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Ossification of Laryngeal Structures as Indicators of Age*

ABSTRACT: As the role of forensic anthropologists expands to the medical examiner setting, their expertise is being applied beyond the traditional dry skeletal material. In such scenarios radiographic techniques can be applied when maceration is not appropriate. This study explores the use of radiographic analysis of laryngeal structures for age-at-death determination. Isolated human laryngeal structures (n = 104) from individuals between the ages of 15 and 89 were removed at autopsy and radiographically examined. The cricoid and individual regions of the thyroid cartilage were scored according to degree of ossification, and the relationship between age and degree of ossification statistically examined. A previously published study on age-determination from thyroid ossification by Černý was assessed for accuracy. The results of the study indicate that although a consistent sequence in the ossification of laryngeal structures exists, variations in timing does not permit narrow age range estimates. Consequently the method presented by Černý is inaccurate and should not be used in the forensic setting.

KEYWORDS: forensic science, forensic anthropology, thyroid cartilage, age determination, hyoid bone, cartilage ossification

Examining multiple skeletal elements and using appropriate methods are critical to obtaining accurate age-at-death estimates from human remains. Although the ossified laryngeal structures are rarely recovered with skeletal remains (1), and therefore may appear insignificant, as more forensic anthropologists find roles in Medical Examiner's Offices, their unique skills may be applied to fleshed remains where the structures are relatively intact. The laryngeal structures are routinely extracted during autopsy and can be radiographed within minutes, allowing the remains to be returned to the body before completion of the autopsy. If a correlation exists between chronological age and ossification of the laryngeal structures, a quick radiographic analysis during autopsy can provide pertinent information to investigators, facilitating the identification of the individual without interfering or altering autopsy procedures. The ossified areas identified radiographically are a direct representation of what would remain after processing. Therefore, once the patterns of ossification of the laryngeal structures are depicted and the degree of correlation with age determined, an age estimation method could be applied in either setting, to fleshed or skeletonized remains.

Currently, when presented with either a partially or completely ossified thyroid cartilage, forensic anthropologists and pathologists are likely to turn to a chart originally produced by Černý (2) and replicated in the well-known text *The Human Skeleton in Forensic Medicine* (pp. 127–129) (1). This chart includes diagrams depicting the patterned areas of ossification of the thyroid cartilage and corresponding narrow age ranges. Written descriptions portray the initial ossification of the posterior branches (15–21 years) followed by the inferior (21–25 years) and then superior horns (25.5–31 years), caudal branch (28–39 years), paramedian bars (37.5–45 years), cranial branch and median bar (51–58 years), and finally completion of the laminae (57–68 years) (1). The completely ossified thyroid cartilage is represented as displaying anterior and posterior windows lacking ossification.

Although originally published in 1983, Černý's (1,2) phase method is still the most common reference used to estimate age from thyroid cartilage ossification, reflecting a lack of more recent literature on the subject. An extensive literature review revealed only a handful of articles on thyroid ossification, most not directly related to age estimation. Within this literature, the overall consensus is that the hyaline cartilage of the thyroid cartilage does progressively ossify with age, and at a different rate between males and females. The authors, however, refrain from providing any correlations with specific ages (3–6) or suggest that these parameters are not significantly correlated (7,8). Those articles that do discuss specific ages correlating to ossification are not readily available to English-speaking professionals, as they are published in nonindexed journals or require translation (8–10), as is the case with the Černý article (2).

While obtaining the information from a secondary source is not ideal, there are more serious concerns regarding the present application of Černý's method. As pointed out by Scheuer and Black (p. 168) (7), this popular age estimation chart is based on a total sample of five ossified thyroids from "White" males with a minimum age of 54 years. Further, as presented in Krogman and Işcan (2), the criteria utilized to demarcate the nine phases are not clear. Finally, their discrete narrow age ranges are not accompanied by any descriptive statistics or associated probabilities.

The purpose of this study was to determine the relationship between age and ossification of the thyroid and cricoid cartilages. Individual thyroid regions of ossification are examined and discussed in terms of ossification trends to assess the potential use of this structure as a reliable age marker in forensic settings. The accuracy and reliability of Černý's method (1,2) of determining age from thyroid ossification was also assessed. Descriptive statistics and confidence intervals for Černý's phases are provided, based on

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a modern forensic radiographic sample (n = 104). The accuracy of the method is evaluated in terms of frequencies of correct classification within the actual age range, as well as of age/phase correlation.

