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Sexual dimorphism in the cervical vertebrae and its potential for sex estimation of human skeletal remains in a white scottish population

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ABSTRACT

Background: Biological sex determination from skeletal human remains is crucial in archaeological and forensic settings. The purpose of the current study was to evaluate the presence of sexual dimorphism in seven (C_1-C_7) cervical vertebrae dimensions and to further establish a reliable sex estimation method using C_1-C_7 for a White Scottish population.
Method: In this study, three morphometric characteristics from the cervical vertebrae were measured; maximum vertebral body height (CHT), maximum anterior-posterior diameter of vertebral foramen (CAP) and maximum transverse diameter of vertebral foramen (CTR). One-hundred and fifty (150) cervical vertebrae from a total of twenty-five (25) human cadavers (13 males, 12 females) ranging in ages 49 to 103 years were studied.
Results: The resulting statistical analysis showed that CHT measurements exhibited the greatest degree of sexual dimorphism at all cervical vertebral level followed by CTR measurements. CAP measurement only exhibited significant sexual dimorphism at the second cervical vertebra (C_2AP). A total of 25 multivariate discriminant functions were generated that were statistically significant and successfully assigned sex with an 81.8%–100% accuracy range. A cross-validation study was also performed to establish the reliability of the 25 functions and only eight out of 25 functions exhibited weak statistical reliability.
Conclusion: Statistically significant sexual dimorphism in the cervical vertebral dimensions (CHT and CTR) was conclusively established with the second cervical vertebrae (C_2) exhibiting the greatest sexual variance in the cervical vertebral column of a White Scottish population. Age-related changes were not observed in the vertebral dimensions of the study sample and this may however be due to the insufficient sample size for each age category.

Introduction

In the field of forensic anthropology, biological sex estimation remains one of the cornerstones of the construction of a biological profile [1]. In humans, typical sexual differences include the structural make-up of the chromosomes (the presence or an absence of a Y-chromosome), levels of sex hormones and variation in sexual reproductive anatomy structures. Sexual differences are also evident in the skeleton, thereby allowing an anthropologist to utilize skeletal elements for the estimation of biological sex. Commonly, the morphological characteristics of the innominate, skull and long bones are examined for sex determination as they exhibit a degree of sexual dimorphism [1]. However, problems arise in cases when the bones, from which forensic anthropologists must construct a biological profile of the remains are missing or have been damaged or fragmented [2], making it necessary to develop new, reliable methodologies and techniques that enable sex estimation using other skeletal elements.

Bones throughout the skeleton have been noted to exhibit sexual dimorphism; however, the vertebral column has not received much attention. A limited number of studies have reported sexual dimorphism in different vertebrae from cervical, thoracic and lumbar regions of the vertebral column [3–8], with various vertebral dimensions shown to have a considerable degree of sexual dimorphism.

The present study focused on evaluating the use of the seven cervical vertebrae (C_1-C_7) for sex estimation in a White Scottish population. The cervical vertebrae are the smallest bones of the vertebral column and exhibit distinct attributes that set them apart from the thoracic and lumbar vertebrae including horizontally oriented spinous process and the presence of transverse foramina. Furthermore, the atypical characteristics of the first (C_1), second (C_2), and seventh (C_7) cervical vertebrae enables quicker anatomical sequencing of the cervical vertebrae [9]. Unlike other skeletal elements that are often used in estimation of sex, the cervical vertebrae have a small exposed surface area which allows them to be less susceptible to damage and more likely to be recovered from a deposition site [10,11].

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Morphological and morphometric sex differences standards in human vertebrae could not be applied universally as they may vary between populations [12]. Data and formulae that prove a certain trait is sexually dimorphic for a given population in question may therefore be less dimorphic in another. This current study is aimed at evaluating the degree of sexual dimorphism of the seven cervical vertebrae (C_1-C_7) and if sexual variation is found to exist, to establish a multivariate discriminative logistic regression model that allows accurate sex estimation in a White Scottish skeletal population.

The objectives of this research are (i) To establish a correlation between sex and the measurements of the cervical vertebral body in a White Scottish population, (ii) To establish a correlation between sex and the measurements of the cervical vertebral foramen in a White Scottish population and (iii) To develop a population specific discriminant function formula based on C_1-C_7 vertebral measurements of a White Scottish population.

Methods and materials

This study utilized wet disarticulated cervical vertebrae obtained from the human cadavers housed at the Anatomy Lab at the Centre of Anatomy and Human Identification (CAHID) at University of Dundee. The seven cervical vertebrae (C_1-C_7) were isolated from the rest of the vertebral column and disarticulated. Remaining soft tissues attached to the vertebrae were removed to ensure the landmarks of measurements were not obstructed. According to their anatomical location, the cervical vertebrae were numerically labeled as C_1 to C_7.

Demographic data

Cervical vertebrae from a total of 25 human cadavers were used in this study, comprising 13 males and 12 females ranging in ages 49 to 103 years. All individuals had donated their remains and therefore, demographic data for each human cadaver were known, such as biological sex (male or female), ancestry (White Scottish population), year-of-death, age-at-death (Table 1) and cause of death.

