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Evaluation of the differences in the localization of the lingula mandibulae according to pubertal development in children: A new anthropological and forensic approach

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ABSTRACT

Objective: This study aims to investigate changes in lingula mandibulae localization before and after puberty and sex differences.
Design: 288 panoramic radiographs evaluated retrospectively were divided into four groups according to pubertal development: under and over 10 years old for females and under and over 11 years old for males. Four anatomic sites were used as reference points: (a) the anterior border of the ramus; (b) the posterior border of the ramus; (c) the deepest point of mandibular notch; and (d) the angulus mandibulae. The ratio of the distances from the lingula mandibulae to points (a) and (b) and the ratio of the distances from the lingula mandibulae to points (c) and (d) were determined. Two-way ANOVA was performed to assess differences between subgroups based on sex and puberty, and a Bonferroni post-hoc test was performed for multiple comparisons.
Results: Puberty and sex has a significant interaction effect on the vertical location of the lingula mandibulae. Postpubertal lingula mandibulae is positioned proportionally more anteriorly by prepubertal localization. In addition, the lingula mandibulae is situated more posteriorly in females compared to males. A significant interaction effect of puberty and sex was observed in relation to the vertical positioning of the lingula mandibulae.
Conclusions: The spatial localization of the lingula mandibulae and its spatial displacement with age can be used both in estimating sex and the onset of puberty. Changes in the localization of lingula mandibulae according to pubertal development and sex can be used in forensic and anthropological contexts.

1. Introduction

Forensic anthropology is a multidisciplinary field that deals with the legal description of human remains, ranging from osteology to human physiology (Cattaneo, 2007; Krishan et al., 2016). In human-made and natural disasters where dead bodies decompose and/or disintegrate beyond recognition, identification of the remains is of great importance for the advancement of legal processes (de Boer et al., 2018). Bone tissue and its characteristics, which are the framework of vertebrate bodies and provide information about the mechanisms of adaptation to the environment, help estimate the identity of the individual by providing information not only about the origin of the individual from which it was obtained but also about population affinity, sex, age, and height (Belcher et al., 2022). In recent years, not only the age and sex estimation of dead bodies but also age and sex estimation in subjects such as adoption, child abuse and neglect, child labor issues, criminal cases, pornography, and immigration, especially in living children, have gained importance (Ubelaker & Longeway, 2019).

Sex estimation is the most important and first step to obtaining a reliable biological profile (Farias Gomes et al., 2019). Accurate sex estimation is critical in estimating age, population affinity, and height, as there may be differences in growth and development and population affinity between the sexes (Apaydin & Ozbey, 2020). Since changes in skeletal morphology between the sexes in the prepubertal period are insignificant, accurate estimation of the sex of an adult individual is easier and more reliable than that of a newborn or prepubertal

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individual. While phalanges, long bones, patella, pelvis, sternum, and cranium are used in the estimation of adult sex in different populations and population affinities around the world, studies on sex prediction in the prepubertal stage have often focused on the pelvis and cranium (Gualdi-Russo, 2007; Raghavendra Babu et al., 2012; Chandrakanth et al., 2014; Trautmann & Trautmann, 2014; Kranioti & Apostol, 2015; Hayashizaki et al., 2015; Krishan et al., 2016; Bonczarowska et al., 2021). This is due to the fact that the reliability and accuracy of sex estimation are directly dependent on the anatomy of skeletal remains and existing bones (Krishan et al., 2016). After the onset of puberty, the differences between male and female craniums become clearer as the male cranium develops some adult features, but the female cranium tends to retain pedomorphic features (Wood, 2015).

Although the human pelvis is the most dimorphic bone that can be used for sex estimation, it cannot be preserved, especially post-mortem, due to its fragility. For this reason, the human mandible, the second most dimorphic bone of the body, is often preferred for sex estimation (Bigoni et al., 2010; Gillet et al., 2020). The use of the human mandible, which is considered to be the most resistant bone of the face, in estimating sex occurs through qualitative and quantitative analyses. Qualitatively, the human male mandible has more robust anatomical features and a lower degree of thinness while being rougher due to greater chewing forces (Carvalho et al., 2013; Mello-Gentil & Souza-Mello, 2021). On the other hand, females produce weaker muscle forces during chewing, resulting in smoother surfaces of muscle attachments and smaller lower jaws (Velemínská et al., 2012). As documented by anthropologists, the human male mandible is generally significantly larger than the human female mandible. This finding, which is also used for medicolegal purposes, has been shown in many studies (Carvalho et al., 2013; Sharma et al., 2016; Sikka & Jain, 2016).

When the organism is examined in general, after puberty, as males exhibit a wider ontogenetic trajectory than females (rate and time hypermorphosis), the difference between the sexes increases, and male and female trajectories also differ in shape space (Bulygina et al., 2006; Coquerelle et al., 2011). For the human mandible, similar size change rates were observed in males and females before and after the pubertal period until the mean age of 9–12 years. While size increase gradually slows down in females during the pubertal period, this slowdown is not observed in males until around the age of 17 (Fan et al., 2019). From puberty to adulthood, males are characterized by a continuation of allometric shape changes, whereas the shape of the human female mandible continues to change even after size has ceased to increase. The female ontogenetic trajectory thus departs from the allometric direction after puberty (Coquerelle et al., 2011).

