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Sex Estimation from the Hyoid Bone in a Contemporary Spanish Population

Aioze Trujillo-Mederos

artrujillo@uloyola.es

Universidad Loyola Andalucía

Juan Francisco García-Vázquez

FISIDEC University Center (Affiliated with the University of Córdoba)

Elena De-La-Barrera-Aranda

University of Córdoba

Pablo Pérez Jorge

Institute of Legal Medicine and Forensic Sciences of Córdoba

Eloy Girela-Lopez

University of Córdoba

Cristina Beltran-Aroca

University of Córdoba

Research Article

Keywords: Hyoid bone, sex estimation, sexual dimorphism, forensic anthropology, osteometrics, Spanish population

Posted Date: June 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9992737/v1

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Additional Declarations: No competing interests reported.

Abstract

The hyoid bone may provide useful complementary information for sex estimation when more informative skeletal elements are unavailable, although population-specific standards remain limited and the external performance of published equations is uncertain. This study investigated sexual dimorphism in hyoid osteometrics in a contemporary sample from southern Spain, developed a population-specific discriminant function, and evaluated the transferability of previously published models. A total of 147 adult hyoid bones from medico-legal autopsies (92 males, 55 females) were measured using six linear variables. Inter-observer agreement was assessed with intraclass correlation coefficients. Sexual dimorphism was evaluated by MANOVA and follow-up univariate analyses. A stepwise linear discriminant analysis was used to derive a parsimonious sex-estimation function, with internal validation performed by leave-one-out cross-validation. Published discriminant functions from other populations were also applied to the sample. All variables showed significantly larger mean values in males than in females, and MANOVA confirmed a significant overall effect of sex on hyoid dimensions (Wilks' $\Lambda = 0.312$, $p < 0.001$). The final model retained body thickness, body length, body height, and greater horn height, yielding an overall accuracy of 83.0% in both the original and cross-validated classifications, with higher accuracy in males (85.9%) than in females (78.2%). A sensitivity analysis restricted to non-fused hyoids produced broadly comparable results. External equations showed heterogeneous and often sex-biased performance. These findings support the presence of sexual dimorphism in the hyoid bone and the potential usefulness of population-specific hyoid osteometrics for sex estimation, although external validation remains necessary.

INTRODUCTION

Sex estimation is a key step in the reconstruction of the biological profile of unidentified individuals in forensic and archaeological contexts. Although the pelvis and skull remain the skeletal regions of choice because of their high discriminatory value at the morphological level, their usefulness is limited when remains are fragmented, incomplete, or poorly preserved. In such circumstances, osteometric approaches based on alternative skeletal elements may provide valuable complementary information [1, 2].

Among these secondary elements, the hyoid bone has attracted growing interest as a potential resource for sex estimation. Previous forensic studies have shown that the hyoid may be useful for this purpose, with classification rates frequently exceeding 70% and, in some cases, 90%, depending on the population examined, the measurement protocol used, and the modelling strategy applied [3]. However, the available evidence remains highly heterogeneous. Differences in sample composition, ancestry, chronology, fusion status, variable definition, metric acquisition techniques, and analytical strategies hinder direct comparison across studies and limit the generalizability of published discriminant functions [3]. Consequently, the hyoid should not be regarded as a universally interchangeable element for sex estimation, but rather as a structure whose forensic value depends largely on methodological consistency and population specificity [4].

In the contemporary Spanish population, specific discriminant functions have been developed for sex estimation from several skeletal regions, including the skull, dentition [5], long bones [6], clavicle [7], patella [8], sternum [9], carpal bones [10], and metacarpals [11]. By contrast, no population-specific discriminant function has yet been developed for the hyoid bone. This gap is forensically relevant, because in the absence of local standards, sex estimation must rely on equations derived from other populations. Yet such transferability cannot be assumed. Forensic anthropological research has shown that discriminant functions may lose accuracy when applied to different populations as a result of interpopulation, secular, and methodological variation [12, 14, 16, 18]. The use of non-local equations may therefore introduce classification bias, often more pronounced in females, and reduce accuracy in real forensic casework [16–18].

Accordingly, the aims of the present study were to assess sexual dimorphism of the hyoid bone in a contemporary sample from southern Spain, to develop a parsimonious population-specific discriminant function, and to evaluate the performance of previously published equations when applied to this series. We hypothesized that: (1) the hyoid bone would show significant sexual dimorphism in this population; (2) a locally derived function would show useful internal performance for sex estimation; and (3) equations developed for other populations would show limited transferability and, in some cases, unbalanced performance between sexes.