Exclusion criteria

Vertebrae that were either, broken, merged or had morphologically altering pathological conditions such as osteoporosis, spina bifida or metastatic disease to the bone were excluded from the study. Bones with any osteophyte growth or bone spurs present that affected placement of the jaws of the caliper and measurements from being obtained were also excluded from the study. The exclusion of one vertebra or measurement however did not omit an individual. In other words, if one out of three variables could not be measured on a cervical vertebra of an individual, the remaining measurable variables were still recorded rather than excluding the individual completely from the sample (Table 2). The first cervical (C_1) vertebra was completely excluded from the study sample prior to statistical analysis as most of the vertebra were found to be damaged resulting in an insufficient number of samples for both sex groups.

Table 1
Frequency statistics of the age categories used to group individuals of the study sample.

Age Category	Age	Females (n)	Males (n)	Total (n)
1	49 – 58.99	2	1	3
2	59 – 68.99	3	2	5
3	69 – 78.99	1	4	5
4	79 – 88.99	2	3	5
5	89 – 98.99	3	3	6
6	99 – 108.99	1	0	1

Table 2
Total number of cervical vertebrae utilized in current study.

Cervical vertebra	Males (n)	Females (n)	Overall (n)
C_2	11	11	22
C_3	11	11	22
C_4	12	11	23
C_5	12	11	23
C_6	12	11	23
C_7	13	12	25
Total	71	67	138

Measurements protocol

The measurement protocol was adapted from Gama et al. [13], Amores et al. [14], Kibii et al. [15], Raxter et al. [16], Tatarek [17], Taitz [18], and Eisenstein [19]. Three (3) morphometric traits were measured for each cervical vertebra (Table 3); the maximum vertebral body height (CHT), and two vertebral foramen dimensions; maximum anterior-posterior diameter (CAP) and maximum transverse diameter (CTR). CHT measurements were taken at the maximum mid-sagittal length of the vertebrae whereas CAP and CTR measurements were obtained from the superior surface of the vertebral foramen with an exception for CAP measurement of the second (C_2) cervical vertebra which was taken on the inferior aspect as the superior placement of the caliper jaws is hindered by the presence of the odontoid process of the C_2 vertebrae. All measurements were performed with a standard digital vernier caliper, rounding to the nearest 0.01 mm. Measurements were initially recorded on data collection forms and were later added into a Microsoft Excel spreadsheet (Figs. 1 and 2).

Intra and inter-observer error

Skeletal measurements for the second (C_2) and fifth (C_5) cervical vertebra were taken from a randomly drawn subset of 12 human cadavers (6 females and 6 males) in order to analyze the intra- and inter-observer measurement error. The three morphometric traits were measured a second time by the author, two days after the first set measurements were taken, in order to compare and assess intra-observer error. A second observer from an anatomy and forensic anthropology background was asked to perform the three measurements on the cervical vertebrae in the identical subset of 12 individuals. In order to assess inter-observer analysis, the data recorded by the second observer were then compared to the first set of measurements undertaken by the author.

Statistical procedure

The collected data were entered in a spreadsheet (Microsoft Excel 2010) and analyzed using statistical analysis software (IBM SPSS Statistics 22). Statistical significance level was kept at 5% (p-value $\leq$0.05) for all test performed.

Descriptive statistics was first calculated for the study sample including age range, average age, median age, birth year, birth median and year of death. These descriptive statistics were also calculated between males and females of the population. The minimum and maximum values, means and standard deviations were then calculated for CHT, CAP and CTR measurements for males and females in the study sample. Univariate normality test and probability plots was done using Shapiro-Wilk test and Q-Q plot, respectively for each of the three variables (CHT, CAP, CTR) within the study sample to examine the data distribution and identify any outliers. Paired *t*-test was performed to evaluate inter- and intra- observer errors and the replicability of the cervical vertebral measurements.

In order to establish and evaluate the presence of sexual differences between males and females of the study sample in terms of the

Table 3
Description of cervical vertebral measurements (CHT, CAP and CTR) taken for this study.

Cervical vertebral measurements	Description	Adapted from
Maximum vertebral body height (C_nHT)	The maximum superior -inferior length of the vertebral body along the anterior border of the vertebra. For the second cervical vertebrae, this measurement includes the dens.	[15,16]
Maximum anterior-posterior diameter (C_nAP)	The maximum mid-sagittal diameter of the vertebral foramen measured anterior-posteriorly.	[15,18,19]
Maximum transverse diameter (C_nAP)	The maximum medio-lateral diameter of the vertebral foramen measured internally from the right and left pedicles.	[17,18,19]

n = chronological number of the vertebra based on anatomical location (1–7).

