

**Determination of Sex from Morphometric Measurements and Pattern of Ossification of Thyroid Cartilage**

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Correspondence Author: Dr. Priyatha Ponnappan, Assistant professor, Dept. of Forensic Medicine, Govt. Medical College, Kozhikode, PIN.673008. Kerala, India.**Conflicts of Interest:** Nil**Abstract**

Establishment of identity is mandated in cases of mutilated bodies, explosion cases, mass disasters, bodies with advanced stages of decomposition etc. Establishing identity from skeletal remains is one of the most challenging tasks faced by the forensic pathologists. Determining sex is one of the major components in that. Various methods are adopted for this purpose including microscopic methods like barr bodies, Davidson bodies, DNA fingerprinting and macroscopic methods like various anthropological measurements in bones. Thyroid cartilage, largest of laryngeal cartilage is known for its sexual dimorphism in living as evidenced by the prominent nature in males. But in case of bodies with advanced decomposition changes and skeletanisation the prominent nature cannot be identified. As the morphology of thyroid cartilage, i.e. the size, the angle between two laminae and weight varies considerably in males and females; the pattern of ossification also varies. The present study is aimed at the gender based difference in morphometric measurements and pattern of ossification in thyroid cartilages of North Kerala population so that it can be utilised for establishing identity along with other methods so as to get more accurate results.

Keywords: Thyroid Cartilage, Sex, Morphometry, Ossification.**Introduction**

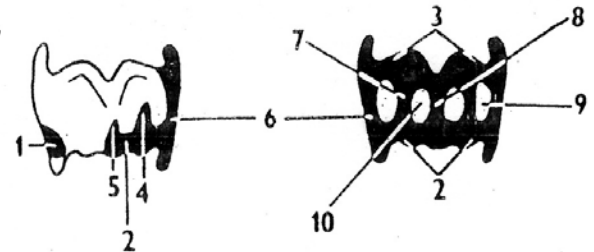
Determination of individuality of a person is known as identification. Question of identification come into consideration for various civil and criminal cases. Identification of living is usually carried out by police, but in case of any disputes medical personal is consulted for the purpose of determination of identity. This job is more challenging if only mutilated body parts or skeletal remains are available for post mortem examination. Determination of sex, race, age, stature by anthropological analysis is the way for establishing the identity in such cases. There are different methods to estimate age and stature. Sex determination usually done by anthropometric measurements taken from pelvic bones and skull. However, cases in which skull and pelvic bones are not available, sex determination becomes more difficult and one has to depend on the other available bones. Long bones like femur and humerus will help in this matter but there exists a huge subjective variability.

Thyroid cartilage, which is the largest of all laryngeal cartilage is well known for its sexual dimorphism in living. It consists of two quadrilateral laminae with anterior borders that fuse along their inferior two-thirds at a median angle to form the subcutaneous laryngeal prominence (Adam's apple)¹. Above, the laminae are separated by a V-shaped anterior thyroid notch or incisura. Posteriorly the laminae diverge, and their

posterior borders are prolonged as slender horns, the superior and inferior cornua. A shallow ridge, the oblique line curves downwards and forwards on the external surface of each laminae. Sterno-thyroid, thyro-hyoid and thyro-pharyngeus (part of the inferior pharyngeal constrictor) are attached to the oblique line. The superior border of each laminae is concave behind and convex in front and thyro-hyoid membrane is attached along this edge. The inferior border of each laminae is concave behind and nearly straight in front and the two parts are separated by the inferior thyroid tubercle. Anteriorly the thyroid cartilage is connected to the cricoid cartilage by anterior crico-thyroid ligament which is a thickened portion of crico-thyroid membrane. The anterior border of each thyroid laminae fuses with its partner at an angle of approximately 90 degree in men and approximately 120 degree in women. The posterior border is thick and rounded. To superior cornu which is long and narrow, the lateral thyro-hyoid ligament is attached. The inferior cornu is short and thick and has a small oval facet for articulation with cricoid cartilage in medial ends.

Histologically the thyroid, cricoid and most of the arytenoids cartilages consist of hyaline cartilage and therefore they undergoes calcification as age advances this process normally starts at about 18 years of age . Initially it involves the lower and posterior part of thyroid cartilage and subsequently spreads to involve the remaining portion of the cartilage. Earlier researches done in this field had showed that the pattern of progression of ossification differ in both sexes. Cerny (1983)² had derived different phase of ossification and he could summarise different age groups for each phase. The Cerny phases were applicable for males only. The later researchers like H MGarvin(2008)⁴ also found out that pattern of ossification in males and females are different but he couldn't find any relationship with age.

