Original Research Article

Age Estimation from Endochondral Ossification Pattern of Thyroid Cartilage in the Population of Punjab

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Abstract

Objective: The present study aims at studying the ossification of thyroid cartilage and to compare it with age of the individual.

Methods: The study was conducted in 300 samples of thyroid cartilage collected during autopsy in the department of Forensic medicine in a tertiary care hospital. Each thyroid cartilage is divided in to thirteen anatomical area and ossification in each defined area was studied through gross and cut section features and classified as ossified and not ossified areas. The samples were grouped in to seven groups of age and ossification features were compared between the age groups.

Results: On analysing the ossification pattern of thyroid cartilage in thirteen defined anatomical areas and comparing it with the age, a peculiar sequence of ossification was noted, and it was also observed that frequency of ossification increases significantly with increasing age groups.

Conclusion: Ossification of thyroid cartilage helps in establishing the age of individual and it can be categorised to an age group of ten years.

Key words: Thyroid cartilage, Age estimation, Ossification, Calcification

Introduction

Forensic medicine experts continue to search for newer methods to estimate the age of individual as it forms an indispensable aspect in establishing the identity of an unknown individual. Most of age estimation methods are based on the ossification of various long bones. The thyroid cartilage being a hyaline cartilage undergoes endochondral ossification with advancement of age. Endochondral ossification of thyroid cartilage is indicated by development of vascular system, invading the perichondrium, seen

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as reddish-brown colouration at the midsection of the cartilage. Development of vascular system into the cartilage is termed as marrow foci. Presence of this vasculature is a prerequisite for determination of ossification. Ossification of hyaline thyroid cartilage is also indicated by hard consistency due to deposition of calcium and phosphate minerals over the dead chondrocytes.[1]

In the past several studies[2-15] have been conducted to explore the use of thyroid ossification as a potential tool for age estimation. Most of such studies were radiological analysis either with x ray (cephalometric and cervical spine radiographs) or computed tomography (multi-slice) to evaluate the degree of ossification of thyroid cartilage. It was mainly based on the computerised measurement of ossification areas in the thyroid cartilage[2,3], quantification of radio-opacity of thyroid cartilage[4] and computerised estimation of volume of ossified thyroid cartilage[5]. One of the drawbacks of these studies was that the thyroid cartilage ossification was not distinguished fromossification of other laryngeal cartilages, calcified lymph nodes, calcified atherosclerotic plaques, foreign bodies, and metastatic calcification.

In the present study, the endochondral ossification of thyroid cartilage was studied through gross and cut section features and analysed for estimation of age of an individual within a target native population.

Objectives:

1. To determine the ossification of various defined areas of thyroid cartilage
2. To calculate any correlation between the ossification pattern of thyroid cartilage and age of the deceased.

Materials and Method

It is a prospective analytical study conducted from July 2012 to December 2013 on 300 cases autopsied in the Department of Forensic Medicine of a tertiary care hospital in Chandigarh. Written informed consent was taken from the legal heirs of the deceased.

Inclusion criteria

- All cases above the age of 18 years.
- Residents of Punjab having at least two generation of ancestors from Punjab.

Exclusion criteria

- Cases with injury to thyroid cartilage.
- Known cases of metastatic calcification in thyroid cartilage.
- Cases with advanced decomposition changes.

Method

The thyroid cartilage was dissected out during autopsy, with great care as not to damage the superior and inferior horns in the process. The entire thyroid cartilage was carefully dissected to remove all muscular and ligamentous attachments. The thyroid cartilage was sliced into thin parallel horizontal sections at equidistant from tip of superior horn to tip of inferior horn. The thyroid cartilage was divided into thirteen defined areas for the study of ossification pattern as described and shown in **Table 1** and **Figure 1**.

Table 1: Various anatomical areas of thyroid cartilage for ossification pattern assessment.

1. Right superior horn (RSH).	8. Right posterior branch (RPB).
2. Left superior horn (LSH).	9. Left posterior branch (LPB).
3. Cranial branch (CRB).	10. Right lower posterior triangle (RLPT).
4. Caudal branch (CAB).	11. Left lower posterior triangle (LLPT).
5. Anterior midline tongue (AMT).	12. Right inferior horn (RIH).
6. Right lamina (RTL).	13. Left inferior horn (LIH).
7. Left lamina (LTL).	