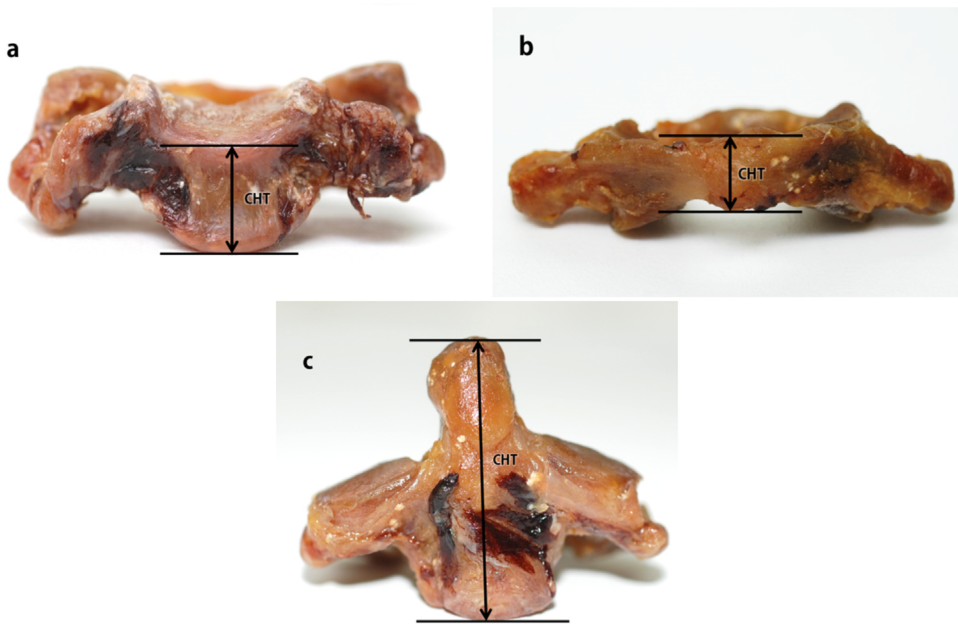

Fig. 1. Anterior view of **(a)** a typical vertebrae, **(b)** the first cervical vertebra (C_1), and **(c)** the second cervical vertebra (C_2) showing the maximum vertebral body height of the cervical vertebra (C_nHT) measurement. (Photo by author).

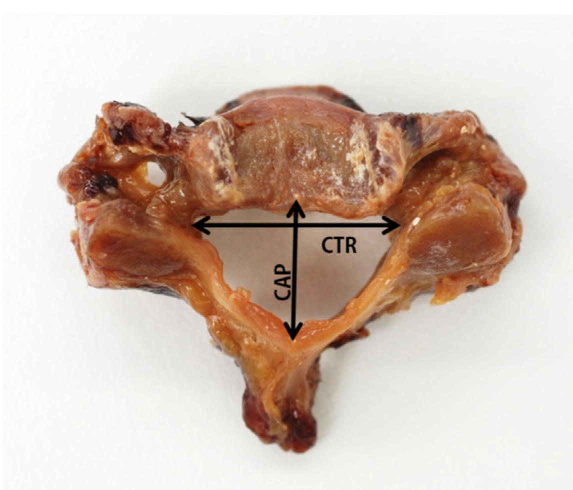

Fig. 2. Superior view of a typical vertebra showing the maximum anterior-posterior diameter (CAP) and maximum transverse diameter (CTR) measurements of the cervical vertebral foramen. (Photo by author).

morphometric characteristics of the cervical vertebrae (CHT, CAP and CTR), an independent (two-sampled) *t*-test was performed on the data. Discriminant functions were created using canonical discriminant functions coefficients in order to estimate sex from cervical vertebral dimensions. Discriminant functions were created first, for each independent cervical vertebrae (C_2 through C_7), second, using all six cervical vertebrae (C_2-C_7), third, utilizing the typical cervical vertebrae (C_3-C_6) and transitional vertebrae (C_7), and fourth, excluding C_7 and only using the typical cervical vertebrae (C_3-C_6). For all four vertebral groups, discriminant functions were created using a combination of traits: 1) all three cervical vertebral measurements (CAP, CHT and CTR); 2) only vertebral foramen measurements (CAP and CTR), 3) the two most dimorphic measurement obtained from the independent *t*-test, in the case of the present study, it was CHT and CTR and finally 4) the most dimorphic measurement and the least dimorphic measurement (CHT and CAP for the present study).

Stepwise discriminant analysis was performed by entering all measurements taken at every cervical vertebral level to create a discriminant function using the most dimorphic variables selected by the stepwise method. Statistically significant discriminant functions with an overall accuracy exceeding 80% were further subjected to cross-validation analysis to assess the reproducibility of the discriminant functions from an independent data set. Fifteen (N = 15) individuals of known sex represent the independent sample.

Results

Intra and inter-observer error

The p-values for the inter-observer measurements showed no statistically significant differences (p-value >0.05) between CHT, CAP and CTR measurements (Table 4). Similar results were obtained for the intra-observer measurements as seen in Table 5. The differences in means

Table 4
Inter-observer error bias and significance for CHT, CAP and CTR measurements of the second (C_2) and fifth (C_5) cervical vertebra.

Inter-observer sample size (N) = 12			
Measurements	t-value	p-value	SD
C_2HT	−0.671	0.516	0.022
C_5HT	0.897	0.389	0.019
C_2AP	1.121	0.286	0.021
C_5AP	−1.817	0.097	0.021
C_2TR	−1.401	0.189	0.027
C_5TR	0.635	0.539	0.023

Statistical significant difference = p-value ≤0.05.
SD = Standard deviation.

Table 5
Intra-observer error bias and significance for CHT, CAP and CTR measurements of the second (C_2) and fifth (C_5) cervical vertebra.

