires an extensive effort and financial means to gain assess to biological samples, which is usually performed in the anthropological museum collection or laboratory. Studies on bone by radiology are more efficient and less invasive to bones for research and identification purposes, which may also be performed on living human subjects. However, Stull et al. (2014) had reported the difference between dry bone and CT images in terms of measurement errors and differences which is about 2 mm approximately. This may bring to suggest that both methods may be useful and acceptable for the anthropologists for their scientific casework investigation (Stull et al. 2014).

Strength and limitation of the review

Studies on vertebrae of human skeletal remains may have promising results in estimating sex for identification in the forensic anthropology casework. To decide which part of the vertebra is important for identification of unknown subjects, a critical review of the methods is highly relevant and warranted. This systematic review had identified 15 research articles. Based on the accuracy score, it was concluded that the 12th thoracic vertebra has a good overview and most accurately scored for sexual dimorphism, although this may be improved by undertaking an advanced meta-analysis in combining the whole vertebrae for further analysis.

Several limitations were identified in this review. While classification according to age groups is important to minimize the identification pool, different age groups were not conducted in these studies. Although a small sample size may not be able to represent a population, different age groups in different populations may be able to produce different effects on the results. Besides, only three search engines were used in the strategy, while missing some relevant studies may still occur among valid publishers.

5. CONCLUSIONS

Finally, it was concluded that the vertebral bones had given good accuracy for sex determination, which means that bone dimensions are population specific. The most frequently used vertebrae for sexual dimorphism were the second cervical (C2), the 12th thoracic (T12) and the first lumbar (L1), and the accuracy seems to increase when used in combination. Studies have shown that the most sexually dimorphic area of the vertebra is the vertebral body, hence it is vital to be used for sex estimation. Further studies may be needed to determine sexual dimorphism in other parts of the vertebrae in an advanced meta-analysis procedure.

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