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.

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                        |

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 346 "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...
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 step-wise 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.

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 |
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 $4.15 \pm 0.72$ cm and $4.46 \pm 0.64$ cm, in females—$3.79 \pm 0.60$ cm and $3.82 \pm 0.37$ cm. The distance between the distal ends of the lower horns in males was $3.88 \pm 0.46$ cm and the base of the lower horns was $3.63 \pm 0.49$ cm. For the female sample, these values corresponded to $3.18 \pm 0.20$ cm and $2.87 \pm 0.24$ 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 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
between the superior point of the anterior intermediate 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):

\[
d = b_1x_1 + b_2x_2 + \ldots + b_nx_n + a,
\]

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

\(b_i\) 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

\[
-22,949 (CC OUTER) + 15,596 (DD INNER) - 3198 (AD SIN) + 3452 (AB SIN) + 4004 (EF) - 0.0001 (Angle) + 3581 (GH SIN) + 10,633 (KL) + 6100 (FP) + 1545 (R) - 5317.
\]

| Variable | Intra-observer error TEM | rTEM | R | Inter-observer error 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 | 0.061                      | 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                    | 0.787| 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| 0.987 | 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 |
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.

| Variable | Males (n = 50) | Females (n = 50) | p | lambda |
|----------|----------------|-----------------|---|---------|
| 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    | 2.3–3.2         | 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
**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.

| 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 | 0.294 | 0.006     | 0.056    | 0.339    | −0.317   |
| R                | 0.2 | 0.5 | 0.296 | 0.006     | 0.056    | 0.090    | 0.479    |
| S                | 0.1 | 0.5 | 0.287 | 0.006     | 0.059    | 0.090    | −0.900   |
| T                | 0.1 | 0.6 | 0.285 | 0.006     | 0.064    | 1.342    | 6.178    |
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 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 6 Correlation between the metric parameter and sex, N = 100

| Parameter      | Correlation coefficient | p   |
|----------------|-------------------------|-----|
| MN             | 0.949                   | 0   |
| KL             | 0.939                   | 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 OUT         | 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|
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

| Equation | Group centroid | Wilks’ lambda | Correct prediction rates (%) |
|----------|----------------|---------------|-----------------------------|
| Univariate analysis | | | |
| 7530 (AA OUTER) – 9257 | 0.274 | 0.274 | 54 58 56 |
| 6230 (AA INNER) – 7428 | 0.342 | 0.342 | 54 56 55 |
| 39,077 (BB OUTER) – 27,833 | 0.589 | 0.589 | 76 70 73 |
| 2775 (BB INNER) – 5752 | 0.463 | 0.463 | 70 60 65 |
| 47,357 (CC OUTER) – 30,110 | 0.940 | 0.940 | 86 90 88 |
| 136,139 (CC INNER) – 52,968 | 1.254 | 1.254 | 90 98 94 |
| 34,980 (DD OUTER) – 21,926 | 0.982 | 0.982 | 82 94 88 |
| 28,701 (DD INNER) – 18,382 | 1.577 | 1.577 | 94 98 96 |
| 3224 (AD DEX) – 9163 | 1.447 | 1.447 | 94 92 93 |
| 10,610 (AD SIN) – 15,087 | 1.267 | 1.267 | 92 88 90 |
| 8919 (AB DEX) – 3144 | 1.144 | 1.144 | 88 88 88 |
| 7840 (AB SIN) – 2354 | 0.686 | 0.686 | 80 86 83 |
| 3040 (CD DEX) + 1687 | 0.355 | 0.355 | 64 66 65 |
| 5198 (CD SIN) + 2292 | 0.645 | 0.645 | 66 84 75 |
| 17,519 (BE DEX) – 19,958 | 2.257 | 2.257 | 98 100 99 |
| 18,411 (BE SIN) – 22,370 | 2.725 | 2.725 | 100 98 99 |
| 12,834 (CF DEX) – 13,040 | 0.783 | 0.783 | 78 86 82 |
| 14,396 (CF SIN) – 13,366 | 0.736 | 0.736 | 78 84 81 |
| 9422 (EF) – 4186 | 1.887 | 1.887 | 100 94 97 |
| 0.001(Angle) – 8164 | 1.990 | 1.990 | 90 100 95 |
| 6439 (GH DEX) – 7839 | 1.936 | 1.936 | 100 98 99 |
| 9418 (GH SIN) – 10,211 | 1.932 | 1.932 | 100 96 98 |
| 15,119 (KL) – 17,778 | 2.780 | 2.780 | 100 98 99 |
| 18,001 (MN) – 21,041 | 3.049 | 3.049 | 100 98 99 |
| 21,171 (DEX thickness) + 17,570 | 0.980 | 0.980 | 70 92 81 |
| 16,691 (SIN thickness) + 14,287 | 0.892 | 0.892 | 70 90 80 |
| 16,338 (FP) – 10,997 | 1.190 | 1.190 | 84 96 90 |
| 6563 (Q) – 7683 | 0.837 | 0.837 | 88 74 81 |
| 8298 (R) + 9046 | 0.717 | 0.717 | 84 72 78 |
| 17,296 (S) + 12,723 | 0.477 | 0.477 | 76 64 70 |
| 7481 (T) – 7855 | 0.491 | 0.491 | 72 64 68 |

