

ESTIMATION OF AGE FROM OSSIFICATION CHANGES IN THYROID CARTILAGE OF NORTH KERALA POPULATION

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ABSTRACT

BACKGROUND

Estimation of age at death is one of the demographic characteristics that has extensively been studied by anthropologists, which has immense forensic importance. Age is a primary identification characteristic of an individual. The various methods employed in age estimation includes odontology, ossification centers, radiological examination, age related changes in bones and various regression formulas, etc.

MATERIALS AND METHODS

The present study is undertaken for the estimation of age from morphological examination of thyroid cartilage for a period of one year at Department of Forensic Medicine, Kozhikode. The study samples included thyroid cartilages from the dead bodies of 200 males and 200 females subjected to post mortem examination. The age of the study sample was taken from the data provided by the police in the requisition for post mortem examination. The observations were statistically evaluated.

RESULTS

The estimation of age from thyroid cartilage from morphological features includes gross examination by serial sectioning for ossification centers, length and breadth of alae, angle between alae and weight of the cartilage and mathematical calculation of these values.

CONCLUSION

However, none of the method stands as a gold standard as a single index as there exists considerable variations between individuals and populations. Hence, combination of different methods are often preferred for more accurate estimation of age. Thyroid cartilage has been used since ages for estimation of age using the parameters like ossification center, calcification, etc.

KEYWORDS

Thyroid Cartilage, Age, Morphology, Ossification.

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BACKGROUND

Human individuals differ from one another in morphometric details, which is also known as personal identity. Establishment of identity of the deceased is one of the objectives of an autopsy.¹ Questions of identity arises every day in medical practice, both in civil and criminal cases. The establishment of "corpus delicti" in criminal cases depends very much on the establishment of identity of the corpse. After death it becomes difficult especially when the body is found severely burnt, mutilated or in a decomposed state.¹ Age, sex, race and stature contribute much to the identity of a person. For determination of sex, race and stature morphometric methods are useful, but for age determination ossification of precursor cartilages, epiphyseal appearance

and fusion in long bones and remodelling and regression changes in bones are to be depended upon mostly, both in living as well as dead. Different traits are used to determine age including microscopic and macroscopic methods. Since all the parameters referred to shows considerable inter-individual variability that is non-pathological or normal, precise and narrow range of age can never be given using a single center. Hence, combination of several methods are to be employed in the calculation of approximate age.

Ossification changes in laryngeal cartilages, especially thyroid cartilage and its association with progression of age was studied in various population using different methods. A classical work in this field was done by Cerny (1983),² who summarised the historical studies and established a specific pattern (phases) of ossification of thyroid cartilage. HM Garvin (2008)³ critically analysed the Cerny's phase method and questioned its usage for the purpose of age assessment. While the studies in Asian populations like Japanese and Chinese had found excellent correlation between the progression of age and pattern of ossification that of European and African did not get the same.

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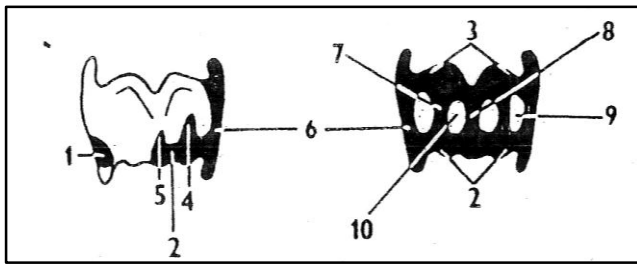


Figure 1. Anatomy of the Thyroid Cartilage (1) Lower Posterior Triangle; (2) Lower (Caudal) Branch; (3) Upper (Cranial) Branch; (4) Paramedian Process; (5) Median Process; (6) Lateral Bar; (7) Paramedian Bar; (8) Median Bar; (9) Posterior Window; (10) Anterior Window

There are few studies of these aspects in the Indian subcontinent. As there were no studies on the local population with regard to the age changes in thyroid cartilage, we decided to take up a study that could be applied to the Kerala population. As the Centre mostly caters for the northern parts of Kerala, the study sample was selected from the north Kerala population.

