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Original Research Article

Autopsy-Based Radiological Assessment of Age-Related Changes in the Hyoid Bone: Experience from S.M.S. Medical College, Jaipur (2023–2024)

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Conflict of interest: Nil

Abstract:

Background: Accurate age estimation remains a cornerstone of forensic science, particularly in medicolegal investigations involving unidentified human remains. Conventional skeletal and dental methods become less reliable after skeletal maturity, prompting the exploration of alternative indicators. The hyoid bone, specifically the fusion of its greater cornu with the body, has emerged as a potential marker of adult age estimation.

Aim: To assess age-related radiological changes in the hyoid bone and evaluate its utility as a supplementary tool for forensic age estimation.

Materials and Methods: This autopsy-based cross-sectional study was conducted on 120 cadavers (60 males, 60 females) at the Department of Forensic Medicine & Toxicology, S.M.S. Medical College, Jaipur, between August 2023 and October 2024. Hyoid bones were dissected, isolated, and examined radiologically. Fusion of the greater cornu with the body was graded according to Harjeet et al. (2010). Data were analyzed using Chi-square tests, Student's t-test, ANOVA, and Spearman's correlation.

Results: Fusion showed a progressive, age-related pattern. Non-fusion was confined to individuals below 20 years, partial fusion predominated in the third decade, and complete fusion was universal by 60 years. The mean age of complete fusion without scar was 59.7 years on the right side and 57.4 years on the left. Spearman's correlation demonstrated a strong positive relationship between age and fusion stage ($\rho = 0.851$ right; $\rho = 0.839$ left; $p < .001$). No significant sex- or side-related differences were observed, though unilateral fusion occurred earlier than bilateral fusion.

Conclusion: Fusion of the hyoid bone demonstrates a strong and predictable correlation with age, making it a useful supplementary marker for adult age estimation in forensic practice. While findings align with prior studies, population-specific variability underscores the need for further multi-centric validation.

Keywords: Forensic age estimation, Hyoid bone, Radiology, Ossification, Fusion, Medicolegal identification.

Introduction

The identification of unknown individuals is a cornerstone of forensic science, particularly in medicolegal investigations, disaster victim identification, and cases involving unclaimed or decomposed remains. Constructing a biological profile typically involves the assessment of age, sex, ancestry, and stature, with age estimation being one of the most widely applied and legally significant parameters for both living and deceased individuals. [1,2]

In living persons, age determination has far-reaching implications in civil, legal, and administrative domains. It is required in contexts such as employment eligibility, participation in competitive sports, pension claims, immigration disputes, adoption proceedings, and legal adjudication in cases involving sexual consent, child protection, and trafficking. [3] In deceased individuals, especially in situations where identity is unknown, accurate age estimation serves to narrow the investigative pool and guide further identification strategies.

Conventional methods for age estimation in juveniles rely on skeletal and dental maturation, such as the development and fusion of ossification centers [4] and the chronology of dental eruption and root formation. [5] Once skeletal maturity is reached, however, reliable markers become scarce. In adults, methods often include cranial suture closure [6], morphological evaluation of the pubic symphysis [7], and analysis of dental wear. [8] These approaches, though valuable, have recognized limitations.

Given these constraints, forensic research has focused on alternative anatomical structures that may provide additional age-related information. The hyoid bone, a U-shaped structure situated in the anterior neck, has gained attention in this regard. Unlike other bones, it does not articulate with adjacent skeletal elements but is suspended by muscles and ligaments. Structurally, it comprises a central body and paired greater and lesser cornua. With advancing age, particularly during adulthood, the greater cornua tend to undergo progressive fusion with the body, a process that can be assessed through both anatomical and radiological methods. [9,10]

However, even in the case of hyoid fusion, inter-individual variability is considerable, and outcomes may be influenced by sex, ethnicity, and population-specific factors. [11,12] Several investigators have examined this process in detail. Jadav et al. [11] reported a significant correlation between chronological age and the degree of hyoid body–cornua fusion in autopsy-based radiological studies, while Kumar et al. [12] observed similar trends in a North Indian cohort but highlighted the importance of accounting for regional variation. Harjeet et al. [13] contributed one of the most extensive datasets, proposing a four-stage grading system for hyoid fusion and recommending its use as a supplementary method of adult age estimation, acknowledging irregularity in fusion timing.

