

Relationship between morphological characteristics of the hyoid bone and mandible in Japanese Cadavers Using three-dimensional computed tomography

Authors

Yoshifumi Ichijo • Yusuke Takahashi • Mahito Tsuchiya • Yoichi Marushita •

Toshio Sato • Hitoshi Sugawara • Shogo Hayashi • Masahiro Itoh • Tsuneo Takahashi

Contact Details

Yoshifumi Ichijo • Yusuke Takahashi • Mahito Tsuchiya • Tsuneo Takahashi

Department of 3D imaging Anatomy, Kanagawa Dental University Graduate School,

82 Inaoka, Yokosuka, Kanagawa, 238-8580, Japan

E-mail: t.takahashi.kdu.ac.jp@gmail.com

Yoichi Marushita

Nippon Engineering College, Department of clinical engineering

5-23-22 Nishikamata, Ota, Tokyo 144-8655, Japan

Hitoshi Sugawara

Department of Physical Therapy, Tokyo University of Technology

5-23-22 Nishikamata, Ota, Tokyo,144-8535 ,Japan

Toshio Sato

Department of Clinical Engineering, Toin University of Yokohama

1614 Kurogane Aoba, Yokohama, Kanagawa, 225-8503,Japan

Shogo Hayashi • Masahiro Itoh

Department of Anatomy, Tokyo Medical University

1-1 Shinjuku 6,Shinjuku,Tokyo,160-0022,Japan

***Corresponding Author**

Yoshifumi Ichijo , Department of 3D imaging Anatomy, Kanagawa Dental University Graduate School,

82 Inaoka,Yokosuka,Kanagawa, 238-8580,Japan.

Telephone number:080-3475-4423

E-mail: ichijo.yoshifumi@gmail.com

Running Title

Hyoid Bone and Mandible Morphology

Abstract

The aim of this study is to obtain a quantitative anatomical description of the hyoid bone and mandible using three dimensional computed tomography. Hyoid bones were obtained from a total of 101 cadavers varying in age from 67 to 102 years. The percentage of symmetrical U-type and asymmetrical type hyoid bones was low compared to symmetrical V type, (14.9, 15.8, 69.3%, respectively) and no significant sex difference was observed. We found bilateral non-fusion in cadavers of advanced age at a rate of 22.8% and bilateral complete fusion at a rate of 51.5%. There were significant differences in the metric variables (length and width) between males and females, but no significant differences in width among the different fusion types. There was no significant interaction effect of sex and degree of fusion. Strong significant associations were observed between size (length and width) of the hyoid bone and mandible in the nonfusion group and the complete fusion group revealed a moderate correlation. We also investigated the hypothesis that the junction between the hyoid body and greater horn plays an important role in the movement of bones that have not yet ossified. However, no statistical difference was observed in the width between the two greater horns. The degree of fusion of the greater horn with the hyoid body may also affect relations of interdependencies between the hyoid bone and mandible, an important component to consider when assessing risk factors in the development of masticatory and swallowing function.

Keywords

Cadavers · Fusion · Hyoid bone · Mandible · Three dimensional computed tomography

Mini Abstract

Three-dimensional CT morphometry of both hyoid bones and mandibles in 101 human cadavers

revealed morphological relations of interdependencies between the two bones by degree of fusion.

Introduction

The hyoid bone, a part of the viscerocranium, unlike other bones in the skeletal system, is articulated to other bones by muscles and ligaments only. It is located between the mandible and thyroid cartilage and is supported by tongue muscles and the hyoid muscle. The hyoid bone consists of the hyoid body, the greater horns and lesser horns protrude in pairs on both the left and right sides. It lies parallel to the inferior border of the mandible and is vertically positioned between the third and fourth cervical vertebrae with its anterior positioned in line with the third molar in the sagittal plane.

The morphological characteristics of the hyoid bone have been extensively researched. Sex-related differences have been observed in the angle between the hyoid body and greater horn and there is a significant relationship between the total anterior-posterior length of the hyoid bone and the height and weight of subjects (Leksan et al. 2005; Kim et al. 2006; Kindschuh et al. 2010; Ito et al. 2012; Fakhry et al. 2013; Urbanová et al. 2013). The hyoid bone can be classified into two common shapes, hyperbolic (U type) and parabolic (V type) (Papadopoulos et al. 1989; Pollanen and Ubelaker 1997; Leksan et al. 2005; Ito et al. 2012). Leksan et al. (2005) suggest that asymmetry in the length of the greater horns is considerably higher in females.

The junction between the hyoid body and greater horn consists of hyaline cartilage, making the hyoid bone extremely supple (Doual et al. 2003; Shimizu et al. 2005). While fusion of the hyoid bone has generally been observed to occur after the age of 50, with the fusion process accelerating with aging, large individual differences have been highlighted. Therefore, the hyoid bone is not suitable for use in

forensic examinations (Miller et al. 1998; Gupta et al. 2008; Balseven-Odabasi et al. 2013).

Moreover, ossification of the thyroid and cricoid cartilage, as well as hyaline cartilage, occurs with aging (Mupparapu and Vuppapapati 2005; Dang-Tran et al. 2010; Claassen et al 2014). Thyroid cartilage ossification can also be a result of inflammation caused by repetitive microtrauma due to muscular overuse (Galline et al. 2005). The hyoid bone is attached to the thyroid cartilage by the thyrohyoid membrane and thyrohyoid ligaments and this structure is anatomically and functionally referred to as the hyoid-larynx complex. Just like thyroid cartilage, the hyaline cartilage in the junction between the hyoid body and greater horn may be susceptible to repetitive microtrauma, possibly caused by swallowing and excessive speaking, leading to ossification.

