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RESEARCH ARTICLE

AGE ESTIMATION FROM RADIOLOGICAL EXAMINATION OF THYROID CARTILAGE IN NORTH KERALA POPULATION

Dr. Ratheesh Punnath Thadathil and *Dr. Priyatha Ponnappan

Department of Forensic Medicine, Govt. Medical College, Kozhikode

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ABSTRACT

Establishment of identity remains the key aims in a medico legal autopsy. Identity of a person can be made from his race, stature, age, sex etc. for living persons the establishing of identity is not a difficult task but not so in cases of dead persons. The problem is more challenging if the skeletal remains only was available for medico legal examination. Determination of age was carried out from various changes in bones and cartilages including fusion of epiphyses, ossification changes, regression changes etc. Thyroid cartilage being the largest cartilage of the laryngeal complex was studied by various researchers for its relationship with age and ossification. However studies on thyroid cartilages for age determination was lacking in North Kerala population. In this study thyroid cartilages from 203 males and 200 females were collected during autopsy conducted in dept of Forensic medicine and was subjected to radiological examination so as to find out its relationship with age. The observations were analyzed with SPSS software. A linear relationship with ossification changes in thyroid cartilage with age was established both in males and females. Characteristic pattern of ossification was derived in both sexes and distinct stages were identified which were different from previous studies.

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INTRODUCTION

Establishment of identity from skeletal remains, mutilated bodies has been a time honored task, in forensic medicine. This includes determination of race, age, sex, stature etc. considering the facts that human beings differ individual by individual and population by population. There are different methods for determining the various parameters. There exist two different types of age for an individual- chronological age and biological age. Chronological age refers to the total number of years lived since birth and is documented individually for each person. Biological age denotes the condition of the individual which is not a constant with person to person of the same age. The conventional methods of age determination like epiphyseal closure, remodeling and regression changes in bones, and teeth are the ones, still widely used. Latest methods like racemization in teeth, dental cementum, lumbar vertebral rim osseus union etc may require more time, advanced technologies and are expensive. None of these methods gives accurate age prediction when used alone. Hence a combination of methods are used to find out the accurate age with maximum precision. The thyroid cartilage is the largest of the laryngeal cartilages. 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). 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. Anteriorly the thyroid cartilage is connected to the cricoid cartilage by anterior crico-thyroid ligament which is a thickened portion of crico-thyroid membrane (Gray's anatomy, 39th edition). 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 ossification as age advances this process normally starts at about 18 years of age. The pattern of ossification in thyroid cartilages were studied by many in the ancient times, but it was Pietro Roncallo (1948), who studied in detail about this subject and given some authentic opinion about the onset and progression of the ossification in the thyroid cartilages.

***Corresponding author:* Dr. Priyatha Ponnappan,**
Department of Forensic Medicine, Govt. Medical College, Kozhikode

But he couldn't find an association between age and ossification. Cerny (1983) studied the ossification changes and divided them into 9 phases (I to IX) and also correlated the phases with particular age groups. But the number of samples was too less to be statistically significant. There are few such studies in this field in our country like works by Harijeet and Jit (1989) who studied calcification of the main laryngeal cartilages in 75 male and 75 female specimens varying in age from 14 to 80 years. The cartilages were divided suitably and x-rayed on dental occlusal films. Calcification was found to commence in the lamina of the thyroid cartilage at its anterior border at 18 years in the males and 16 years in females. Munir Turk and Hogg (1993) studied various changes in the human laryngeal cartilages by naked eye, radiologically and Histologically in 28 dissecting room specimens and 20 autopsy specimens ranging from 14 to 101 years in Caucasian population. They found that it is possible to estimate the age of an adult from the extent of calcification of the main laryngeal cartilages. Sugiyama *et al*. (1995) stated that increasing thyroid cartilage ossification was a reliable method of age determination. The thyroid cartilage of 501 Japanese men and 513 Japanese women were radiographed at autopsy and computerized measurement of ossification areas was performed to obtain an ossification rate for each specimen. Since the studies on radiological means of age estimation from thyroid cartilage wereless, such a study was taken up in the centre. The study group selected was North Kerala population as the centre receives cases mainly from Northern part of Kerala state.