Intra-observer sample size (N) = 12			
Measurements	t-value	p-value	SD
C_2HT	0.89	0.392	0.016
C_5HT	−2.916	0.410	0.015
C_2AP	0.616	0.551	0.019
C_5AP	−1.959	0.076	0.016
C_2TR	−1.465	0.171	0.014
C_5TR	0.000	1.000	0.015

Statistical significant difference = p-value ≤0.05.
SD = Standard deviation.

for all three measurements (CHT, CAP and CTR) with the original data set ranged from 0.01 mm to 0.04 mm for both intra- and inter-observer values.

Descriptive analyses

A total of one hundred and fifty (N = 150) individual cervical vertebra were included in the analysis. Descriptive statistics for males and females are presented in Table 6. Significant sex differences were present for all CHT measurements at every cervical vertebral level, as determined by the independent two-sample *t*-test (Table 6). However, only the CAP diameter of the second cervical vertebra (C_2AP) and CTR measurements of the second (C_2TR), third (C_3TR) and fourth (C_4TR) cervical vertebrae showed significant statistical sexual dimorphism in the study sample.

Estimating sex from a single vertebra

Overall, sex estimation from a single vertebra gave an accuracy range of 77.3% to 100% (72.7% to 100% in males; 81.8% to 100% in females) using all three measurements (CHT, CAP and CTR). Sex estimation from CAP and CTR measurements of the vertebral foramen gave an accuracy range of 60% to 100% (58.3% to 100% in males; 54.5% to 100% in females). The two most dimorphic measurements (CHT and CTR), gave an accuracy range of 86.4% to 100% (75% to 100% in males; 90.9% to 100% in females). CHT and CAP measurements estimated sex with an accuracy of 81.8% to 100% (81.8% to 100% in both males and females) using a single vertebra. The second (C_2) cervical vertebrae gave the highest overall accuracy (100%) in all its discriminant functions.

Estimating sex from all cervical vertebrae (C2−C7)

Discriminant functions were created by the same four set of combinations involving the three vertebral measurements (CHT, CAP and CTR) using all six cervical vertebrae (C_2−C_7). Discriminant function generated using all three measurements (CHT, CAP and CTR) and all six cervical vertebrae (C_2−C_7) resulted in 100% accuracy for both males and females. CTR measurement for the fifth (C_5TR), sixth (C_6TR) and seventh (C_7TR) vertebra however was excluded by SPSS in generating the function (Table 7; Function 25) due to it being a weak variable in predicting sex. However, similar overall accuracy (100%) was obtained from the remaining set of combinations of measurements utilizing all six cervical vertebrae (C_2−C_7).

Estimating sex from typical cervical vertebrae (C3−C6) and C7

The same four combinations of the three vertebral measurements (CAP, CTR and CHT) using the transitional seventh cervical vertebra (C_7), and the remaining four typical vertebrae (C_3−C_6) were used to create discriminant functions to estimate sex. All four functions generated gave an accuracy rate exceeding 80%, indicating that measurements from vertebrae C_3 to C_7 can successfully assign sex. Two functions utilizing all

Table 6
Descriptive statistics of the vertebral dimensions measured for each sex and independent *t*-test output to assess and evaluate the significance of the differences or similarities in measurements between females and males of the study sample at every cervical vertebral level.

Measurements	n		Minimum		Maximum		Mean ($\bar{x}$)		t-value	p-value
	Female	Male	Female	Male	Female	Male	Female	Male		
C_2HT	11	11	35.24	40.11	44.23	49.55	37.95	43.72	4.55	0.000*
C_3HT	11	11	12.02	13.66	13.65	18.43	12.72	15.15	4.87	0.000*
C_4HT	11	11	10.94	13.17	13.34	17.54	12.20	14.42	5.19	0.000*
C_5HT	11	11	10.18	12.23	13.33	15.58	12.22	13.82	3.84	0.001
C_6HT	11	12	11.13	12.48	13.36	16.39	12.30	14.22	4.51	0.000*
C_7HT	12	13	10.16	14.75	14.67	19.1	13.70	16.57	5.64	0.000*
C_2AP	11	11	14.45	17.01	16.88	20.74	15.90	18.40	5.10	0.000*
C_3AP	11	11	12.63	11.7	15.95	17.04	14.49	14.89	0.64	0.530
C_4AP	11	12	12.28	10.37	15.45	16.87	13.63	13.97	0.51	0.618
C_5AP	11	12	12.74	11.23	15.31	17.74	13.93	14.13	0.30	0.767
C_6AP	11	12	10.66	11.78	15.19	16.75	13.17	14.08	1.46	0.159
C_7AP	12	13	11.23	11.74	15.46	16.53	13.43	13.69	0.49	0.632
C_2TR	11	11	21.82	25.11	24.87	27.24	23.79	26.03	5.75	0.000*
C_3TR	11	11	22.33	22.13	24.59	26.16	23.47	24.78	3.31	0.003*
C_4TR	11	12	21.78	23.19	24.73	27.48	24.16	25.28	2.67	0.014
C_5TR	11	12	23.05	23.99	25.83	30.78	25.02	25.99	1.59	0.126
C_6TR	11	12	24.19	23.33	26.65	31.04	25.32	26.19	1.27	0.219
C_7TR	12	13	22.13	23.41	27.37	28.88	24.50	25.13	1.11	0.280

All measurements were recorded in millimeters (mm).
^{n} = number of vertebrae studied.
SD = standard deviation.
$\bar{x}$ = Sample mean.
Statistical significant difference = p-value ≤0.05.