MATERIALS AND METHODS

Sample

A total of 147 adult hyoid bones from individuals of known sex, recovered during medico-legal autopsies performed at the Institute of Legal Medicine of Córdoba (Spain), were included in the study. The sample comprised 92 males (71 non-fused and 21 fused) and 55 females (43 non-fused and 12 fused). According to the available medico-legal records, all individuals were classified as belonging to a contemporary sample from southern Spain. Age at death ranged from 20 to 95 years (Table 1). Hyoids showing macroscopic pathology, traumatic alteration affecting the measured regions, or post-mortem damage interfering with osteometric assessment were excluded.

Table 1
Distribution of the hyoid sample by sex, fusion status, and age

Sex	Fusion status	n	Mean age (SD)	Range
Male	Non-fused	71	60.2 (16.7)	20–95
	Fused	21	63.6 (7.1)	48–79
Female	Non-fused	43	64.7 (19.0)	20–95
	Fused	12	66.8 (14.0)	41–85
Total	Non-fused	114	61.9 (17.7)	20–95
	Fused	33	66.8 (14.0)	40–85

Linear measurements

Six linear osteometric variables were recorded in millimetres following previously published protocols [14, 16] (Table 2). All measurements were obtained using a digital caliper (Stainless-Hardened®, 150 mm; Mauá, São Paulo, Brazil).

Table 2
Description of measurements

Measurement	Description
BH	Body height measured as the distance between the upper and lower edge of the body in the midsagittal plane.
BL	Body length measured as the distance between the lateral edges of the hyoid body.
BT	Upper anteroposterior body thickness, measured as the distance between the most anterior and most posterior points of the hyoid body, usually at its upper portion.
CHI (L – R)	Height of the greater horn measured as the distance between the upper and lower edge at the anterior end of the greater horn (non-fused bones) or, alternatively, the distance from the upper to lower edge of the fusion line connecting the hyoid body with the right/left greater horn (fused bones).
CL (L – R)	Length of the greater horn measured as the distance from the central part of the anterior end to the distal tip (non-fused bones) or, alternatively, the distance from the central part of the fusion line to the distal tip of the greater horn (fused bones).
CWI	Inferior width of the greater horn at its junction with the hyoid body.

Inter-observer reliability

Inter-observer reliability was assessed in a random subsample of 25 hyoid bones. Two observers independently (a forensic anthropologist and a forensic pathologist) recorded all variables following the same standardized protocol in sessions separated by two weeks. Agreement was evaluated using the

intraclass correlation coefficient (ICC), based on a two-way mixed-effects model for absolute agreement and single measurements, with 95% confidence intervals.

Sample size estimation

An a priori sample size estimation was performed in G*Power 3.1.9.3 for the multivariate comparison of hyoid measurements between sexes. Assuming two groups, six dependent variables, $\alpha = 0.05$, power = 0.80, and a medium multivariate effect size ($f^2(V) = 0.15$), the minimum required total sample size was estimated at 88 individuals. This calculation referred to the MANOVA component of the study and not to the predictive performance of the discriminant function, which was evaluated separately by internal cross-validation.

Statistical analysis

Statistical analyses were performed in IBM SPSS Statistics version 21.0. Two-tailed p-values < 0.05 were considered statistically significant.

Distributional properties were assessed separately within each sex group using the one-sample Kolmogorov–Smirnov test ($p > 0.05$). As no marked departures from normality were detected, parametric procedures were considered acceptable for the purposes of the study.

Right and left measurements were recorded independently for all bilateral variables. To assess potential asymmetry of the greater horns, paired-samples t-tests were performed for CWI, CHI, and CL. As no statistically significant side-to-side differences were observed, bilateral mean values were calculated and used in subsequent analyses and model development.

The sample was not stratified according to fusion status because the fused subgroup was relatively small (33/147), which would have generated sparse cells and unstable estimates in multivariate analyses and internal validation procedures.

Sex-related differences in hyoid dimensions were first assessed using a one-way multivariate analysis of variance (MANOVA), with sex (male/female) as the between-subject factor and BT, BL, BH, CWI, CHI, and CL as dependent variables. When the multivariate test was significant, follow-up univariate analyses of variance (ANOVAs) were performed for each variable.

A stepwise linear discriminant analysis (LDA) was then performed to derive a parsimonious sex-classification function from the hyoid osteometric variables. Variable selection followed Wilks' lambda criterion, with $F \geq 3.84$ for entry and $F \leq 2.71$ for removal. Model performance was assessed using the eigenvalue, canonical correlation, group centroids, standardized and unstandardized discriminant coefficients, and classification accuracy.