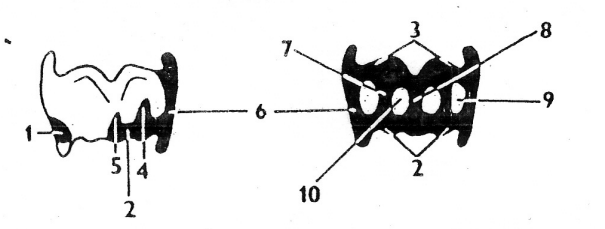

Figure 1. Anatomy of the thyroid cartilage (1) lower posterior triangle; (2) lower (caudal) branch; (3) upper (cranial) branch; (4) paramedian process; (5) median process; (6) lateral bar; (7) paramedian bar; (8) median bar; (9) posterior window; (10) anterior window (Krogman and Iscan, 1986)

Aims

To observe the pattern and progression of ossification changes occurring in thyroid cartilage with advancement of age and the differences of these in the male and female .

Objectives

1. To collect thyroid cartilages from at least 200 male and 200 female dead bodies in the age range of 15 – 70 yrs. and conduct radiographic examination to observe the phases of ossification

MATERIALS AND METHODS

The study was a series observation of 403 thyroid cartilages, collected from dead bodies autopsied in the Govt. Medical College, Kozhikode mortuary during the period of August 2009 to September 2010 and subjected to radiological examination at Department of Radio Diagnosis, Govt. Medical College Kozhikode.

Exclusion criteria

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

Method of sample collection:

The thyroid cartilage is dissected out, by routine autopsy technique and macerated by immersion in 10% Sodium Hydroxide solution for 3 days and then cleaned with plain water. The cleaned specimen is stored in labeled plastic jars containing 10% formalin. After getting a bunch of specimen, they are arranged and tied on to serially numbered plastic board. 16- 20 specimens of same age and sex group were arranged on one board. The X- rays were taken using 14 x 16 inch Kodac X- ray film. Soft x rays are taken using radiation of 6- 8 mAs and 45 – 55 kvp. Areas of opacities were looked for and documented. The data thus obtained are statistically analyzed using SPSS software with the help of a statistician.

RESULTS

A total of 403 cases, from north Kerala were included in the study. Thyroid cartilages collected from 203 male and 200 female persons were studied using soft tissue radiography. The lowest was 16 years of age for male group and 15 for female group, and maximum age was 85 for both the sexes.

Ossification changes

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 21 years had begun ossification changes. Lower branch was not ossified in 15 % of the case examined. And their mean age was 21 years. A partly ossified lower branch (lateral half only) was seen in 13 % of the cases (mean age 24.78). 72 % had a completely ossified lower branch.Upper branch ossification was absent in 83% of the males; the mean age was 37.53yrs. (SD 16.31). 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 and their mean age was 51 years. A well ossified Para median bar was absent in 39 % of the males (Mean age 26yrs). The remaining 61 %, in which a Para median process present had a mean age of 49.97 yrs. (SD14.83). Median process was not seen in 71 cases. The rest of the cases in which a median process was present, had a mean age of 49.20yrs. (SD 14.69). The minimum age, in which an ossified median process seen was 20 years. Lateral bar, on each side of the laminae had an ossification earlier, following LPT. 14 % of the cartilages in which no ossified lateral bar was seen, had a mean age of 18.97 yrs. 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, and their mean age was 24.73 yrs. A completely ossified lateral bar was found

in 73 % of the cases. Para median bar was well ossified in 27 % of the males and their mean age was 52.85 yrs. Median bar is found to be ossified in 53 % of the cases. Their mean age was 52.13yrs. and minimum age was 22 yrs. 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 and their mean age was 18.84 and their maximum age was 25yrs. Most of the cases had (87 %) an ossified inferior horn and their mean age was 43.70 yrs. Superior horn tends to ossify in the middle ages. 72 % of the examined male cartilages had a superior horn ossified. The mean age for them was 48.06yrs. All the cartilages of the age above 30 years had an ossified superior horn.

48.25 yrs. (SD 17.68). Para median bar was absent in86 % of the cases. A fully ossified. Para median bar was found in 74 of the cases having a mean age of 67.71 yrs. 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 with a mean age of 46.74 yrs. 41.5 % of the female cartilages had an ossified superior horn, and their mean age was 50.40 yrs. For statistical analysis, only ossification changes are considered for the deduction of best (statistically significant) model. A four model method, using parameters like ossification of Median Bar, Para median Process, Inferior Horn and Posterior Window for males, and ossification of Para median Process, Para median Bar, Median Process and Inferior Horns for females were the most appropriate one for the prediction of age. The four model method has got an adjusted

Table 1. Ossification changes noted in specific areas and their mean age in males and females