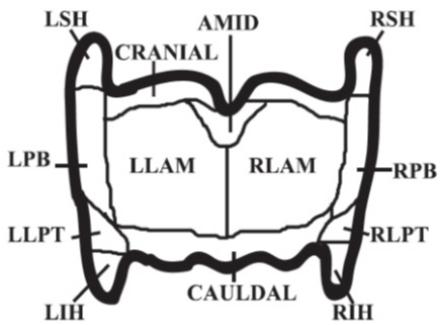

Figure 1: Anatomical areas of thyroid cartilage for assessment of ossification pattern

Each defined area of thyroid cartilage was assessed and classified as ossified or not ossified as follows. For each defined area of thyroid cartilage four factors namely consistency, colour, sensation of cutting, colour of cut surface were studied. presence of firm to hard consistency of cartilage, chalky white colour of cartilage, gritty sensation on cutting and brownish to red colour of cut section were taken as indicator of ossification.

All the observations were made independently by three observers and amean observation was taken as final observation of the given sample area and accordingly the sample areas were classified as either ossified or as not ossified sample area. For each defined area, the earliest age at which it shows ossification was noted. The total number of ossified and not ossified areas of each defined area and percentage of ossification for each defined area were also calculated. All the 300 samples were then categorized into different age groups as follows.

Age group 1 – 18 to 20 years

Age group 2 - 21 to 30 years

Age group 3 – 31 to 40 years

Age group 4 – 41 to 50 years

Age group 5 – 51 to 60 years

Age group 6 – 61 to 70 years

Age group 7 – 71 to 80 years

The total number of samples (S) for each age group was calculated. Then total study areas (A) of each group were determined by multiplying the total number of samples (S) in that age group with number of defined areas of each sample (13).

- Total study areas of an age group (A) = Total no of samples of that age group (S) * 13

The number of ossified areas (A1) and not ossified study areas (A2) for each group was calculated. The frequency of ossification of each age group was calculated by dividing number of ossified areas (A1) by total study areas (A) in that age group and expressed as percentage.

- Frequency of ossification = no of ossified areas (A1) / total study areas(A) *100

All the data were tabulated using Microsoft excel version 2010 and all analysis were carried out with the help of IBM SPSS Statistics package (version 20.0). In all analysis, a p value < 0.05 with 95 % confidence interval was considered statistically significant.

Results

For each of the thirteen defined anatomical area, the presence or absence of ossification was studied in all three hundred cases and then analysed with individual age. The number of ossified and not ossified areas of each defined area, percentage of ossification of each defined area, the earliest age at which each area ossified were tabulated in **Table 2**.

Table 2: Number of ossified and not ossified samples, percentage, earliest age of ossification.

AREA	NO OF OSSIFIED SAMPLES	NO OF UNOSSIFIED SAMPLES	PERCENTAGE OF OSSIFICATION	EARLIEST AGE AT WHICH OSSIFIED (years)
RTSH	119	181	39.6%	44
LTSH	123	177	41.0%	42
CRB	62	238	20.6%	53
CAB	118	182	39.3%	45
AMT	11	289	3.6%	63
RTL	16	284	5.3%	63
LTL	18	282	6.0%	62
RPB	182	118	60.6%	34
LPB	189	111	63.0%	32
RLPT	285	15	95.0%	18
LLPT	286	14	95.3%	18
RTIH	184	116	61.3%	33
LTIH	188	112	62.6%	32

On comparing the percentage of ossification of each defined area and earliest age at which each defined area ossified, a peculiar sequence of ossification with initiation from posterior and inferior areas and then progressing to superior and anterior areas was also noted. The ossification started in Posterior lower triangle areas followed by other areas and ended in Anterior Midline Tongue as follows.

- Right and left Posterior triangle
  –1st to ossify (started at 18 years)
- Right and left Inferior horns
  – 2nd to ossify (started at 32 & 33 years)
- Right and left posterior branch
  – 2nd to ossify (started at 32 & 34 years)
- Right and left superior horns
  – 3rd to ossify (started at 42 & 44 years)
- Caudal branch
  - 3rd to ossify (started at 45 years)
- Cranial branch
  – 4th to ossify (started at 53 years)
- Right and left lamina
  – last to ossify (started at 62 & 63 years)
- Anterior midline tongue
  – last to ossify (started at 63 years)

In each age group, the total number of samples (S), total number of study areas (A), the number of ossified (A1) and not ossified areas (A2), and frequency of ossification in each age group were studied and shown in **Table 3**.

Table 3: Number of samples, number of study areas, number of ossified and unossified areas, frequency in each age group of thyroid cartilage.

