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Sex estimation based on the anthropometric measurements of thyroid cartilage using discriminant analysis

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Abstract

Background: The morphometric analysis of the individual bones of the human skeleton can be used to estimate the sex of unidentified corpses. Our aims were as follows: to test whether thyroid cartilage can be used for forensic purposes as a predictor of biological sex; to establish the level of sexual dimorphism of the thyroid cartilage in a sample of adult subjects from a population of European Russia; and to test the accuracy of the morphometric parameters obtained from the thyroid cartilage.

Results: The thyroid cartilage from 100 adults of known age (50 males and 50 females) was obtained during forensic examination; morphometric tests were conducted using Vernier Digital ROKTOOLS ABS DIN 862 0-200/6 inch with measurement accuracy ± 0.01 mm. The measured parameters were $N = 31$ for each subject. Intra- and inter-observer reproducibility was tested. Multivariate statistical analysis was applied to the measurements. To check the data set for normal distribution, the Kolmogorov-Smirnov test was used. Finally, to estimate the sex of the observed individuals, a stepwise discriminant analysis was conducted, using the Wilks' lambda selection method. The most significant parameters were the outer distance between bases of inferior horn; the inner distance between distal ends of inferior horns; distance between distal ends of left superior and inferior horns; left superior horn length (distance between left superior horn distal end and base); distance between superior and inferior notches; thyroid angle; left lamina height (vertical line along left lamina middle); horizontal distance between anterior intermedium line and the right lamina posterior edge; distance between inferior thyroid notch and line connecting left and right thyroid laminae; and left superior horn thickness at mid-line. The stepwise discriminant analysis resulted in an equation with ten parameters.

Conclusions: The results of the current study indicated that in the European Russian population, the equation obtained in the stepwise discriminant analysis makes it possible to predict sex with a probability of 100% on the validation set. On the test set, the resultant accuracy was 100% for females and 100% for males. Our findings confirm the scientific evidence that the thyroid cartilage has a pronounced sexual dimorphism.

Keywords: Forensic anthropology, Human identification, Population data, Sex estimation, Thyroid cartilage, Russia

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Background

Sex estimation is essential for completing an individual's biological profile (Krishan et al. 2016; Asadujjaman et al. 2019). Assessing sex is an important part of the human identification process in the forensic field. Almost all elements of the human skeleton are characterized by sexual dimorphism to varying degrees and with different levels of reliability (Gonçalves et al. 2013; Dawson et al. 2011). In most cases, the pelvis (Torimitsu et al. 2017), skull (Manjunath et al. 2017), and long bones (Akhlaghi et al. 2012; Siddapur 2017) are used to estimate sex. If these bones are missing (fragmentation due to purposeful dismemberment, high-impact trauma, exposure to adverse taphonomic conditions, or the use of poor recovery protocols) or significantly destroyed, assessing sex may be difficult (Lovell 2007). In these contexts, it is necessary to use other elements of the skeleton, such as the thyroid cartilage, if it is preserved. The thyroid cartilage is the largest cartilage of the human larynx. It is usually easy to be identified, quite resistant to postmortem changes, and, if intact, might show sexual dimorphism (Kaur et al. 2015; Kovac et al. 2010; Patel et al. 2016; Monica and Usha 2010). Morphometric characteristics of the thyroid cartilage in adults differ depending on ethnic group and region of residence (Monica and Usha 2010).

In this research, the anthropometric characteristics and sexual differences of the thyroid cartilage in an adult population from European Russia were studied. The aims were as follows: to test whether thyroid cartilage can be used for forensic purposes as a predictor of biological sex; to assess the level of sexual dimorphism of the thyroid cartilage in the sample; to test the accuracy of the morphometric parameters obtained from the thyroid cartilage; and to develop equations using discriminant analysis that take into account the group characteristics of this population.

Methods

The study was conducted during the forensic examination of corpses in cases where an identity document was available. No more than 48 h had elapsed since the time of death and discovery of the body. The circumstances of death were established according to the protocol of examination of the corpse during the autopsy.

The research adhered to a standard protocol, which included the registration of the forensic autopsy number, the date of death and autopsy, and the cause of death.

The analysis was conducted on the thyroid cartilage obtained from 100 corpses of males and females, aged between 20 and 93 years: 50 males (with a mean age of 49.74 ± 19.87 years) and 50 females (with a mean age of 52.46 ± 20.51 years). All thyroid cartilages included in the study were perfectly intact. Thyroid cartilages of subjects over 60 years were partially ossified. The distribution of the sample by age group at the stage of anthropometry is presented in Table 1.

Exclusion criteria were fractures or trauma of the thyroid cartilage, neck injuries, burns, repair and reconstruction due to cancer resection, stenosis, developmental disruptions, and acquired or congenital anomalies. All the larynges were removed along with the hyoid bone and trachea, up to the third tracheal ring. The muscles and ligaments attached to the larynx were also carefully removed before the thyroid cartilage was fixed in a 5% formaldehyde solution for 1–2 days (Order of the Ministry of health and social development of the Russian Federation of May 12, 2010 N 346n "on approval of the Procedure for organizing and conducting forensic medical examinations in state forensic institutions of the Russian Federation"). Then, a soft tissue sample was prepared, layer by layer, from the detached perichondrium. This procedure was followed by an anatomo-morphometric study. For each cartilage, 30 linear measurements were performed (Table 2) using a digital caliper with 0.03 mm precision (Electronic Digital Caliper DIN 862 0-200/6, with measurement accuracy ± 0.1 mm/0.01). The thyroid angle was determined with an optical goniometer with 0.08° accuracy. The measurements were taken with the help of Digital Vernier Caliper to the nearest 0.01 mm (Electronic Digital Caliper DIN 862 0-200/6, ± 0.1 mm/0.01). The cartilages were weighed on Single Pan Electronic Balance (Shimadzu BL series 2204; sensitive to 0.01 g).

The choice of points for anthropometric measurements was based on the main anatomical landmarks of the thyroid cartilage. Variables or parameters were chosen to reflect features of the laryngotracheal cartilage in general and of the thyroid cartilage mineralization in particular. This last structure showed distinct sexual differences in ossification (Sabnis and Mane 2020).