Materials and Methods

The sample consisted of 104 radiographs of isolated throat sections from 68 men and 36 women of "White" and "Black" ancestries, ranging in age from 15 to 89 years (Table 1). The throat sections were removed during routine autopsies at the Broward County Medical Examiner's Office, FL, during the summer of 2006, and radiographed in anatomical position using a UX Universal Uni-Matic 325 (Universal/Allied Inc.) open x-ray machine, set at 50 A, 40 kVp for 0.5 sec. The radiographs were randomly numbered and the matching demographic data stored in a separate database, so that the biological profile of the individual was unknown to the researcher during radiograph examination and data recording. As Černý (1,2) developed his method from a sample of "White" males, all subsequent analyses were performed separately on both the "White" male and pooled samples.

Individual anatomical regions of the thyroid cartilage (Fig. 1) were scored according to the degree of ossification: "0" if no ossification was present, "1" if ossification had begun, and "2" if ossification was complete. Left and right ossification regions were scored independently to compensate for asymmetry. The cricoid cartilage was included in this examination, but treated as a whole instead of regionally.

The degree of ossification of the thyroid cartilage was also scored using Černý's method (1,2) and the corresponding phase assigned to each radiograph. Because Černý Phase 1 describes the first appearance of ossification, a phase of "0" was assigned when no ossification was observed. The parameters of the actual age distribution, including 68% and 95% confidence intervals, were then estimated for each phase and for the total sample.

The accuracy of Černý's method was first assessed in terms of percent correct classification. To account for sample size effects, the likelihood of obtaining the observed number of correct age diagnostics in each phase was estimated for different theoretic correct classification rates. This was approximated using a binomial distribution model in which the theoretical percent correct rate was set as the probability of success (p). Finally, a Spearman rank correlation between Černý's phase and actual age was estimated.

TABLE 1—*Demographics of sample obtained at autopsy.*

	Sex		
Ancestry	Females	Males	Ancestry Total
"White"	29	52	81
"Black"	7	16	23
Sex Total	36	68	104

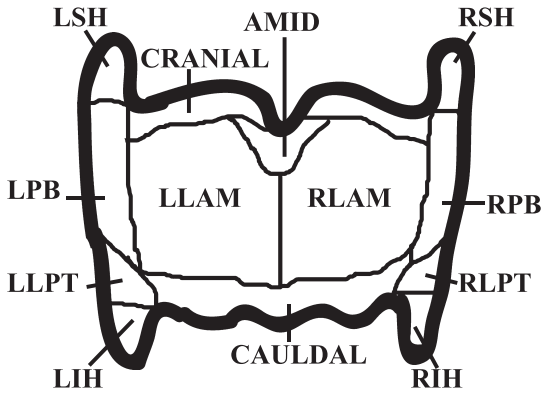

FIG. 1—*Diagram of the individual regions of the thyroid cartilage scored for ossification. LLPT/RLPT, left and right lower posterior triangle; LIH/RIH, left and right inferior horns; LPB/RPB, left and right posterior branches; CRANIAL, cranial branch; CAUDAL, caudal branch; LSH/RSH, left and right superior horns; LLAM/RLAM, left and right laminae; AMID, anterior midline tongue.*

Results

Ossification Sequences

The standard deviations obtained for the isolated areas of thyroid ossification ranged from 8.66 to 20.77 years, suggesting that 95% age confidence intervals would span between 35 and 84 years. Although this range is too large and overlapping to suggest a direct correlation between specific areas of ossification and age, a consistent sequence in ossification was noted. This sequence of ossification is similar to the sequence presented by Černý (1); however, paramedian and median processes were not observed and ossification windows were inconsistent. As displayed in Fig. 2, 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 ossify next. The remaining anatomical regions were more variable in their sequence of ossification and their mean ages fall within a few years of each other. The anterior midline tongue was, on average, the last to begin ossification, and was not ossified in individuals younger than 30 years of age. Overall, no thyroid ossification was noted in individuals under 19 years old, at which time ossification became variable. Complete ossification of the laminae and cranial branches of the thyroid cartilage were not observed in individuals younger than 39 years of age.

When comparing the ossification patterns of males and females, it was noted that, in general, females never exhibit complete ossification in the laminae, cranial branches, or anterior midline tongues. Similarly, none of the black male individuals displayed ossification of the cranial branch, and only four exhibited any signs of ossification in the laminae.

The ossification of the cricoid cartilage was also highly variable with individuals up to the age of 80 years lacking any ossification. The commencement of ossification was seen as young as 25 years, and 26 years of age was the minimum age of complete ossification.