Age group (in years)	Total no of samples (S)	Total no of study areas (A)	Number of ossified areas (A1)	Number of unossified areas (A2)	Frequency of ossification
1 (18 to 20)	23	299	24	275	8.02%
2 (21 to 30)	81	1053	155	898	14.71%
3 (31 to 40)	64	832	343	489	41.22%
4 (41 to 50)	65	845	549	296	64.97%
5 (51 to 60)	46	598	455	143	76.08%
6 (61 to70)	19	247	230	17	93.11%
7 (71 to 80)	2	26	25	1	96.15%

It was observed that the frequency of ossification increased significantly in each group as age increases with minimum frequency of ossification observed in age group one 1 and maximum frequency of ossification observed in age group 7.

Discussion

A number of studies in the past has used thyroid cartilage for estimation of stature[16-18]orgender.[18-20]Several radiological studies[6-8] have observed the ossification of thyroid other laryngeal cartilages and classified them into various stages of ossification. In the year 1958, Keen and Wainwright [6]were the earliest to define, recognizable stages of radiopacity of the thyroid, cricoid, and arytenoid cartilages in male and female subjects in accordance with the age. The ossification pattern of thyroid cartilage was analysed with age by Grand Maison et al,[4]Ajmani et al,[9] Cerny,[10]Mupparapu and Vuppalapati,[11] Jurik AG,[12] Hately et al,[13]Gravin et al[14] and Turkmen et al.[15] In all the above studies the ossification pattern varies in accordance with both sex and age. In the present study the ossification pattern was studied only in accordance with age and not with sex as the number of male samples and number of female samples varied grossly(238 male samples and 62 female samples).

In the present study, Ossification of thyroid cartilage was first seen at the age of 18 years and the completely not ossified thyroid cartilage was last seen at the age of 23 years. These findings were consistent with the study documented by Hately et al,[13] Garvin

et al,[14] and Turkmen et al.[15] In Hately et al [13]study, a total of 516 cases of which 259 were males and 257 were females were analysed. According to this study the first ossified thyroid cartilage was seen at the age of 18 years among males and at the age of 16 years among females. Garvin et al [14] conducted their study on 104 isolated human laryngeal structures (68 men and 36 women) This study also documents that the first ossified sample of thyroid cartilage at the age 19 years. The study conducted by Turkmen et al[15] was done in 300 lateral cervical radiography of which 139 males and 161 females, also documents the first ossified sample of thyroid cartilage in the age group of 10 – 19 years of age.

In the present study, above 20 years of age all cartilages were ossified except for three cases. This observation was consistent with the study conducted by Hately et al,[13]where all the cartilages were ossified above the age of 20 years except for two cases.

In the present study the ossification starts at the posterior and inferior parts of thyroid cartilage and then proceeds from to superior and anterior parts of thyroid cartilage. The right and left posterior triangle were the first regions to ossify followed by ossification of inferior horns and posterior branches of both sides. This observation was similar to the findings of the work reported by Gravin et al[14]and the study conducted by Turkmen et al[15].

The study conducted by Gravin et al[14] in the year 2008 observed that the left and right posterior triangles are the first to ossify, documented in individuals as young as 19 years of age. The inferior horns and the posterior branches are the next to ossify. Complete ossification of the laminae and cranial branches of the thyroid cartilage were not observed in individuals younger than 39 years of age.

The study conducted by Turkmen et al[15] analysed the ossification of thyroid cartilage at four parts. (postero-inferior, postero-superior, centro-lateral and anterior). It also documented that the ossification started in the postero-inferior parts of thyroid cartilage in both males and females, in the 10–19 years age group.

The anterior midline portion of thyroid cartilage was the last portion to be ossified in the present study. This area was first seen to be ossified at the age of 63 years. This finding was consistent with the work done by Gravin et al[14] and Turkmen et al[15]. The pattern of ossification proceeding from posterior and inferior parts to superior and anterior parts could be due to vascular pattern of thyroid cartilage which invaginates the posterior and inferior parts of thyroid cartilage and then as age progresses it invaginates to superior and anterior areas of thyroid cartilage.

In the present study the frequency of ossification increased from age group 1 to age group 7. This finding was consistent with the study conducted by Mupparapu and Vuppalapati[11] in the year 2005. In the study conducted by Mupparapu and Vuppalapati[11] the frequency of ossification was observed to be 59% in males and 16% in females in age group 3 (31 to 40 years), 75% in males and 37% in females in age group 4 (41 to 50 years) and 95% in males and 54% in females in age group 5 (51 to 60 years). In our study the samples varied from 18 years to 80 years and so we were able to observe the increase in frequency of ossification in six age groups from 8% in age group 1 to 96% in age group 7.