Before LDA, multivariate outliers were screened using Mahalanobis distances. The maximum observed Mahalanobis distance ($D^2 = 18.912$) did not exceed the critical χ^2 value for 6 degrees of freedom at $p = 0.001$ ($\chi^2 = 22.46$), indicating the absence of extreme multivariate outliers. Homogeneity of covariance matrices across sex groups was assessed using Box's M test. Given the sensitivity of this test to deviations from normality and unequal group sizes, its result was interpreted cautiously; the observed value ($p = 0.050$) was considered acceptable for proceeding with discriminant analysis. As a sensitivity analysis, the stepwise discriminant procedure was repeated after restricting the sample to non-fused hyoids only.

The overall significance of the discriminant function was evaluated using Wilks' lambda and chi-square statistics. Classification performance was assessed using both the original classification and leave-one-out cross-validation (LOOCV), to obtain an internal estimate of model stability and reduce the risk of overfitting. Intercorrelations among predictors were examined prior to discriminant modelling and were not considered high enough to preclude their inclusion in the multivariate procedures.

External validation of published discriminant functions

Previously published discriminant functions for sex estimation from the hyoid bone were applied to the present sample in order to assess their external performance (Table 3).

Table 3
Published discriminant functions selected for external validation and their reported original performance

Author	Function No.	Discriminant function	Specimen condition / applicability	Original reported accuracy (%) total	Male (%)	Female (%)
Kindschuh et al. [14]	1	$D = 0.210 \cdot BL + 0.416 \cdot BH + 0.498 \cdot CHI + 0.118 \cdot (A^*) - 13.047$	Unfused	84.3	80.5	89.1
	2		Unfused	84.3	80.5	89.1
	3	$D = 0.311 \cdot BL + 0.498 \cdot BH + 0.027 \cdot (A^*) - 12.850$	Body only	82.8	80.6	89.1
	4		Body only	82.8	80.6	89.1
	5	$D = 0.240 \cdot BL + 0.413 \cdot BH + 0.430 \cdot CHI - 13.006$	Unfused	85.2	82.2	89.1
	6	$D = 0.313 \cdot BL + 0.495 \cdot BH - 12.827$	Body only	82.8	80.6	85.4
D'Anastasio et al. [18]	1	$D = 0.681 \cdot BH + 0.651 \cdot ThMed - 10.440$	Body only	75.9	77.5	72.2
	2	$D = 0.598 \cdot BLMax - 13.522$	Body only	80.0	76.5	87.5
Logar et al. [12]	1	$D = 0.197 \cdot BL + 0.453 \cdot BH + 0.894 \cdot CHI - 0.636 \cdot CWI + 0.028 \cdot CL - 13.555$	Unfused	90.1	87.8	92.0
	2	$D = 0.160 \cdot BL + 0.511 \cdot BH + 0.729 \cdot CHI - 13.916$	Unfused	89.0	85.4	92.0
	3	$D = 0.279 \cdot BL + 0.624 \cdot BH - 13.497$	Unfused	89.0	87.8	90.0
Urbanová et al. [16]	1	$D = 0.24993 \cdot BH + 0.16218 \cdot BL + 0.61905 \cdot CHI + 0.08990 \cdot CI + 0.10756 \cdot BT - 13.97004$	Unfused	84.93	85.0	84.85
Komenda and Černý [19]	1	$D = 63.14 - 0.56 \cdot BH - 1.17 \cdot BL - 0.92 \cdot CL$	Unfused	95.20	NR	NR
	2	$D = 32.69 - 1.36 \cdot BL$	Body only	91.30	NR	NR
Torimitsu et al. [20]	1	$D = 0.383 \cdot BL - 9.730$	Unfused	86.1	85.0	87.1

Author	Function No.	Discriminant function	Specimen condition / applicability	Original reported accuracy (%) total	Male (%)	Female (%)
Tyagi et al. [21]	1	D = −7.909 + 0.225·BL + 0.302·BH	Unfused	75.1	76.3	73.3
Reesink et al. [22]	1	D = −8.51 + 0.56·MMH + 1.92·ATP + 2.46·MTD	Fused and unfused	**76**	**82**	**72**

Table note. BL, body length; BH, body height; BT, body thickness; CHI, greater horn height; CL, greater horn length; CWI, width of the greater horn at its junction with the body; NR, not reported separately by sex in the original publication.

* In the discriminant functions of Kindschuh et al. [14], A corresponds to the ancestry coding used in the original study, where A = 1 for African individuals and A = 2 for European individuals.

When alternative equations were available according to fusion status, preference was given to those developed for non-fused hyoids, because the fused subgroup in the present sample was too small to allow meaningful stratified comparison. Equations based exclusively on body measurements were also included when they were methodologically comparable with the variables recorded in this study.

Each function was applied according to the metric definitions and decision rules described in the corresponding publication. For each equation, the number and percentage of correctly classified males and females were calculated separately.