Figure 1 shows the contours of the thyroid cartilage, indicating the measurement points. The measured parameters ($n = 31$) are listed in Table 2.

Statistical analysis

Intra- and inter-observer reproducibility was assessed by calculating technical error of measurement (TEM), relative technical error of measurement (rTEM), and coefficient of reliability (R). Research and measurements were

Table 1 Distribution of sample by age group

| Age group (years) | Number of observations | | | |
| | Male (mean 49.74 ± 19.87) | | Female (mean 52.46 ± 20.51) | |
	N	%	N	%
20–35	12	24	11	22
36–60	25	50	22	44
Over 61	13	26	17	34
Total	50	100	50	100

Table 2 Thyroid cartilage measurements

Number	Variables	Measurement
1	AA OUTER	Outer distance between distal ends of superior horns
2	AA INNER	Inner distance between distal ends of superior horns
3	BB OUTER	Outer distance between bases of superior horns
4	BB INNER	Inner distance between bases of superior horns
5	CC OUTER	Outer distance between bases of inferior horns
6	CC INNER	Inner distance between bases of inferior horns
7	DD OUTER	Outer distance between distal ends of inferior horns
8	DD INNER	Inner distance between distal ends of inferior horns
9	AD DEX	Distance between distal ends of right superior and inferior horns
10	AD SIN	Distance between distal ends of left superior and inferior horns
11	AB DEX	Right superior horn length (distance between right superior horn distal end and base)
12	AB SIN	Left superior horn length (distance between left superior horn distal end and base)
13	CD DEX	Right inferior horn length (distance between right inferior horn distal end and base)
14	CD SIN	Left inferior horn length (distance between left inferior horn distal end and base)
15	BE DEX	Distance between superior point of the anterior intermedium line and base of right superior horn
16	BE SIN	Distance between superior point of the anterior intermedium line and base of left superior horn
17	CF DEX	Distance between inferior point of the anterior intermedium line and base of right superior horn
18	CF SIN	Distance between inferior point of the anterior intermedium line and base of left superior horn
19	EF	Distance between superior and inferior notches
20	GH DEX	Right lamina height (vertical line along right lamina middle)
21	GH SIN	Left lamina height (vertical line along left lamina middle)
22	KL	Horizontal distance between anterior intermedium line and the right lamina posterior edge
23	MN	Horizontal distance between anterior intermedium line and left lamina posterior edge
24	DEX thickness	Right lamina thickness along the mid-line
25	SIN thickness	Left lamina thickness along the mid-line
26	FP	Distance between inferior thyroid notch and line connecting left and right thyroid laminae
27	Q	Right superior horn thickness at mid-line
28	R	Left superior horn thickness at mid-line
29	S	Right inferior horn thickness at mid-line
30	T	Left inferior horn thickness at mid-line
31	α	Thyroid angle prior to cricothyroid joint dissection and thyroid cartilage separation from other laryngeal sections

carried out by a forensic medical expert (1 researcher), has a special training in medical criminology, 21 years of work experience, and an anthropologist (2 researchers), 15 years of work experience. The time interval between two measurements by the same observer was at least 15 days. The second researcher analyzed 25% of randomly selected thyroid cartilage, 3 weeks after the first. To validate, derivative function was used to test dataset that did not participate in the construction of the discriminant function. Test dataset consisted of males and females, aged between 22 and 94 years: 5 males (with a mean age of 56.80 ± 32.81 years) and 5 females (with a mean age of 51.00 ± 17.29 years). To check the data set for normal distribution, the Kolmogorov-Smirnov test was used.

To estimate the sex of the observed individuals, a stepwise discriminant analysis (Renjith et al. 2019) was conducted, using the Wilks' lambda selection method (Renjith et al. 2019). The discriminating function was constructed on a sample of 100 subjects. To validate the correctness of the constructed equation, additional testing was performed on an independent sample of 10 subjects. A multivariate statistical analysis was conducted using Statistica 13.5.0 software (TIBCO 2018). The basic values (mean, standard deviation, and spread) were calculated for all variables significant for sex estimation.
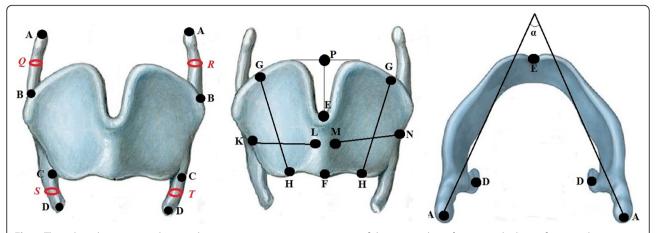
Fig. 1 Thyroid cartilage contour showing the main measurement points. **a**-apex of the superior horn; **b**-point at the base of superior horn; **c**-point at the base of inferior horn; **d**-apex of inferior horns; E-superior point of anterior intermedium line; **f**-inferior point of the anterior intermedium line; **g**-superior point of lamina middle line; **h**-inferior point of lamina middle line; **k**-point in the middle of right lamina's posterior edge; **l**-point in the middle of right lamina's anterior edge; **m**-point in the middle of left lamina's anterior edge; **n**-point in the middle of left lamina's posterior edge; P-middle of the line connecting the upper edge of the left and right thyroid plates; Q-middle of the right upper horn; R-middle of the left upper horn; S-middle of the right lower horn; T-middle of the left lower horn; α-thyroid angle prior to cricothyroid joint dissection and thyroid cartilage separation from other laryngeal parts

Finally, discriminant analysis was applied to estimate sex by relying on osteometric parameters.

Results

Estimations of inter- and intra-observer error using TEM, rTEM, and R are listed in Table 3. The rTEMs for intra-observer and inter-observer errors ranged from 0.637 to 2.365% and from 1.037 to 2.759%, respectively. The R values ranged from 0.986 to 0.999 for intra-observer and ranged from 0.981 to 0.999 for inter-observer.