More recently, the morphological characteristics of the hyoid bone have been highlighted by clinical medicine. It has been shown that the position of the hyoid bone, along with the surrounding components of the hyoid-larynx complex, is affected by obesity and age which in turn can lead to obstructive sleep apnea (OSA), (Sforza et al. 2000; Tangugsorn et al. 2000; Iked et al. 2001; Paoli et al. 2001; Chi et al. 2011; Feng et al. 2014; Ryu et al. 2015). We believe that it is not only the position of the hyoid bone but also the size, shape and degree of fusion of the hyoid body and greater horn that contributes to function. Therefore it is important to research and document the morphological characteristics of the hyoid bone, especially as most studies to this point have used the dissection of cadavers (Leksan et al. 2005; Kim et al. 2006; Kindschuh et al 2010; Urbanová et al. 2013). One weakness in such studies is that, in cases where there is no fusion between the hyoid body and greater horn, the total length of the hyoid bone

and the distance between the distal portions of the greater horns of the hyoid bone cannot be measured.

This means that we do not have a complete understanding of the morphological characteristics of the hyoid bone. Using three-dimensional computed tomography(CT), it is possible to measure the size and morphological characteristics, including different degrees of fusion, of the hyoid bone without removing it from the body.

The hyoid bone and mandible are connected by the suprahyoid muscle, so these two structures interact anatomically, functionally and developmentally forming an integrated system. Therefore it is important to understand the relationship between the hyoid bone and mandible. However, after an extensive search of the literature, we were only able to find one related study looking at the relationship between the hyoid bone and mandible of the pharyngeal arches in young subjects, suggesting research is sparse, especially in older subjects (Mitani and Sato 1992).

Therefore, the purpose of this study was to (1) clearly measure the morphological characteristics of the hyoid bone in older cadavers using three-dimensional computed tomography, and (2) investigate the relationship between the morphological characteristics of the hyoid bone and mandible with the hypothesis that the hyoid bone and mandible are functionally related.

Materials and Methods

Subjects

This study was carried out using Japanese cadavers used for dissection from the year 2011 to 2014 in

the Department of Anatomy in the School of Dentistry, Kanagawa Dental University. The average age of the cadavers was 84.5 years (ranging from 67 to 102 years), 48 males and 53 females.

All 101 cadavers were systemically analyzed with computed tomography (CT) (Asteion 4; Toshiba, Tokyo, Japan) before the preservation treatment. The hyoid bones and mandibles from persons of known age and sex used in this study were selected randomly.

The Kanagawa Dental University Institutional Review Board approved the use of human cadavers in this study (#140).

Computed tomography assessments

Multislice CT scans performed using a four-slice CT with the following parameters: 120 kV, 100 mAs, reconstruction slice thickness of 1 mm. The CT scans were performed parallel to the Frankfort horizontal plane. CT scan images (DICOM format) were assessed, and all measurements were performed on digital data in OsiriX 5.5.6.

Classification of hyoid bone by shape and degree of ossification

First, the shape of hyoid bones was classified into three types using the method of Laksan et al. (2005). symmetrical hyperbolic (U-type), and symmetrical parabolic (V-type), and asymmetrical. It was determined that bones with a bone angle of less than 25° between the long axis of the bilateral greater horn belonged to the symmetrical U type, while those with an angle greater than 25° belonged to the

symmetrical V type. Those with a difference of over 10% in the length of the greater horns on the opposite(right and left) sides were determined to be of asymmetrical type.

Second, the degree of ossification between the hyoid body and the greater horn of the hyoid bone was classified [fusion of the greater horn with the body of the hyoid bone was determined by partially modifying the classification used by Ito et al. (2012), (Fig.1)]. Subjects were further divided into 3 groups to take into account the degree of ossification at the junction between the hyoid body and the greater horns: a first group with complete bilateral fusion (complete fusion group), a second group with complete unilateral fusion (one side only) or a condition of unilateral or bilateral incomplete fusion (incomplete fusion group), and a third group with bilateral nonfusion (nonfusion group)(Fig.1). The mandibles were also divided into the same groups based on the degree of fusion of the hyoid bone.

Measurement methodology

Similar to Fakhry et al. (2013) and Whyms et al. (2013), five landmarks of the hyoid bone and seven landmarks of the mandible were determined in multiplanar reformation images from the axial, sagittal, and frontal planes three dimensionally (Fig.2). Each measurement site is shown in Fig.3. The measurement of angle β was defined as the angle formed between the gonion and menton.

Data analysis

The data obtained were analyzed using SPSS 15.0 software and are expressed as mean \pm standard deviation (SD).

Chi-square tests were performed to analyze the association between sex (males and females) and shape type (symmetrical U type, symmetrical V type and asymmetrical type). Chi-square tests were also performed to analyze the relationship between age group and degree of fusion (complete fusion, incomplete fusion, and nonfusion) in each sex and between age group and sex (males and females) in each degree of fusion type.

Statistical analyses included a two-way analysis of variance (ANOVA) to compare sex (males and females) and degree of fusion (complete fusion, incomplete fusion, and nonfusion). Where significant main effects were identified, simple main-effect post hoc tests with Bonferroni corrections were further applied.

The Pearson correlation coefficient (r) was used to analyze the relationship of the metric measurements between the hyoid bone and mandible in the complete fusion and non-fusion groups.

Results

Distribution of shape types of hyoid bone by sex

The sex characteristics of shape type in the study population are summarized in Table 1. No significant difference existed between sex and shape type according to the Chi-square test. The symmetrical V type was most common in males and females.