Aims and Objectives

1. To estimate age from thyroid cartilage by gross examination for ossification changes.
2. To compare the ossification changes in male and female populations.

MATERIALS AND METHODS

The study was a series observation of 400 thyroid cartilages, collected from dead bodies autopsied in the Kozhikode Medical College mortuary during a period of August 2009 to September 2010.

Inclusion 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 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. Prominence of larynx seen externally was also recorded.

Dissection of Cartilage- After the anterior midline incision was made during the autopsy, the skin and platysma of neck was reflected to either side, sternomastoids and the anterior ribbon muscles were reflected gently from below upwards. A cartilage knife was inserted behind the submandibular salivary gland of the left side and then moved along the lower jaw border to separate the muscles. Holding the tip of the tongue with the forceps exerting moderate pull and cutting tissues between the hard and soft palates, the midline neck structures were pulled out. The thyroid cartilage is then dissected out using forceps and scissors for blunt dissection. Relative size of the thyroid gland and ossification of thyrohyoid ligament was also recorded.

Cleaning and Processing- 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 NaOH 10% to macerate. It was taken out after 3 days and cleaned thoroughly.

The cleaned specimen is stored in plastic jars containing 10% formalin according to their ages and sex. 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 paramedian bar was measured using a vernier caliper. The weight was measured using an electronic weighing machine. All these morphometric data are recorded in a serially numbered proforma.

Gross Examination- Gross examination was conducted by serially sectioning the thyroid cartilage at various levels and naked eye examination for ossification changes. Each cartilage after noting its number is kept on the wooden dissecting board. Different areas of the cartilage are transected using a sharp knife and the cross-sections were examined for ossification on both halves of cartilage separately. The ossified areas appeared as chalky white or brown in colour and also produced a gritty feeling on cutting. The findings were recorded in the corresponding proforma.

Statistics- All the data thus obtained are statistically analysed with the help of a statistician. The SPSS software was used for this purpose.

RESULTS

A total of 400 cases from north Kerala were included in the study. Age of the cases ranged from completed 16 years to 85 years with a mean age of 41.94.

Age Groups	Number of Male Cases	Number of Female Cases
< 20	2	20
20 – 29	44	54
30 – 39	29	35
40 – 49	53	27
50 – 59	40	24
60 – 69	26	19
> 70	6	21
Total	200	200

Table 1. Number of Cases in each Age Group

a) Morphological Findings

Prominence of the larynx was medium in 64% of the male cartilages examined, whereas 30.5% were highly prominent. In females, majority had a minimum prominence (83%). Apparent size of the thyroid cartilage was medium in 76% of the males and was small in 60% of the females. Apparent size of the thyroid gland was medium in majority (83%) of the males, whereas it was small in 59% of the females. A complete ossification of thyrohyoid ligament was not seen in any of the males or females. There was progression of ossification in 7 males and 3 females.

b) Ossification Changes

Males- Lower posterior triangle was the first area to ossify. All the 200 cases examined had an ossified LPT. But there were only 2 cases of less than 20 yrs. Lower branch was seen ossified in 83.5% of the males and their mean age was 46.90 yrs. and minimum age was 21 yrs. A partially ossified lower branch was seen in 15% of the cases and their mean age was 24.67 yrs.

Upper branch was not ossified in most of the males. A partially ossified upper branch was seen in 6 cases. Fully ossified upper branch was seen in 8.5% of the cases and their mean age was 60.88 yrs. (SD 10.98). Paramedian process was found to be ossified in 75% of the cases examined and their mean age was 48.81 yrs. and minimum age was 21 yrs.

A well ossified median process was seen in 71% of the cases and their mean age was 49.23 (SD 11.35). Lateral bar tends to ossify much earlier following LPT. There were 17 cases of partially ossified lateral bar and their mean age was 22.29 yrs. A fully ossified lateral bar was seen in 88.5% of the males.