Figure: 1:Anatomy of the thyroid cartilage (1) lower posterior triangle; (2) lower (caudal) branch; (3) upper (cranial) branch; (4) paramedian process; (5) median process; (6) lateral bar; (7) paramedian bar; (8) median bar; (9) posterior window; (10) anterior window¹⁰



Pietro Roncallo(1948)³ studied the thyroid cartilage and found out that ossification begins at about 20 years, probably little before in men than in women, and begins in the lower posterior zone. The way of progression of ossification showed remarkable degree of individual variability. According to him complete ossification occurred sometimes in men but rarely in women. Ossification process as a rule was observed to begin as a rule in well determined points , that are almost the same in both sexes, while in the advanced stages notable differences had occurred both in the persons of the same sex and of different sexes. The angle was found to be narrower in men than in women. He also found that complete ossification of the cartilage can occur in men, whereas never in women.

There may be several reasons for the difference in progression of ossification in males and females. The power and contractibility of neck muscles may be one factor. Weight bearing and other uses will be more in males.

After death, the so called Adam's apple is not well identifiable in decomposed or skeletonised bodies. So if we could get the morphometric measurements and ossification status of thyroid cartilage, the gender of the person can be determined with more precision. This could

go a long way in establishing the identity of the deceased and hence helping the police in solving the crime behind the case.

The present study aims in finding out the morphometric sexual dimorphism and difference in pattern of ossification in both sexes by studying the thyroid cartilages collected from dead bodies during autopsy conducted in the Dept. of Forensic Medicine, Govt. Medical College Kozhikode by their morphological and radiological examination.

Aims and Objectives

Aims

To observe the morphometric measurements and pattern and progression of ossification of thyroid cartilage and the differences of these in the male and female.

Objectives

1. To collect thyroid cartilages from 203 male and 200 female dead bodies in the age range of 15 – 70 yrs and take morphometric measurements of each.
2. To conduct radiographic examination of collected cartilages and look for their pattern of ossification
3. To derive cut off values and indices for identification of sex.

Materials and Methods

The study was a series observation of 403 thyroid cartilages, collected from dead bodies autopsied in the Kozhikode Medical College mortuary during the 13 month period from August 2009 to September 2010.

Selection Criteria

The materials are selected from cases personally attended by the principal researcher. Materials were collected from dead bodies whose identity and age were certain as per police enquiries from first degree relatives with a random number of them further cross checked later with records showing age (School leaving certificates).

Exclusion criteria

1. Dead bodies of persons whose relatives could not be contacted **or** of non Kerala origin.
2. Dead bodies of <15 years of age.
3. Bodies with apparent physical or endocrinal abnormalities or chronic illness
4. Bodies which had features of gross trauma to neck

Collection and preparation of specimen

The dead body data from police was checked for sex and age, the near relative who identified the body for legal purpose was interviewed regarding source and nature of verification of age possible and the nativity and district of domicile. Height, weight, build and nourishment were observed.

Collection of cartilage:

After the routine autopsy examination thyroid cartilage is dissected out from the larynx using scalpel. The cartilage is cleaned by removing as much of the soft tissues as possible with a thumb forceps. A tag with post mortem number is tied on any of the horns and the specimen was left in water containing Sodium Hydroxide 10% to macerate. It was taken out after three days and cleaned thoroughly.

The cleaned specimen is stored in properly labelled plastic jars containing ten percentage formalin. After several days, when the cartilage was well fixed in formalin, it was taken out and the morphometric measurements were taken.

Measurements

The cartilage was kept on a wooden dissection board, immobilised and the length and breadth of the alae were measured using a ruler. The average of three readings was taken as the value. The angle between two laminae was measured using a protractor. The thickness of the posterior window and Para median bar was measured using a vernier calliper. The weight was measured using

an electronic weighing machine. All these morphometric data are recorded in a serially numbered proforma.

Radiological Examination

After getting a bunch of specimen, they are arranged and tied on to a plastic board after breaking them in the midline to facilitate observation of both alae recording the case series number. 16- 20 specimens of same age and sex group were arranged on one board.

These were then submitted for radiological examination. The X- rays were taken using 14 x 16 inch Kodak X- ray film. Soft x rays are taken using radiation of 6- 8 mAs and 45 – 55 kvp. The individual images are given their corresponding number in the X- ray sheet also.