Table 7
25 statistically significant discriminant functions that correctly assessed sex with more than 80% accuracy using dimensions of the cervical vertebrae and its respective cross-validation accuracies.

Function	Formula	Sectioning point			Overall Accuracy	Cross-Validation Accuracy
1	$y = 0.228 (C_2HT) + 0.536 (C_2AP) + 0.445 (C_2TR) – 29.604$	Female<	−0.9135	<Male	100%	100%
2	$y = 0.450 (C_2AP) + 0.743 (C_2TR) −26.222$	Female<	−0.6970	<Male	100%	100%
3	$y = 0.203 (C_2HT) + 0.849 (C_2TR) −29.446$	Female<	−0.7680	<Male	100%	95.5%
4	$y = 0.268 (C_2HT) + 0.740 (C_2AP) −23.630$	Female<	−0.8490	<Male	100%	100%
5	$y = 0.673 (C_3HT) + 0.179 (C_3AP) + 0.433 (C_3TR) - 22.467$	Female<	−0.5685	<Male	90.9%	86.4%
6	$y = 0.278 (C_3AP) + 1.091 (C_3TR) −30.406$	Female<	−0.3850	<Male	81.8%	77.3%[a]
7	$y = 0.701 (C_3HT) + 0.378 (C_3TR) −18.891$	Female<	−0.5495	<Male	95.5%	90.9%
8	$y = 0.850 (C_3HT) + 0.132 (C_3AP) −13.785$	Female<	−0.5290	<Male	90.9%	90.9%
9	$y = 0.932 (C_4HT) + 0.195 (C_4AP) + 0.391 (C_4TR) - 24.769$	Female<	−0.6475	<Male	90.9%	86.4%
11	$y = 0.898 (C_4HT) + 0.430 (C_4TR) −22.564$	Female<	−0.6165	<Male	95.5%	90.9%
12	$y = 1.021 (C_4HT) + 0.229 (C_4AP) −16.774$	Female<	−0.5935	<Male	90.9%	86.4%
15	$y = 0.986 (C_5HT) + 0.340 (C_5TR) −21.489$	Female<	−0.4715	<Male	86.4%	72.7%[a]
16	$y = 1.022 (C_5HT) + 0.165 (C_5AP) −15.627$	Female<	−0.4230	<Male	81.8%	81.8%
17	$y = 0.962 (C_6HT) + 0.262 (C_6AP) + 0.150 (C_6TR) - 20.240$	Female<	−0.0480	<Male	82.6%	73.9%[a]
19	$y = 0.257 (C_6HT) + 0.963 (C_6TR) −19.426$	Female<	−0.0455	<Male	87%	78.3%[a]
20	$y = 0.966 (C_6HT) + 0.334 (C_6AP) −17.404$	Female<	−0.0470	<Male	87%	82.6%
21	$y = 0.784 (C_7HT) + 0.113 (C_7AP) + 0.134 (C_7AP) - 16.790$	Female<	−0.0475	<Male	88%	88%
23	$y = 0.774 (C_7HT) + 0.202 (C_7TR) −16.781$	Female<	−0.0471	<Male	88%	88%
24	$y = 0.796 (C_7HT) + 0.209 (C_7AP) −14.928$	Female<	−0.0469	<Male	92%	92%
25	$y = 0.972 (C_2HT) – 1.39 (C_2AP) + 1.985 (C_2TR) – 4.175 (C_3HT) – 0.343 (C_3AP) + 6.699 (C_3TR) + 9.478 (C_4HT) + 0.065 (C_4AP) – 4.505 (C_4TR) + 1.805 (C_5HT) + 2.335 (C_5AP) – 2.026 (C_6HT) + 3.351 (C_6AP) – 1.15 (C_7HT) – 4.601 (C_7AP) – 171.987$	Female<	0.4705	<Male	100%	41.2%[a]
26	$y = 3.082 (C_2AP) – 1.223 (C_2TR) – 2.757 (C_3AP) + 2.366 (C_3TR) + 0.67 (C_4AP) + 2.2 (C_4TR) + 1.462 (C_5AP) – 2.357(C_5TR) + 0.158 (C_6AP) – 0.443 (C_6TR) – 0.695 (C_7AP) + 0.685 (C_7TR) – 61.714$	Female<	−1.8890	<Male	100%	72.2%[a]
27	$y = 0.557 (C_2HT) – 0.004 (C_2TR) – 0.575 (C_3HT) + 1.319 (C_3TR) – 1.081 (C_4HT) – 1.918 (C_4TR) + 1.461 (C_5HT) + 1.497 (C_5TR) – 0.047 (C_6HT) – 0.816 (C_6TR) + 1.737 (C_7HT) + 0.129 (C_7TR) – 78.003$	Female<	0.2619	<Male	100%	82.4%
28	$y = 0.346 (C_2HT) + 0.896 (C_2AP) – 0.3 (C_3HT) – 0.64 (C_3AP) - 0.618 (C_4HT) + 0.371 (C_4AP) + 0.711 (C_5HT) – 0.564 (C_5AP) + 0.175 (C_6HT) + 0.004 (C_6AP) + 1.815 (C_7HT) + 0.427 (C_7AP) – 49.874$	Female<	0.1948	<Male	100%	70.6%[a]
33	$y = - 1.555 (C_3HT) – 4.102 (C_3AP) + 1.049 (C_3TR) + 2.615 (C_4HT) + 0.538 (C_4AP) + 8.141 (C_4TR) + 4.853 (C_5HT) + 3.674 (C_5AP) – 4.834 (C_5TR) – 4.003 (C_6HT) + 1.648 (C_6AP) – 0.738 (C_6TR) – 130.996$	Female<	−1.6120	<Male	100%	72.2%[a]
37	$y = 0.894 (C_4HT) + 0.779 (C_2TR) – 31.184$	Female<	0.1000	<Male	100%	100%