In this context, the present study was conducted at the Department of Forensic Medicine & Toxicology, S.M.S. Medical College, Jaipur, with the objective of documenting radiological age-related changes in the hyoid bone in a local population. Specifically, it aimed to determine the approximate age of fusion of the greater horn with the hyoid body, to evaluate sex-based variation in fusion, and to assess possible differences between the right and left sides. By pursuing these objectives, the study seeks to strengthen the evidence for hyoid fusion as an adjunct method of adult age estimation, while also contributing clinically relevant insights for radiologists and otolaryngologists engaged in head and neck imaging.

Materials and Methods

Study Design and Setting: This was an autopsy-based descriptive observational study with a cross-sectional design. It was carried out in the Mortuary of the Department of Forensic Medicine & Toxicology, S.M.S. Medical College and Attached Hospitals, Jaipur, from August 2023 to October 2024.

Ethical Approval: The study protocol was reviewed and approved by the Institutional Ethics Committee of S.M.S. Medical College and Attached Hospitals, Jaipur (Ref. No. 123/MC/EC/2023; Receipt No. 1568 dated 05-10-2023). Written informed consent was obtained from legal relatives of the deceased, and confidentiality was maintained throughout.

Study Population and Sampling: The study included medicolegal autopsy cases conducted at the Mortuary of S.M.S. Medical College. A total of 120 cadavers fulfilling the inclusion criteria were examined. Cases were stratified into six age groups (10–<20, 20–<30, 30–<40, 40–<50, 50–<60, and ≥60 years) with equal distribution of males and females.

Inclusion Criteria:

- Cadavers aged above 10 years.
- Availability of valid legal documentation of age.
- Deceased domiciled in Jaipur.

Exclusion Criteria:

- Cadavers with injuries, deformities, or pathological defects in the neck region.
- Unidentified cadavers.
- Cases where relatives did not provide consent.

Cadaver Dissection and Bone Retrieval: Eligible cadavers were positioned with a wooden block under the shoulders to extend the neck. Standard autopsy dissection was performed to remove the tongue, pharynx, larynx, trachea, and upper esophagus en masse with the hyoid bone. The hyoid was then carefully isolated by blunt dissection to avoid fracture. Each specimen was labelled with a unique identification number (age, sex, and postmortem case number) and preserved in 10% formalin until radiological examination.

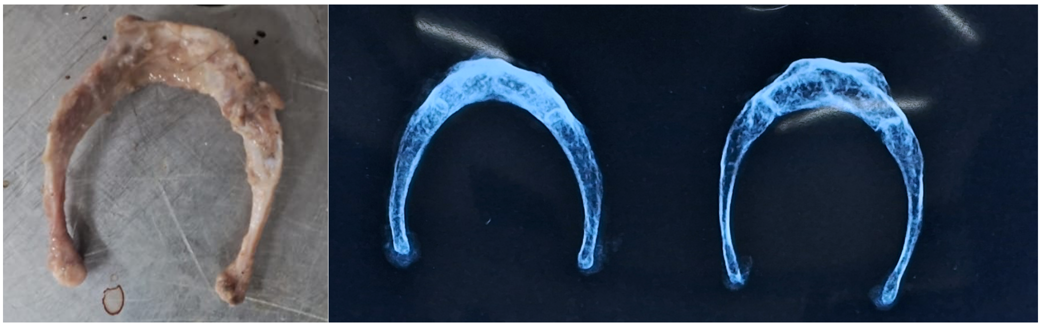


Image 1: (A) Retrieved Hyoid Bone (B) X-ray Imaging of Hyoid Bone

Radiographic Examination: Each hyoid bone was radiographed with its superior surface facing the X-ray tube, and a lead token marked the specimen number for identification. Up to 16 specimens were radiographed simultaneously. Radiographs were evaluated for the degree of fusion between the greater cornu and the hyoid body.