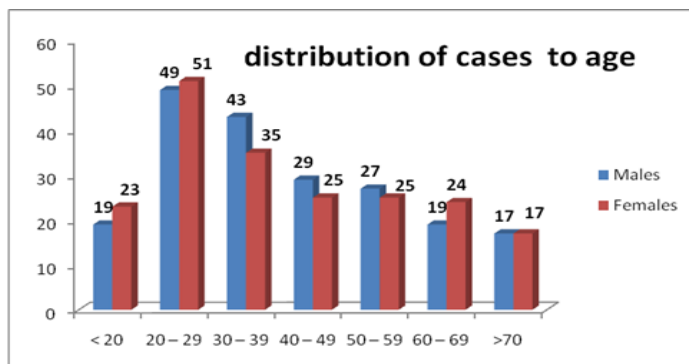
Statistics

All the data thus obtained are statistically analysed with SPSS software with the help of a statistician.

Results

A total number of 403 cartilages were studied of which 203 were males and 200 were females.

Figure 2: Number of cases in different age group



Morphology

22 % of the males had a highly prominent larynx, and their mean age was 51 years. Only three cases from females had a highly prominent larynx. Prominence of larynx is found to be increasing with age in males.

Apparent size of the thyroid cartilage was medium in 76 % of the males, and it was small in 60 % of the females

examined. 21 % of the males and 5 % of the females had an apparently large cartilage.

Almost all the cases had a medium sized thyroid gland. Thyro-hyoid ligament was completely ossified in three male persons only. In 83 % of the males and 99 % of the females, there was no ossification at all in thyro-hyoid ligament.

Morphometry

The mean length (vertical height) of lamina of the cartilage was 2.688 (SD 0.229) for males and 2.058 (SD 0.174) for females.

The mean width (transverse) of lamina of the cartilage was 3.873 (SD 0.264) in males and 2.862 (SD 0.232) for females.

Both the length and width of the lamina showed a linear correlation with age in both the sexes. An index was calculated from the length and width (L/W X100) of the lamina, and the mean value was around 70 in both the sexes.

The mean weight of the cartilage was 9.673 gm (SD 1.741) for males and was 4.893 gm (SD 0.928) for females. In both the sexes, weight of the cartilages had a positive correlation with the age.

The mean angle between the two laminae of thyroid cartilage was found to be 84.97 degree (SD 14.27) in male cases and 108.59 (SD 11.64) degree in case of females. The angle was found to be decreasing in the males as the age advances.

The mean thickness of the lamina in the region of the anterior window was 2.588mm in males and 2.236mm in females. Thickness of paramedian bar was 4.407 for males and 3.838mm in females.

Pattern and progression of ossification

A. Males

Beginning of the ossification was noted in the lower posterior triangle (LPT) of the cartilage. 9 % of the males

cartilage examined had no signs of ossification within them. The rest of the cartilages showed ossified LPT and had a mean age of 42.82yrs (SD 16.66). All the cartilages of more than twenty one years had begun ossification. Lower branch was not ossified in 15 % of the case examined

A partly ossified lower branch (lateral half only) was seen in 13 % of the cases. 72 % had a completely ossified lower branch.

Upper branch ossification was absent in 83% of the males and 11% of the males had a fully ossified upper branch and that too were in the advanced age group. A partly ossified (medial half only) upper branch was seen in 5% of the cases.

A well ossified Para median bar was absent in 39 % of the males and 61 % showed an ossified Para median process. Median process was not seen in 71% cases.

Lateral bar, on each side of the laminae had an ossification earlier, following LPT. 14 % of the cartilages in which no ossified lateral bar were seen. All the cartilages above 25 year had started ossification in lateral branch. A partly ossified lateral bar (lower half only) was seen in 13 % of the cases. A completely ossified lateral bar was found in 73 % of the cases.

Para median bar was well ossified in 27 % of the males and Median bar is found to be ossified in 53 % of the cases. Posterior window was well formed in 9% of the males and that too was in advanced ages. Anterior window was seen in 18% of the male cases and it tends to be formed in earlier than the posterior window.

Ossification in the inferior horn was seen started in early ages following the LPT. Only 12 % had an un ossified inferior horn. Most of the cases had (87 %) an ossified inferior horn. Superior horn tends to ossify in the middle ages. 72 % of the examined male cartilages had a superior horn ossified.

1. Females

In females also ossification had apparently started in lower posterior triangle. Upper branch of the female cartilages had more tendencies to get ossified, when compared to males. 17.5 % of the cases in which beginning of ossification (lateral half) present. A fully ossified upper branch was seen in 12 cases and that too was in the advanced ages.