Distribution of age, sex and degree of fusion in hyoid bone

Age data for males and females for the complete fusion group ($n = 52$, 51.5 % of total, males 56.3 %, females 47.2 %), incomplete fusion group ($n = 26$, 25.7 % of total, males 27.1 %, females 24.5 %) and nonfusion group ($n = 23$, 22.8 % of total, males 16.7 %, females 28.3 %) are presented in Table 2. In regard to the relationship between age group and degree of fusion in each sex, Chi-square tests revealed that there was no significant difference in male and female groups, ($p = 0.22$ and $p = 0.45$, respectively). Chi-square tests also revealed no significant difference between age group and sex in each degree of fusion (complete fusion group $p = 0.64$, incomplete fusion group $p = 1.0$ and non-fusion group $p = 0.30$, respectively).

Comparison of anthropometric measurements by sex and degree of fusion

Table 3 presents the dimensions and angle of the hyoid bone according to the sex and degree of fusion. The metric variables (length A , B , C , D , E , F , and G and width H and I) were significantly different between males and females [$F(2,95) = 28.22$, $p < 0.01$, $F(2,95) = 23.35$, $p < 0.01$, $F(2,95) = 33.91$, $p < 0.01$, $F(2,95) = 24.61$, $p < 0.01$, $F(2,95) = 78.69$, $p < 0.01$, $F(2,95) = 51.44$, $p < 0.01$, $F(2,95) = 40.75$, $p < 0.01$, $F(2,95) = 47.15$, $p < 0.01$, $F(2,95) = 23.04$, $p < 0.01$], but no effect was observed in relation to angle α [$F(2,95) = 0.04$, $p = 0.83$]. The length A , C , D , E and G were significantly different among the complete fusion group, incomplete fusion group and nonfusion group [$F(2,95) = 4.83$, $p < 0.05$, $F(2,95) = 3.81$, $p <$

0.05, $F(2,95) = 3.44$, $p < 0.05$, $F(2,95) = 3.96$, $p < 0.05$, $F(2,95) = 3.48$, $p < 0.05$]. There was no significant interaction effect of length, width and angle α (table 3).

Post hoc tests revealed that the lengths C , D , E , F , G and width H in complete fusion and nonfusion were longer in men than women ($p < 0.01$). In complete fusion width I was greater in men than women ($p < 0.01$), but, there was no difference between men and women in nonfusion. In men, length C was greater in nonfusion than in complete fusion ($p < 0.05$). Also in men, length E and G were greater in nonfusion than in incomplete fusion ($p < 0.05$ and $p < 0.01$, respectively). In women, there was no difference in length and width based on degree of fusion.

Table 4 presents the dimensions and angle of the mandible in relation to the degree of fusion of the hyoid bone. The metric variables (length J , K , M , N , O , P , Q , R , S , width U , V , W and angle β) were significantly different between males and females [$F(2,95) = 10.09$, $p < 0.01$, $F(2,95) = 4.61$, $p < 0.05$, $F(2,95) = 6.51$, $p < 0.05$, $F(2,95) = 5.91$, $p < 0.05$, $F(2,95) = 8.82$, $p < 0.01$, $F(2,95) = 27.06$, $p < 0.01$, $F(2,95) = 22.5$, $p < 0.01$, $F(2,95) = 38.9$, $p < 0.01$, $F(2,95) = 27.27$, $p < 0.01$, $F(2,95) = 6.30$, $p < 0.05$, $F(2,95) = 24.69$, $p < 0.01$, $F(2,95) = 10.54$, $p < 0.01$, $F(2,95) = 6.21$, $p < 0.05$]. The length M , P and Q were significantly different among the complete fusion, incomplete fusion, and nonfusion groups [$F(2,95) = 3.15$, $p < 0.05$, $F(2,95) = 7.61$, $p < 0.01$, $F(2,95) = 4.28$, $p < 0.05$], but no significant difference was observed regarding width H and I among the groups [$F(2,95) = 0.18$, $p = 0.82$, $F(2,95) = 0.06$, $p = 0.93$]. There was no significant interaction effect of length, width and angle α (table 3).

Post hoc tests revealed that the lengths P and Q and widths V and W in nonfusion were greater in men

than women ($p < 0.01$, $p < 0.01$, $p < 0.05$ and $p < 0.01$, respectively). In complete fusion length P , Q , R and S , and Width V , W and angle β were greater in men than women ($p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.05$ and $p < 0.01$, respectively). In men, lengths M , P and Q were greater in nonfusion than in incomplete fusion ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively). In women, there was no difference in length and width based on degree of fusion.

Correlations between hyoid bone and mandible in the metric measurements

Table 5 presents the correlations between the metric variables (Length G and P , Q , T . width H , I and V , W . and angle α and β) of the hyoid bone and mandible for the complete fusion group and nonfusion group.

No significant associations were observed between length G and T in the complete fusion or nonfusion groups ($r = 0.11$, $r = 0.32$, respectively). However, strong significant associations were observed between lengths G and P , and lengths G and length Q in the nonfusion group ($r = 0.73$, $r = 0.68$, respectively), while the complete fusion group revealed a moderate correlation between these variables ($r = 0.54$, $r = 0.56$, respectively).

Strong significant associations were observed between widths H and V ; widths H and W in the nonfusion group ($r = 0.61$, $r = 0.60$, respectively). Moderate significant association was observed between widths H and V in the complete fusion group ($r = 0.48$), and weak significant association was observed between widths H and W in the complete fusion group ($r = 0.27$). In the nonfusion group, a significant

positive correlation was found between widths I and W ($r = 0.49$), but not in the complete fusion group ($r = 0.14$).

In the complete fusion group, significant negative correlations were observed between width I and length T ; angle α and length T ($r = -0.37$, $r = -0.49$, respectively). As in the nonfusion group, no significant associations were observed between width I and length T ; angle α and length T ($r = -0.17$, $r = -0.28$, respectively). A negative correlation between angle α and length P , and angle α and length Q was observed in the nonfusion group and complete fusion group ($r = -0.46$, $r = -0.45$; $r = -0.32$, $r = -0.39$, respectively).