According to the Kolmogorov-Smirnov test results, only three parameters had a normal distribution: AA OUTER (p value 0.200), AA INNER (p value 0.134), BB INNER (p value 0.200). Box-Cox transformations were applied to transform non-normal dependent variables into a normal shape. For all parameters was found the lambda that maximizes the log-likelihood function (Table 4).

For all parameters, except for the thyroid angle, differences between males and females were statistically significant; visual differences between the parameters were clearly distinguishable. In all cases, the superior horns of the thyroid cartilage were larger than the inferior ones: 2.2 times longer in males and 2.08 times longer in females. The thyroid angle was significantly greater in females and varied between 88°–101° compared to 66°–93° in males. The distance between the distal ends of the upper horns and the base of the upper horns in males was 4.15 ± 0.72 cm and 4.46 ± 0.64 cm, in females—3.79 ± 0.60 cm and 3.82 ± 0.37 cm. The distance between the distal ends of the lower horns in males was 3.88 ± 0.46 cm and the base of the lower horns was 3.63 ± 0.49 cm. For the female sample, these values corresponded to

3.18 ± 0.20 cm and 2.87 ± 0.24 s. That is, for both sexes, the following structural feature was observed: in the upper horns, the distance between the distal ends was less than the distance at the base of the horns; for the lower horns, on the contrary, the distance at the base of the horns prevailed over the distance of their tops. The height of the laminae of the thyroid cartilage in all cases was less than their width, with a ratio of 1:1.37 for the right side and 1:1.34 for the left side in males, and 1:1.32 and 1:1.28, respectively, in females.

The average width of the right and left plates of the thyroid cartilage in the male sample was 3.81 ± 0.23 and 3.86 ± 0.20; the height of the plate had the following values: 2.77 ± 0.19 and 2.86 ± 0.17. In females, these indicators had a lower value: the average value of the plate height was 2.02 ± 0.20 on the right and 2.14 ± 0.20 on the left, the plate width was 2.37 ± 0.20 and 2.74 ± 0.17, respectively.

The data set consisted of observations made on 50 male objects and 50 female subjects. Each subject was characterized by 31 variables. Table 4 lists the main values (mean, standard deviation, and spread) for all variables relating to sex.

All variables showed significant differences between the two groups separated by sex. Maximum and minimum values, mean values, standard deviation, skewness, and kurtosis were calculated (Table 5).

A strong correlation was found between the metric parameters and sex (Table 6).

The most significant parameters were the horizontal distance between the anterior intermedium line and the posterior edges of the left and right laminae; the distance

Table 3 The technical error of measurement (TEM), relative technical error of measurement (rTEM) and the coefficient of reliability (R) (n = 25)

Variable	Intra-observer error			Inter-observer error		
	TEM	**rTEM**	**R**	**TEM**	**rTEM**	**R**
AA OUTER	0.033	0.831	0.998	0.057	1.436	0.993
AA INNER	0.025	0.698	0.999	0.046	1.284	0.995
BB OUTER	0.032	0.771	0.997	0.059	1.422	0.991
BB INNER	0.029	0.762	0.998	0.05	1.314	0.994
CC OUTER	0.029	0.821	0.997	0.049	1.387	0.99
CC INNER	0.024	0.802	0.998	0.039	1.304	0.994
DD OUTER	0.027	0.831	0.998	0.045	1.385	0.993
DD INNER	0.025	0.874	0.998	0.037	1.293	0.995
AD DEX	0.032	0.807	0.997	0.058	1.463	0.99
AD SIN	0.031	0.785	0.997	0.06	1.52	0.988
AB DEX	0.011	0.751	0.998	0.023	1.569	0.992
AB SIN	0.013	0.902	0.998	0.023	1.595	0.994
CD DEX	0.005	0.741	0.999	0.007	1.037	0.999
CD SIN	0.004	0.637	0.999	0.009	1.434	0.997
BE DEX	0.028	0.784	0.998	0.046	1.288	0.994
BE SIN	0.028	0.787	0.998	0.048	1.349	0.993
CF DEX	0.025	0.839	0.994	0.041	1.376	0.985
CF SIN	0.025	0.838	0.995	0.042	1.407	0.986
EF	0.015	0.947	0.998	0.022	1.389	0.996
Angle (α)	0.628	0.724	0.996	1.167	1.345	0.985
GH DEX	0.023	0.961	0.997	0.036	1.504	0.993
GH SIN	0.022	0.879	0.997	0.034	1.359	0.993
KL	0.029	0.894	0.998	0.042	1.295	0.995
MN	0.028	0.846	0.998	0.049	1.481	0.993
DEX thickness	0.006	2.365	0.986	0.007	2.759	0.981
SIN thickness	0.006	2.277	0.988	0.007	2.656	0.984
FP	0.019	0.819	0.998	0.031	1.337	0.994
Q	0.005	1.705	0.993	0.006	2.046	0.99
R	0.006	2.029	0.989	0.007	2.368	0.984
S	0.006	2.099	0.99	0.007	2.449	0.986
T	0.006	2.106	0.991	0.007	2.457	0.988

between the superior point of the anterior intermedium line and the base of the left and right superior horns; lamina height; thyroid angle; the distance between the superior and inferior horns ($r = 0.9$); the distance between the distal ends of the right superior and inferior horns; and the inner distance between the distal ends of the inferior horns ($r = 0.7$).

The key tasks of discriminant analysis are to develop the decision rules for classifying a subject into a group and to give the weight of each variable for distributing subjects into specific groups. These tasks require the calculation of an appropriate discriminant function (d):

$$d = b_1 x_1 + b_2 x_2 + \ldots + b_n x_n + a,$$

where x_n is the value of the variable corresponding to the case under consideration;

b_n is the estimated coefficient of discriminant analysis.

One of the subtasks of discriminant analysis is to determine the list of necessary variables. With a small number of variables, a forced inclusion method can be applied to calculate the discriminant function for all the variables at once, or each variable may be analyzed separately. An alternative option is stepwise discriminant analysis, where the variables are entered sequentially one after another, depending on their capability to distinguish between groups. The results of the univariate and stepwise discriminant analyses of the anatomic measurements of the thyroid cartilages are presented in Table 7.

