

Gender & Age Related Morphometric Assessment of Hyoid Bone Using Cone Beam Computed Tomography (CBCT): A Retrospective Study

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ABSTRACT

Background:

Age and gender determination are fundamental components of forensic identification. Cone Beam Computed Tomography (CBCT) enables three-dimensional, accurate, and non-invasive evaluation of the hyoid bone. The hyoid bone, owing to its unique anatomical position, developmental pattern, and resistance to post-mortem destruction, serves as a valuable skeletal element in forensic and anthropological investigations.

Objectives:

To determine the age-related morphological changes in the hyoid bone using CBCT scans and to establish sexual dimorphism in hyoid bone by evaluating different morphometric parameters.

Methods:

A retrospective analysis was performed on 300 CBCT scans of individuals aged 16–99 years. Statistical analysis was carried

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out using Mann–Whitney U test was used to compare gender differences, and Spearman's correlation coefficient was applied to assess the relationship between fusion stage and chronological age.

Results: The mean age of bilateral fusion was approximately 53 years in both males and females. All linear morphometric measurements of the hyoid bone were significantly greater in males compared to females ($p < 0.05$), indicating marked sexual dimorphism. Angular measurements showed minimal variation between sexes. A statistically significant positive correlation was noted between complete fusion with advancing age.

Conclusion: CBCT is important non-invasive imaging modality for evaluating hyoid bone morphology and fusion status, and may serve as a useful adjunct in forensic age and sex estimation when combined with other skeletal indicators

Keywords: Hyoid bone, Cone Beam Computed Tomography, Age estimation, Sexual dimorphism, Forensic radiology.

Introduction

Age estimation along with the determination of ancestry, sex, and stature forms the cornerstone of human identification ¹. In medicolegal investigations, accurate estimation of age is essential for identification of human remains ². Dental age estimation is based on the stages of development, calcification and eruption of deciduous and permanent teeth, which follow a relatively predictable chronological pattern ³. The hyoid bone represents a valuable anatomical structure for evaluating skeletal maturation. Owing to its integral role in the hyoid–laryngeal complex, the hyoid bone holds considerable clinical as well as forensic importance. Structurally, the greater and lesser horns of hyoid bone are typically connected to its body by fibrous tissue or synovial joints during early life. With increasing age, these articulations commonly undergo a progressive physiological process of ankylosis, resulting in partial or complete fusion of the horns with the body ⁴. The distance between the condyle of body and greater cornu of hyoid bone grows similarly between males and females (Werner et al., 2021) ⁵.

The present study was undertaken to determine the age-related morphological changes in the hyoid bone using CBCT scans and to establish sexual dimorphism in hyoid bone by evaluating different morphometric parameters on CBCT scans.

Materials and Methods:

Ethical Approval: Not Applicable

Study Design and Ethical Approval:

This retrospective study was conducted on 300 CBCT scans with the field of view (FOV) 10*5 cm and 10*10 cm extracted from the archives. The study was conducted over a period of 18 months commencing from January 2024 till June, 2025 in the Department of Oral Medicine and Radiology at I.T.S Dental College, Hospital & Research Centre, Greater Noida, Uttar Pradesh, India. The study was approved by the Institutional Ethics Committee of ITS Dental College, Hospital & Research Centre, Greater Noida, Uttar Pradesh, India (IEC/OMDR/01/24) and conducted in compliance with standards of the Declaration of Helsinki (2013 revision).

INCLUSION CRITERION: CBCT scans of the patients in which complete extent of the hyoid bone was visible and CBCT scans of the patients with known chronological age and gender.

EXCLUSION CRITERION: CBCT scans of the patients in which complete extent of hyoid bone was not visible, scans of the patients with age less than 16 years, scans of the patients with history of any injury, deformity or defect in the neck region, scans with artifacts

Sample Size Estimation: Sample size estimation for multiple linear regression analysis was performed using the following formula:

Methodology:

All CBCT scans archived were performed after taking consent from the patient with the help of CS 9300S scanner (manufactured by Carestream Health Inc., 150, Verona Street, Rochester, New York, USA). The demographic data of the patient and scan information was recorded in a pre-structured proforma. The archived CBCT scans were reformatted using CS3D viewer software on LED 19"5' inch monitor screen with 1600×900 pixels resolution under dim light condition. Axial, sagittal, coronal, and panoramic views were evaluated at the slice thickness of 1 mm. Density and contrast of the images were adjusted to improve the visibility of hyoid bone. Fusion between the body and the greater horns of the

$$n = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{d^2}$$

Where:

$Z_{1-\alpha/2} = 1.96$ for a 95% confidence level,

$Z_{1-\beta} = 0.84$ corresponding to 95% statistical power,

$d = 0.4$ representing a moderate anticipated effect size and

Statistical analysis was performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA).

hyoid bone was assessed according to classification given by Harjeet et al., 2010.⁶

Fusion Staging (fig.1):

Stage 1: Non-fusion

Stage 2: Commencement of fusion – Fusion has just started either in middle or at either side. The extent of fusion is less than a quarter of the total contact area.