Assessment of Černý's Phase Method

The descriptive statistics and confidence intervals obtained for each phase in the pooled sample are displayed in Table 2. The mean ages do not even successively increase with phase (Fig. 3). For example, the mean age of the individuals assigned to Phase 3 was 29.64 years, while Phase 2 (supposedly applying to younger individuals) has a mean age of 37.17 years. The mean age of Phase 7 is actually younger than the mean age of Phase 4.

Furthermore, a very large degree of overlap, in both the pooled and "White" male samples, exists in confidence intervals and maximum/minimum values of the phases. The overlap of the pooled confidence intervals as compared to Černý's age ranges is also presented in Fig. 3.

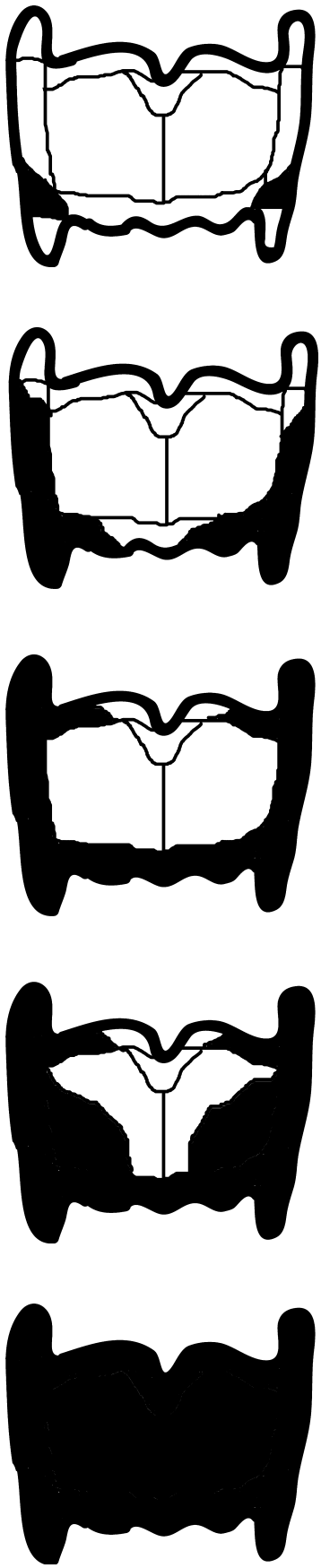

FIG. 2—*Illustration of the observed sequence of ossification of the thyroid cartilage regions. Sequence is from top to bottom with colored areas representing ossification.*

TABLE 2—*Age distributions and confidence intervals obtained for each Černý phase (1,2) when the pooled sample was applied.*

Phase Assigned	*n*	Min Age	Max Age	Mean	SE	1 SD	2 SD
0	7	15	21	18	0.78	2.06	4.12
1	2	21	36	28.5	7.50	10.61	21.21
2	6	21	68	44.5	6.92	16.95	33.90
3	11	19	47	33	2.69	8.91	17.83
4	5	31	71	51	6.46	14.45	28.89
5	29	22	89	55.5	3.53	19.02	38.04
6	18	23	87	55	4.78	20.26	40.52
7	5	30	59	44.5	5.05	11.28	22.57
8	15	39	79	59	3.69	14.30	28.60
9	6	49	79	64	4.16	10.19	20.38

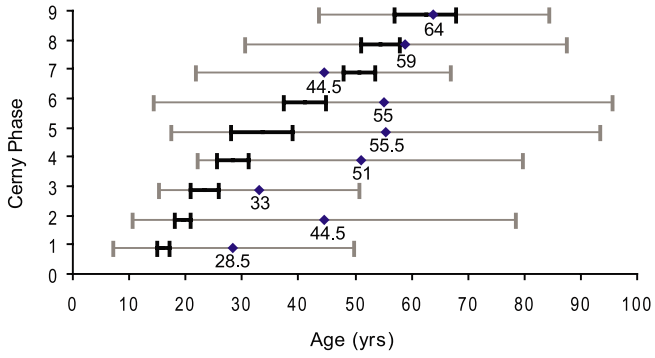

FIG. 3—*Pooled distribution of age ranges assigned to Černý's phases (grey lines indicate 2 SD with mean value labeled) and original age ranges provided by Černý (solid black lines) (1,2).*

TABLE 3—*Percentage of individuals correctly classified into their age range Černý's phase method (1).*

	Sex		
Ancestry	Females (%)	Males (%)	Ancestry Total (%)
"White"	10.34	28.84	22.22
"Black"	14.29	18.75	17.39
Sex Total	11.11	26.47	21.15

TABLE 4—*Percentage of individuals (pooled sample) correctly classified in each Černý phase (1).*

Phase	# Classified	# Correct	% Correct
0	7	1	14.29
1	2	0	0.00
2	6	1	16.67
3	11	2	18.18
4	5	1	20.00
5	29	5	17.24
6	18	4	22.22
7	5	2	40.00
8	15	2	13.33
9	6	4	66.67

The total percentages of correct classification within the actual age range are shown in Table 3. As expected, "White" males show the highest percent correct at 28.84%. The percent correct for the pooled sample was slightly lower at 21.15%.