One of the main parameters in discriminant analysis is Wilks' coefficient, which indicates whether the mean values of the discriminant function in the groups of the data set are significantly different. This value is associated with the F value involved in stepwise discriminant analysis. If $F > 3.84$, the predictor is included in the regression equation, but if $F < 2.71$, it is excluded. The distance between the centroids of the groups characterizes the completeness of separation: the longer the distance, the more complete the separation between the groups. The sectioning points are all zero, that means that the value greater than the sectioning point is classified as a male, and a value less than the sectioning point is classified as a female.

The quality of the forecasting model is characterized by the classification accuracy of the subjects and is defined as the ratio between correctly classified subjects and incorrectly classified ones. The results of the univariate analysis allow each parameter to be evaluated separately. If necessary, combinations of several parameters may be tested with the greatest accuracy. Table 6 presents the results for each parameter. Several parameters demonstrated a higher percentage of correct predictions, and prediction accuracy was particularly high for the following parameters: BE DEX, BE SIN, GH DEX, KL, and MN (99% for both males and females); GH SIN (98%); and EF (97%).

The stepwise discriminant analysis resulted in an equation with ten parameters, involving CC OUTER, DD INNER, AD SIN, AB SIN, EF, ANGLE, GH SIN, KL, FP, and R.

The developed equation was – 22,949 (CC OUTER) + 15,596 (DD INNER) – 3198 (AD SIN) + 3452 (AB SIN) + 4004 (EF) – 0.0001 (Angle) + 3581 (GH SIN) + 10,633 (KL) + 6100 (FP) + 1545 (R) – 5317.

Table 4 Description of the variables collected. *P* indicates the significance of differences in variables in the two groups based on the test of equality of group averages; lambda indicates parameters for Box-Cox transformation

Variable	Males (*n* = 50)		Females (*n* = 50)		*p*	lambda
	Mean ± SD	Range	Mean ± SD	Range		
AGE	49.74 ± 19.87	20–93	52.46 ± 20.51	20–93	0.502	–
AA OUTER	4.15 ± 0.72	2.6–6.4	3.79 ± 0.60	2.7–4.9	0.007	− 0.153
AA INNER	3.78 ± 0.65	2.9–5.5	3.37 ± 0.64	2.5–4.5	0.002	− 0.083
BB OUTER	4.46 ± 0.64	3.1–6.1	3.82 ± 0.37	3.2–4.5	< 0.001	− 1.105
BB INNER	4.07 ± 0.64	2.4–5.6	3.52 ± 0.54	2.5–4.6	< 0.001	0.626
CC OUTER	3.88 ± 0.46	2.7–5	3.18 ± 0.20	2.8–3.6	< 0.001	− 1.232
CC INNER	3.36 ± 0.44	2.3–4.5	2.61 ± 0.11	2.4–2.9	< 0.001	− 2.356
DD OUTER	3.63 ± 0.49	2.4–4.6	2.87 ± 0.24	2.5–3.7	< 0.001	− 1.189
DD INNER	3.31 ± 0.40	2.4–4.2	2.42 ± 0.18	2.1–2.8	< 0.001	− 0.993
AD DEX	4.44 ± 0.28	3.8–5	3.48 ± 0.38	3.0–4.7	< 0.001	0.951
AD SIN	4.38 ± 0.33	3.6–5.2	3.52 ± 0.36	3.1–4.7	< 0.001	0.061
AB DEX	1.66 ± 0.20	1.1–2.1	1.27 ± 0.15	1.0–1.7	< 0.001	− 0.151
AB SIN	1.60 ± 0.28	0.9–2.3	1.27 ± 0.18	0.9–2.0	< 0.001	− 0.636
CD DEX	0.74 ± 0.21	0.4–1.3	0.61 ± 0.13	0.3–0.9	< 0.001	− 0.107
CD SIN	0.72 ± 0.16	0.4–1	0.53 ± 0.14	0.3–0.9	< 0.001	0.434
BE DEX	4.13 ± 0.31	2.8–4.6	3.01 ± 0.17	2.6–3.5	< 0.001	− 0.158
BE SIN	4.11 ± 0.24	3.7–4.6	3.00 ± 0.17	2.6–3.6	< 0.001	− 0.049
CF DEX	3.19 ± 0.31	2.5–3.7	2.77 ± 0.22	2.3–3.3	< 0.001	− 0.121
CF SIN	3.19 ± 0.30	2.5–3.7	2.77 ± 0.27	2.3–3.9	< 0.001	− 0.293
EF	1.88 ± 0.19	1.6–2.8	1.28 ± 0.14	1.0–1.6	< 0.001	0.083
Angle (α)	78.36 ± 5.54	66–93	94.95 ± 2.73	88–101	< 0.001	2.637
GH DEX	2.77 ± 0.19	2.4–3.1	2.02 ± 0.20	1.6–2.8	< 0.001	0.740
GH SIN	2.86 ± 0.17	2.5–3.2	2.14 ± 0.20	1.8–3.1	< 0.001	0.381
KL	3.81 ± 0.23	3.3–4.3	2.67 ± 0.20	2.4–3.6	< 0.001	0.026
MN	3.86 ± 0.20	3.3–4.3	2.74 ± 0.17	2.5–3.6	< 0.001	− 0.012
DEX thickness	0.29 ± 0.04	0.2–0.35	0.22 ± 0.03	0.1–0.3	< 0.001	0.807
SIN thickness	0.30 ± 0.05	0.2–0.36	0.23 ± 0.04	0.1–0.3	< 0.001	0.727
FP	2.63 ± 0.33	1.8–3.2	2.00 ± 0.16	1.5–2.5	< 0.001	− 0.712
Q	0.33 ± 0.05	0.25–0.45	0.26 ± 0.04	0.2–0.4	< 0.001	− 0.054
R	0.33 ± 0.05	0.2–0.45	0.26 ± 0.04	0.2–0.35	< 0.001	0.203
S	0.31 ± 0.05	0.2–0.45	0.26 ± 0.06	0.1–0.4	< 0.001	0.940
T	0.31 ± 0.06	0.2–0.6	0.26 ± 0.05	0.1–0.4	< 0.001	0.311

Age is expressed in years, measurements in cm

The remaining 21 parameters were removed from the model, either because they did not provide sufficient information for classification into groups, or because they showed a strong correlation with other parameters and would therefore fail to improve classification accuracy. Table 6 demonstrates that the stepwise discriminant analysis allows for the prediction of sex with 100% probability. "Leave-one-out classification" cross-validation method was used; the results are presented in Table 8.