Stage 3: Partial fusion – The extent of fusion is more than a quarter but not showing complete fusion.

Stage 4a: Complete fusion with visible radiopaque white scar at the site of fusion.

Stage 4b: Complete fusion without visible scar at the site of fusion



Figure 1: X-ray images of hyoid bone fusion stages. Hyoid bone showing fusion stage 1 (non-fusion) as marked by white arrows. Hyoid bone showing fusion stage 2 (commencement of fusion) as marked by white arrows. Hyoid bone showing fusion stage 3 (partial fusion) as marked by white

arrow, and fusion stage 4b (complete fusion without the visible scar) as marked by the red arrow. Hyoid bone showing fusion stage 4a (complete fusion with the presence of a scar) as shown by white arrows.

Morphometric Variations

Using the linear measurement tool & angle tool available in the software different linear and angle measurements were performed to assess the sexual dimorphism. Following linear and angular measurement was performed. Gender evaluation was done by using metric and angular 10 parameters on the hyoid bone- Hyoid body major axis (A-A'), Hyoid body minor axis (B-B'), Hyoid body thickness (C-C'), Right greater horn length

(D-D'), Left greater horn length, The distance between right and left lesser horns (F-F'), The distance between right and left greater horn centers (G-G'), Posterior distal distance between right and left greater horn (D-E), The angle made by the right greater horn and the hyoid body (GHI), The angle made by the left greater horn and the hyoid body (G'H'I) (Fig.2)