[a] Indicates cross-validation accuracies less than the original predicted accuracy of the function and less than 80%.

three measurements (CHT, CAP and CTR) and the two most dimorphic measurements (CHT and CTR) respectively, gave the highest sex predicting accuracy of 100% for both males and females.

Estimating sex from four typical cervical vertebrae (C3-C6)

The transitional seventh cervical vertebra (C_7) was then excluded to create discriminant functions utilizing only the combinations of the vertebral measurements (CAP, CTR and CHT) of the four typical vertebrae ($C_3 - C_6$) to estimate sex. All four functions generated gave an accuracy rate of more than 80%. Two functions utilizing all three measurements (CHT, CAP and CTR) and the two most dimorphic measurements (CHT and CTR) respectively, gave the highest sex predicting accuracy of 100% for both males and females.

Stepwise discriminant function analysis

Stepwise discriminant analysis was performed on SPSS to select the most dimorphic variables for sex estimation. Only two variables; the CHT measurement of the fourth (C_4) cervical vertebra and the CTR diameter of the second (C_2) cervical vertebra, indicated a high sexual dimorphic potential. A function was generated by SPSS using these two measurements (Table 7; Function 37).

Sex estimation accuracy of discriminant functions

A total of 32 discriminant functions achieved an overall accuracy more than 80%, the minimum percentage of accuracy required to successfully assign sex (34). Wilk's Lamda test however showed a high discriminatory ability in only 25 out of the 32 discriminant functions. Furthermore, significance (p-value) of the 25 functions was less than 0.05 which indicated that the discriminant function does better than chance at estimating sex. Table 7 shows 25 statistically significant discriminant functions that were able to successfully estimate sex using measurements of the cervical vertebrae. The functions were also subjected to cross-validation and the accuracy obtained was compared to the original predicted accuracies to further validate the accuracy and reliability of the functions (Table 7). Results from cross-validation analysis showed that eight out of 25 functions had cross validation accuracy less than the SPSS predicted accuracy and less than 80%, the minimum percentage of accuracy required to successfully assign sex.

Discussion

Sexual dimorphism in cervical vertebral dimensions (CHT, CAP and CTR)

In this present study, measurements involving the cervical vertebral foramen (CAP and CTR) and vertebral height (CHT) were analyzed to assess the degree of sexual dimorphism between them. Previous studies on the vertebral foramen found a significant sexual dimorphism in vertebral foramen dimensions between males and females [15,17–19]. However, these differences were more focused on the clinical aspects of the vertebral foramen [17,18] and not solely on estimating sex from human skeletal remains. Research on sex estimation using the cervical vertebrae has mostly focused on the vertebral body dimensions including the length and width of the vertebra and its articulating facets [3,11,13]. Despite being sexually dimorphic, measurements of these characteristics were more likely to be affected by taphonomic damage to, and fragmentation of, the spinous process and articular facets compared to the three morphometric characteristics measured in this present research (CHT, CAP and CTR). The CAP and CTR diameters of the vertebral foramen are protected by the vertebral arches whereas the CHT measurement is taken on the dense vertebral body that is resilient to architectural and mechanical stresses as well as taphonomic damage. Thus, these three morphometric characteristics; CHT, CAP and CTR, are more likely to be present and reliable for sex estimation analyses in a forensic setting.

It was observed that the mean value of the three measurements (CHT, CAP and CTR) was generally higher in males than in females at every cervical vertebral level. The first (C_1) cervical vertebra was excluded from the sample prior to statistical analysis as most of the samples found on the cadavers had broken posterior arches which resulted in an insufficient sample size in males and females. Therefore, sexual dimorphism of C_1 was not able to be evaluated in the present study. Further research can still be conducted to validate findings focused on the sexual dimorphism of the first (C_1) cervical vertebrae in a White Scottish population if an appropriately sized data set were available.

Intra- and inter-observer error rates were low. Results from two-sample *t*-test performed indicated that all CHT measurements were sexually dimorphic (p-value <0.05). Only three CTR diameters (C_2TR, C_3TR and C_4TR) in the study sample exhibit statistically significant differences between males and females. The CAP diameter of the vertebral foramen is not sexually dimorphic with the exception of the second (C_2) cervical vertebra, which showed a significant dimorphism between males and females. Therefore, the most dimorphic vertebral measurement is the maximum height of the vertebral body (CHT) followed by the maximum transverse diameter of the vertebral foramen (CTR) at only the second (C_2), third (C_3) and fourth (C_4) cervical vertebral level. These findings were in accordance with a number of previous studies in the field [15,20,21]. Bethard and Seet [22] and Marlow and Pastor [11] tested Wescott's [23] method of sex estimation using the second cervical vertebra from an American and White European population, respectively and found that the CHT length exhibited 'highly significant' dimorphism between males and females.