Grading of Fusion: Fusion status was assessed using the classification proposed by Harjeet et al. (2010) [13]

Stage	Description	Score
Stage 1	Non-fusion	0
Stage 2	Commencement of fusion (<¼ of contact area)	1
Stage 3	Partial fusion (>¼ but incomplete)	2
Stage 4a	Complete fusion with radiopaque scar	3
Stage 4b	Complete fusion without visible scar	3

Data Recording and Analysis: Findings were documented in a structured proforma, including case number, age, sex, side of fusion (right/left), and fusion stage. Quantitative data were expressed as mean ± standard deviation, while qualitative data were presented as frequencies and percentages. Statistical analysis was performed using Chi-square test, Student's t-test, and ANOVA where appropriate. A p-value <0.05 was considered statistically significant, and p <0.001 highly significant.

Results

Case Characteristics: A total of 120 cadavers (60 males, 60 females) were included in the study. Equal distribution across six age groups ensured uniform representation by both age and sex. The mean age ranged from 16.6 ± 2.01 years in the youngest group to 67.2 ± 3.53 years in the ≥60 years group (Table 1).

Table 1: Baseline Characteristics of Cadavers (n = 120)

Age Group (Years)	Males (n, %)	Females (n, %)	Total (n, %)	Mean Age ± SD
10 – <20	10 (16.7)	10 (16.7)	20 (16.7)	16.6 ± 2.01
20 – <30	10 (16.7)	10 (16.7)	20 (16.7)	24.6 ± 2.87
30 – <40	10 (16.7)	10 (16.7)	20 (16.7)	34.5 ± 2.95
40 – <50	10 (16.7)	10 (16.7)	20 (16.7)	44.5 ± 2.95
50 – <60	10 (16.7)	10 (16.7)	20 (16.7)	54.5 ± 2.95
≥ 60	10 (16.7)	10 (16.7)	20 (16.7)	67.2 ± 3.53
Total	**60 (50.0)**	**60 (50.0)**	**120 (100)**	—

Fusion Stages by Age – Right Side: On the right side, non-fusion (Stage 1) was confined to the 10–<20 years group, while commencement (Stage 2) and partial fusion (Stage 3) were mostly seen in the second and third decades. Complete fusion (Stages 4a/4b) predominated after 40 years and was universal by 60 years (Table 2).

Table 2: Distribution of Fusion Stages by Age Group — Right Side (n = 120)

Age Group (Years)	Stage 1	Stage 2	Stage 3	Stage 4a	Stage 4b
10 – <20	9	9	2	0	0
20 – <30	0	5	10	5	0
30 – <40	0	3	7	10	0
40 – <50	0	0	2	14	4
50 – <60	0	0	0	12	8
≥ 60	0	0	0	6	14
Total	**9**	**17**	**21**	**47**	**26**

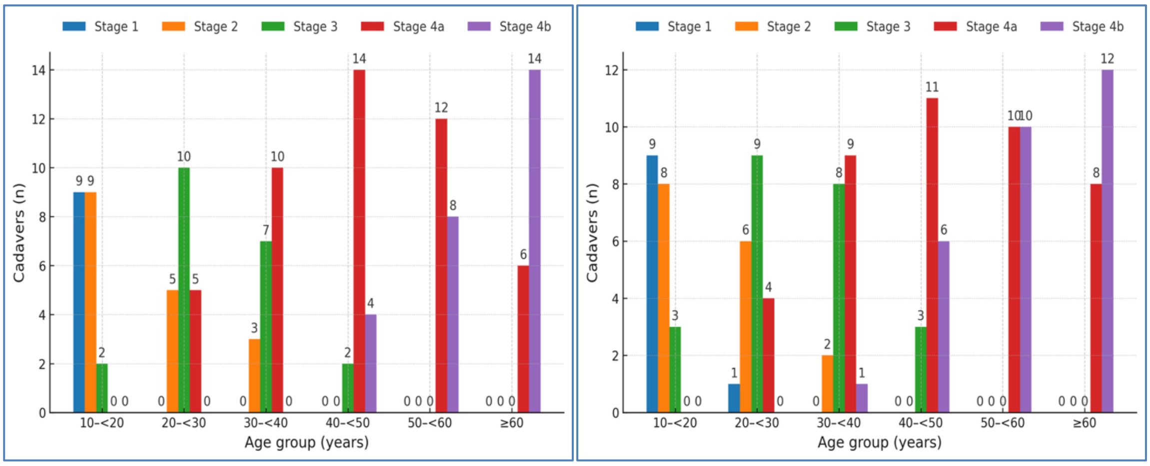
χ^2 (20, N = 120) = 147, $p < .001$

Fusion Stages by Age – Left Side: The left side showed a similar pattern of progression. Non-fusion was restricted to the youngest age group, while complete fusion was predominant after 40 years and universal by ≥60 years (Table 3).