Percent correct values were also calculated per phase (Table 4). Phases 9 and 7 had the highest values, 66.67% and 40.00%,

respectively. Interestingly, only "White" males were assigned to these phases. All other percent correct values per phase were under 23%. Percent correct values were slightly higher when only analyzing the "White" male sample, but the sample size is highly restricted.

The binomial probabilities for the pooled sample show that for Phases 3, 5, 6, and 8 it is not even reasonable to assume a 50% probability of correct classification. Only Phase 9 may have an actual probability of correct classification of 90%, and none of the observed percent correct values is consistent with a 95% rate of correct classification.

Still, a significant Spearman correlation was found between age and the assigned Černý phases ($r = 0.52$; $p < 0.001$).

Discussion

When individual anatomical areas of ossification were analyzed, a general sequence was noted. As documented by the maximum ages lacking ossification, specific areas of ossification can be easily grouped. The ossification of the posterior triangles began long before, and always preceded, any of the other areas. Complete ossification of the inferior horns and beginning ossification of the posterior branches were the next group to ossify, and likewise preceded the remaining areas of the thyroid cartilage. The final areas to ossify, including the cricoid cartilage, were more variable in sequence. However, the anterior midline tongue was always the last to ossify and ossification was not noted before 30 years of age. This is particularly interesting because the anterior midline portion of the thyroid cartilage is one of the few areas that lacks any major muscle attachments (11,12) and suggests that the functions of the laryngeal muscles may play a role in advancing ossification.

Although a general sequence is observed, the ossification of specific areas was not highly correlated with age. This suggests that individual factors affect ossification, beyond the aging process. Consistent with the conclusions presented by Jurik (8), the results suggest that although the degree and frequency of ossification of the thyroid cartilage will increase with age, there is no direct correlation. Because the first signs of ossification are noted around 18–20 years of age, this attribute may at best be used to conclude that an individual is an adult. Similarly, because all individuals 37 years and older displayed complete ossification of the posterior triangles and inferior horns, and 97% exhibited completed ossification of the posterior branches, lack of ossification in these areas may suggest that an individual is less than 37 years of age. However, any further estimation of age does not seem possible based on ossification as scored in this study, due to the high degree of variation observed in the variables considered.

Consistent with previous studies, a difference in the patterns of male and female ossification was noted. In particular, females generally lacked ossification in the laminae and cranial branches. The high degree of ossification in the later decades of males compared to females has been previously documented in other studies (3,4,6,8,13). The patterns of ossification of males and females, in general, diverge around the fifth decade of life. Interestingly, "Black" males displayed a similar pattern of ossification to females, lacking complete ossification.

The large, and in some cases completely, overlapping age ranges obtained in this study when applying Černý's phases (1,2) show that, at least for the population under consideration, the age intervals assigned by Černý are highly unrealistic and far from the 95% or 90% probability intervals considered acceptable in forensic settings. Incorporation of groups not considered by Černý had little or no effect on modifying the age intervals.

The lack of precision and accuracy of Černý's method for estimating age-at-death is confirmed by the percent correct values. Even when restricted to the "White" male sample, consistently low percent correct values were observed for all phases, and the overall percent correct value of 28.84%, indicating conclusively that this method is not appropriate for forensic applications. Even when sample size is taken into account, the binomial probabilities show that only a few phases would show higher percent correct rates if larger samples were considered, and only the oldest phase could be expected to show successful classification rates above the 60–70% range.

Conclusion

The use of the ossification of the laryngeal structures for age-at-death estimation is limited to providing a general indication of age. While Černý (2) may have noted a specific correlation in age and ossification from his restricted sample, when applied to a large, modern forensic sample the method proves highly inaccurate and not applicable. This study reveals that at best, laryngeal ossification trends may be used to differentiate broad age groups, such as juveniles, young adults, and middle-aged adults. The lack of high correlation between age and ossification of the laryngeal structures suggests that other variables known to influence bone, such as pathology, nutrition, hormones, mechanical stress, or genetics, may be involved (6,14,15).

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