Conclusion

In our study on comparing the ossification pattern of thirteen defined areas a peculiar sequence of ossification of thyroid cartilage was found and the frequency of ossification was found to increase significantly with increase of age in decades. As per our study, by analysing the ossification pattern of a thyroid cartilage, we can estimate the age of the individual and group it into any one of the age groups defined. Ossification pattern in the present study though was analysed with gross and cut section features, it only classifies the thyroid cartilage areas as ossified and not ossified. And it fails to recognise various degrees of ossification of the thyroid cartilage. Further studies if done with histological methods to observe various degree of ossification, it may help to narrow down the age estimates to increased precision.

Ethical Clearance: This study was conducted after the approval of Institutional Ethics Committee clearance of Post Graduate Institute of Medical Education and Research with reference number 8857/PG-2Trg/2011/3445-46 dated 05.02.2013.

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Conflict of Interest: Nil

References

1. Williams PL, Warwick R, Dyson M, Bannister LH. Gray's Anatomy. 37th Ed. Edinburgh, Churchill Livingstone, 1989;1250-51
2. Sugiyama S, Tatsumi S, Noda H, et al. Estimation of age from image processing of soft X-ray findings in Japanese male thyroid cartilages. Nihon Hoigaku Zasshi 1995;49(4):231-235.
3. Sugiyama S, Tatsumi S, Noda H, et al. Estimation of age from soft X-ray findings in Japanese female thyroid cartilages. Nihon Hoigaku Zasshi 1995;49(4):236-241.
4. Grandmaison de la GL, Banasr A, Durigon M. Age estimation using radiographic analysis of laryngeal cartilage. Am J Forensic Med Pathol. 2003; 24:96-9
5. Dang-Tran KD, Dedouit F, Joffre F, et al. Thyroid cartilage ossification and multislice computed tomography examination. A useful tool for age assessment? J Forensic Science 2010;55(3):677-683.
6. Keen JA, Wainwright J. Ossification of the thyroid, cricoid and arytenoid cartilages. S Afr J Lab Clin Med 1958; 4: 83-108.
7. Vleck.E. [Estimation of age from skeletal material based on the degree of thyroid cartilage ossification] Soud Lek 1980;25 (1): 6-11
8. Turk LM, Hogg DA. Age changes in the human laryngeal cartilages. Clin Anat 1993; 6(3); 154-62
9. Ajmani ML, Jain SP, Saxena SK. A metrical study of laryngeal cartilages and their ossification. Anat Anz. 1980; 148:42-8.
10. Cerny M. Our experience with estimation of an individual's age from skeletal remains of the degree of thyroid cartilage ossification. Acta Univ Palacki Olomuc 1983; 3:121–44.
11. Mupparapu M, Vuppalapati A. Ossification of laryngeal cartilages on lateral cephalometric radiographs. Angle Orthod 2004; 75:196–201.
12. Jurik AG. Ossification and calcification of the laryngeal skeleton. Acta Radiol 1984; 25:17–22.
13. Hately W, Evison G, Samuel E. The pattern of ossification in the laryngeal cartilages: a radiological study. Br J Radiol 1965; 38:585–91.
14. Garvin HM. Ossification of laryngeal structures as indicators of age. J Forensic Sci. 2008; 53:1023-7
15. Turkmen S, Cansu A, Turedi S, Eryigit U, Sahin A, Gunduz A, Shavit I. Age-dependent structural and radiological changes in the larynx. Clinical Radiology 2012; 67: e22–26.
16. Fayoux P, Marciniak B, Devisme L, Storme L. Prenatal and early postnatal morphogenesis and growth of human laryngotracheal structures. J Anat. 2008; 213:86-92
17. Subramanyam S, Singh D, Bansal YS, Mandal SP. Determination of Stature from Anthropometric Measurements of Thyroid Cartilage in the Population of Punjab. Indian Journal of Forensic Medicine & Toxicology. 2020;14(1):67
18. Monica J, Usha D. Morphometry of the thyroid and cricoid cartilages in adults. J Anat Soc India. 2008; 57:119-123.
19. Ajmani ML. A metrical study of the laryngeal skeleton in adult Nigerian. J Anat. 1990; 171:187-91.
20. Subramanyam S, Murali G, Mandal SP, Bansal YS, Singh D. Sex determination from anthropological measurements of thyroid cartilage in the population of Punjab. Journal of Indian Academy of Forensic Medicine. 2014;36(4):367-70.