Anatomical formations on the human mandible may also show dimensional and positional changes depending on the effect of anatomical differences and adaptation, both in different sexes and depending on pubertal status (Alarcón et al., 2016; Nuzzolese et al., 2019). Condylar and coronoid processes, mental and mandibular foramen, lingula mandibulae, lower, upper, anterior, and posterior borders and corners of the human mandible, and alveolar processes vary spatially and dimensionally in different sexes and depending on pubertal status and age (Koudelová et al., 2015; Patcas et al., 2017). Quantitative analyses are based on the principle of metric evaluation of anatomical formations in different parts of the human mandible. The most important point of these analyses is that the results differ for individuals of different population affinities and sexes living in different geographical regions. For this reason, studies suggest that similar measurements are made in different population affinities and regions to obtain results for relevant populations (Carvalho et al., 2013; Vinay et al., 2013).

Although the change in the localization of the lingula mandibulae has been the subject of studies on mandibular block anesthesia due to the entry point of the mandibular nerve into the human mandible (Ahn et al., 2020; Choi & Hur, 2021), no studies have been found on the localization of the lingula mandibulae in relation to puberty in the forensic and anthropological context.

Therefore, the aim of this study was to evaluate the changes in the localization of the lingula mandibulae on panoramic radiographs of a group of children before and after puberty and to investigate the differences in these changes between the sexes. The first hypothesis of the study was that there was no significant change in the localization of the lingula mandibulae before and after puberty in the same sex. The second hypothesis of the study was that there was no change in the localization of the lingula mandibulae before and after puberty in males and females.

2. Materials and methods

2.1. Ethical approval and study population

This retrospective and cross-sectional study was approved by the Çanakkale Onsekiz Mart University School of Medicine Clinical Research Ethics Committee (protocol no. 2022–120). For this study, panoramic radiographs from Caucasian Turkish children living in the same region (Northwestern Anatolia) (ages range from 6 to 15 years) were selected from the archives of the Department of Maxillofacial Radiology, School of Dentistry, Çanakkale Onsekiz Mart University. Panoramic radiographs used in this study were taken between November 2021 and November 2022 for diagnosis and treatment planning within the appropriate indication. Radiographs of children with a previous diagnosis of developmental delay or progression and with systemic disease that may affect growth and development were not included in the study.

2.2. Sample size calculation

The sample size was calculated based on a pilot study that was conducted by our team with 100 panoramic radiographs using G*Power software (v. 3.1.9.7, Universitat Kiel, Kiel, Germany) with the following assumptions: permissible error $\alpha = 0.05$, study power of 0.95, mean ratio from lingula mandibulae to mandibular gonion and from lingula mandibulae to deepest point of the mandibular notch for before and after puberty in the total sample equals 0.82 and 1.14, standard deviations equals 0.43 and 0.58. The effect size was determined to be 0.6267. For paired t-tests, it was concluded that the total sample size should be at least 136 for each sex.

2.3. Sampling criteria

Puberty is the transitional period between childhood and adulthood characterized by the development of secondary sexual characteristics, gonadal maturation, and the acquisition of reproductive capacity (Abreu & Kaiser, 2016). Usually, the first sign of puberty is breast development in girls and testicular enlargement in boys. This is followed by the development of genital, pubic, and armpit hair, the first ejaculation, and menarche. Pubertal milestones are affected differently by environmental and genetic factors and thus are observed at different times in different populations (van den Berg et al., 2006; Yang et al., 2021). In studies conducted in the Turkish population, it was determined that the mean age of onset of puberty was 10.1 ± 1.0 years for girls (Bundak et al., 2008) and 11.6 ± 1.2 years for boys (Bundak et al., 2007). For this reason, the panoramic radiographs included in the study, considering the chronological age of the patients, were under 10 years old and over 10 years old for girls, and under 11 years old and over 11 years old for boys.

2.4. Panoramic radiographs

Digital panoramic radiographs were taken with a Morita device (VeraView IC5, J. Morita MFG. Corp., Kyoto, Japan) with an exposure time of 8.8 s at 60–70 kV and 7.5 mA at the Department of Maxillofacial Radiology, School of Dentistry, Çanakkale Onsekiz Mart University. Panoramic radiographs were classified as 1 = diagnostically excellent, 2

= diagnostically acceptable, and 3 = diagnostically unacceptable, according to the United Kingdom National Radiological Protection Board (Public Health England (PHE) and Faculty of General Dental Practice, 2020) criteria. Grade 1 radiographs do not contain exposure, positioning, or rendering errors, while grade 2 radiographs may have some exposure, positioning, or rendering errors. Grade 3 radiographs contain exposure, positioning, or processing errors, and it is strongly recommended that these radiographs not be evaluated. In addition, the guidelines suggest that at least 70% of radiographs should be grade 1 for the validity of the assessment. In this context, only Grade 1 radiographs were evaluated in terms of validity and reliability.