RESULTS

Inter-observer reliability

Inter-observer agreement was good to excellent across variables, with ICCs ranging from 0.762 to 0.996 (all $p < 0.001$) (Table 4). The highest reproducibility was observed for CWI (ICC = 0.996; 95% CI: 0.990–0.998), followed by CL-R (ICC = 0.963; 95% CI: 0.939 − 0.978), BH (ICC = 0.948; 95% CI: 0.922–0.965), CL-L (ICC = 0.933; 95% CI: 0.882–0.959), and BL (ICC = 0.931; 95% CI: 0.897–0.953). The lowest ICC was observed for BT (ICC = 0.762; 95% CI: 0.647–0.839).

Table 4
Intraclass Correlation Coefficients (ICC) for Interobserver Reproducibility of Hyoid Bone Measurements

Measurement	ICC	F value	95% CI (Lower)	95% CI (Upper)	p-value
BT	0.762	4.200	0.647	0.839	< 0.001
BL	0.931	14.449	0.897	0.953	< 0.001
BH	0.948	19.101	0.922	0.965	< 0.001
CHI-R	0.871	7.733	0.808	0.913	< 0.001
CHI-L	0.854	6.837	0.783	0.901	< 0.001
CL-R	0.963	27.394	0.939	0.978	< 0.001
CL-L	0.933	15.036	0.882	0.959	< 0.001
CWI	0.996	242.061	0.990	0.998	< 0.001

Descriptive statistics and sexual dimorphism

Descriptive statistics for the total sample are presented in Table 5, and sex-specific descriptive statistics are shown in Table 6. Mean values were higher in males than in females for all recorded variables. Paired-samples t-tests showed no statistically significant right–left differences for CWI ($t = 0.577$, $p = 0.565$), CHI ($t = 1.570$, $p = 0.118$), or CL ($t = -0.929$, $p = 0.354$). Bilateral mean values were therefore used in subsequent analyses. The highest percentages of sexual dimorphism were observed for BT (33.10%), CHI (25.21%), and CWI (24.60%).

Table 5
Descriptive statistics for the total sample

Measurement	Side	n	Mean	Median	SD	Range	Minimum	Maximum
BT	-	147	3.35	3.24	0.93	3.99	1.65	5.64
BL	-	147	22.97	23.09	3.23	17.07	14.66	31.73
BH	-	147	11.58	11.40	1.49	6.76	8.55	15.31
CWI	R	147	3.80	3.69	0.78	4.33	1.95	6.28
	L	147	3.74	3.68	0.71	3.54	2.01	5.55
	Mean	147	3.75	3.68	0.71	4.36	1.29	5.65
CHI	R	147	6.17	6.15	1.22	5.31	3.61	8.92
	L	147	6.01	5.92	1.30	6.94	3.03	9.97
	Mean	147	6.09	6.02	1.22	5.74	3.45	9.19
CL	R	147	28.68	29.35	4.10	26.02	13.26	39.28
	L	147	29.30	29.79	4.16	25.41	13.41	38.82
	Mean	147	29.11	29.48	3.81	25.49	13.34	38.83

MANOVA showed a significant multivariate effect of sex on the set of hyoid measurements (Wilks' $\Lambda = 0.312$; $F = 51.494$; $p < 0.001$). Follow-up univariate ANOVAs confirmed significant sex differences for all variables included in the analysis (BT, BL, BH, CWI, CHI, and CL; all $p < 0.001$) (Table 7).

Table 6
Sex-specific descriptive statistics for selected hyoid bone measurements (in mm)

Variable	Male, mean ± SD	n	Female, mean ± SD	n	p-value	% sexual dimorphism
BT	3.65 ± 0.91	92	2.85 ± 0.73	55	< 0.001	33.10
BL	24.34 ± 2.88	92	20.66 ± 2.35	55	< 0.001	20.72
BH	12.17 ± 1.44	92	10.59 ± 0.98	55	< 0.001	18.86
CWI	3.97 ± 0.69	92	3.40 ± 0.61	55	< 0.001	24.60
CHI	6.50 ± 1.16	92	5.39 ± 0.97	55	< 0.001	25.21
CL	30.38 ± 3.95	92	26.98 ± 2.34	55	< 0.001	17.94

SD: standard deviation. Sexual dimorphism was calculated as the percentage difference between male and female mean values.