In cross validation, each case is classified by the functions derived from all cases other than that case. One hundred percent of original grouped cases and 100% of cross-validated grouped cases have been correctly classified. The coefficients of the discriminant function obtained using stepwise discriminant analysis and the combination based on them were tested on an independent sample of 10 individuals. As a result, for 10 individuals, the sex was estimated correctly.

Table 5 The statistical parameters of angles and sizes, $N = 100$ (cm)

Parameter	Min	Max	Mean	Std.Error	Std.Dev.	Skewness	Kurtosis
AA OUTER	2.6	6.4	3.968	0.068	0.682	0.698	1.334
AA INNER	2.5	5.5	3.575	0.067	0.674	0.457	− 0.087
BB OUTER	3.1	6.1	4.139	0.061	0.611	0.790	0.546
BB INNER	2.4	5.6	3.798	0.065	0.650	0.164	− 0.111
CC OUTER	2.7	5.0	3.530	0.049	0.495	0.559	− 0.470
CC INNER	2.3	4.5	2.987	0.050	0.496	0.807	− 0.337
DD OUTER	2.4	4.6	3.249	0.054	0.542	0.586	− 0.647
DD INNER	2.1	4.2	2.861	0.054	0.543	0.597	− 0.620
AD DEX	3.0	5.0	3.962	0.058	0.584	− 0.062	− 1.419
AD SIN	3.1	5.2	3.947	0.055	0.551	0.124	− 1.308
AB DEX	1.0	2.1	1.463	0.026	0.264	0.306	− 0.939
AB SIN	0.9	2.3	1.439	0.029	0.288	0.710	− 0.066
CD DEX	0.3	1.3	0.674	0.018	0.184	0.908	1.036
CD SIN	0.3	1.0	0.627	0.017	0.174	0.263	− 0.784
BE DEX	2.6	4.6	3.570	0.061	0.613	0.133	− 1.616
BE SIN	2.6	4.6	3.553	0.060	0.595	0.112	− 1.597
CF DEX	2.3	3.7	2.979	0.034	0.337	0.311	− 0.305
CF SIN	2.3	3.9	2.981	0.036	0.355	0.374	− 0.248
EF	1.0	2.8	1.581	0.035	0.345	0.383	− 0.043
Angle (α)	66	101	86.655	0.940	9.401	− 0.286	− 1.417
GH DEX	1.6	3.1	2.392	0.042	0.424	− 0.009	− 1.327
GH SIN	1.8	3.2	2.499	0.041	0.407	0.046	− 1.510
KL	2.4	4.3	3.241	0.061	0.611	0.087	− 1.676
MN	2.5	4.3	3.299	0.059	0.594	0.072	− 1.753
DEX thickness	0.1	0.3	0.254	0.005	0.051	0.108	− 0.390
SIN thickness	0.1	0.4	0.264	0.006	0.056	0.139	− 0.630
FP	1.5	3.2	2.315	0.041	0.406	0.464	− 0.933
Q	0.2	0.5	02.94	0.006	0.061	0.523	− 0.002
R	0.2	0.5	0.296	0.006	0.056	0.339	− 0.317
S	0.1	0.5	0.287	0.006	0.059	0.090	0.479
T	0.1	0.6	0.285	0.006	0.064	1.342	6.178

Discussion

In this study, the most statistically significant parameters for assessing sexual dimorphism are the angle of closure of the plates, their width, and their height. It should be noted that in previous studies of thyroid cartilage, digital anthropometric data were provided for males and females, and comparisons made between populations, but correlation coefficients between the studied parameters and sex were not given.

The results of our study prove that it is possible to use thyroid cartilage as a predictor of sex. Sexual dimorphism of the thyroid cartilage has been demonstrated in many previous papers (Kaur et al. 2015; Kovac et al. 2010; Patel et al. 2016; Ajmani 1990; Jotz et al. 2014).

Researchers have agreed that sexual differences are found in size characteristics, and inter-population comparisons have been conducted. In a study by Kovac et al. (2010), for example, 12 metric parameters of thyroid cartilage in a Croatian sample were studied and compared with 5 different studies on samples from other countries. This research provided detailed morphometric descriptions of the thyroid cartilage in the population of Eastern Croatia. The size characteristics of the thyroid cartilage of the Western Indian population were described by Mohini et al. (2015), who compared all dimensional characteristics with previous studies, based on the measurement of 17 parameters, including the weight of the cartilage.

Table 6 Correlation between the metric parameter and sex, N = 100

Parameter	Correlation coefficient	p
MN	0.949	0
KL	0939	0
BE SIN	0.937	0
BE DEX	0.916	0
GH DEX	0.891	0
GH SIN	0.888	0
ANGLE	0.887	0
EF	0.874	0
AD DEX	0.825	0
DD INNER	0.822	0
AD SIN	0.782	0
FP	0.769	0
CC INNER	0.764	0
AB DEX	0.751	0
DD OUTER	0.707	0
DEX thickness	0.705	0
CC OUTER	0.702	0
SIN thickness	0.673	0
Q	0.635	0
CF DEX	0.624	0
R	0.595	0
CF SIN	0.591	0
AB SIN	0.576	0
CD SIN	0.545	0
BB OUTER	0.521	0
S	0.433	0
T	0.430	0
BB INNER	0.426	0
CD DEX	0.354	0
AA INNER	0.311	0.002
AA OUTER	0.266	0.007

However, most studies test a different number of parameters. The difference in anthropometric points and sizes makes it difficult to compare researchers' data to identify inter-population differences. The development of a universal set of parameters and a scheme of anthropometric points for such studies would make them more universal. In our research, we established a list of features that provide the highest possible level of accuracy of results for sex diagnostics. The developed prognostic model included the following most significant indicators: CC OUTER, DD INNER, AD SIN, AB SIN, EF, ANGLE, GH SIN, KL, FP, and R. These can be used

to form an inter-population database of morphometric indicators of thyroid cartilage.