2.5. Measurements

The panoramic radiographs were transferred to a computer-aided measurement program (ImageJ version 1.49 v, U.S. National Institutes of Health, Bethesda, Maryland, USA). All radiographs were evaluated double-blind by two academic dentists who had previously worked on panoramic radiographs and knew how to use a program in a quiet room with dim ambient lighting. On the panoramic radiographs, the localization of the lingula mandibulae was evaluated relative to the plane parallel to the occlusal plane, passing through the deepest point of the coronoid notch (Jung et al., 2018). Four anatomic sites on the panoramic radiographs were used as reference points: (a) the anterior border of the ramus; (b) the posterior border of the ramus; (c) the deepest point of the mandibular notch; and (d) the angulus mandibulae. Two measurements were made at each of these four points from the lingula mandibulae. For validity and reliability, the mean of the two measurements made for each distance was noted. To normalize possible differences in magnification and angulation between radiographs, the ratio of the distances from the lingula mandibulae to points (a) and (b) and the ratio of the distances from the lingula mandibulae to points (c) and (d) were determined. Thus, the localization of the lingula mandibulae with respect to the midpoint of the ramus in both horizontal and vertical directions was determined. The value of the lingula mandibulae-a/lingula mandibulae-b ratio greater than 1 showed anterior localization, while the value of the lingula mandibulae-c/lingula mandibulae-d ratio smaller than 1 means superior localization according to the midpoint of the ramus in both horizontal and vertical directions (Fig. 1).

2.6. Statistical analyses

Data differences in age groups and sexes were analyzed using SPSS Statistics Version 27.0 (IBM Corp., Armonk, New York, USA). Descriptive statistics (mean and standard deviation) were utilized to describe the data. All the data showed a normal distribution. To assess variations among subgroups based on sex, puberty, and puberty*sex, two-way ANOVA was conducted, followed by Bonferroni's post-hoc test for multiple comparisons. Statistical significance was determined at a threshold of $p < 0.05$.

Measurements were made by two different, well-trained examiners (B.S., Y.D.). Three weeks after the initial measurement, for the purpose of calibration for examiners, 50 randomly chosen panoramic radiographs were scored twice, two weeks apart, by examiners. Reproducibility measurements and statistics were evaluated with the intraclass correlation coefficient. Intraexaminer reliability was assessed using Cohen's Kappa test.

3. Results

The intra-examiner Kappa values of the examiners ranged from 0.84 to 0.88. The high degree of agreement was retained during the re-examination periods. In the radiographic measurements, the intra- and inter-examiner agreements had intraclass correlation coefficient values of 0.96 and 0.93, respectively. There was no statistically significant difference, and the intraclass correlation coefficient values were found to be quite strong and reliable.

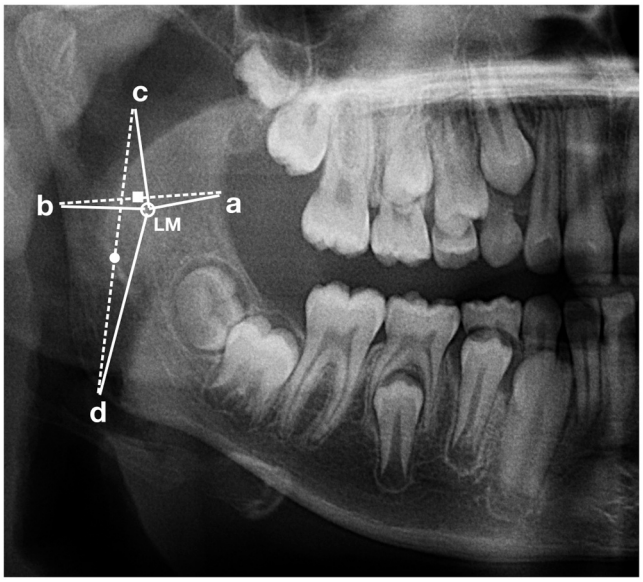


Fig. 1. Representation of lingula mandibulae, points a, b, c, and d and the distances between these points on a panoramic radiograph of a post-pubertal male individual. (Solid lines represent the distances from points a, b, c, and d to the lingula mandibulae; dashed lines represent the distance between points a and b, and points c and d. The white solid square represents the midpoint of the a-b distance, and the white solid circle represents the midpoint of the c-d distance.).

Table 1 shows the descriptive data of lingula mandibulae localization between sexes according to midpoint of the ramus in both horizontal and vertical directions. According to the Table 1, lingula mandibulae was located in more posterior localization in female prepubertal group. Besides, the mean ratios of postpubertal lingula mandibulae-a/lingula mandibulae-b and lingula mandibulae-c/lingula mandibulae-d ratios in terms of sex showed that lingula mandibulae was located in more posterosuperior localization in postpubertal female group than male group.

Puberty and sex were together examined for their effect on the position of the lingula mandibulae using two-way ANOVA analysis, as presented in Table 2. The analysis revealed a significant interaction effect of puberty and sex on the vertical location of the lingula mandibulae ($p < 0.05$), while no significant interaction effect was observed for the horizontal position of the lingula mandibulae ($p > 0.05$). When the effect of puberty on the position of the lingula mandibulae without sex distinction was evaluated, it was found that the ratio of the distances between lingula mandibulae-a and lingula mandibulae-b was statistically significantly greater in prepubertal group than postpubertal group ($p < 0.05$) (Table 2). The ratio of the lingula mandibulae-a and lingula mandibulae-b distances was measured as 1.219 and 1.137 for prepubertal and postpubertal, respectively. This means that although both prepubertal and postpubertal ratios are greater than 1, which means the lingula mandibulae is located posteriorly according to the horizontal midpoint of the ramus, after puberty the lingula mandibulae is positioned proportionally more anteriorly by prepubertal lingula mandibulae localization. Besides, the ratio of lingula mandibulae-a to lingula mandibulae-b distances was measured as 1.240 for females and 1.115 for males. Given the significant effect of sex on the horizontal position of the lingula mandibulae as indicated by the two-way ANOVA, it can be inferred that, on average, the lingula mandibulae is situated more anteriorly in females compared to males. In addition, there was no statistically significant difference in the change of ratio in lingula mandibulae-c and lingula mandibulae-d distances according to puberty,

Table 1
Descriptive data of lingula mandibulae localization between sexes according to midpoint of the ramus in both horizontal and vertical directions.