Discussion

In the present study, the morphological characteristics of the hyoid bone and mandible from the same cadaver, in 101 cadavers in total, were classified by sex, age, shape and degree of fusion of the junction between the hyoid body and greater horn, using three-dimensional CT.

1. Sex difference of hyoid bone and mandible

A main effect was observed in relation to the length and width of the hyoid bone between males and females, which is in agreement with previous studies (Shimizu et al. 2005; Leksan et al. 2005; Kim et al. 2006; Kindschuh et al 2010; Ito et al. 2012; Fakhry et al. 2013) . No significant sex relationship was

found in regard to the angle between the two greater horns (angle α , male 37.2° degrees, female 36.7°). This result is in concordance with Leksan et al. (2005, male 25.2° , female 24.2°), however in their study anthropometric parameters were investigated. On the other hand, also studying anthropometric parameters, Kim et al. (2006) found a significant sex difference, (male 37.8° , female 29.3°). Observations by Fakhry et al. (2013) with three dimensional imaging also show a significant difference between the sexes,(male 38.8° , female 44.1°), but this time angle α was larger in females. As can be seen from the discussion above, results are highly variable depending on the researcher which may be due to the methods and age of the cadavers used.

Leksan et al. (2005) have classified the shape of the hyoid bone into two types using angle α . These types are: greater than 25 degrees symmetrical V type; less than 25 degrees symmetrical U type. One more type, asymmetrical, was determined when the difference between greater horn length was higher than 10%. They found that 45.7% of men and 42.9% of females had the symmetrical U type, 48.6% of men and 34.3% of females had the symmetrical V type and 5.7% of men and 22.9% females had the asymmetrical type. When considering the two symmetrical types together versus the asymmetrical type using the Chi-square test they found a significant difference between sex and shape. In our study the percentage of symmetrical U type and asymmetrical type hyoid bones was low compared to symmetrical V type, 69.3%, and no significant sex difference was observed. From this result we propose that the symmetrical V type hyoid bone may be more common in the Japanese population.

Previous studies have shown that the length and width of the mandible is larger in males than in

females (Humphrey et al. 1999; G V et al. 2013; Bhardwaj et al. 2014). On the other hand, Bayome et al. (2013) failed to observe any difference in the expanded angle (angle β), measured in the horizontal plane between the gonion and menton of the mandible, between males and females in young adult subjects. Our results show that the length and width of the mandible and the size of angle β are all generally larger in males than in females. As the present study was carried out on elderly cadavers it is possible that this result may be due to changes in the morphological plasticity of the mandible over time. We believe that it is essential that all researchers make use of the 'expanded angle' when considering the relationship between the hyoid bone and mandible.

2. Degree of fusion in hyoid bone and mandible

Gupta et al. (2008) and Balseven-Odabasi et al. (2013) observed bilateral complete fusion in all cadavers over 61 years old (male: $n=7$, female: $n=3$ and male: $n=11$, female: $n=7$, respectively). In the present study bilateral complete fusion was observed at a rate of 56.3% in males and 47.2% in females, much lower than in previous studies. However, Harjeet et al. (2010) observed bilateral non-fusion in cadavers over 61 years of age at a rate of 42.1% and bilateral complete fusion at a rate of 21.1%. The difference between the results of these studies may be due to racial differences in the cadavers used.

Length C of the hyoid bone was longer in non-fusion than in complete fusion in men. Kindschuh et al. (2010) found that the length of the hyoid bone is longer in non-fused bone than in fused bone as in our study. On the other hand, Urbanová et al. (2013) observed that the hyoid bone was slightly shorter in

nonfused bone than in fused bone. Urbanová et al. (2013) state that determining the endpoints between the lateral edges of the body and anterior end of the greater horns was complicated due to connective tissue forming a protruded bridge over both ends. However, in our study we determined the length of the greater horn by measuring from the posterior end to the lesser horn, eliminating the possibility of error and increasing repeatability.

Fusion of the greater horn with the body of the hyoid bone usually begins after 30 years of age and the incidence of this phenomenon increases with age. This condition is believed to affect the mobility of this junction and in turn masticatory and swallowing functions (Kanetaka et al, 2011). Fakhry et al. (2013) suggest that hyoid bones of narrow width are a risk factor in dysphagia due to a reduction in the pharyngeal volume. There was no significant difference between width H and angle α of the hyoid bone. We hypothesized that the width and angle of the hyoid bone would be related to the degree of fusion however no such relationship was observed. This is most likely because when there is no fusion of the junction between the hyoid bone and greater horn the width and angle of the hyoid bone can be controlled by muscle tension. No significant difference was found in relation to width and angle in complete fusion and nonfusion groups because the present research was conducted on cadavers where no muscle tension was present. Movement analysis studies in the horizontal plane have yet to be published on the junction between the hyoid body and greater horn however our research group has made computer simulation models of this movement under muscle tension, the results we intend to present in a future paper.

Interesting results were observed regarding the morphological characteristics of the mandible. Length P

and Q of the mandible was longer in non-fusion than in incomplete fusion. We also found that the ramus of the mandible (Length R and Length S) seemed to depend on the degree of fusion of the hyoid bone with longer lengths in non-fusion type compared to complete fusion type. de Oliveira et al. (2015) found a positive correlation between age and ramus length. Furthermore, Franklin and Cardini (2007) revealed ramus length (Length R and S) is related to the discrimination of the sexes in adolescence but not in childhood.