The maximum set of linear parameters (n = 31) was used. This made it possible to conduct a full comparative analysis using the results of the morphometric study of the European Russian sample combined with the data obtained from other studies focused on different populations, to determine the similarities and differences between them. The average values of the most important and informative parameters in the samples studied from various populations are shown in Table 9.

The most important parameters were selected using a statistical data sample, and they had a strong correlation coefficient with sex.

Based on this comparative study, similarities and differences between the measured variables were established. One similarity is the finding that the larynx in males is 10–30% larger than in females, which was observed by Zrunek et al. (1988) and confirmed by our study. Furthermore, all of the researchers recorded that the thyroid cartilage is larger in the adult male. With regard to the differences between the five studies, some of these are related to the length of the upper horns and the interlaminar angle (Ajmani 1990). The upper horns of the thyroid cartilage were larger than the lower ones in all cases studied, a finding that was also reported by Krogman et al. (1986), but the observed difference in size varies between studies. According to our data, the length of the upper horns in males was 2.3 times longer than the lower ones, and 2.0 times longer in females. However, Kovac et al. (2010) observed that the upper horns were 1.3 times longer than the lower ones in both males and females; for Ajmani (1990), this difference was 1.1 times longer in males and 1.2 times longer in females, whereas Kaur et al. (2015) found an equal difference in length between the upper and lower horns in both sexes, a ratio of 1.8:1. For Patel et al. (2016), the difference was 1.6 times longer in males and 1.5 times longer in females. There is also a significant difference in the linear size of the upper and lower horns in the different samples studied (Table 7). We found that the angle between the thyroid laminae was significantly greater in females and varied between 88°–105°, and 66°–90° in males. However, in studies of Europeans, this indicator was 61.5°–122.25° for females and 60.4°–103.3° for males (Kovac et al. 2010). In the North Indian sample, the angle sizes ranged between 85°–126° for females and 43°–100° for males (Kaur et al. 2015) and in the Nigerian sample these values ranged between 78°–134.7° and 73.2°–103.6° respectively (Ajmani 1990).

Sex differences on human larynx and therefore also on the thyroid cartilage can be associated with increased testosterone circulation during puberty, according to the existing literature (but further research is needed to

Table 7 Univariate and stepwise discriminant analyses

Equation		Group centroid		Wilks' lambda	Correct prediction rates (%)		
		Male	Female		Male	Female	Total
Univariate analysis	7530 (AA OUTER) − 9257	0.274	− 0.274	0.929	54	58	56
	6230 (AA INNER) − 7428	0.342	− 0.342	0.893	54	56	55
	39,077 (BB OUTER) − 27,833	0.589	− 0.589	0.739	76	70	73
	2775 (BB INNER) − 5752	0.463	− 0.463	0.821	70	60	65
	47,357 (CC OUTER) − 30,110	0.940	− 0.940	0.526	86	90	88
	136,139 (CC INNER) − 52,968	1.254	− 1.254	0.384	90	98	94
	34,980 (DD OUTER) − 21,926	0.982	− 0.982	0.504	82	94	88
	28,701 (DD INNER) − 18,382	1.577	− 1.577	0.283	94	98	96
	3224 (AD DEX) − 9163	1.447	− 1.447	0.319	94	92	93
	10,610 (AD SIN) − 15,087	1.267	− 1.267	0.379	92	88	90
	8919 (AB DEX) − 3144	1.144	− 1.144	0.428	88	88	88
	7840 (AB SIN) − 2354	0.686	− 0.686	0.675	80	86	83
	3804 (CD DEX) + 1687	0.355	− 0.355	0.886	64	66	65
	5198 (CD SIN) + 2292	0.645	− 0.645	0.702	66	84	75
	17,519 (BE DEX) − 19,958	2.257	− 2.257	0.161	98	100	99
	18,411 (BE SIN) − 22,370	2.725	− 2.725	0.117	100	98	99
	12,834 (CF DEX) − 13,040	0.783	− 0.783	0.615	78	86	82
	14,396 (CF SIN) − 13,366	0.736	− 0.736	0.644	78	84	81
	9422 (EF) − 4186	1.887	− 1.887	0.216	100	94	97
	0.001(Angle) − 8164	− 1.990	1.990	0.198	90	100	95
	6439 (GH DEX) − 7839	1.936	− 1.936	0.207	100	98	99
	9418 (GH SIN) − 10,211	1.932	− 1.932	0.208	100	96	98
	15,119 (KL) − 17,778	2.780	− 2.780	0.113	100	98	99
	18,001 (MN) − 21,041	3.049	− 3,049	0.095	100	98	99
	21,171 (DEX thickness) + 17,570	0.980	− 0.980	0.505	70	92	81
	16,691 (SIN thickness) + 14,287	0.892	− 0.892	0.552	70	90	80
	16,338 (FP) − 10,097	1.190	− 1.190	0.409	84	96	90
	5963 (Q) − 7683	0.837	− 0.837	0.583	88	74	81
	8298 (R) + 9046	0.717	− 0.717	0.656	84	72	78
	17,296 (S) + 12,723	0.477	− 0.477	0.811	76	64	70
	7481 (T) − 7855	0.491	− 0.491	0.803	72	64	68
Stepwise analysis	− 22,949 (CC OUTER) + 15,596 (DD INNER) − 3198 (AD SIN) + 3452 (AB SIN) + 4,004 (EF) − 0.0001 (Angle) + 3581 (GH SIN) + 10,633 (KL) + 6100 (FP) + 1545 (R) − 5317	6.053	− 6.053	0.026	100	100	100

explain the inter-population differences) (Jotz et al. 2014). By analogy with the studied features of the hyoid bone structure (Pollard et al. 2011), it can be assumed that the development of sexual characteristics may be influenced by the contraction and strength of the muscles (Pollard et al. 2011; Logar et al. 2016). In males, the muscles around the larynx and the two sternocleidomastoid muscles are more developed than in females (Starostina and Hikolenko 2011). The average length of the true vocal cords in males is longer than in females

Zviagin and Sineva 2009); in males, the distance between the attachment points of the ends of the true vocal cords is greater than in females, which leads to the formation of a more acute angle.