	Ossification changes		Age of ossification			
			Male		Female	
S.No.	Area	Ossification	Mean	Minimum	Mean	Minimum
		Nil	18.22	16	25.50	15
1	Lpt	Ossified	42.82	18	45.01	18
		Nil	21	16	25.91	15
2	Lower	Lateral half	24.78	19	41	19
	Bar	Fully ossified	47.79	19	57.69	32
		Nil	37.53	16	34.85	15
3	Upper	Lateral half	61	52	56.80	30
	Bar	Medial half	51.10	28	39.67	19
		Fully ossified	57	22	74	65
		Nil	25.99	16	31.08	15
4	Paramedianprocess	Ossified	49.97	20	59.73	28
		Nil	24.70	16	37.65	15
5	Median process	Ossified	49.20	20	63.26	27
		Nil	18.97	16	26.55	15
6	Lateral	Lower half only	24.73	19	30.60	22
	Bar	Fully ossified	47.68	20	48.25	19
		Nil	36.09	16	36.78	15
7	Para median bar	Ossified	52.85	22	67.11	25
		Nil	27.57	16	39.71	15
8	Median bar	Ossified	52.13	22	70.78	58
	Posterior	Nil	38.89	16	40.36	15
9	Window	Present	58.61	22		
	Anterior	Nil	37.20	16	40.02	15
10	Window	Present	55.55	22		
	Inferior horn	Nil	18.84	16	25.49	15
11		Ossified	43.70	19	46.74	19
	Superior	Nil	22.o9	16	28.01	15
12	Horn	Ossified	48.06	19	50.40	20

A.Females

In females also ossification had apparently started in Lower Posterior Triangle. 20 % of the female cartilages had no ossification changes within them and their mean age was 25.50 yrs. Whereas 80 % of the case. 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 had a mean age of 56.80 yrs. A fully ossified upper branch was seen in 12 cases and that too was in the advanced ages (mean age 74yrs). Para median process was not seen in 65 % of the cases. The rest of the cases in which a Para median process seen had a mean age of 59.73 yrs. Median process seldom gets ossified in females. Only 13.5 % had a median process and that too was in later ages. The mean age was 63.26 (SD 11.61). Lateral bar tends to ossify earlier. Ossification was not seen 14.5 % of the cases and their mean age were 26.55 yrs. A partly ossified lateral bar was seen in 26 cases (mean age 24.73yrs). A fully ossified lateral bar was seen in rest of the cases and their mean age was

R square value of 0.591 for males and 0.652 for females, which means that age can be predicted precisely in 59.1 % of the males and 65.2% of the females which is definitely a good number.

Table 2. Model summary

SEX	Model	Adjusted R Square
male	1	.495
	2	.560
	3	.577
	4	.591
female	1	.529
	2	.579
	3	.625
	4	.652

Table 3. Coefficients of the model and their significance

Coefficients[a]

SEX	Model		Unstandardized Coefficients B	Sig.
male	4	(Constant)	-21.731	.000
		OS_MB	14.390	.000
		OS_PP_RT	9.941	.000
		IH_RT	8.324	.002
		PW_RT	7.916	.006
female	4	(Constant)	-25.581	.000
		OS_PP_RT	17.010	.000
		IH_RT	9.984	.000
		OS_PB_R	13.032	.000
		OS_MP	10.201	.000

a. Dependent Variable: AGE

OS_MB – ossification of median bar
OS_PP_RT – ossification of Para median process
IH_RT – inferior horn right
PW_RT – posterior window right
OS_PB_R – ossification of right Para median bar
OS_ MP – ossification of median process

calcification are the same. 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 osseus nuclei. 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 purpose.

Ossification changes

The present study also found that thyroid cartilage in both the sexes undergoes ossification as age advances. The details of the ossification changes are discussed in following headings.

1) Age of onset of ossification

Pietro Roncallo (1958) had suggested that thyroid cartilages undergoes ossification from certain ossification centers at the age of around 20 years, in both the male and females. Hately (1965) also got similar observation, and he concluded the age of onset of ossification as 15 to 20 years for both sexes. Cerny, by his phase method, had described age of onset of ossification