Paramedian bar was found to be ossified in 44% of the cases. Their mean age was 52.66. The cartilages in which a paramedian bar was absent had a mean age of 35.89 yrs. Median bar was found to be ossified in 37% of the cases and their mean age was 54.43 and minimum age was 30 yrs. A well-formed posterior window was seen in 9 cases of advanced age. An anterior window was seen in 17.5% of the cases and they had a mean age of 58.94 yrs. and a minimum age of 45 yrs. Inferior horn ossifies much earlier; 99% of the males had an inferior horn ossified and their mean age was 43.36 yrs. Superior horn, which tends to ossify in the middle age group was seen ossified in 77.5% of the cases and their mean age was 48.59 yrs.

Females- In females also, beginning of ossification was seen in the lower posterior triangle. 88.5% of the examined female cartilages had an ossified LPT. The remaining group had a mean age of 18.96 yrs.

Lower branch was seen partially (lateral half only) ossified in 50% of the females and their mean age was 45.19; 23.5% cases in which a fully ossified lower branch was seen had a mean age of 52.72 yrs. and minimum age of 30 yrs. Upper branch tends to ossify more and much earlier in females when compared to males. 25% of the cartilages had a partially ossified (lateral half only) upper branch and their mean age was 57.72. Only 7 cases had a completely ossified upper branch. A well-ossified paramedian process was seen in 34.5% of the females. The rest in which no paramedian process was seen had a mean age of 30.81 yrs. Median process ossification was not seen in almost all (97%) cases of females. Lateral bar ossifies following LPT in earlier age. 66% of the cases had a fully ossified lateral bar and their mean age was 50.09 yrs. (SD 15.76). A partially ossified lateral bar was found in 10.5% of the cases and their mean age was 25.29 yrs.

A well-ossified paramedian bar was not seen in 87% of the females. The rest had a mean age of 73.31 yrs. This study cannot find a medial bar in any of the female cartilages. Window formation was also absent in almost all cases, except one case with age of 70 yrs. Inferior horn was seen ossified in 77% of the females and their mean age was 46.57 (SD 17.02), whereas 23% in which an ossified inferior horn was not seen had a mean age of 20.61 yrs. Superior horn tends to ossify in middle ages. 58.5% had an ossified superior horn and the rest 41.5% showed a mean age of 23.63 yrs. and a minimum of 18 yrs.

Sl. No.	Ossification Changes		Age of Ossification			
	Area	Ossification	Male		Female	
			Mean	Minimum	Mean	Minimum
1	LPT	Nil			18.96	16
		Ossified			43.41	17
2	LB	Nil	27	23	21.15	16
		Lateral half	24.67	18	45.19	20
		Fully ossified	46.90	21	52.72	30
3	UB	Nil	41.43	18	32.98	16
		Lateral half	44	24	57.72	28
		Medial half	63	63		
		Fully ossified	60.88	45	74	60

4	PP	Nil	26.64	18	30.81	16
		Ossified	48.81	21	59.19	28
5	MP	Nil	28.82	18	40.17	16
		Ossified	49.23	24	54.50	46
6	Lateral Bar	Nil	24.50	18	20.79	16
		Lower half only	22.29	19	25.29	18
		Fully ossified	45.92	21	50.01	23
7	PB	Nil	35.89	18	35.77	16
		Ossified	52.66	24	73.31	60
8	MB	Nil	36.71	18		
		Ossified	54.43	30		
9	PW	Nil	42.48	18	40.45	15
		Present	60	28	70.00	70
10	AW	Nil	39.95	18		
		Present	58.94	30		
11	IH	Nil	34	21	20.61	16
		Ossified	43.36	18	46.57	18
12	SH	Nil	24.93	18	23.63	16
		Ossified	48.59	26	52.64	24

Table 2. Comparison of Mean and Minimum Age of Ossification with regard to Specific Areas in Males and Females

Statistical Analysis Data - A seven model method was created for males and females, which is given in the table. Since it is gross examination, some morphological parameters which are statistically significant are also considered for the deduction of the best method. The seven model method has got an adjusted R square value of .710 for males and .867 for females. This signifies that age can be derived precisely in 71% of the males and 86.7% of the females.