With regard to our comparative study, in all cases, the distance between the distal ends of the horns was greater than between their bases, and this is observed much more often in the upper horns than in the lower ones. The height of the plates (KL, MN) of the thyroid cartilage in our study on the European Russian sample was

Table 8 Classification results with stepwise analysis

		Sex	Predicted Group Membership		Total
			Male	Female	
Original	Count	Male	50	0	50
		Female	0	50	50
	%	Male	100	0	100
		Female	0	100	100
Cross-validated	Count	Male	50	0	50
		Female	0	50	50
	%	Male	100	0	100
		Female	0	100	100

less than their width (GH), and the ratio in males was 1:1.38, and in females 1:1.32. In the work of Patel et al. (2016), the height of the plates is also greater than the width, but the ratio of these indicators is almost equal—1:1.28 (M); 1:1.29 (F). In the Nigerian sample, according to Ajmani (1990), the height of the plates exceeds the width: 1:0.92 (M); 1:0.91 (F). In addition, we found that in both sexes, the morphometric parameters of the right cartilage plate in most cases did not coincide with the parameters of the left plate, but these differences were not significant.

The difference between our results in comparison to those obtained by other researchers can be explained by the fact that the measurements were taken on samples obtained from different populations, which only emphasizes the necessity to obtain data that will provide descriptions of thyroid cartilage morphology in the population of our selected region.

The dependence of the ossification process on sex has been previously established (Garvin 2008; Claassen et al. 2014; Cerny 1983). Enchondral ossification is characterized by sexual differences; it develops at different rates and shows different distribution patterns in males and females. A number of studies indicate a close relationship between ontogenetic development and the structural and functional features of the development of the thyroid cartilage and hyoid bone [Torimitsu et al. 2017; Urbanová et al. 2013a, 2013b; Logar et al. 2016]. Urbanová et al. (2013a, b) suggested the possibility of a joint assessment of the anthropometric characteristics of hyoid cartilage depending on sex and body size (sexually dimorphic variation was revealed for age-dependent changes in both size and shape of hyoid bones).

Since the thyroid cartilage and hyoid bone form the single backbone of the larynx, the peculiarities in the structure of the larynx in males and females can be explained by different levels of development and the strength of the attached neck muscles. A comprehensive assessment of morphometric parameters of anatomical structures of the larynx could increase the accuracy and reliability of the results (Urbanová et al. 2013).

Table 9 Comparison of thyroid cartilage dimensions (mm) with previous studies (mm)

Study		Kovac et al. (2010) (Eastern Croatia)	Ajmani (1990) (Nigeria)	Kaur et al. (2015) (North India)	Patel et al. (2016) (Gujarat Region)	The present study (European Russian Region)
Parameter	Sex	Male 39 Female 29	Male 28 Female 12	Male 25 Female 5	Male 24 Female 21	Male 50 Female 50
Height of lamina	M	–	37.92 ± 4.42	24.03 ± 2.89	31.22 ± 3.73	27.7 ± 1.8
	F		35.25 ± 3.1	21.17 ± 0.43	24.04 ± 3.71	20.0 ± 3.1
Width of lamina	M	–	34.98 ± 4.08	–	40.07 ± 4.64	38.1 ± 2.3
	F		32.17 ± 4.27		30.95 ± 4.97	26.5 ± 3.6
Length of posterior border	M	–	44.82 ± 6.45	–	44.56 ± 6.03	31.9 ± 3.2
	F		37.25 ± 7.47		35.60 ± 2.77	26.5 ± 4.0
Length of superior horn	M	18.22	20.70 ± 2.99	13.93 ± 2.69	16.78 ± 2.38	16.5 ± 2.0
	F	15.32	20.92 ± 3.01	11.84 ± 1.02	12.16 ± 1.74	12.2 ± 2.3
Length of inferior horn	M	13.99	18.35 ± 3.11	7.89 ± 1.70	10.65 ± 2.09	7.3 ± 2.1
	F	11.97	17.35 ± 2.67	6.75 ± 1.22	7.96 ± 1.32	6.1 ± 1.6
Angle between thyroid laminae	M	78.83 ± 12.05	89.92 ± 13.67	86 ± 5.95	86.13 ± 7.69	78.76 ± 5.64
	F	94.19 ± 15.86	106.38 ± 28.36	108 ± 8.37	100.24 ± 7.62	94.64 ± 3.64

It has been documented that there is no correlation between body growth and thyroid cartilage when males and females are evaluated separately. The logistic regression model presented in this article confirms the conclusion that sex has a more significant influence on the measurement of the larynx (Enver et al. 2020).

The main purpose of previously published studies on thyroid cartilage was to describe and compare the size characteristics of males and females in a sample from one population and compare the data obtained with the results of other studies. One of the aims of our research was to determine which metric parameters depended most strongly on sex and, based on the data obtained, to develop an equation that allowed us to estimate sex from the available skeletal remains.

Using step-by-step discriminant analysis, we developed an equation which makes it possible to predict male and female sex with a probability of 100% on the validation set. On the test set, the accuracy was 100% for female (5 of 5 samples were classified correctly), and 100% for males (5 of 5 samples were classified correctly). In addition, the results of our comparative study showed differences in size characteristics between five population samples. Therefore, the validity of these findings needs to be checked by other studies on inter-population variation. The limitations of this study could only be linked to an altered integrity of the thyroid cartilage (i.e., in case of violent death with injurious involvement of the neck region), or to unknown congenital/acquired malformations of the larynx. Finally, we shall consider applying our method to estimate sex for different forensic purposes in which it is necessary to identify corpses (mass disasters, bodies found in burned areas) and for presentations in the court of law, in the context of expert witness testimony, in conjunction with techniques and tools used by the scientific community. Indeed, our method could be considered an innovative approach that fulfills the criteria of scientific proof, including reliability, validity, critical review, and specificity.