| Equation | Group centroid | Wilks’ lambda | Correct prediction rates (%) |
|----------|----------------|---------------|-----------------------------|
| 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 | 100 100 100 |
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 8 Classification results with stepwise analysis

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

### 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 | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| Height of lamina              | M         | 37.92 ± 4.42 | 35.25 ± 3.1 | 24.03 ± 2.89 | 21.17 ± 0.43 | 31.22 ± 3.73 | 24.04 ± 3.71 | 27.7 ± 1.8 | 20.0 ± 3.1 |
|                             | F         | 34.98 ± 4.08 | 32.17 ± 4.27 | 40.07 ± 4.64 | 30.95 ± 4.97 | 38.1 ± 2.3 | 26.5 ± 3.6 | 31.9 ± 3.2 | 26.5 ± 4.0 |
| Width of lamina              | M         | 44.82 ± 6.45 | 37.25 ± 7.47 | – | – | 44.56 ± 6.03 | 35.60 ± 2.77 | 31.9 ± 3.2 | 26.5 ± 4.0 |
|                             | F         | 18.22 | 15.32 | 20.70 ± 2.99 | 13.93 ± 2.69 | 16.78 ± 2.38 | 13.12 ± 1.74 | 16.5 ± 2.0 | 11.2 ± 2.3 |
| Length of posterior border   | M         | 18.35 ± 3.11 | 20.32 ± 3.11 | 7.89 ± 1.70 | 7.76 ± 1.32 | 10.65 ± 2.09 | 8.04 ± 1.77 | 7.3 ± 2.1 | 6.1 ± 1.6 |
|                             | F         | 13.99 | 11.97 | 17.35 ± 2.67 | 16.35 ± 2.67 | 7.96 ± 1.32 | 6.7 ± 1.6 | 6.1 ± 1.6 | 6.1 ± 1.6 |
| Length of superior horn      | M         | 78.83 ± 12.05 | 89.92 ± 13.85 | 86 ± 5.95 | 86.13 ± 7.69 | 86.13 ± 7.69 | 86.13 ± 7.69 | 78.76 ± 5.64 | 78.76 ± 5.64 |
|                             | F         | 94.19 ± 15.86 | 106.38 ± 28.36 | 108 ± 8.37 | 100.24 ± 7.62 | 100.24 ± 7.62 | 100.24 ± 7.62 | 94.64 ± 3.64 | 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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Authors’ contributions

RC conceived the key points of the work. GZ acquired the data and made analysis. IK conducted statistical analysis. RS contributed to conception of the work and verified the structure of the manuscript. YP supervised the findings of this work and verified the analytical method. All authors contributed to the final manuscript.

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All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The research was approved by the Ethics Committee of Sechenov University (Protocol No. 10-19 of 17.07.2019). Consent to participate is deemed unnecessary according to the current legislation of the Russian Federation (Federal law on The State Forensic Activity In The Russian Federation of 31.05.2001 N 73-FZ).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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