Table 7
Follow-up univariate ANOVAs for hyoid measurements by sex

Variable	df1	df2	F	p-value
BT	1	145	46.887	< 0.001
BL	1	145	123.010	< 0.001
BH	1	145	102.692	< 0.001
CWI	1	145	89.156	< 0.001
CHI	1	145	57.406	< 0.001
CL	1	145	174.295	< 0.001

Discriminant function analysis

To assess the ability of hyoid measurements to discriminate between sexes, a linear discriminant analysis was performed using the osteometric variables as predictors. Prior to analysis, the assumptions for discriminant analysis were evaluated and considered acceptable, as described in the Methods section.

Tests of equality of group means showed that all variables contributed significantly to sex discrimination, with BL showing the highest discriminative power (Wilks' $\lambda = 0.694$, $F = 64.015$, $p < 0.001$), followed by BH, CHI, CL, BT, and CWI (Table 8).

Table 8
Tests of equality of group means (Wilks' lambda and F-statistics)

Variable	Wilks' Lambda	F-value	df1	df2	*p*-value
BT	0.826	30.619	1	145	< 0.001
BL	0.694	64.015	1	145	< 0.001
BH	0.735	52.226	1	145	< 0.001
CWI	0.853	24.928	1	145	< 0.001
CHI	0.805	35.073	1	145	< 0.001
CL	0.812	33.505	1	145	< 0.001

Stepwise discriminant analysis was then performed using Wilks' lambda as the selection criterion. Four variables were retained in the final model: BL, CHI, BT, and BH (Table 9). The discriminant function had an eigenvalue of 0.727, a canonical correlation of 0.649, and a Wilks' lambda of 0.579 ($\chi^2 = 78.138$, $df = 4$, $p < 0.001$).

Table 9
Stepwise discriminant analysis: variables entered by Wilks' lambda criterion

Step	Variable Entered	Wilks' Lambda	F-value	df1	df2	p-value
1	BL	0.694	64.015	1	145	< 0.001
2	CHI	0.630	42.321	2	144	< 0.001
3	BT	0.597	32.125	3	143	< 0.001
4	BH	0.579	25.811	4	142	< 0.001

Note: Entry criterion = F ≥ 3.84; Removal criterion = F ≤ 2.71.

The standardized canonical discriminant function coefficients were 0.465 for BL, 0.416 for CHI, 0.325 for BT, and 0.316 for BH. The unstandardized discriminant function was:

$$D = 0.383 \cdot BT + 0.172 \cdot BL + 0.245 \cdot BH + 0.380 \cdot CHI - 10.389$$

Positive scores were associated with male classification, whereas negative scores were associated with female classification. Group centroids were 0.655 for males and − 1.095 for females.

The overall classification accuracy of the discriminant function was 83.0% in both the original classification and leave-one-out cross-validation. The corresponding 95% confidence interval for overall accuracy was 76.1%–88.2%. Correct classification rates were 85.9% for males and 78.2% for females, yielding a balanced accuracy of 82.1% (Table 10).

Table 10
Classification results for the discriminant function in the original sample and leave-one-out cross-validation

Classification method	Actual sex	Predicted male	Predicted female	Total	Correctly classified (%)
Original	Male	79	13	92	85.9
	Female	12	43	55	78.2
	Overall			147	83.0
Cross-validated (LOOCV)	Male	79	13	92	85.9
	Female	12	43	55	78.2
	Overall			147	83.0

Table note. Overall accuracy was 83.0% (122/147; 95% CI: 76.1%–88.2%) in both the original and cross-validated classifications. Balanced accuracy was 82.1%.

As a sensitivity analysis, the stepwise discriminant procedure was repeated after restricting the sample to non-fused hyoids. The resulting model retained three variables (BT, BL, and CHI), whereas the full-sample model retained four (BT, BL, BH, and CHI). Discriminant performance remained broadly

comparable to that observed in the complete sample, with an eigenvalue of 0.701, a canonical correlation of 0.642, and a Wilks' lambda of 0.588 ($\chi^2 = 59.227$, $df = 3$, $p < 0.001$). Classification accuracy was 84.3% in the original sample and 83.5% after leave-one-out cross-validation, with correct classification rates of 87.3% and 85.9% for males, and 79.5% and 79.5% for females, respectively. These findings suggest that exclusion of fused specimens did not materially alter the overall pattern of sex discrimination.

External validation of published discriminant functions in the studied sample

The application of previously published discriminant functions to the present Spanish sample yielded heterogeneous results, with marked variation in performance across equations and frequent asymmetry between sexes (Table 11).