Conclusions

The anthropometric study found that the thyroid cartilage has a pronounced sexual dimorphism, and the metric parameters that depend most strongly on sex are the thyroid angle and the width and height of the laminae. Since the equation developed in the stepwise discriminant analysis was intended for this specific population, it may produce less accurate results for other population groups. Stepwise discriminant analysis clearly produces better results compared to one-factor analysis. In the future, to deepen our scientific research, it could be useful to increase the number of samples, test the discriminant function in populations from different countries, and study the dependence of sex and age on anthropometric data.

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References
Ajmani M (1990) A metrical study of the laryngeal skeleton in adult Nigerians. J Anat 171:187–191

Akhlaghi M, Sheikhazadi A, Ebrahimnia A, Hedayati M, Nazparvar B, Saberi Anary SH (2012) The value of radius bone in prediction of sex and height in the Iranian population. J Forensic Leg Med 19(4):219–212

Asadujjaman M, Ali Molla MB, Al Noman SN (2019) Stature estimation from hand anthropometric measurements in Bangladeshi population. J Forensic Leg Med 65:86–91

Cerny M (1983) Our experience with estimation of an individual's age from skeletal remains of the degree of thyroid cartilage ossification. Acta Univ Palacki Olomuc Fac Med 3:121–144

Claassen H, Schicht M, Sel S, Paulsen F (2014) Special pattern of endochondral ossification in human laryngeal cartilages: X-ray and light-microscopic studies on thyroid cartilage. Clin Anat 27(3):423–430

Dawson C, Ross D, Mallet X (2011) Sex determination. In: Black S, Ferguson E (eds) Forensic Anthropology: 2000 to 2010. CRC Press, Boca Raton

Enver N, Doruk C, Kara E, Kaşali K, Asliyuksek H, Basaran B (2020) Does size matter in laryngology? Relation between body height and laryngeal morphometry. J Voice. https://doi.org/10.1016/j.jvoice.2019.08.001

Garvin HM (2008) Ossification of laryngeal structures as indicators of age. J Forensic Sci 53(5):1023–1027

Gonçalves D, Thompson TJ, Cunha E (2013) Osteometric sex determination of burned human skeletal remains. J Forensic Leg Med 20(7):906–911

Jotz GP, Stefani MA, Pereira da Costa Filho O, Malysz T, Soster PR, Leão HZ (2014) A morphometric study of the larynx. J Voice 28:668–672

Kaur R, Kumar Singla R, Laxmi V (2015) Morphology and morphometry of adult human cricoid cartilage: a cadaveric study in North Indian population. Int J Anat Res 3(1):910–914

Kovac T, Popovié B, Marjanovic K et al (2010) Morphometric characteristics of thyroid cartilage in people of Eastern Croatia. Coll Antropol 34(3):1069–1073

Krishan K, Chatterjee PM, Kanchan T, Kaur S, Baryah N, Singh RK (2016) A review of sex estimation techniques during examination of skeletal remains in forensic anthropology casework. Forensic Sci Int 261:165 e1-8

Krogman WM, Iscan MY (1986) The Human Skeleton in Forensic Medicine. Charles C. Thomas, Springfield.

Logar CJ, Peckmann TR, Meek S, Walls SG (2016) Determination of sex from the hyoid bone in a contemporary white population. J Forensic Leg Med 39:34–41

Lovell NC (2007) Analysis and Interpretation of Skeletal Trauma. In Biological Anthropology of the Human Skeleton (eds M.A. Katzenberg and S.R. Saunders).

Manjunath TH, Raghavendra R, Umesh SR (2017) Study of identification of sex of adult human skull by cranial circumference. Indian J Forensic Med Toxicol 11(1):92–95

Mohini MJ, Sharada SJ, Subhash DJ (2015) Morphometric study of thyroid cartilages in Western India. Int J Anat Res 3(2):1028–1033

Monica J, Usha D (2010) Morphometry of the thyroid and cricoid cartilages in adults on C.T. Scan. J Anat Soc India 59(1):19–23

Patel S, Bhardwaj R, Parmar P, Vaniya V (2016) Morphometric characteristic of thyroid cartilage in Gujarat region: A cadaveric study. J Res Med Den Sci 4(2): 150–154

Pollard J, Piercecchi-Marti MD, Thollon L et al (2011) Mechanisms of hyoid bone fracture after modelling: evaluation of anthropological criteria defining two relevant models. Forensic Sci Int 212(1-3):274 e1-5

Renjith G, Mary DP, Soe K, Wan MY, Beh H-C, Phuah WH, Tseu ATHT (2019) Sex estimation by discriminant function analysis using anatomical location of mental foramen. Forensic Science International: Reports. 1:100018

Sabnis A, Mane P (2020) A case report on ossified thyroid cartilage. J Media Res 6(1):7–8

Siddapur K (2017) Study on anthropometric parameters of dry tibia of human origin to aid sex identification in forensic investigation. Arab J Forensic Sci & J Forensic Med 1(5):532–539

Starostina SV, Hikolenko VN (2011) Stereomorphometric characteristics of hyoid bone in adult humans with different variants of neck structure. Morfologiia 140(4):51–56

TIBCO Statistica® 13.5.0. 2018. https://docs.tibco.com/products/tibco-statistica-13-5-0. Accessed 13 Jan 2020.

Torimitsu S, Makino Y, Saitoh H et al (2017) Sex determination based on sacral and coccygeal measurements using multidetector computed tomography in a contemporary Japanese population. J Forensic Radiol Imaging 9:8–12

Urbanová P, Hejna P, Zátopková L, Šafr M (2013a) What is the appropriate approach in sex determination of hyoid bones? J Forensic Leg Med 20(8): 996–1003

Urbanová P, Hejna P, Zátopková L, Šafr M (2013b) The morphology of human hyoid bone in relation to sex, age and body proportions. Homo 64(3):190–204

Zrunek M, Happak W, Hermann M, Streinzer W (1988) Comparative anatomy of human and sheep laryngeal skeleton. Acta Otolaryngol 105:155–162

Zviagin VN, Sineva IM (2009) Identification of male somatotype based on osteometric characteristics of the upper and lower extremities. Sud Med Ekspert 52(5):6–11

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