Sex		N	Measurement ratios	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval	
							Lower	Upper
Prepuberty	Female	72	LM-a/LM-b	1.306	0.366	0.043	0.589	2.023
			LM-c/LM-d	0.596	0.460	0.054	0.301	1.501
	Male	72	LM-a/LM-b	1.131	0.211	0.025	0.718	1.544
			LM-c/LM-d	0.573	0.143	0.017	0.293	0.853
Postpuberty	Female	72	LM-a/LM-b	1.174	0.223	0.026	0.737	1.612
			LM-c/LM-d	0.580	0.153	0.018	0.281	0.879
	Male	72	LM-a/LM-b	1.100	0.186	0.022	0.736	1.464
			LM-c/LM-d	0.700	0.142	0.017	0.423	0.978

N: number; LM: lingula mandibulae; Std: standard.

Table 2
Changes of lingula mandibulae localization with puberty*sex according to midpoint of the ramus in both horizontal and vertical directions.

Ratios	Groups	Subgroups	Means	df	Mean Square	F-value	p-value†	Partial Eta Square
LM-a/LM-b	Puberty	Prepuberty	1.219	1	0.486	7.394	**0.007**	0.25
		Postpuberty	1.137					
	Sex	Female	1.240	1	1.097	16.699	**< 0.001**	0.56
		Male	1.115					
	Puberty*Sex			1	0.193	2.938	0.088	0.10
LM-c/LM-d	Puberty	Prepuberty	0.587	1	0.241	3.492	0.063	0.012
		Postpuberty	0.640					
	Sex	Female	0.590	1	0.156	2.269	0.133	0.008
		Male	0.637					
	Puberty*Sex			1	0.348	5.045	**0.025**	0.018

LM: lingula mandibulae; df: degrees of freedom; F-value: ratio of two mean squares. †Two-way ANOVA. Bold font: p-value < 0.05.

which measured 0.587 and 0.640 for prepubertal and postpubertal, respectively. Thus, there was no relationship between the localization of the lingula mandibulae and puberty with respect to the midpoint of the ramus height, and both prepubertal and postpubertal lingula mandibulae are potentially located in superior positions with respect to the vertical midpoint of the ramus (lingula mandibulae-c/lingula mandibulae-d < 1).

Table 3 is derived from post hoc Bonferroni's test of two-way ANOVA and represents the descriptive statistics of lingula mandibulae localization between pubertal status and sex according to midpoint of the ramus in horizontal direction. When examining the impact of puberty on the horizontal position of the lingula mandibulae in females, it was observed that the lingula mandibulae-a/lingula mandibulae-b ratio decreased from 1.306 in prepubertal individuals to 1.172 in postpubertal individuals (Table 3). This change signifies a statistically significant difference, which is attributed to the female dataset, as revealed by the two-way ANOVA analysis (Fig. 2). On the other hand, when the horizontal changes in lingula mandibulae localization with puberty were examined in males, the lingula mandibulae-a/lingula mandibulae-b ratio was measured 1.131 and 1.100 for the prepubertal and postpubertal groups, respectively, and it can be estimated that this result did not make a difference in the horizontal direction in males, unlike females (Fig. 3). Additionally, lingula mandibulae-a/lingula mandibulae-b ratios were measured as 1.306 and 1.131 in prepubertal female and male groups, respectively, which can be concluded that lingula mandibulae was located more posteriorly in the female prepubertal group.

Table 3
Descriptive statistics of lingula mandibulae localization between pubertal status and sex according to midpoint of the ramus in horizontal direction.

Ratios	Pubertal status	N	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Female	Prepuberty	72	**1.306**	0.030	1.247	1.365
	Postpuberty	72	1.172	0.030	1.112	1.232
	Total	144	**1.240**	0.021	1.197	1.281
Male	Prepuberty	72	1.131	0.030	1.071	1.191
	Postpuberty	72	1.100	0.030	1.041	1.160
	Total	144	1.115	0.021	1.073	1.158

N: number; Std: standard. Bonferroni's post hoc test of Two-way ANOVA at 95% confidence interval. Bold font means estimated differences.

Table 4 is derived from post hoc Bonferroni's test of two-way ANOVA and represents the descriptive statistics of lingula mandibulae localization between pubertal status and sex according to midpoint of the ramus in vertical direction. When the vertical changes in localization of lingula mandibulae with puberty were examined in females, it was seen that there was no difference in the lingula mandibulae-c/lingula mandibulae-d ratio, so there is no significant change in the vertical direction. (Fig. 4). On the other hand, the lingula mandibulae-c/lingula mandibulae-d ratios were respectively measured as 0.573 and 0.700 in the prepubertal and postpubertal groups in males, and it was observed that the lingula mandibulae shifted vertically to inferior in the postpubertal group (Table 4). Therefore, the lingula mandibulae is thought to be relocated to a more inferior localization in males by puberty (Fig. 5). Besides, the mean ratios of postpubertal lingula mandibulae-c/lingula mandibulae-d in terms of sex were measured as 0.584 and 0.700 for females and males, respectively, showed that lingula mandibulae was located in a more superior localization in the postpubertal female group than the male group.