In the present study, the degree of fusion of the hyoid bone is related to the size of the mandible. It may be possible to consider that this is due to the change in the degree of fusion of the hyoid bone and the change in the size of the mandible during the developmental phase of the subjects in their younger years. However, the length of the mandible may be related to the number and condition of remaining teeth in later years however these factors were not investigated in the present study. Future studies should consider these factors and also the shape of the articular surfaces of the head of the mandible.

3. Relationship between hyoid bone and mandible dimensions in complete fusion and incomplete fusion groups

There was a strong correlation between Length G and Length P , Length G and Length Q , Width H and Width V , and Width H and Width W , in the non-fusion group. A negative correlation was also observed between Width I of the hyoid bone and Length T of the mandible. A strong correlation between the hyoid bone and mandible size reflects the ability of the suprahyoid muscles, connecting the hyoid bone to the

mandible, to accurately maintain length-tension function. In the complete fusion group there was a weak correlation between the size of the hyoid bone and mandible. There is a possibility this may lead to a reduction in the functionality of surrounding muscle.

A negative correlation between angle α and Length P , and angle α and Length Q in both complete fusion type and nonfusion type was observed. This may also reflect the ability of the suprahyoid muscles to accurately maintain length-tension function. Mitani and Sato (1992) found no significant relationship between the length of the hyoid bone (in this study Length G) and the length of mandible (in this study Length P and Length Q). In the present study we found a relationship between Length G and Length P , and Length G and Length Q . It must be noted however that Mitani and Sato (1992) performed their study on 9 to 14 year old students whereas our research was performed on elderly cadavers.

It has been reported that the size of the hyoid bone and mandible plays a role in Sleep Apnea Syndrome (SAS). Ha et al. (2013) found that in subjects with severe SAS the angle of the two greater horns (in this study angle α) and the width between the greater horns (in this study Width H) are both smaller than in normal subjects. Chi et al. (2011) found that the length of the mandible (in this study Length T) was larger in male subjects with SAS and the width of the mandible (in this study Width V) was shorter in female subjects with SAS. Okubo et al. (2006) found that mandibular divergence (close to angle β in this study) was larger in subjects with SAS and the length of the mandible (in this study Length T) was shorter in subjects with SAS. In our study we observed a negative correlation between angle β and Length T in both complete fusion type and nonfusion type. We did not set out to investigate the effects of the

morphological characteristics of the hyoid bone and mandible on SAS however these results, in conjunction with those of previous studies, should be considered in this field of research in the future.

Conclusion

In this study we analyzed the morphological characteristics of the hyoid bone and mandible in 101 cadavers using three-dimensional CT and divided the bones into three groups (complete fusion group, incomplete fusion group and non-fusion group) depending on the condition of the junction between the hyoid body and greater horn. There was a strong correlation between the length and width of the hyoid bone and mandible in the nonfusion group. Our results suggest that there is a possibility that the cooperation of movement between the hyoid bone and mandible is dependent on the condition of the junction.

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Conflict of interest

None

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Appendices

None

Figure Legends

Figure 1. The classification of changes in ossification at the junction between the hyoid body and greater horns.

A: complete fusion; high-density areas between the hyoid body and greater horns, B: incomplete fusion; ossification commences on one side between the hyoid body and the greater horns, and on the other side with complete fusion, C: non-fusion; bone fusion absent between the hyoid body and greater horns.

Figure 2. CT imaging and illustration mapping measurement points of the hyoid bone and mandible in this study.

This figure shows the CT imaging (upper plates : A, B, C) and illustration mapping of measurement points (lower plates: D, E,F). Cranial view of the hyoid bone is shown in A and D. Point 1 was positioned in the middle of the anterior section of the hyoid body (landmark D1). Points 2 and 3 were positioned in the middle of the basal section of the lesser horn (one on each side of the hyoid bone, landmarks D2 and D3). Points 4 and 5 were positioned on the distal parts of the greater horns of the hyoid bone (one on each side of the hyoid bone, landmarks (D4 and D5). Right view of the mandible is shown in B and E. Points 4 and 5 were positioned on the gonion (one on each side of the mandible, landmarks E4 and E5). Points 6 and 7 were positioned in the middle section of the head of the mandible (one on each side of the mandible, landmarks E6 and E7). Caudal view of the mandible is shown in C and F. Point 1 was positioned in the lowermost section of the menton (landmark F1). Points 2 and 3 were positioned in the middle section of

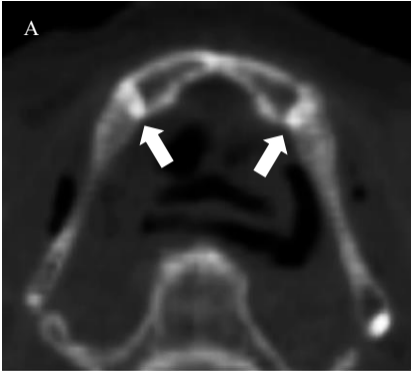
the mental foramen (one on each side of the mandible, landmarks F2 and F3). Points 4 and 5 were positioned on the gonion (one on each side of the mandible, landmarks F4 and F5). Points 6 and 7 were positioned in the middle section of the head of the mandible (one on each side of the mandible, landmarks F6 and F7). All landmarks in the lower plates (D, E and F) are indicated by white arrows in the upper plates (A, B and C).

Figure 3. Anthropometric variables used in metric analyses.

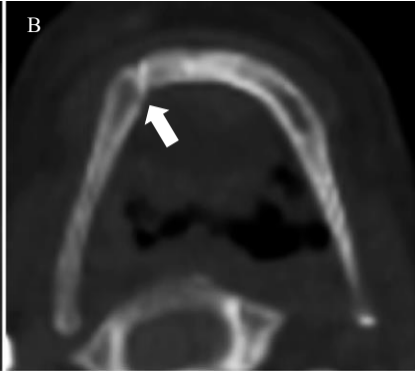
(A) Cranial view of hyoid bone, (B) right view of mandible and (C) caudal view of mandible.