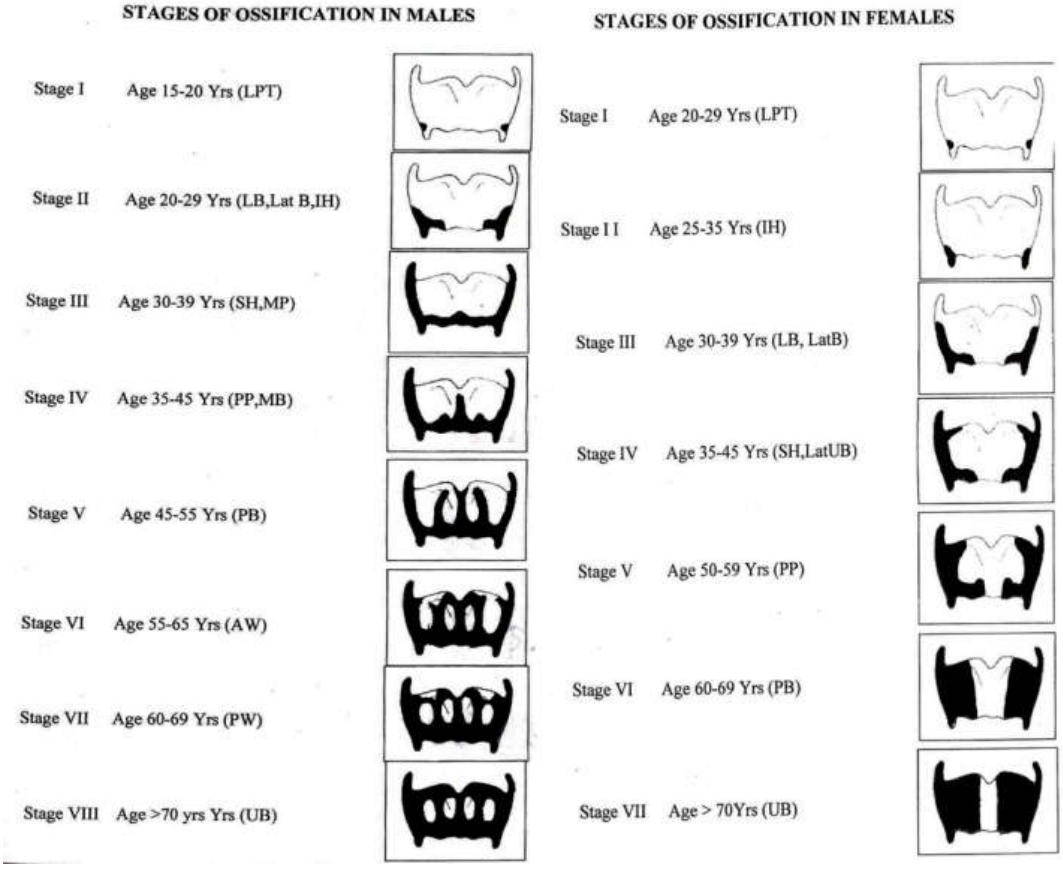

Figure 2. Different stages of ossification derived in the present study

DISCUSSION

Ossification changes and its relation with age in laryngeal cartilages especially thyroid cartilage, , was studied by a many forensic and physical anthropologists by using different techniques, i.e. gross examination, radiographic, histological, and recently using Computed Tomography examination. Before the emergence of X rays anatomical studies were undertaken, but the results were fragmentary or incomplete. Earlier many authors believed that the ossification and as 15 to 21 years for males. Garvin (2008) pointed towards 18 to 20 years. No other researcher could conclude a different opinion. From this study, it was found that age of onset of ossification was around 20 years in males and 20 to 29 years in females.

2) Area of onset of ossification

Roncallo (Harijeet, Jit 1989) 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. Hately *et al* 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 Turk and Hogg (1993), 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 Dan Tran *et al*. (2010) also confirmed this.

3) Progression of ossification

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 Hately *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 by Cerny, we found that anterior window forms earlier than the posterior. And also median process tends to ossify in the middle ages.

4) Complete ossification

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 Grandmaison *et al*. (2003), as they got one completely ossified female cartilage. Keen and Wainwright (1958) found that front part of the laminae and midline area remains cartilaginous to a more advanced age in the females which was confirmed by Hately *et al*. 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.

5) Relation with age

Although many authors had studied the ossification in the thyroid cartilage, its use in determination of age was not recommended by many. Cerny (Gray's anatomy 39th edition) introduced phase method for the age determination in males, but it was critically analyzed by 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. Latest CT based study also confirmed that methods based on thyroid cartilage ossification were not accurate enough for individual age assessment. Grandmaison *et al* had got a positive correlation of age and degree of ossification, but their standard error was too high (12.6). But studies conducted on Chinese Han population by Cheng *et al*. (2003) and on Japanese population by Sugiyama *et al* 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 statistically better when compared to the previous authors. Different stages of the ossification and their age of occurrence were also studied. The stages of the ossification in males and female are given above.

Summary and Conclusion

Establishment of identity from skeletal remains has been a time honored tasks, in Forensic Medicine. Thyroid cartilage undergoes ossification changes as any other hyaline cartilage. Onset and progression of ossification was studied in detail in both sexes using radiological 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. An extended study may be attempted in another population or with the help of histology and or advanced techniques like computed tomography in the same population, for a better evaluation and correlation.

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