According to the differences between the lingula mandibulae-a/lingula mandibulae-b prepubertal groups and lingula mandibulae-c/lingula mandibulae-d postpubertal groups obtained from the post-hoc Bonferroni test given in Table 3 and Table 4, which are thought to cause statistically significant differences observed by two-way ANOVA in Table 1 ($p < 0.05$), sex may affect the horizontal localization of the lingula mandibulae in prepubertal groups and its vertical localization in postpubertal groups.

4. Discussion

Lingula mandibulae emerges as an important anatomical structure in major surgical procedures and inferior alveolar nerve blockage. Therefore, knowing the anatomical localization of the lingula mandibulae is

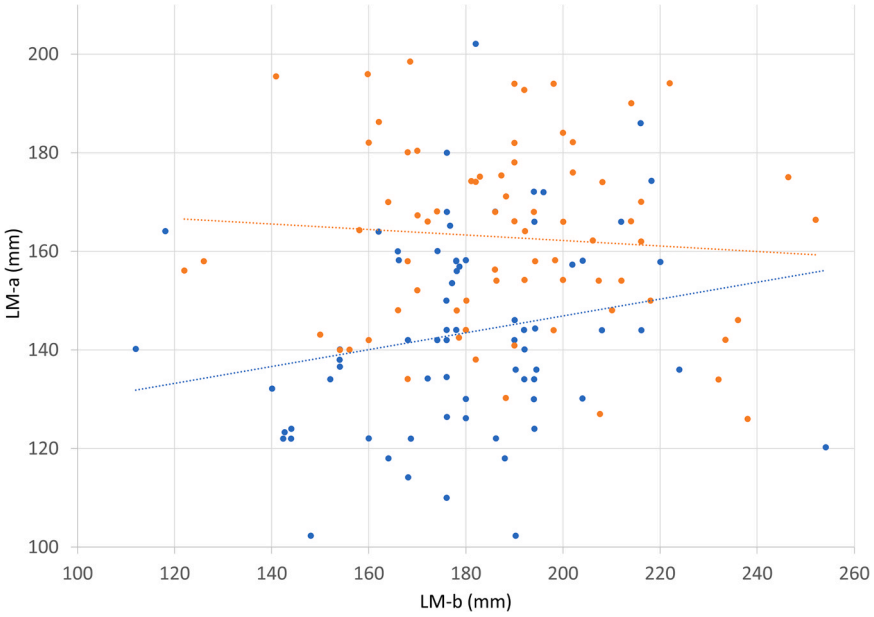


Fig. 2. LM-a and LM-b distances in female group before and after puberty. The blue dots separated underside in the graph indicate that the lingula mandibulae is located more anteriorly in the postpubertal female group than in the prepubertal group. (LM: Lingula mandibulae; blue dots: LM-a/LM-b ratios of prepubertal group; orange dots: LM-a/LM-b ratios of postpubertal group) (All numerical values are expressed in millimeters.).

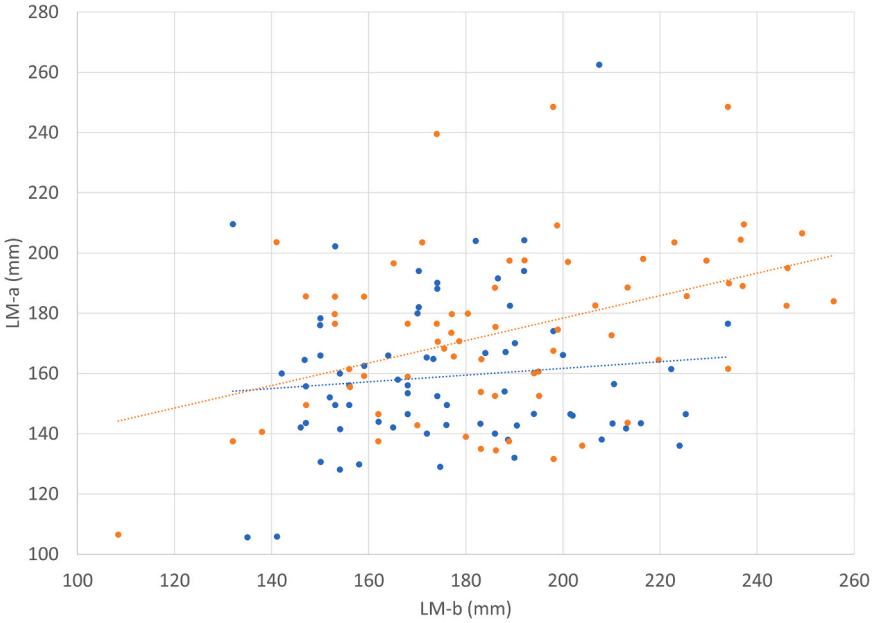


Fig. 3. LM-a and LM-b distances in male group before and after puberty. The absence of a clear distinction in the graph between the blue and orange dots indicates that the LM-a/LM-b ratio does not cause a statistically significant difference with puberty in male group. (LM: Lingula mandibulae; blue dots: LM-a/LM-b ratios of prepubertal group; orange dots: LM-a/LM-b ratios of postpubertal group) (All numerical values are expressed in millimeters.).