Table 3: Distribution of Fusion Stages by Age Group — Left Side (n = 120)

Age Group (Years)	Stage 1	Stage 2	Stage 3	Stage 4a	Stage 4b
10 – <20	9	8	3	0	0
20 – <30	1	6	9	4	0
30 – <40	0	2	8	9	1
40 – <50	0	0	3	11	6
50 – <60	0	0	0	10	10
≥ 60	0	0	0	8	12
Total	**10**	**16**	**23**	**42**	**29**

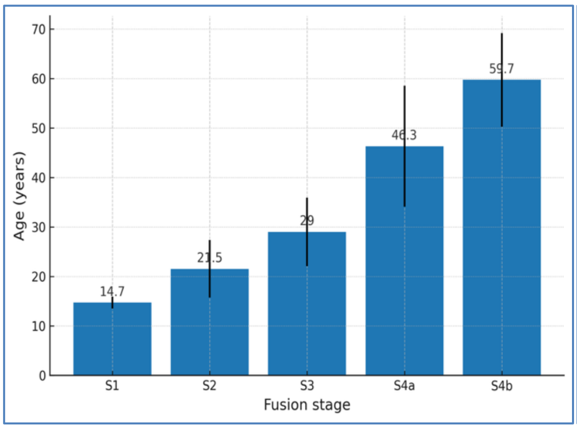
χ^2 (20, N = 120) = 123, $p < .001$

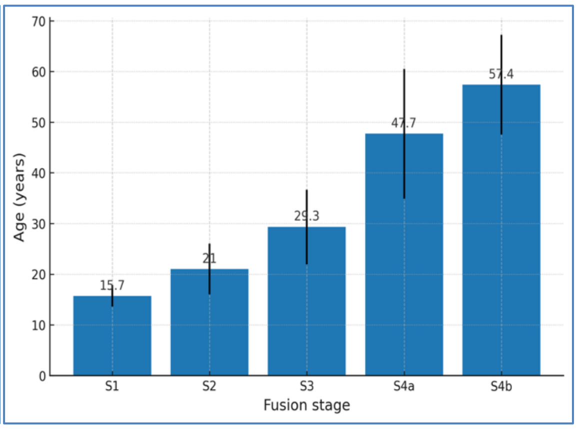


Mean Age of Fusion Stages: The mean ages corresponding to each fusion stage are presented in Table 4. Fusion generally began in the second decade, advanced through the third and fourth decades, and was typically complete by the sixth decade.

Table 4: Mean Age of Fusion for Each Stage (Right vs. Left Side)

Fusion Stage	Right Side (Mean ± SD, years)	Left Side (Mean ± SD, years)
Stage 1	14.7 ± 1.22	15.7 ± 2.11
Stage 2	21.5 ± 5.82	21.0 ± 5.03
Stage 3	29.0 ± 6.92	29.3 ± 7.37
Stage 4a	46.3 ± 12.23	47.7 ± 12.82
Stage 4b	59.7 ± 9.48	57.4 ± 9.84





Sex-wise Distribution: When compared by sex, fusion patterns were broadly similar (Table 5). Males showed a slightly higher frequency of Stage 4a, while females showed marginally more Stage 4b. However, the differences were not statistically significant.

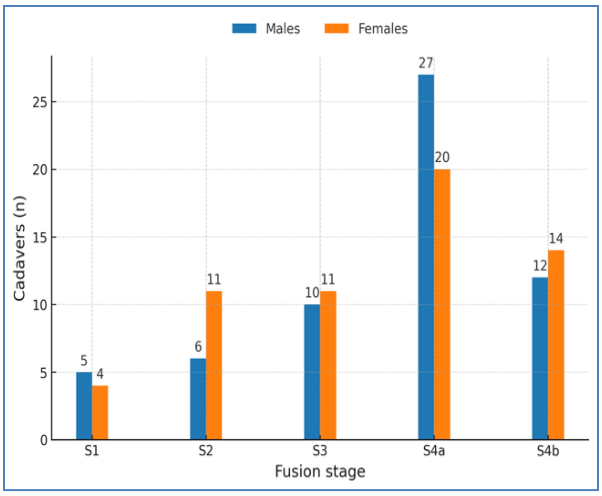
Table 5: Sex-wise Distribution of Fusion Stages (Combined Sides, n = 120)