Sex	Model	Parameter	Unstandardised Coefficient	Significance
Male	7	(Constant)	-26.837	.000
		SH_Rt	12.173	.000
		AW_Rt	6.806	.000
		OS_MB	6.111	.000
		Pro_L	3.486	.001
		OS_PP_Rt	6.301	.002
		Size TG	3.646	.015
		PW_Rt	6.321	.019
Female	7	(Constant)	-20.002	.013
		SH_Rt	14.063	.000
		OS_PP_Rt	8.218	.000
		OS_PB_Rt	19.327	.000
		OS_UB_Rt	3.345	.000
		OS_LB_Rt	3.095	.000
		Angle	-.123	.026
		OS_MP	6.617	.027

Table 3. Coefficients of Seven Model Method

- SH_Rt – Right Superior Horn.
- AW_Rt – Right Anterior Window.
- OS_MB – Ossification of Median Bar
- Pro_L – Prominence of Larynx
- OS_PP_Rt – Ossification of Right Paramedian Process
- Size TG – Size of Thyroid Gland
- PW_Rt – Right Posterior Window
- SH_Rt – Right Superior Horn
- OS_PP_Rt – Ossification of Right Paramedian Process
- OS_PB_Rt – Ossification of Right Paramedian Bar
- OS_UB_Rt – Ossification of Right Upper Branch
- OS_LB_Rt – Ossification of Right Lower Branch
- Angle – Angle between two laminae
- OS_MP – Ossification of Median Process

DISCUSSION

Ossification changes in laryngeal cartilages especially thyroid cartilage and its relation with age was studied by a few forensic and physical anthropologists by using different modalities. Earlier view was that the ossification and calcification are the same process. Later, there were several attempts to differentiate the two. In the present study it is found that as all hyaline cartilages, thyroid cartilage also undergoes ossification from particular osseous nuclei.⁴ Dystrophic calcification can also occur in the cartilage, if present it is seen as islands of calcified masses. It is very difficult to differentiate the two grossly or radiologically. Histology can solve the problem to some extent. Since the calcification incidence is very minimal, this study considered

them together for the ossification changes. The present study found that thyroid cartilage in both the sexes undergoes ossification as age advances. It was found that age of onset of ossification was around 20 years in males and 20 to 29 years in females. The age of onset of ossification observed was in males, was fit with the observation made by pioneers in this field like Pietro Roncallo (1948),⁵ W. Hately (1965)⁶ and HM Garvin (2008),³ but in females it was much slower when compared to their observation.

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. The present study found that it was lower posterior triangle of the cartilage, in which the ossification first begins. Latest CT based study by KD Dan Tran et al⁷ also confirmed this.⁷ Progression of ossification was different in different studies. The present study found that ossification progresses in three directions from the point of onset, LPT (lower posterior triangle). It was through lateral bar, lower branch and to the inferior horn. In females the progression was at a slower rate, as pointed out by many earlier. In males more progression was through the lower branch and from there

to the lamina as paramedian 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. 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. Cerny introduced phase method for the age determination in males, but it was critically analysed by HM Garvin and he clarified that that method was highly inaccurate and not applicable for age determination. The present study also did not show correct Cerny phases. Studies conducted on Chinese Han population by Cheng J et al (2003)⁸ and on Japanese population by Sugiyama et al⁹ had proposed that thyroid cartilage ossification can be used for determination of age at death. The present study demonstrated a positive correlation with the age. This study has got very significant adjusted R-square values for both sexes, which was far better when compared to the previous authors. Different stages of the ossification and their age of occurrence were also detected. The stages of the ossification in males and females are given below.