clinically important (Lupi et al., 2021). It is also known that the lingula mandibulae localization changes with increasing age in paediatric patients (Aps et al., 2018). However, no study has been found in the literature showing that lingula mandibulae localization can be used to define the puberty period. From this point of view, the relationship of lingula mandibulae localization with puberty and sex was investigated in this study. There are a few studies determining localization of lingula mandibulae, however, these studies were carried out due to importance of lingula mandibulae localization in terms of mandibular surgery and that give results on metric measurements (Jansisyanont et al., 2009; Samanta & Kharb, 2012; Shenoy et al., 2012; Sekerci & Sisman, 2014; Senel et al., 2015). Shenoy et al. (2012) found the distance between anterior border and lingula mandibulae as 16 mm. Samanta and Kharb (2012) found that mean distance from lingula mandibulae to the anterior border of mandibular ramus was 20.0 mm, to posterior border of the ramus was 15 mm and to mandibular notch was 15.4 mm, whereas Senel et al. (2015), were found the same distances as 18.5 mm, 16.9 mm, and 18.1 mm, respectively. Studies performed by Sekerci & Sisman (2014) and Jansisyanont et al. (2009) reported similar data about lingula mandibulae localization and mean distances from lingula mandibulae to anterior border of the mandibular ramus were found as 16.8 mm and 20.6 mm, from lingula mandibulae to the mandibular

Table 4
Descriptive statistics of lingula mandibulae localization between pubertal status and sex according to midpoint of the ramus in vertical direction.

Ratios	Pubertal status	N	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Female	Prepuberty	72	0.596	0.031	0.535	0.656
	Postpuberty	72	0.584	0.031	0.523	0.645
	Total	144	0.590	0.022	0.547	0.633
Male	Prepuberty	72	0.573	0.031	0.512	0.634
	Postpuberty	72	**0.700**	0.031	0.640	0.761
	Total	144	0.637	0.022	0.593	0.680

N: number; Std: standard. Bonferroni's post hoc test of Two-way ANOVA at 95% confidence interval. Bold font means estimated differences.

notch were found as 15.3 mm and 16.6 mm, respectively. As seen in these studies on lingula mandibulae localization, either dry mandible or cone beam tomographic images were used and measurements were made on direct or on 3D images. Although the results of these studies are important when evaluated clinically, they are studies conducted on adult patients and cannot explain the spatial displacement tendency of the lingula mandibulae during adolescence. The main purpose of our study was whether puberty and sex influence the spatial displacement of the lingula mandibulae. For this purpose, in our study, metric measurements were compared to each other, and a result was tried to be obtained over the ratios. Thus, it has been revealed in which direction/directions the lingula mandibulae is spatially displaced when the midpoint of the ramus is referenced. Accordingly, when the results of our study are evaluated, lingula mandibulae localization is spatially displaced by being affected by both the onset of puberty and sex in a statistically significant way. So, lingula mandibulae localization was found as posteriorly according to horizontal midpoint of ramus regardless of sex and puberty, and lingula mandibulae repositioned proportionally more anteriorly by prepubertal lingula mandibulae localization after puberty. Besides, lingula mandibulae is located more anteriorly in the postpubertal group than the prepubertal group, and also, lingula mandibulae is relocated in a more inferior localization in males by puberty. In addition, lingula mandibulae is located in more posterior localization in female prepubertal group and in more superior localization in postpubertal female group than male groups. All these statistically significant results which allows us to measure the spatial displacement of lingula mandibulae were actually obtained from the interpretation of metric measurements by proportioning each other, not directly. That is, the evaluation method used in our study has indeed been a useful method to help us understand the spatial displacement of lingula mandibulae. Therefore, first and second null hypothesis of the study were rejected.

The human mandible contains various potential morphological anatomical structures that can be used for forensic and anthropological purposes (Iliescu et al., 2022). One of these anatomical structures is lingula mandibulae, however, the differences in the shape of the lingula mandibulae were generally emphasized (Tuli et al., 2000). Since the mandibular ramus and condyle are regions of extensive morphological change that contribute to sexual dimorphism, sex estimation is commonly performed by evaluating metrically the distance and angles of ramus and condyle to different anatomical regions of the human mandible (Dong et al., 2015; Gamba et al., 2016; Lopez et al., 2017; Kelly et al., 2017; Nguyen et al., 2022). On the other hand, although there is no existing study showing the change in the localization of the lingula mandibulae according to pubertal status in different sexes, a few studies have focused on the localization of the lingula mandibulae in different sexes and population affinities. In their study, Alves & Deana (2015) investigated the location and shape of the lingula mandibulae and the relationship between rapopulation affinity and sex in males and females of Amerindians and Caucasians. Considering the anteroposterior position, they noted that the lingula mandibulae is positioned slightly behind the center of the mandibular ramus width. Also, regarding the superoinferior position, they stated that the lingula mandibulae is located in the upper or middle third of the ramus. In addition, as a result of the study, which detected various significant relationships between population affinity, sex, shape, and position of the lingula mandibulae and various distances, it was emphasized that the findings are important in terms of surgical procedures and inferior alveolar nerve anesthesia (Alves & Deana, 2015). In another study (Jansisyanont et al., 2009) evaluating the shape, height, and position of the lingula mandibulae in Thai human mandibles, it was determined that the lingula mandibulae was located above the occlusal plane in most of the human mandibles examined. It has been stated that the