Each length, width and angle were measured based on the landmarks in Fig. 2. Length G is the distance from the middle of the anterior section of the hyoid body to a hypothetical line connecting the distal sections of the greater horns. Angle α is the angle between the two greater horns. Length T is the distance from the menton to a hypothetical line connecting the distal sections of the head of the mandible. Angle β is the angle between the gonion and menton.

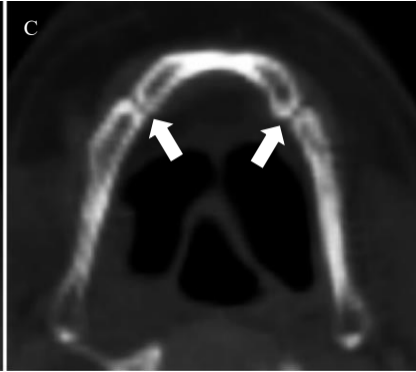
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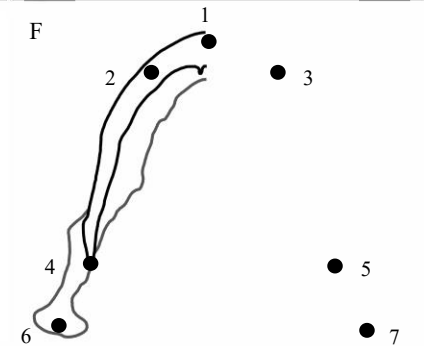
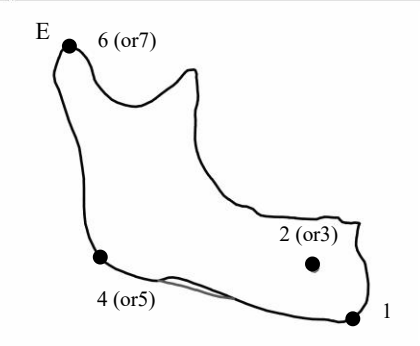
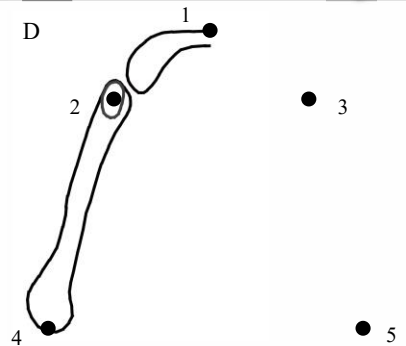
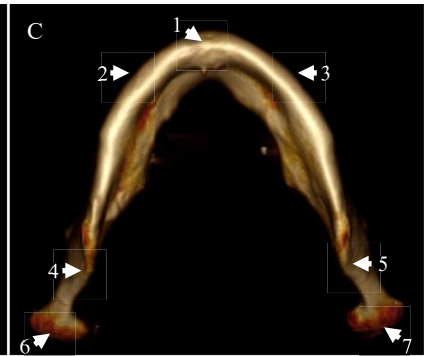
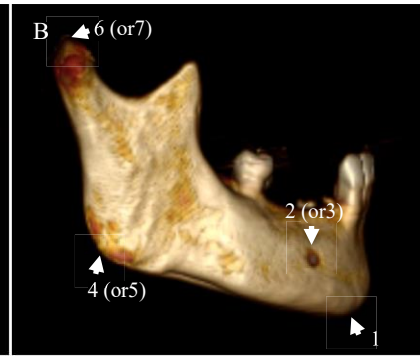
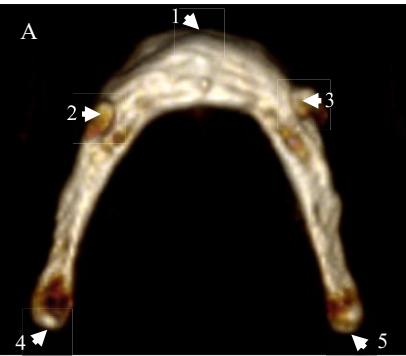


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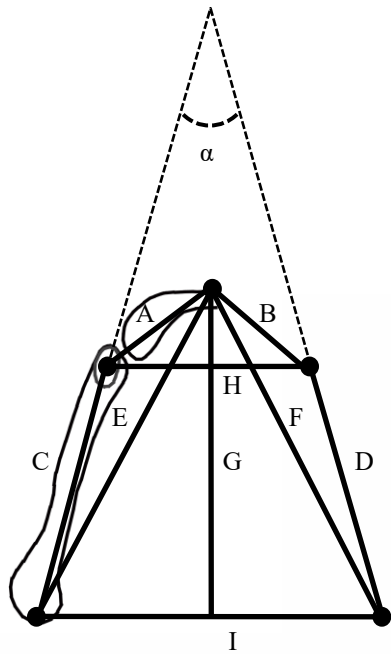


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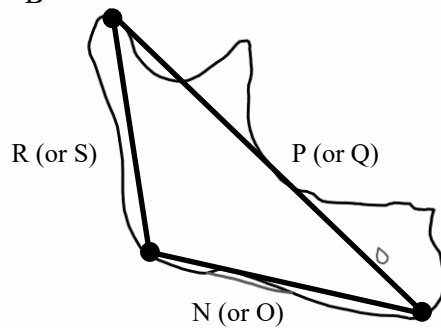




A



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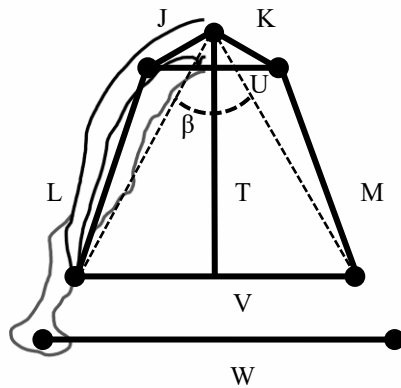