In the present study, statistically significant difference and sexual dimorphism regarding CTR diameter was only observed in the second (C_2), third (C_3) and fourth (C_4) cervical vertebrae. Sexual dimorphism in the CTR diameter at different cervical vertebral level was also reported by a number of authors that shared certain similar observation with the present study [15,17,18]. Tatarek [17] also observed that the CTR measurement was the widest at the sixth cervical vertebrae (C_6) and narrowest at either the second (C_2) or third (C_3) cervical vertebra, which concurred with the findings of the present study.

The CAP diameter showed no significant statistical difference and sexual dimorphism between males and females with the exception of the CAP diameter of the second cervical vertebra (C_2AP). The absence of sexual dimorphism of CAP diameters in this study agrees with other researchers who had also observed little or no dimorphism in CAP diameters in their study [13,15,18,24]. Liguoro and associates [21] reported a significant variation in the CAP diameters of the second cervical vertebra (C_2) between males and females when compared to the rest of the cervical vertebrae of a French population. A similar finding was also obtained by Marlow and Pastor [11] upon examining the second cervical vertebrae (C_2) of a White European population. The CAP diameter of the second cervical vertebra (C_2) was reported to exhibit a 'very significant' sex differences among the set of nine variables. In contrast to the findings of the present study, there have also been studies that have reported the existence of sexual dimorphism in CAP diameters of cervical vertebrae in different populations [3,15,17,25].

In the current study, only the second (C_2) cervical vertebra was observed to exhibit significant sexual dimorphism in all three morphometric characteristics measured (CHT, CAP and CTR). The distinct morphology of the second (C_2) cervical vertebrae and its functional relationship with the first (C_1) cervical vertebra may be the contributing factors to sexual variation between males and females. Furthermore, unlike the typical cervical vertebrae (C_3-C_7), the second (C_2) cervical vertebra is not associated with movement related mechanical constrictions [21]. It was also observed that there were many contrasting results between every study of the cervical vertebrae to another in various populations including the current study. A discriminating variable in one population may not be relevant in another due to population and human variation. This inter-population variability of human morphometric is due to differences in ancestral and genetical origins, and environmental triggers [26,27]. Moreover, the choice of methodological procedure and statistical analysis by researchers in every study also has an influence on the variability in morphometric measurements of the cervical vertebrae.

In the present study, two cervical vertebral dimensions (CHT and CTR) were found to exhibit sexual dimorphism in a White Scottish population. The maximum height of the cervical vertebral bodies (CHT) showed the greatest degree of dimorphism at all cervical vertebral level between males and females of the study sample. The maximum transverse diameter of the cervical vertebral foramen (CTR), specifically at the second (C_2TR), third (C_3TR) and fourth vertebral level (C_4TR) was observed to be the second most dimorphic variable for sex estimation. The maximum anterior-posterior diameters of the cervical vertebral foramen (CAP) exhibited no potential sexual dimorphism except for the second cervical vertebra (C_2AP).

The degree of sexual dimorphism of a vertebra is reported to be directly proportional to the duration of its growth and development [28]. That is, the longer the time taken for the growth of the vertebrae to be established; the more sexual dimorphism is expressed in the terms of its vertebral dimensions. The growth of the mid-sagittal length of a cervical vertebra, in other words, its anterior-posterior diameter (CAP) is rapid after birth and comes to a halt at about the four years of age [28]. Studies on cervical radiographs [29,30] have also observed that CAP diameters in young adolescents (11–19 years of age) were almost identical to those in adults (>20 years of age) with the differences not being statistically significant. These findings were also in accordance with Porter and Pavitt [31] who found that the full adult length of CAP diameter is established prior to puberty, at approximately four or five years of age. Therefore, the growth of CAP diameter is not effected by secondary sexual development and sex hormones which are usually present at puberty at about 8–12 years of age [28,31]. Thus, this may be the reason for the absence of sexual dimorphism in measurements of the CAP diameters in the present study.

The differing conclusions for the presence of sexual dimorphism in the CAP diameters among varying population studies may also be related to the methodologies employed by respective researchers in their studies. The present study did not observe any sexual dimorphism in the CAP measurement at every vertebral level except for the second cervical vertebra (C_2AP). This finding is contrary to the results found by [3,15,17,25]. Of the four studies that show the existence of sexual dimorphism in CAP diameter, three were performed on dry bone samples [3,15,17] and one was performed using radiographs (imaging technique) [25]. The use of cervical bones directly obtained from human cadavers (wet bones) was exclusive to the present research. Additionally, of the six studies that show the non-existence of sexual dimorphism in CAP diameters, four were conducted on dry bones [11,13,15,18] and two were performed using radiographs [21,24]. Studies have reported that methods utilizing imaging techniques can result in a 1 mm to 3 mm difference in CAP diameter as compared to measurement from dry bone due to magnification errors [15,19,32,24]. As a result, concerns over the statistical significance of vertebral dimensions arise when magnification errors are considered.

Unlike CAP, the growth and development of CHT and CTR vertebral dimensions are slower and extends over a longer period of time [28].