Table 11
Classification performance of published discriminant functions when applied to the present Spanish sample

Author	Function no.	Correctly classified males, n/N	Male (%)	Correctly classified females, n/N	Female (%)
Kindschuh et al. [14]	1	68/92	73.91	48/55	87.27
	2	67/92	72.83	46/55	83.64
	3	70/92	76.09	46/55	83.64
	4	67/92	72.83	50/55	90.91
	5	68/92	73.91	49/55	89.09
	6	73/92	79.35	46/55	83.64
D'Anastasio et al. [18]	1	60/92	65.22	50/56	89.29
	2	80/92	86.96	40/56	71.43
Logar et al. [12]	1	63/92	68.48	49/55	89.09
	2	68/92	73.91	50/55	90.91
	3	69/92	75.00	48/56	85.71
Urbanová et al. [16]	1	49/92	53.26	52/56	92.86
Komenda and Černý [19]	1	39/65	60.00	41/43	95.35
	2	41/92	44.57	51/56	91.07
Torimitsu et al. [20]	1	30/92	32.61	54/56	96.43
Tyagi et al. [21]	1	11/92	11.96	56/56	100.00
Reesink et al. [22]	1	92/92	100.00	0/56	0.00

Table note. Denominators vary across equations because not all published functions were applicable to the full study sample. Differences reflect equation-specific requirements regarding anatomical comparability, variable availability, and specimen condition.

Among the externally tested models, the equations of Kindschuh et al. [14] showed the most stable performance overall, although some variation was observed across equations. In the variants incorporating ancestry coding, differences between codings were present but not uniform. For the equation based on BL, BH, and CHI, the A = 1 variant yielded slightly higher classification rates than A = 2 in both males (73.91% vs 72.83%) and females (87.27% vs 83.64%). In contrast, for the body-only equation based on BL and BH, the A = 1 variant performed better in males (76.09% vs 72.83%), whereas the A = 2 variant performed better in females (90.91% vs 83.64%). These findings indicate that the

ancestry coding included in the original Kindschuh et al. [14] formulation affected external performance in the present sample, although without a consistent directional effect across equations or sexes. Particularly marked asymmetry was observed for the equations of Urbanová et al. [16], Torimitsu et al. [20], Tyagi et al. [21], and Reesink et al. [22], which showed strongly divergent classification rates between males and females. The most extreme results were obtained for the equation of Tyagi et al. [21], which correctly classified 11.96% of males and 100.00% of females, and for Reesink et al. [22], which correctly classified 100.00% of males and 0.00% of females.

DISCUSSION

The present study confirms the existence of sexual dimorphism in the hyoid bone in a contemporary sample from southern Spain and highlights the relevance of this structure as a complementary source for sex estimation when skeletal regions with greater diagnostic value are not available. In line with previous literature, mean values were higher in males than in females for all variables analysed, and both the multivariate analysis and the univariate tests showed statistically significant differences between the two sexes [16, 18, 20, 27, 31]. Taken together, these findings support the hypothesis that certain hyoid dimensions, particularly those related to the body and the greater horns, show sexual dimorphism from an osteometric perspective.

This finding is particularly relevant in an anatomical structure that rarely occupies a central place in biological profile reconstruction, yet has proved useful for sex estimation when other skeletal regions are unavailable or poorly preserved [3, 24, 25, 27]. The forensic usefulness of the hyoid does not depend solely on the existence of sexual dimorphism, but also on whether the variables employed can be clearly defined and measured with an acceptable degree of reproducibility. In our series, inter-observer agreement was generally good to excellent, supporting the consistency of the measurement protocol used. Likewise, the absence of significant right–left differences in the paired variables justifies the use of bilateral averages and places our results within a methodological framework consistent with previous literature [16].

On this basis, discriminant analysis made it possible to obtain a parsimonious function composed of four variables (BL, CHI, BT, and BH). This result suggests that sexual differentiation of the hyoid does not depend on a single isolated dimension, but rather on the combination of measurements from both the body and the greater horns. The overall classification accuracy of the discriminant function was 83.0% in both the original classification and leave-one-out cross-validation. The corresponding 95% confidence interval for overall accuracy ranged from 76.1% to 88.2%. Correct classification rates were 85.9% in males and 78.2% in females, yielding a balanced accuracy of 82.1% (Table 10). This level of accuracy falls within the range reported in the literature for discriminant functions applied to the hyoid and supports the potential practical value of the model in contexts comparable to that of the analysed sample [3, 26]. However, these results should be interpreted with caution. The better performance observed in males may be influenced, at least in part, by the imbalance between the two groups in the sample, whereas the agreement between the original classification and leave-one-out cross-validation

should be understood as an indicator of internal stability rather than proof of external validity. Accordingly, the proposed function should be regarded as specific to the population analysed and potentially useful in comparable settings, although its broader forensic applicability will need to be confirmed through evaluation in independent samples.