Table 1. Shape types of males and females

Sex	asymmetrical type		symmetrical U-type		symmetrical V-type	
	No.	%	No.	%	No.	%
male	6	12.5	8	16.7	34	70.8
femal	10	18.9	7	13.2	36	67.9
total	16	15.8	15	14.9	70	69.3

Table 2. Fusion of hyoid bones in males and females

age	male						female					
	complete fusion		incomplete fusion		non-fusion		complete fusion		incomplete fusion		non-fusion	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
50-59											1	1.9
60-69	1	2.1					1	1.9				
70-79	5	10.4	1	2.1	4	8.3	4	7.5	1	1.9	4	7.5
80-89	13	27.1	8	16.7	4	8.3	16	30.2	8	15.1	6	11.3
90-99	8	16.7	3	6.3			4	7.5	3	5.7	4	7.5
100-			1	2.1					1	1.9		
total	27	56.3	13	27.1	8	16.7	25	47.2	13	24.5	15	28.3

Table 3. Dimension means (mm) and angle (degree) of hyoid bone for sex and degree of fusion

Measurement	male				female				main effect		inter-action	
	complete fusion	incomplete fusion	non-fusion	total	complete fusion	incomplete fusion	non-fusion	total	sex	degree of fusion		
Length A	mean	16.0 ^{aa}	15.0 ^{bb}	16.3 ^{cc}	15.8	14.2	12.6	14.1	13.7	$p < 0.01$	$p < 0.05$	0.79
	SD	1.7	1.3	2.0	1.7	2.2	1.9	2.0	2.1			
Length B	mean	15.8 ^{aa}	15.5 ^{bb}	15.2 ^c	15.6	13.5	13.0	13.2	13.3	$p < 0.01$	0.63	0.90
	SD	2.7	1.8	1.8	2.4	1.8	2.1	1.9	1.9			
Length C	mean	31.1 ^{aa}	31.1	33.9 ^{cc,f}	31.6	27.4	29.3	28.5	28.2	$p < 0.01$	$p < 0.05$	0.09
	SD	3.9	2.3	2.3	3.4	2.6	2.4	2.0	2.5			
Length D	mean	31.3 ^{aa}	30.8	33.9 ^{cc}	31.6	27.5	29.3	29.2	28.4	$p < 0.01$	$p < 0.05$	0.19
	SD	4.3	2.6	1.3	3.6	3.0	2.7	2.4	2.8			
Length E	mean	44.4 ^{aa}	43.1 ^{bb,c}	46.6 ^{cc}	44.4	39.1	39.0	40.0	39.3	$p < 0.01$	$p < 0.05$	0.31
	SD	3.6	2.1	1.8	3.2	2.6	3.1	1.9	2.5			
Length F	mean	44.1 ^{aa}	42.8 ^{bb}	45.0 ^{cc}	43.9	38.7	38.8	39.9	39.1	$p < 0.01$	0.20	0.64
	SD	3.8	3.3	1.8	3.5	2.6	3.3	2.7	2.8			
Length G	mean	37.2 ^{aa}	35.4 ^{ee}	39.7 ^{cc}	37.1	33.2	33.2	33.5	33.3	$p < 0.01$	$p < 0.05$	0.07
	SD	4.0	2.3	2.2	3.6	2.6	3.2	2.0	2.6			
Width H	mean	27.3 ^{aa}	27.3 ^{bb}	27.5 ^{cc}	27.3	23.2	22.9	23.7	23.3	$p < 0.01$	0.83	0.92
	SD	3.3	2.0	2.3	2.8	2.6	2.7	2.8	2.6			
Width I	mean	47.4 ^{aa}	48.5 ^{bb}	45.5	47.4	40.4	40.2	43.0	41.1	$p < 0.01$	0.93	0.18
	SD	6.6	5.9	4.7	6.1	4.1	5.3	6.9	5.4			
Angle α	mean	37.9	39.8	30.7	37.2	36.6	34.1	39.1	36.7	0.84	0.67	0.07
	SD	11.8	9.4	8.0	10.8	9.0	11.8	10.6	10.2			

SD: standard deviation

^{aa} indicates significant difference between sex in complete fusion group ($p < 0.01$).

^{bb} indicates significant difference between sex in incomplete fusion group ($p < 0.01$).

^c indicates significant difference between sex in non-fusion group ($p < 0.05$).

^{cc} indicates significant difference between sex in non-fusion group ($p < 0.01$).

^e indicates significant difference between incomplete fusion and non-fusion in men ($p < 0.05$).

^{ee} indicates significant difference between incomplete fusion and non-fusion in men ($p < 0.01$).

^f indicates significant difference between non-fusion and complete fusion in men ($p < 0.05$).