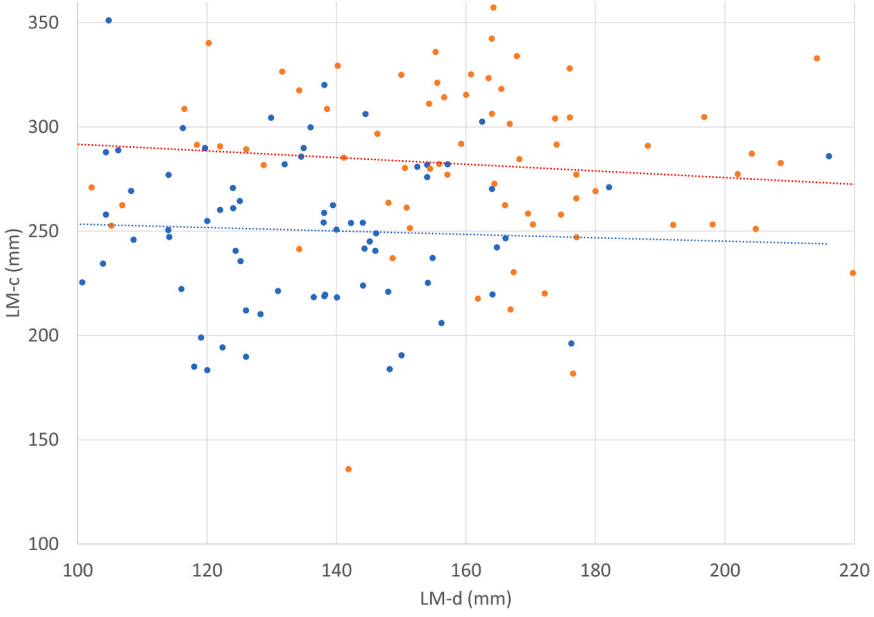


Fig. 4. LM-c and LM-d distances in female group before and after puberty. The absence of a clear distinction in the graph between the blue and orange dots indicates that the LM-c/LM-d ratio does not cause a statistically significant difference with puberty in female group. (LM: Lingula mandibulae; blue dots: LM-c/LM-d ratios of prepubertal group; orange dots: LM-c/LM-d ratios of postpubertal group) (All numerical values are expressed in millimeters.).

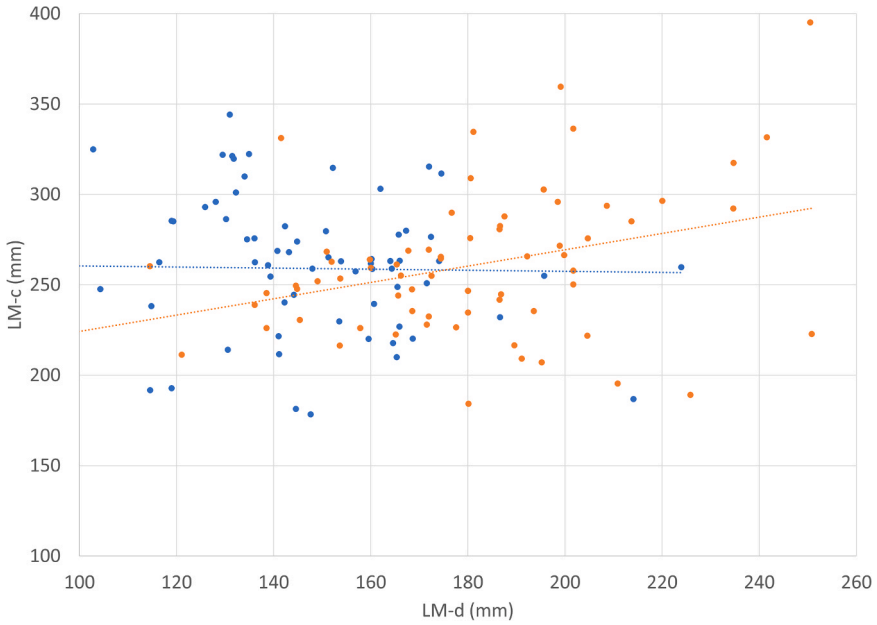


Fig. 5. LM-c and LM-d distances in male group before and after puberty. A distinction in the graph between the blue and orange dots indicates that the LM-c/LM-d ratio causes a statistically significant difference with puberty in male group. (LM: Lingula mandibulae; blue dots: LM-c/LM-d ratios of prepubertal group; orange dots: LM-c/LM-d ratios of postpubertal group) (All numerical values are expressed in millimeters.).