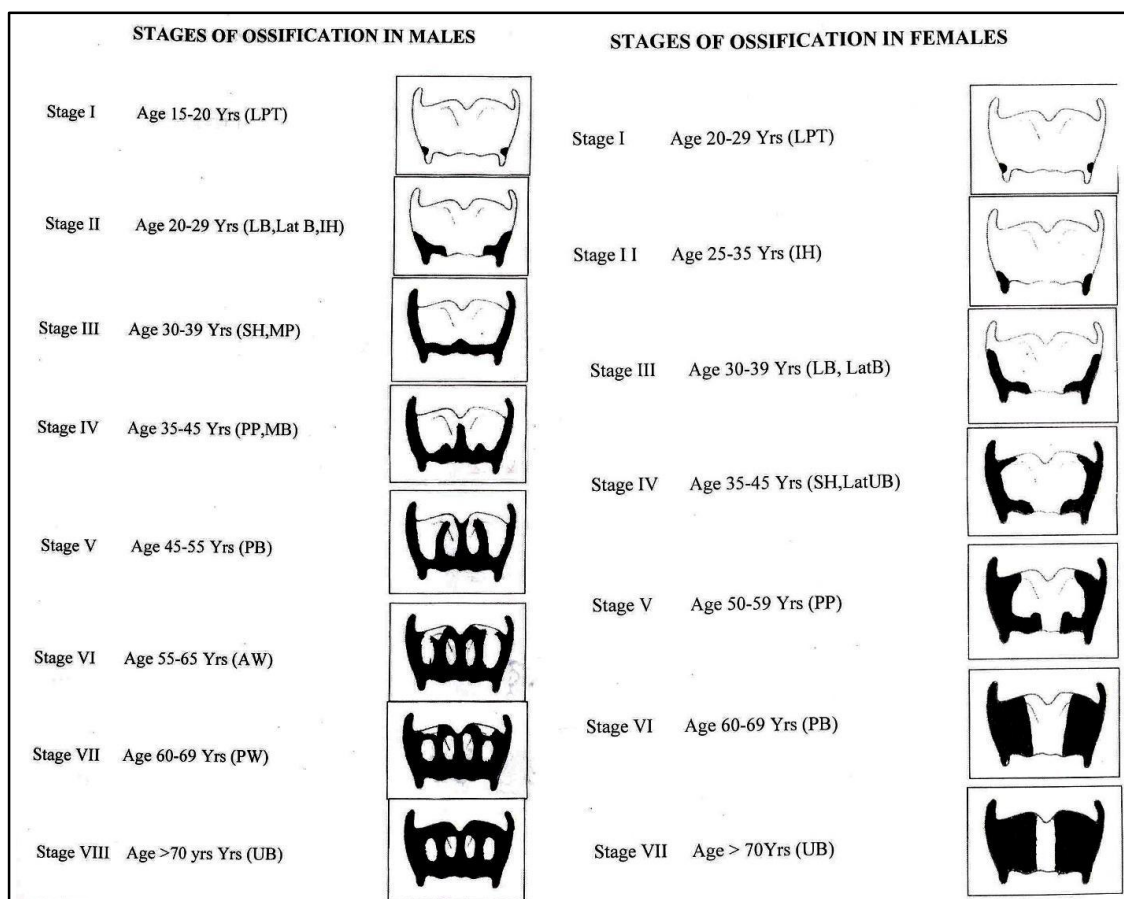


Figure 2. Stages of Ossification and their Age Group Derived in the Present Study

CONCLUSION

Onset and progression of ossification was studied in detail in both sexes using morphological and gross examination. It is found that although males and females have considerable difference in the pattern of ossification, age can be

determined from thyroid cartilage ossification to a certain extent using the different stages derived from this study. Since there is inter-individual variation, this method should be used as a supportive aid for other age determination methods.

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