Table 4. Dimension means (mm) and angle (degree) of mandible in relation to the degree of fusion of hyoid bone

Measurement		male				female				main effect		inter-action
		complete fusion	incomplete fusion	non-fusion	total	complete fusion	incomplete fusion	non-fusion	total	sex	degree of fusion	
Length J	mean	30.3	30.3	32.5 ^{cc}	30.7	29.5	28.9	29.6	29.4	$p < 0.01$	0.09	0.24
	SD	2.4	3.0	2.8	2.7	1.9	2.2	2.7	2.2			
Length K	mean	29.9	31.4	30.6	30.4	29.3	30.1	29.4	29.5	$p < 0.05$	0.09	0.72
	SD	1.7	2.2	2.9	2.1	2.1	2.6	1.9	2.2			
Length L	mean	59.5	58.1	61.5	59.5	59.2	56.7	58.2	58.3	0.11	0.15	0.50
	SD	5.0	3.4	6.5	4.9	4.5	4.5	5.6	4.8			
Length M	mean	60.6	57.8 ^c	63.2 ^{cc}	60.3	59.6	56.9	57.2	58.2	$p < 0.05$	$p < 0.05$	0.10
	SD	4.5	4.3	5.1	4.8	4.5	3.5	6.5	5.0			
Length N	mean	82.1	80.8	85.2 ^c	82.3	81.1	78.6	80.5	80.3	$p < 0.05$	0.10	0.37
	SD	4.7	2.9	6.4	4.8	5.0	5.4	6.0	5.4			
Length O	mean	83.1	81.4	85.5 ^{cc}	83.1	81.4	79.9	80.0	80.6	$p < 0.01$	0.23	0.24
	SD	4.3	3.3	4.8	4.3	4.6	4.1	6.0	4.9			
Length P	mean	124.6 ^{aa}	121.2 ^{cc}	129.1 ^{cc}	124.4	118.3	117.6	121.5	119.0	$p < 0.01$	$p < 0.01$	0.41
	SD	5.2	6.4	4.0	5.9	4.8	4.9	5.4	5.2			
Length Q	mean	124.1 ^{aa}	121.7 ^c	128.2 ^{cc}	124.2	118.4	118.6	120.9	119.1	$p < 0.01$	$p < 0.05$	0.38
	SD	5.4	6.2	4.3	5.8	4.5	4.9	6.1	5.1			
Length R	mean	62.1 ^{aa}	63.0 ^{bb}	64.1 ^c	62.7	55.5	54.3	59.4	56.3	$p < 0.01$	0.05	0.37
	SD	6.3	5.8	2.9	5.7	3.4	4.5	4.7	4.5			
Length S	mean	60.6 ^{aa}	62.9 ^{bb}	63.4	61.7	54.8	54.1	59.1	55.8	$p < 0.01$	0.05	0.34
	SD	6.7	6.2	1.9	6.0	4.3	5.2	6.3	5.5			
Length T	mean	65.8	64.5	68.4	65.8	66.3	64.5	64.5	65.4	0.30	0.31	0.20
	SD	4.7	2.7	5.7	4.5	4.5	4.0	6.8	5.2			
Width U	mean	47.1	47.5	48.0	47.4	46.2	45.1	46.4	46.0	$p < 0.05$	0.60	0.63
	SD	3.1	3.4	3.0	3.1	2.8	3.2	2.9	2.9			
Width V	mean	99.8 ^{aa}	98.3 ^{bb}	102.0 ^c	99.7	93.7	91.9	95.1	93.7	$p < 0.01$	0.15	0.97
	SD	6.8	6.2	5.8	6.5	5.5	6.1	4.8	5.5			
Width W	mean	108.6 ^a	105.7	111.1 ^{cc}	108.2	104.9	105.2	103.0	104.4	$p < 0.01$	0.55	0.09
	SD	7.3	4.3	5.4	6.5	5.2	4.9	5.8	5.3			
Angle β	mean	74.4 ^{aa}	74.6	73.6	74.3	70.5	70.9	73.1	71.4	$p < 0.05$	0.79	0.39
	SD	5.6	4.6	4.7	5.1	3.7	4.0	6.5	4.8			

SD: standard deviation

^a indicates significant difference between sex in complete fusion group ($p < 0.05$).

^{aa} indicates significant difference between sex in complete fusion group ($p < 0.01$).

^{bb} indicates significant difference between sex in incomplete fusion group ($p < 0.01$).

^c indicates significant difference between sex in non-fusion group ($p < 0.05$).

^{cc} indicates significant difference between sex in non-fusion group ($p < 0.01$).

^e indicates significant difference between incomplete fusion and non-fusion in men ($p < 0.05$).

^{cc} indicates significant difference between incomplete fusion and non-fusion in men ($p < 0.01$).

Table 5. Correlation matrix of relationship between hyoid bone and mandible

		complete fusion								
non-fusion	Length G	0.44 **	0.19	-0.48 **	0.54 **	0.56 **	0.11	0.36 **	0.44 **	0.19
	0.45 *	Width H	0.58 **	-0.08	0.40 **	0.37 **	0.00	0.48 **	0.27 *	0.36 **
	-0.03	0.57 **	Width I	0.66 **	0.10	0.04	-0.37 **	0.52 **	0.14	0.66 **
	-0.58 **	-0.02	0.75 **	Angle α	-0.32 *	-0.39 **	-0.49 **	0.12	-0.17	0.45 **
	0.73 **	0.31	-0.03	-0.46 **	Length P	0.88 **	0.39	0.51 **	0.26	0.10
	0.68 **	0.13	-0.11	-0.45 **	0.93 **	Length Q	0.45 **	0.40 **	0.25	-0.24
	0.32	-0.07	-0.17	-0.28	0.71 **	0.76 **	Length T	0.08	0.06	-0.66 **
	0.45 *	0.61 **	0.33	-0.08	0.40	0.38	0.21	Width V	0.41 **	0.68 **
	0.53 **	0.60 **	0.49 *	0.46	0.42 *	0.27	0.08	0.55 **	Width W	0.26
	-0.06	0.42 *	0.38	0.25	-0.44 **	-0.51 **	-0.81 **	0.37	0.23	Angle β

The data above Length, Width and Angle names shows Pearson correlation coefficients between hyoid bone and mandible in complete fusion group. The data below Length, Width and Angle names shows non-fusion group Pearson correlation coefficients.

*Indicates significant correlations between width, length and angle ($p < 0.05$).

**Indicates significant differences between width, length and angle ($p < 0.01$).