shape and metric properties of the lingula mandibulae in relation to the surrounding structures differ in Thais from other population affinities (Jansisyanont et al., 2009). Hormonal changes observed during puberty cause structural changes in the human mandible as well as in the whole body. The difference in pubertal changes in male and female body results in the human mandible showing different changes in the different sexes (Bejdová et al., 2013). Because coronoid processes and mandibular condyles are longer and ramus is higher, lingula mandibulae is located higher in adult males than in females (Bejdová et al., 2013). However, this situation does not fully express the proportional displacement. Whereas the ratio of the distances of the lingula mandibulae to the ramus boundaries reveals spatial displacement as opposed to metric displacement. Although there are many methods used in forensic and anthropological sex estimation, scientific authorities recommend the use of the least invasive imaging techniques (Krishan et al., 2016). Considering the importance of radiological approaches in the examination of historical skull remains and/or severely damaged dead body finds, the use of relatively robust bones such as the human mandible is of great importance. Accordingly, not only the estimation of the sex, but also the estimation of the pubertal status of the found remains are valuable both forensic and anthropological approaches. Therefore, in our opinion, regardless of individual characteristics, lingula mandibulae can be used as a forensic and anthropological marker by interpretation of metric measurements by proportioning each other.

Recent interest and policy development have led to a massive increase in the forensic examination of evidence in crimes such as rape, child abuse, murder, human trafficking, pornography, and/or child labor. Although the results are more meaningful in subadult individuals, especially in cases of sexual abuse, with the development of technology in the field of forensic examination, documenting basic medical findings and collecting relevant evidence are the difficulties encountered in such cases (Kaur et al., 2022). Especially in the forensic context, sex estimations are considered an important step in the biological profile assessment of unknown human remains, not only in sociological phenomena but also in severely physically damaged remains (as a result of major disasters, fire, etc.) (Garcovich et al., 2022). Considering all these factors, especially in sociological cases, the stage of pubertal development of the individual can be determined by using various bones of the body and different anatomical landmarks in these bones. Sex estimation methods are often unreliable in subadult groups due to the incomplete development of dimorphic sexual characteristics (Estévez Campo et al., 2018). In contrast, Gonzalez (2012) showed promising accuracies of lateral cephalometric measurements of population-specific 78% to 89% in subadults. Although even the most reliable bone of the human skeleton in adult individuals showed low reliability in subadults, Estévez Campo et al. (2018) noted the reliability due to low sexual dimorphism in the ischium and pubis. In addition, although other bones such as the human ilium or human mandible offer promising results in predicting forensic sex, it has been emphasized that more validation studies are needed on these (Cardoso & Saunders, 2008; Blake, 2019). In this context, the results of our study present forensic confirmatory and scientific findings.

The patient population in this study consisted of children and adolescents aged 6 to 15 years, and it was necessary to have sufficient samples before and after puberty. In addition, since the study was planned retrospectively, it was difficult to find sufficient number of CBCTs that included the entire mandible in this age group, and the study was carried out using panoramic radiographic images (Hajem et al., 2020). As it is known, CBCT is used in a limited way, especially in children and adolescent patients, since the radiation dose is higher than panoramic radiography (European Commission, 2012). In addition, for the same reasons, images of the patient which are produced in appropriate quality and obtained in time are used for imaging methods needed in forensic events. Thus, panoramic radiography is an easily accessible, cheap, and dose-sparing imaging method that has become the first-line tool for examining the teeth and jaws (Tassoker et al., 2019). From this point of view, when considering a real event, panoramic imaging is a much more common imaging method in forensic cases than CBCT. Additionally, the images used in our study were checked according to the United Kingdom National Radiological Protection Board and included in the study. Briefly, the study was carried out on panoramic images in order to reflect the situation that may occur in reality and to examine a much larger number of appropriate images.

There were also some limitations in our study. The most important limitation is that it was not possible to estimate exactly whether the patients in the sample were in the puberty period when the images were taken, due to the fact that it was a retrospective study. For this reason, studies previously published in the literature on the mean age of onset of

puberty for males and females in the Turkish population were taken as a basis (Bundak et al., 2007; Bundak et al., 2008). Therefore, the adequacy and reliability of the method should be tested by questioning the actual conditions of the patients face to face in a prospective manner. Another limitation is that it contains errors that may arise from panoramic imaging. However, as explained above, panoramic imaging is still the cheapest and fastest available imaging method. and in real conditions, it is an inevitable fact that panoramic images are mostly used.

In conclusion, lingula mandibulae-a/lingula mandibulae-b ratio was decreased by approximately 10.1% and 2.7% in female and male groups, respectively, with the onset of the puberty which means the proportional change in horizontal direction is more considerable in females. Besides, the position of the lingula mandibulae is located proportionally more anteriorly with the onset of puberty, regardless of sex, and is situated further posteriorly in females, irrespective of puberty. Additionally, while lingula mandibulae-c/lingula mandibulae-d ratio was decreased by approximately 2.8% in female groups, across that, it was increased by approximately 22.3% in male groups with the onset of the puberty which means the proportional change in vertical direction differs more clearly in males. Furthermore, the vertical location of the lingula mandibulae is influenced by the interaction between puberty and sex. The spatial localization of the lingula mandibulae and its spatial displacement with age can be used both in sex predilection and in estimating the onset of puberty. The contribution of the change in the localization of the lingula mandibulae according to the pubertal development in the forensic and anthropological context to the literature should be supported by studies to be carried out in a larger sample group and different populations in the future.

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CRediT authorship contribution statement

Deniz Yeşim: Writing – review & editing, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Sezer Berkant:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Eren Hakan:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data Availability

The data used in this study are available upon reasonable request to the corresponding author.

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