Para median process was not seen in 65 % of the cases. Median process seldom gets ossified in females. Only 13.5 % had a median process and that too was in later ages. Lateral bar tends to ossify earlier. Ossification was not seen 14.5% of the cases. A partly ossified lateral bar was seen in 26 cases. A fully ossified lateral bar was seen in rest of the cases. Para median bar was absent in 86 % of the cases. A fully ossified. Para median bar was found in 74 of the cases. Median bar was not ossified in most of the females.

Window formation was not found in any of the female cases. Ossification in inferior horn occurred much earlier in females also. 73.5 % had an ossified inferior horn. 41.5 % of the female cartilages had an ossified superior horn, and their mean age was 50.40 yrs.

Discussions

The morphological features and pattern of ossification of the thyroid cartilage were the subjects of study. As many researchers found out early, the morphology of the male and female cartilages differ considerably, particularly in the parameters like length and width of the laminae, weight of the cartilage, and angle between the two laminae. Pietro Roncallo (1958)³ after thoroughly revising the ancient studies on thyroid cartilage, had concluded in his study that angle between the two laminae was same in two sexes during first decade, later it becomes narrower in men, whereas in female, it is same or becomes wider as age advances. M L Ajmani (1990)⁵ was also concluded

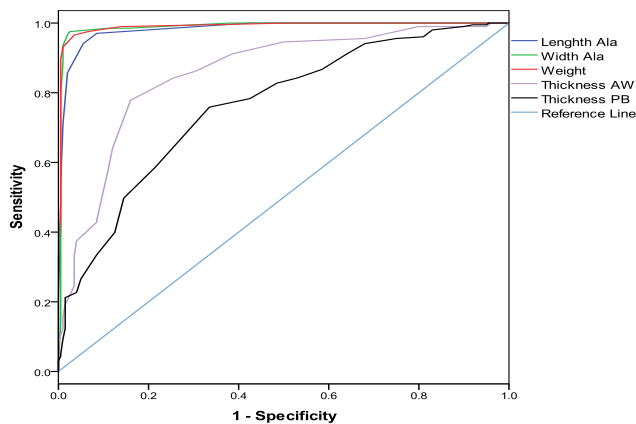
that The differences in dimension between male and female laryngeal cartilages are conspicuous and mostly significant.

In the present study, some cut off values were arrived at for some parameters, so that sex of the person can be determined from these values, with significant sensitivity and specificity. The parameters include length and width of the each laminae, weight of the cartilage, and angle between the two laminae. The cut off values are given in the table and a receiver operative curve (ROC) is created with them.

Table:1; Coordinates of the Curve for figure 3

Test Result Variable(s)	Positive if Greater Than or Equal To	Sensitivity	1 - Specificity
Length of ala(cm)	2.250	.970	.085
Width of ala(cm)	3.250	.975	.025
Weight(gm)	6.250	.975	.065
Thickness AW(mm)	2.550	.778	.160
Thickness PB(mm)	4.150	.759	.335

Figure 3: ROC curve 1

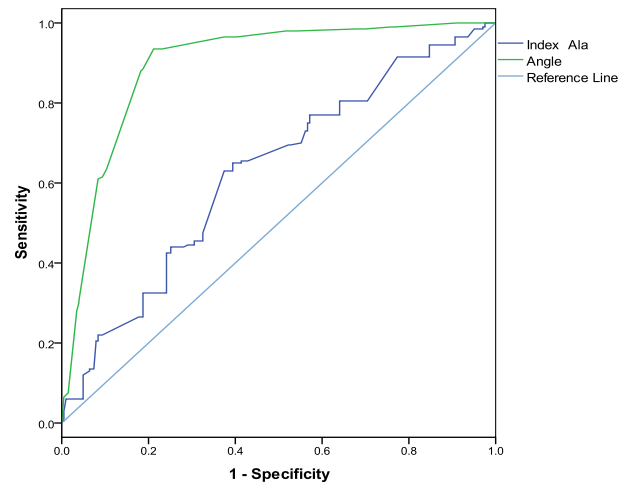


In the above ROC curve, all the positive values indicate male thyroid cartilages. From the position of the curves it is clear that length and width of the alae and weight of the cartilage are the most ideal parameters to identify the sex of an individual.

Table- 2: Coordinates of the Curve for figure: 4.