Fusion Stage	Males (n, %)	Females (n, %)	Total (n, %)
Stage 1	10 (8.3)	9 (7.5)	19 (15.8)
Stage 2	13 (10.8)	20 (16.7)	33 (27.5)
Stage 3	21 (17.5)	23 (19.2)	44 (36.7)
Stage 4a	48 (40.0)	41 (34.2)	89 (74.2)
Stage 4b	28 (23.3)	27 (22.5)	55 (45.8)

Side-wise Distribution: Fusion was largely symmetrical on both sides (Table 6). Stage 4a was slightly more frequent on the right, while Stage 4b was slightly more common on the left.

Table 6: Comparison of Fusion Stages on Right vs. Left Side (n = 240 observations)

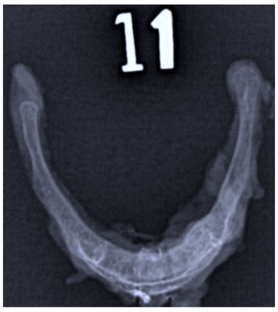
Fusion Stage	Right Side (n, %)	Left Side (n, %)
Stage 1	9 (7.5)	10 (8.3)
Stage 2	17 (14.2)	16 (13.3)
Stage 3	21 (17.5)	23 (19.2)
Stage 4a	47 (39.2)	42 (35.0)
Stage 4b	26 (21.7)	29 (24.2)



Correlation with Age: Spearman's correlation confirmed a strong positive relationship between age and fusion stage on both sides (Table 7).

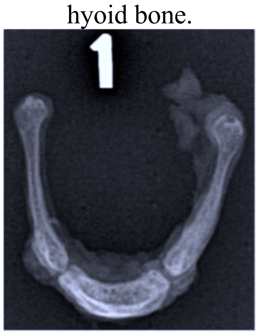
Table 7: Correlation Between Age and Fusion Stage

Variable	Spearman's rho (ρ)	p-value	Significance
Age vs. Fusion Stage (Right Side)	0.851	< .001	Significant
Age vs. Fusion Stage (Left Side)	0.839	< .001	Significant



Radiograph A: Photo radiograph demonstrating **bilateral complete fusion** of the hyoid bone.

Radiograph B: Photo radiograph demonstrating **bilateral non-fusion** of the hyoid bone.



Radiograph C: Photo radiograph demonstrating **commencement of fusion** on the right side and **non-fusion** on the left side of the hyoid bone.

Radiograph D: Photo radiograph demonstrating **partial fusion** on the right side and **non-fusion** on the left side of the hyoid bone.

Discussion

In the present study, a total of 120 cadavers were examined, with equal distribution of 60 males and 60 females, stratified across six age groups. This ensured a balanced representation, minimizing bias and allowing valid comparisons. Previous studies such as Miller et al. (1998) [14] and Urbanová et al. (2013) [15] also analyzed large collections of hyoid bones, although their samples were not always evenly distributed by sex or age. The uniform distribution in the present study strengthens the reliability of our findings compared to those earlier works.

Age-related Fusion Patterns: Fusion of the greater cornu with the body of the hyoid bone in our study showed a clear age-related progression. Non-fusion was confined to the 10–<20 years group, fusion commenced in the second decade, partial fusion predominated in the third decade, and complete fusion was universal by 60 years. The mean age of complete fusion without scar was 59.7 years on the right and 57.4 years on the left. Correlation analysis showed a strong positive relationship between age and fusion stage (Spearman's rho = 0.851 on the right and 0.839 on the left, $p < .001$). These results are consistent with Shimizu et al. (2005) [16], who confirmed progressive fusion with age both radiologically and histologically, and with Jadav et al. (2021) [11], who also reported a statistically significant correlation between chronological age and fusion stage. Similarly, Gupta et al. (2008) [17] observed complete fusion in all bones beyond 60 years. However, Harjeet et al. (2010) [13] reported variability, including cases of non-fusion even in individuals above 60 years, questioning the reliability of hyoid fusion as an age marker. The discrepancies may be explained by methodological differences (gross bone excision versus radiology), sample size, and population genetics.

