



### A3 Sex-Specific Patterns of Ossification in Macerated Thyroid Cartilages

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After attending this presentation, attendees will be aware that the sequence of ossification of the thyroid cartilage differs between the sexes.

This presentation will impact the forensic science community by introducing sequences of sex-specific patterns of ossification in the thyroid cartilage, providing a novel component for use in sex estimation in individuals.

Previous studies of the ossification process of the thyroid cartilage using radiographs, CT scans, and macerated samples focused on potential correlations between age-at-death and patterns of ossification.<sup>1-8</sup> Recent research reveals there is no correlation between age and ossification of the thyroid cartilage, but there does appear to be a difference in the pattern of ossification between the sexes.<sup>8</sup> This finding suggests implications for the use of this structure in sex estimation. Through examination of a large sample of thyroid cartilages, significant differences in pattern of ossification between the sexes were demonstrated. Presented here are sex-specific sequences of ossification and accuracy rates for this new method of sex estimation.

This study was conducted in two parts using macerated thyroid cartilages. To observe and establish expected sequences of ossification for males and females, 390 ossified thyroid cartilages from individuals of known sex and age were examined. A subsample of 48 ossified thyroid cartilages was then employed in independent, blind tests of sex estimation.

A significant difference in the pattern of ossification was observed between males and females, specifically in the relationship between the ossification of the superior horns (cornua) and the anterior superior margin (laryngeal prominence). Ossification begins along the lateral inferior margins of the thyroid cartilage in both sexes. Ossification then extends to the superior horns and along the anterior inferior margin in females. In males, ossification extends along the anterior inferior and anterior superior margins and the laminae prior to any development of the superior horns. Thus, the superior horns appear to be the last portion of the cartilage to ossify in males, occurring after the complete ossification of the laryngeal prominence at the anterior superior margin, and at times appear not to ossify at all. In contrast, the superior horns appear early in the ossification process in females, prior to the ossification of the laryngeal prominence, which is rarely observed as ossified in females. In the subsample of 48 ossified thyroid cartilages, 17 of 28 males (60%) exhibited some degree of ossification of the superior horns (cornua) and of these, 17 of 17 (100%) also exhibited ossification of the anterior superior margin (laryngeal prominence). Twenty of 20 females (100%) exhibited ossification of the superior horns and of these, 0 of 20 (0%) exhibit ossification of the anterior superior margin.

Using these criteria, a series of independent blind tests were performed to evaluate the degree of inter-analyst correspondence and the accuracy of sex determination. Under macroscopic observation, sex was accurately estimated with a 96% two-way correspondence and 97% accuracy. These results demonstrate that the sex-specific patterns of ossification observed in this research may be employed as supplementary support in estimates of sex in individuals.

Recent immunohistochemical research demonstrates sex-specific differences in cartilage mineralization of the laryngeal structures.<sup>9-11</sup> These studies suggest that apoptosis and HADH-TR positive chondrocytes play a role in

the ossification of cartilage in males and females, and that the chondrocytes “stay alive” longer in females than males.<sup>11</sup> This research also proposes that hormonal changes in men and women contribute to the ossification processes.<sup>9</sup> Still, these studies remain tied to the concept of age as the driving factor behind ossification, hindering interpretations of the process and the establishment of any clear sex-specific ossification sequences.

The patterns of ossification observed here appear to affirm the hypothesis that multiple cartilage types are responsible for ossification of the components of the thyroid cartilage, and those elements respond in a sex-specific manner during ossification. Further, this research demonstrates a very high accuracy rate for sex estimation using the ossified thyroid cartilage. Finally, the results of this study have implications for the forensic pathology community as well, as understanding the female pattern of superior horn ossification may alter standard autopsy procedures to better capture potential injury in strangulation cases.

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### Thyroid Cartilage, Ossification Patterns, Sex Estimation