Test Result Variable(s)	Positive if Greater Than or Equal To	Sensitivity	1 - Specificity
Index	70.660	.650	.399
Angle (degree)	94.50	.935	.212

Figure: 4: ROC curve 2



In the above curves, positive values indicate female cartilages. As the index of the alae and angle between the laminae greater than the cut off value, as given in the table, the chance of the cartilage to be female is very high. Pattern and progression of ossification in males and females

Roncallo had found that ossification begins in the junction between the posterior bar and inferior horn in males and a little above, in the middle of posterior bar in females. W. Hatley et al⁶ suggested inferior portion of posterior one third of the laminae as the area of onset. Many other researchers, including Cerny also, had a similar opinion that LPT is the area of onset of ossification in both the sexes. A different thought was put forwarded by M Turk and D A Hogg (1993)⁷, they thought it was inferior horn. The present study found that it was lower posterior triangle of the cartilage, in which the ossification first begins. Latest CT based study by K D Dan Tran et al⁸ also confirmed this.

Roncallo believed that progression of ossification was similar in both sexes in the early period and then it tends to decrease in females. He also said that inter individual variation is very common. W Hatley et al found that ossification progresses along the posterior and inferior borders of the laminae and it was similar in males and

females. He also said that superior horn ossifies in later stages and windows formation exists. Turk and Hogg believed that ossification progresses from inferior horn to posterior one third of the laminae along the inferior border, and it was much slower in females. H M Garvin thought that up to fifth decade ossification progresses in similar manner in males and females. And median process was the one which ossifies last in males. He also said that no ossification present in laminae and cranial branch of the cartilage. Cerny also thought in similar fashion and in his phase method for males, he included the median process and upper branch in the last phases.

The present study found that ossification progresses in three directions from the point of onset, LPT. It was through lateral bar, lower branch and to the inferior horn. In females the progression was at a slower rate, as pointed out many in earlier. In males more progression was through the lower branch and from there to the lamina as Para-median and median process and bar. In females, ossification progresses to the upper branch at earlier period. The region of median process and the median bar rarely get ossified in females posterior and anterior window formation seen occurred in males.

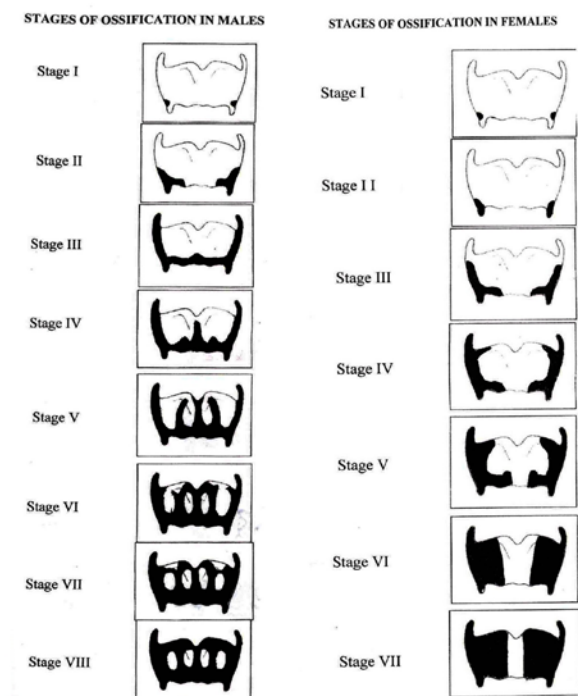
On contrary to the phase method of Cerny, we found that anterior window forms earlier than the posterior. And also median process tends to ossify in the middle ages.

Most of the authors found that a complete ossification occur in male cartilages only ,that too was in the advanced age groups. Almost all researchers believed that female cartilages never get ossified completely, except G L Grandmaison et al⁹, as they got one completely ossified female cartilage. Keen JA , Wainwright (1958)¹¹ found that front part of the laminae and midline area remains cartilaginous to a more advanced age in the females which was confirmed by W Hately et al⁶.

The present researcher also found that male thyroid cartilages undergo complete ossification with formation of the windows in the advanced ages. But a window formation or complete ossification had never occurred in females.

Diagrams showing pattern and progression of ossification changes in thyroid cartilage of both the sexes are represented as different stages is given below.

Figure; 5: Pattern and progression of ossification in both sexes derived in the present study given in different stages.



Summary and conclusions

Morphology and morphometric measurements of thyroid cartilage differ considerably in males and females so as to be distinctive. Average cut off values for all the morphometrical parameters were deducted statistically by which sex can be determined.

Pattern and progression of ossification was studied in detail in both sexes using radiology. It is found that males and females have considerable difference in the pattern of

ossification. Since there is inter individual variation, this method should be used as a supportive aid for other sex determination methods.

An extended study may be attempted in another population or in living subjects with the help of advanced techniques like computed tomography and morphometric difference in males and females and difference in their pattern and progression of thyroid cartilages can be established.

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