Sex-related Differences in Fusion: In the present study, no major sex differences were observed. On the right side, males showed slightly higher Stage 4a fusion (45%) while females showed higher Stage 4b fusion (23.3%). On the left, Stage 4a was equal in both sexes (35%), with males showing marginally more Stage 4b. These results indicate that sex does not significantly influence fusion timing. Miller et al. (1998) [14] similarly reported no difference in non-fusion rates between sexes, while Balseven-Odabasi et al. (2013) [18] also noted no substantial sex-based differences. In contrast, Gupta et al. (2008) [17] found that females fused about five years earlier than males, and Harjeet et al. (2010)

[13] reported higher incidence of fusion in elderly females. Urbanová et al. (2013) [19] further observed that age-related changes were more pronounced in males. The variations may be due to differences in sample composition, hormonal influences, or regional genetic factors.

Side-wise Differences in Fusion: The present study demonstrated near symmetry in fusion between right and left sides. Stage 4a was slightly higher on the right (39.2%), whereas Stage 4b was slightly higher on the left (24.2%), but these differences were not statistically significant. This finding aligns with Gupta et al. (2008) [17], who reported no significant side differences, and with Shimizu et al. (2005) [16], who confirmed symmetrical fusion histologically. Thus, fusion appears to be a largely bilateral and synchronous process, with only minor side-specific variations.

Unilateral vs. Bilateral Fusion: Unilateral fusion occurred earlier in the present study, at a mean age of 25.8 years in males and 29.0 years in females, while bilateral fusion was later, at 47.0 years in males and 45.0 years in females. Bilateral fusion predominated after 40 years and was universal beyond 50 years. These findings agree with Gupta et al. (2008) [17], who also observed earlier unilateral fusion compared to bilateral, and with Shimizu et al. (2005) [16], who documented progressive ossification with age. By contrast, Harjeet et al. (2010) [13] found irregular timing, including unfused cases after 60 years. This difference may reflect methodological variation (radiographic versus gross anatomical examination) and population-specific skeletal maturation rates.

Forensic Relevance of Fusion: The strong correlation between fusion and chronological age in the present study confirms its forensic utility. With Spearman's rho values of 0.851 on the right and 0.839 on the left, fusion was shown to increase predictably with age. Jadav et al. (2021) [11] reported similar findings using radiology, further validating hyoid fusion as an age marker. Fisher et al. (2016) [20] also noted that fusion, combined with bone density, was a strong predictor of age, while Balseven-Odabasi et al. (2013) [18] observed sharp increases in fusion after 60 years. These studies support the conclusion that hyoid bone fusion is a useful supplementary parameter in forensic age estimation, especially when other skeletal or dental indicators are unavailable.

Limitations and Future Scope: This study, while comprehensive, has certain limitations that should be acknowledged. First, the sample size, though adequate for statistical analysis, was limited to a single geographic region (Jaipur), which may restrict the generalizability of findings to other populations with different genetic and environmental backgrounds. Second, radiological evaluation was the sole method employed; histological confirmation of ossification stages was not performed, which could have provided additional validation. Third, potential confounding factors such as nutritional status, metabolic disorders, or systemic diseases influencing bone metabolism were not systematically controlled.

Future research should therefore focus on larger, multi-centric studies across diverse populations to establish population-specific standards. Integration of radiological, histological, and advanced imaging techniques such as CT or MRI could yield more precise staging criteria. Longitudinal studies following individuals across life stages would further strengthen the reliability of hyoid fusion as an age estimation tool. Additionally, correlating hyoid fusion with other skeletal and dental markers may enhance the accuracy of forensic profiling, particularly in complex identification scenarios.

Conclusion

The present study demonstrated a clear, progressive, and statistically significant relationship between chronological age and the fusion of the greater cornu with the body of the hyoid bone. Fusion commenced in the second decade, progressed through partial fusion in the third, and was universally complete by 60 years. No major sex- or side-related differences were observed, although unilateral fusion was noted to precede bilateral fusion. These findings support the forensic utility of hyoid bone fusion as a supplementary marker for adult age estimation, especially when other skeletal or dental parameters are unavailable. While variability across populations necessitates caution, the present study strengthens the evidence that radiological assessment of hyoid fusion provides reliable adjunctive data for medicolegal identification.

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