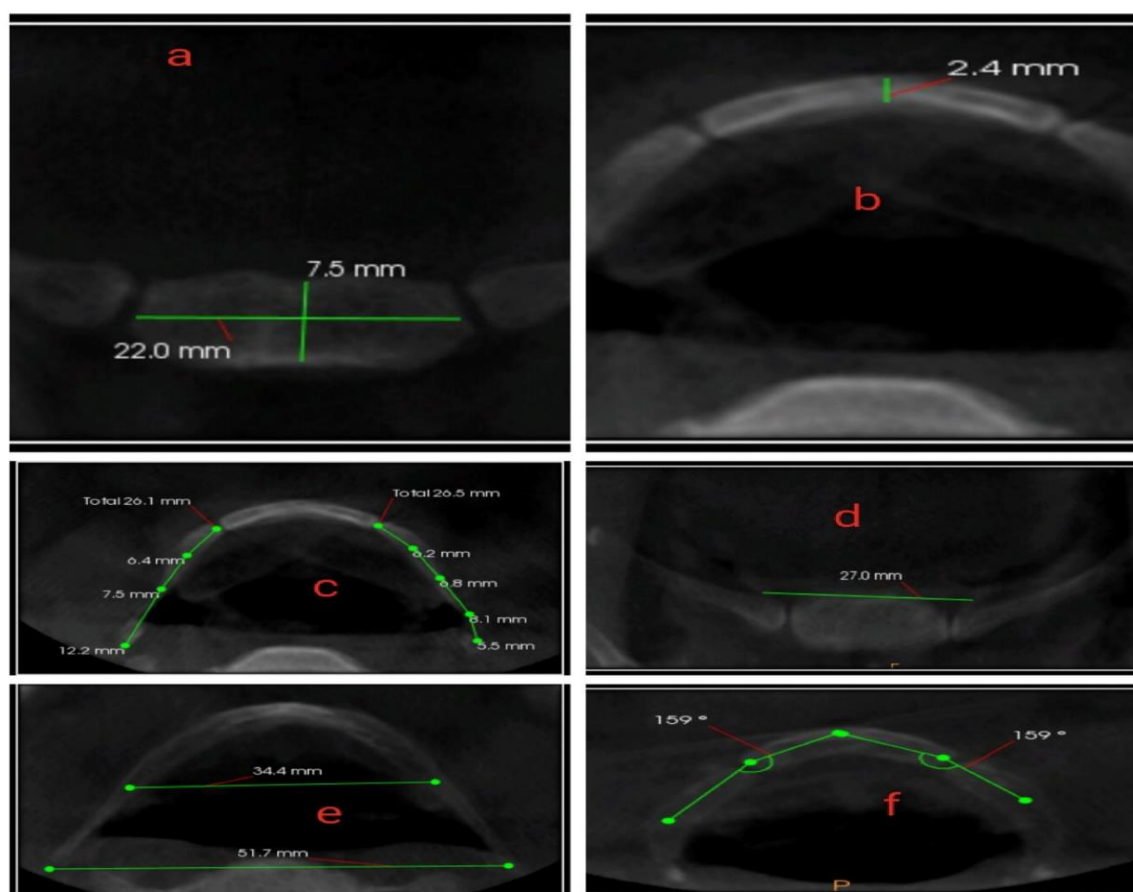


Figure 2, a: Hyoid body major axis (A-A') & minor axis (B-B'), b: Hyoid body thickness (C-C'), c: Right (D-D') & Left (E-E') greater horn length, d: The distance between right and left lesser horns (F-F'), e:- The distance between right and left greater horn

centers (G-G') & Posterior distal distance between right and left greater horn (D-E), f: The angle made by the Right Greater Horn and the Hyoid Body (GHI) & Left Greater Horn and the Hyoid Body (G'HI).

Results:

A total of 300 CBCT scans archived from the records were evaluated in the present study, comprising 101 male scans and 199 female scans. In different age groups, the majority of the scans included were of the patients in the age group of 50–59 years (22.3%). Out of the total 300 patients included in the study, the distribution of fusion stages on the right side and left side were analyzed separately for males and females. Amongst male patients on right side, Stage 2 was observed in 32.7% cases and Stage 4b in 5.9% cases. On left side Stage 1 was observed in 29.7% cases and Stage

4b in 5.9% cases. Amongst female patients on the right side, Stage 1 was noted in 31.7% cases, and Stage 4b in 4.5% cases, while in female patients on the left side, Stage 1 was noted in 36.7% cases and Stage 4b in 4.5% cases. There was an age-related progression of right-side hyoid bone fusion, with non-fusion/incomplete fusion stages predominating in younger individuals and partial/complete fusion stages becoming increasingly common with advancing age in both males and females.

Table 1: Distribution of fusion stages in males and females on right side

Age Group (Years)	Males										Females									
	Stage 1		Stage 2		Stage 3		Stage 4a		Stage 4b		Stage 1		Stage 2		Stage 3		Stage 4a		Stage 4b	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
10 -19	1	50.0	1	50.0	0	0.0	0	0.0	0	0.0	5	83.3	1	16.7	0	0.0	0	0.0	0	0.0
20 -29	8	50.0	4	25.0	2	12.5	2	12.5	0	0.0	19	63.3	5	16.7	3	10.0	2	6.7	1	3.3
30 -39	2	16.7	5	41.7	3	25.0	2	16.7	0	0.0	16	47.1	10	29.4	4	11.8	4	11.8	0	0.0
40 -49	3	18.8	6	37.5	5	31.3	2	12.5	0	0.0	6	16.7	15	41.7	9	25.0	3	8.3	3	8.3
50 -59	4	16.7	8	33.3	5	20.8	5	20.8	2	8.3	10	23.3	17	39.5	6	14.0	8	18.6	2	4.7
60 -69	3	14.3	6	28.6	7	33.3	2	9.5	3	14.3	7	18.9	8	21.6	9	24.3	10	27.0	3	8.1
70 -79	0	0.0	3	37.5	3	37.5	1	12.5	1	12.5	0	0.0	4	40.0	2	20.0	4	40.0	0	0.0
80 -89	0	0.0	0	0.0	1	50.0	1	50.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0
90 -99	0	–	0	–	0	–	0	–	0	–	0	0.0	1	50.0	1	50.0	0	0.0	0	0.0
Total	21.0	20.8	33.0	32.7	26.0	25.7	15.0	14.9	6.0	5.9	63.0	31.7	62.0	31.2	34.0	17.1	31.0	15.6	9.0	4.5