One of the most relevant contributions of the present study lies not only in the construction of a local discriminant function, but also in the evaluation of the transferability of previously published equations when applied to a contemporary sample from southern Spain. Overall, our results show that such transferability is limited and often asymmetric between the sexes. The literature has consistently shown that interpopulation differences in hyoid morphology and morphometry can substantially affect the performance of sex-estimation models [12, 14, 16, 18, 24, 26, 31]. This caution remains pertinent even in recent studies which, despite reporting high levels of accuracy, still emphasize that their models should be regarded as population-specific and require external validation before being extrapolated to other series [26].

Within the set of external models analysed, the equations of Kindschuh et al. [14] showed the most stable performance when applied to our sample, although some variability was observed across equations. This pattern may be related, at least in part, to the relative descriptive similarity between the two series in variables such as BL, BH, and CL, which in our sample show values close to those reported for the Terry Anatomical Collection. By contrast, other dimensions, such as CHI and CWI, show more noticeable differences, which may have contributed to the partial loss of accuracy and to the classification bias observed when these equations were transferred to our material. It should also be noted that some of the original equations of Kindschuh et al. [14] include ancestry coding in their formulation, and that in our study both original variants were applied. The results show that this coding did not have a uniform effect: in the equation based on BL, BH, and CHI, the A = 1 variant produced slightly better performance in both sexes, whereas in the equation based only on BL and BH the pattern was divergent, with better male performance for A = 1 and better female performance for A = 2. This finding suggests that the ancestry coding built into the original model may modify the external behaviour of the function when applied to a different sample, but does not by itself allow a direct correspondence to be inferred between that coding and the population composition of our series.

A partly similar pattern was observed in Logar et al. [12], whose functions showed moderate performance, with better results in females than in males. In this case as well, the relative similarity in variables such as BL, BH, and CHI may favour a certain degree of comparability between the two samples, whereas discrepancies in CWI and, above all, in CL suggest differences in the development of the greater horns that may limit full transferability of the model. Likewise, the function of Urbanová et al. [16] showed marked asymmetry when applied to our series. Although some body variables, such as BL and BH, remain relatively similar between the two populations, the systematically lower values in our sample for CHI, CL, and BT may have shifted the function towards a preferential assignment to the female sex. In D'Anastasio et al. [18], which focused exclusively on metric variables of the hyoid body in an Italian archaeological sample, the behaviour of the equations depended more strongly on the variable

used. The function based on BLMax showed a relatively better fit to our sample than the one incorporating BH and ThMed, suggesting partial comparability in certain body dimensions, but not a fully balanced transfer of the model. Taken together, these findings indicate that the transfer of equations across populations does not guarantee equivalent performance, even when the variables employed are anatomically comparable and the original models showed good levels of accuracy in their respective samples.

The most extreme results were observed in the equations of Komenda and Černý [19], Torimitsu et al. [20], Tyagi et al. [21], and Reesink et al. [22]. In the case of Komenda and Černý [19], this behaviour may be related to the strong dependence on very specific linear variables and to the difficulty of transferring their cut-off points to a different sample. In Torimitsu et al. [20], population differences and the fact that the measurements were derived from computed tomography may have contributed to the poor performance observed in males. The equation of Tyagi et al. [21] is particularly illustrative because, despite using anatomically comparable variables, it showed extreme asymmetry, suggesting that the joint distribution of BL and BH and the cut-off point derived from the Indian sample are not directly transferable to our series. Conversely, Reesink et al. [22] showed the opposite pattern, with complete classification in males and none in females, a result that probably reflects the combination of a small original sample, variables that are not strictly equivalent, and a radiography-based methodology. In general terms, this pattern confirms that the usefulness of a discriminant function depends to a large extent on the population on which it was developed and the population to which it is subsequently applied.

The explanation for this limited transferability is probably not attributable to a single cause. On the one hand, it is reasonable to assume that interpopulation and secular differences in hyoid morphometry alter the position of the cut-off point when an equation is transferred to another sample. This issue is particularly relevant in the hyoid, since its small size means that apparently minor mean differences, on the order of 1–2 mm, may carry considerable relative weight and substantially shift the cut-off point of a discriminant function. Unlike what occurs in larger bones, such variations in the hyoid may translate into meaningful changes in the final classification.

On the other hand, comparability across studies is hindered by the lack of universal standardization of osteometric variables. Not all measurements with similar names are strictly equivalent from an anatomical or metric point of view, and heterogeneity increases further when studies based on radiographs [22], digital photographs [28, 29], computed tomography [20, 26, 30], and direct measurements on dry bone [12, 14, 18, 27, 32] are considered together. In addition, published samples are not always comparable in terms of fusion status, skeletal integrity, or the type of variables used. Consequently, the external validation of previously published equations should not be interpreted only as a comparison between populations, but also between measurement protocols and distinct methodological frameworks.