Therefore, these measurements are more likely to be influenced by secondary sexual development and sex hormones as well as environmental triggers. The vertebral foramen reaches 90% of adult size by the age of six [31] and is usually susceptible to biomechanical forces during this development period. The mechanical force alters the shape of the vertebral foramen that was originally circular to a more triangular shape in order for it to be morphologically stable to protect the spinal cord [26]. Up till about 10 years of age, the CTR diameter of the vertebra undergoes an increase in dimension [28]. The height of the vertebral bodies (CHT) has the longest development period compared to CTR and reaches its full adult length at around 20 years of age [28]. Thus, CHT dimensions are more influenced by secondary sexual development and hormones due to its extended development period and are therefore more likely to express sexual dimorphism. This corroborates with the findings of the present study in which CHT measurements at all cervical level was found to exhibit the greatest degree of sexual dimorphism in the study sample.

Sex estimation using cervical vertebrae

Discriminant functions utilizing all three vertebral measurements (CHT, CAP and CTR) estimated sex with an accuracy range of 77.3% to 100% at each vertebral level. Accuracies for sex estimation using only CAP and CTR measurements ranged from 60% to 100%. Combination of CHT and CTR measurements in discriminant functions resulted in an accuracy range of 86.4% to 100% at every vertebral level. When CHT and CAP were utilized, accuracies ranged between 81.8% and 100%. Sex was estimated with an accuracy of above 80% at every vertebral level with C_2 giving the most accurate sex estimation of 100% in all four combinations of vertebral measurements. The combination of the two most sexually dimorphic dimensions (CHT and CTR) gave the highest accuracy range among the four vertebral measurements combinations. The results indicate that a single cervical vertebra does have a strong potential in estimating sex in a White Scottish population. These findings were in agreement with studies of other researchers who have successfully estimated sex from a single cervical vertebra [3,13,22,23].

Furthermore, an increase in the accuracy ranges was observed when all three measurements and more than one vertebral level were utilized in the discriminant functions. Discriminant function using all cervical vertebrae (C_2-C_7) and all three vertebral measurements (CHT, CAP and CTR) resulted in an accuracy rate of 100% in sex estimation. Similar accuracy range was also achieved when only the typical vertebrae (C_3-C_6) and C_3-C_7 was used in discriminant functions utilizing all three vertebral measurements (CHT, CAP and CTR). This observation of the current study concurs with Wescott [23] who also reported that an increase in sex estimating accuracy directly correlates with the increase in measurements utilized in a discriminant function.

Twenty-five functions from a total of 37 discriminant functions created were significantly discriminatory and achieved a sex predicting accuracy of more than 80%, the minimum percentage of accuracy required to successfully assign sex, considering intra-observer error is at a minimum [33]. Furthermore, cross-validation was performed on the 25 functions to re-evaluate the reliability of the discriminant functions in sex estimation [34]. Cross-validation of 25 functions showed that eight functions (Function 6, 15, 17, 19, 25, 26, 28 and 33) achieved accuracies lower than 80% and less than their predicted accuracies. The difference between the predicted accuracies and cross validation accuracies were 4.5%, 13.7%, 8.7%, 8.7%, 58.8%, 27.8%, 29.4% and 27.8% for Functions 6, 15, 17, 19, 25, 26, 28 and 33, respectively. The variance in accuracies are high and therefore discriminant functions 6, 15, 17, 19, 25, 26, 28 and 33 are not as reliable and accurate at estimating sex as compared to the remaining functions. However, according to Christensen and Crowder [34], a discriminant function with accuracies of less than 80% may still be of valuable use in a forensic setting as a corroborating evidence when limited skeletal material are available for forensic anthropologist for analysis. Moreover, statistical variation of accuracies can also occur due to different sample sizes used to test each discriminant function which in turn may have affected the response of each function [35]. The remaining 17 discriminant functions in the current research however can successfully predict sex with a predicted and cross validated accuracy of more than 80%. Among the total of 18 variables measured in this study, stepwise discriminant analysis (Function 37) indicated that the CHT measurement of the fourth cervical vertebra (C_4HT) and the CTR diameter of the second cervical vertebrae (C_2TR) exhibited the greatest degree of sexual dimorphism between males and females in a White Scottish population.

Conclusion

The results of this study show that the human cervical vertebrae have potential for use in sex estimation due to its skeletal morphological variation between males and females in the White Scottish population. The CTR and CHT measurements were found to contribute the greatest to biological sex variation in White Scottish population. The findings of the present study concur with several previous literatures that have mentioned CHT to be the most dimorphic variable followed by CTR and then CAP diameter. Variations in degree of sexual dimorphism exhibited by cervical measurements in this study may be due to duration of growth and the role played by sexual hormones during the development of these vertebral dimensions (CHT, CAP and CTR).

This study also developed 25 multivariate discriminant functions that successfully classified individuals as male or female with an accuracy greater than 80%. C_4HT and C_2TR was selected as the most sexually dimorphic variables in the present study, giving an accuracy of 100% in sex estimation. Results obtained from this study are preliminary due to a small sample size (N = 25) and a limited time frame. Therefore, additional research involving cervical vertebral dimensions using a larger and more equally distributed sample in terms of sex and age groups would be useful to further validate the findings of this study.

Declaration of Competing Interests

The authors declare no competing interests.

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