Table 2: Distribution of fusion stages in males and females on left side

Age Group (Years)	Males										Females									
	Stage 1		Stage 2		Stage 3		Stage 4a		Stage 4b		Stage 1		Stage 2		Stage 3		Stage 4a		Stage 4b	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
10 -19	1	50.0	1	50.0	0	0.0	0	0.0	0	0.0	5	83.3	1	16.7	0	0.0	0	0.0	0	0.0
20 -29	9	56.3	2	12.5	3	18.8	2	12.5	0	0.0	22	73.3	5	16.7	0	0.0	2	6.7	1	3.3
30 -39	3	25.0	4	33.3	3	25.0	2	16.7	0	0.0	18	52.9	8	23.5	4	11.8	4	11.8	0	0.0
40 -49	6	37.5	3	18.8	4	25.0	3	18.8	0	0.0	7	19.4	15	41.7	7	19.4	4	11.1	3	8.3
50 -59	6	25.0	4	16.7	7	29.2	5	20.8	2	8.3	13	30.2	11	25.6	9	20.9	8	18.6	2	4.7
60 -69	5	23.8	4	19.0	7	33.3	2	9.5	3	14.3	7	18.9	10	27.0	7	18.9	10	27.0	3	8.1
70 -79	0	0.0	3	37.5	3	37.5	1	12.5	1	12.5	1	10.0	3	30.0	2	20.0	4	40.0	0	0.0
80 -89	0	0.0	0	0.0	1	50.0	1	50.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0
90 -99	0	–	0	–	0	–	0	–	0	–	0	0.0	1	50.0	1	50.0	0	0.0	0	0.0
Total	30.0	29.7	21.0	20.8	28.0	27.7	16.0	15.8	6.0	5.9	73.0	36.7	54.0	27.1	31.0	15.6	32.0	16.1	9.0	4.5

(Table 1 and Table 2) The mean age of hyoid bone fusion increased progressively with advanced fusion stages in both sexes. Early fusion stages were

observed at younger ages, while advanced stages occurred predominantly at older ages.

Table 3:- Mean age of hyoid bone fusion stages in males and females

	Mean Age (Years)	SD
Males		
Stage 1	38.5	16.2
Stage 2	47.9	15.2
Stage 3	53.4	15.3
Stage 4a	51.1	16.1
Stage 4b	60.3	7.9
Total	48.6	16.2
Females		
Stage 1	37.4	14.8
Stage 2	49.3	15.3
Stage 3	51.5	15.6
Stage 4a	53.8	14.0
Stage 4b	51.2	11.7
Total	46.7	16.1

(Table 3) Mean values of all linear parameters (A–A', B–B', C–C', D–D', E–E', F–F', G–G' and D–E) were higher in males compared to females. Statistically significant gender differences were observed for most of these parameters, indicating that the male hyoid bone was generally larger in size than that of females. The minimum values for A–

A', B–B', C–C', D–D', E–E', F–F', G–G' and D–E were lower in females than in males, while the maximum values were higher in males for the majority of measurements. Significant differences between male and female minimum values were observed for different parameters.

Table 4: Linear and angular measurements of hyoid bone in males and females

		A-A' [mm]	B-B' [mm]	C-C' [mm]	D-D' [mm]	E-E' [mm]	F-F' [mm]	G-G' [mm]	D-E [mm]	GHI [°]	G'HI [°]
Male	Mean	25.8	8.9	3.6	21.8	21.0	32.8	33.8	39.4	154.8	154.1
Male	SD	3.0	1.8	1.2	5.5	5.2	4.3	5.4	4.3	7.2	7.1
Male	Min	20.0	5.2	1.6	11.3	10.3	12.4	20.8	25.6	138.0	138.0
Male	Max	32.5	19.5	7.6	39.2	32.4	41.9	71.7	51.7	174.0	171.0
Female	Mean	21.8	8.1	3.3	21.9	21.6	28.6	30.7	36.5	154.3	154.1
Female	SD	2.1	1.3	1.3	4.3	4.5	2.9	3.6	4.1	7.0	6.8
Female	Min	17.3	4.8	1.4	9.6	8.9	20.0	20.5	20.9	130.0	134.0
Female	Max	27.4	11.0	13.5	31.7	31.4	34.9	44.0	47.1	176.0	174.0

(Table 4) The angles formed by the greater horns and the hyoid body (GHI and G'HI) showed comparatively smaller differences between males and females. There was a clear sexual dimorphism in the morphometric measurements of the hyoid bone, with males exhibiting greater dimensions than females in most parameters, while angular measurements showed relatively minimal differences between the sexes. For several linear measurements, a proportion of female values were found to be smaller than the minimum values

Discussion:

The present study evaluated the radiographic assessment of the fusion between the body and greater horns of the hyoid bone along with the morphometric linear assessments, confirming the bone as a reliable source for predicting the age and gender of an individual, certaining its utility in forensic research. The results indicated that individual age increases, fusion score of hyoid bone increases. Significant correlation was observed

observed in males, indicating clear gender-related size differences in the hyoid bone.