From a methodological standpoint, our results highlight that global accuracy, considered in isolation, may provide an incomplete picture of the true performance of a discriminant function, especially when the classifier behaves very differently in males and females. Several external equations showed apparently acceptable performance in one group but clearly deficient performance in the other. For this reason, the evaluation of sex-specific behaviour should be regarded as a minimum requirement in studies of sex estimation based on the hyoid and, more broadly, in any discriminant proposal intended for real forensic contexts.

Despite the interest of these findings, the study has limitations that should be acknowledged explicitly. The sample derives from a specific population and therefore does not necessarily represent the full variability of the contemporary Spanish population. Moreover, although the sample size was sufficient for the objectives of the study according to the a priori power analysis, there is some imbalance between the number of males and females. This circumstance, which is common in medico-legal autopsy series for epidemiological reasons, may influence classifier behaviour and contribute to the better performance observed in males. This possibility requires the overall accuracy of the model to be interpreted with caution and reinforces the usefulness of accompanying it with metrics that are less sensitive to class imbalance, such as balanced accuracy.

The analysis was also not stratified by fusion status, owing to the small size of the fused subgroup, although this decision introduces a potential source of metric heterogeneity that should be taken into account. Some studies have suggested that fusion status may influence the behaviour of certain osteometric variables [20, 21], whereas others have shown that some measurements retain discriminative value across different degrees of fusion [12, 14]. In our case, the combined inclusion of both types of specimen allowed us to make use of the available sample size, but it did not entirely rule out the possibility that fusion status may have influenced variable selection or classifier performance. However, the sensitivity analysis restricted to non-fused hyoids showed a discriminant structure broadly comparable to that obtained in the full sample. The main discriminating variables remained largely the same, and the differences observed in eigenvalue, canonical correlation, Wilks' lambda, and classification rates were small. Taken together, these findings suggest that the inclusion of fused hyoids did not substantially alter the overall pattern of sex discrimination in the series analysed. On this basis, the function derived from the complete sample was retained as the main model, since it makes use of the largest available dataset and is therefore likely to provide more stable estimates. Even so, the sensitivity analysis remains relevant because it shows that the main findings do not depend exclusively on the inclusion of fused specimens.

Taken together, the data obtained suggest that sex estimation from the hyoid bone may clearly benefit from models developed and evaluated in the reference population itself. At the same time, they show that the uncalibrated application of equations derived from other series may introduce substantial bias, especially when performance is interpreted solely on the basis of overall accuracy. Accordingly, in forensic contexts comparable to the one analysed here, the proposed function should be understood as

a complementary tool with potential practical value, whose applicability will need to be confirmed through external validation in independent and methodologically comparable samples.

CONCLUSIONS

The present study confirms the existence of sexual dimorphism in the hyoid bone in a contemporary sample from southern Spain and shows that hyoid osteometrics can provide useful complementary information for sex estimation. The population-specific discriminant function developed in this study showed acceptable internal performance, but its forensic applicability should still be regarded as provisional until externally validated in independent samples. The heterogeneous and frequently sex-asymmetric behaviour of previously published equations in our series reinforces the need for population-specific and methodologically comparable standards in hyoid-based sex estimation. Accordingly, the hyoid should be considered a complementary skeletal element whose forensic value depends on locally derived and externally validated models.

Declarations

Conflict of interest

The authors declare that they have no competing interests.

Ethics approval

This study was approved by the Research Ethics Committee of Córdoba (Committee reference: 5676; approval granted on 25 July 2023, Act No. 351) and was conducted in accordance with the applicable Spanish legal framework and the ethical principles set out in the Declaration of Helsinki. In addition, the reporting of the study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

Informed consent

The study was conducted on biological material obtained from deceased individuals during medico-legal autopsies. Informed consent requirements were addressed in accordance with the protocol approved by the Research Ethics Committee of Córdoba and the applicable Spanish legal and ethical framework.

Funding

The authors received support for open access charge from University of Córdoba/CBUA and Universidad LOYOLA Andalusia.

Author Contribution

A.T.M: Conceptualization, methodology, writing – original draft, supervision, corresponding author.C.M.B.A: Conceptualization, data curation, methodology, writing – review and editing, supervision.J.F.G.V: Formal analysis, methodology, writing – review and editing.E.B.A: Writing – review and editing.E.G.L: Writing – review and editing, supervision.

Acknowledgement

The authors thank the Institute of Legal Medicine of Córdoba (Spain) for access to the study material and the staff involved in the medico-legal management of the cases.

Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to ethical and legal restrictions related to medico-legal autopsy material and participant confidentiality, but are available from the corresponding author on reasonable request and with permission from the relevant ethics committee and the Institute of Legal Medicine of Córdoba.

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