Overall, the findings demonstrated that linear dimensions of the hyoid bone showed considerable overlap between males and females, although males tend to exhibit larger measurements. The angular parameters show minimal to no overlap, suggesting limited gender differentiation for these measurements.

between linear morphometric measurements performed on the hyoid bone amongst males and females. Ossification of hyoid bone occurs at the junction between the hyoid body and greater horns. Ossification commences in adults, and gets complete with increasing age. Harjeet et al ⁶ D'Souza et al ⁷, Gupta et al ⁸. have described the role of male and female hormones, cumulative muscular traction in neck region, genetic heterogenicity, nutritional deficiencies, minor sex

related differences in habitual muscle use in ossification amongst males and females.^{6,7,8} In this study, the hyoid bone was evaluated using 3-dimensional CBCT data for the assessment of fusion between hyoid body and the greater horns using the stages of fusion given by Harjeet et al.⁶ The present research showed that the mean age increases in different stages of fusion i.e. from (non-fusion to complete fusion with or without visible radiopaque scarring) in both males and females confirming the fact that the cartilaginous joints ossify with advancing age and hence ossification can be considered as the predictor for the assessment of age amongst individuals. Our findings were consistent with the observations of Miller⁹, Shimizu et al.³, Fisher et al.¹⁰, Gupta et al.⁸, Ito et al.⁷, and D'Souza et al.⁷, where the mean age of the complete ossification of the hyoid body with the greater horns was observed in the older age group that is between 50 to 60 years. Jadav et al.¹¹, Harjeet et al.⁶, and Balseven-Odabaşı et al.¹², did not found statistically significant relationship between increasing age and ossification of the greater cornu and body of hyoid bone for which they have attributed unequal sample size distribution as a major cause. The results of the current study is found similar amongst both sexes, although some studies by Jadav et al.¹¹, D'Souza et al.⁷, reported that females attain skeletal maturity earlier than males, leading to earlier onset of fusion^{11,7}. Ito et al.⁴, observed that the hyoid bone fusion occurred earlier on the left side as compared to right side. Gupta et al.⁸, has contrasting results where unilateral fusion of hyoid bone occurred first on right side followed by the left side. In our study, both the right and left side fusion happened synchronously. Fisher et al.¹⁰, and D'Souza et al.⁷, also reported that no differences exist between right and left side. The present study demonstrated that the mean values of all linear morphometric measurements performed on the hyoid bone were significantly greater in males when compared to females, thereby confirming sexual dimorphism. The length of the hyoid bone body (AA'), length of

the right (DD') and left greater horns (EE') were found to be significantly increased amongst males over females and is in accordance with the study conducted by different authors (Miller KWP et al.⁹, Leksan I et al.¹³, Kindschuh SC et al.¹⁴) from different geographical locations. Total hyoid bone width measured from center (G-G') and distal ends (DE) was similar to the observations of Kindschuh SC et al.¹⁴, and Walls et al.¹⁵, who found significant differences between males and females from white European biological affinity. The height of the body (BB') is found to be significantly different in our study amongst males and females which was similar to that observed by Miller et. et al.⁹, Kindschuh et al.¹⁴, Mukhopadhyay et al.¹⁶, while this parameter was not found to be significantly altered in both the genders as observed by Seham A. G EL et al.¹⁷, and Walls et al.¹⁵. The difference is attributed to the method of the removal of hyoid bone from the dead bodies and how the morphological features were preserved thereafter. Metric analysis conducted on 3-dimensional CBCT images in the present study are more precise and can serve as a good alternate to traditional clinical assessment. The angles formed between the greater horn and the body of hyoid bone on both sides in the present study did not show statistically significant differences between males and females. Our results align with the findings of Kyoichi Ito et al.⁴.

Limitations: Owing to significant population differences, future research involving larger sample sizes from varied geographical locations is necessary to evaluate the significance of the hyoid bone in determining the age and gender related changes for forensic identification

Future Prospects:

This research provides a baseline for understanding typical hyoid bone fusion and gender based morphological variation which can be utilized in the fields of forensics and craniofacial research.

Conclusion:

Hyoid bone fusion shows a significant age-related progression with increase in fusion observed as age advances. CBCT is a reliable and non-invasive imaging modality for evaluating hyoid bone

morphology and fusion status, and may serve as a useful adjunct in forensic age and sex estimation when combined with other skeletal indicators.

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