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Relationship Between Styloid Process and Lesser Horn of Hyoid Bone Lengths, and Sex Determination Using Hyoid Bone Weight in a Thai Population

Relación Entre la Longitud del Proceso Estiloides y el Asta Menor del Hueso Hioides y la Determinación del Sexo Mediante el Peso del Hueso Hioides en una Población Tailandesa

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SUMMARY: The styloid process connects to the lesser horn of the hyoid bone via the stylohyoid ligament, playing a role in controlling vocal muscles. However, the relationship between the lengths of these bones has not been studied before. Additionally, the weight of the hyoid bone has been found useful in forensic anthropology. Therefore, this study aimed to investigate the relationship between the lengths of the styloid process and the lesser horn of the hyoid bone and to explore using the hyoid bone weight for determining sex in the Northeastern Thai population. The study used 80 complete dry skeletons from the Unit of Human Bone Warehouse for Research, Khon Kaen University. The lengths of the bones were measured using a digital vernier caliper, and the hyoid bones were weighed using a digital scale. The data were analyzed for length relationships and sex differences. Results showed that no relationship was found between the lengths of the styloid process and the lesser horn of the hyoid bone in both sexes. The weight of the hyoid bone can help estimate sex, as males have significantly heavier hyoid bones than females. The hyoid bone weight in males tends to decrease significantly with age. In conclusion, while the styloid process and the lesser horn of the hyoid bone are connected, there is no relationship between their lengths. However, the weight of the hyoid bone can be a useful parameter for basic sex determination in forensic science for the Northeastern Thai population.

KEY WORDS: Styloid process; Hyoid bone; Lesser horn; Sex determination.

INTRODUCTION

The styloid process is a slender, elongated structure that protrudes from the temporal bone, serving as an attachment point for several important muscles and ligaments, such as the styloglossus, stylohyoid, and stylopharyngeus muscles, which play crucial roles in swallowing and tongue movement. In clinical anatomy, when the styloid process exceeds 30 millimeters in length, it is referred to as an elongated styloid process, which is associated with Eagle's syndrome, a condition that causes recurring pain in the head, face, ear, and neck regions (Kapur *et al*., 2022). The hyoid bone is unique, as it is the only bone in the human body that does not articulate with any other bone. It plays a vital role in speech and swallowing. The lesser horn of the hyoid bone is a part of the hyoid bone that projects laterally and is connected to the styloid process via the stylohyoid ligament (Kapur *et al*., 2022). Anatomical observations suggest that the mechanism of the connected muscles and ligaments may influence the lengths of these two bony structures.

Previous studies have shown that the length of the styloid process varies among different populations, with demographic factors playing a significant role (Sakaew *et al*., 2016; Baena-Caldas *et al*., 2017). Moreover, the styloid

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process has been shown as a bony part for applying in sex estimation (Saheb & Shepur, 2011; Rathva *et al*., 2013; Chaurasia *et al*., 2017; Wahane & Nandanwar, 2019). However, there is a lack of clear data on whether the lengths of the styloid process and the lesser horn of the hyoid bone are correlated, and there is no comprehensive statistical data for the Thai population. Therefore, this study aims to analyze the relationship between the lengths of the styloid process and the lesser horn of the hyoid bone, and to investigate the factors influencing the length of the styloid process and the weight of the hyoid bone. This information could serve as a baseline for sex determination in the Northeastern Thai population. Although conducting this study is not complicated, its success could greatly benefit forensic medicine and medical practice.

MATERIAL AND METHOD

Sample collections. This study utilized dry skeletons from the unit of human bone warehouse for research (UHBWR), Anatomy department, Faculty of Medicine, Khon Kaen University. The inclusion criteria required skeletons with both styloid processes and lesser horns of the hyoid bone intact on both sides. Exclusion criteria included skeletons with incomplete or damaged styloid processes or lesser horns of the hyoid bone. The researchers initially considered a population of 80 skeletons.

The sample size was calculated using the Pearson correlation statistical formula, with a confidence level of 95 % and an acceptable margin of error of 15 %. The correlation coefficient was set at 0.5. The calculation resulted in a required sample size of 388 skeletons. Although the skeleton repository contained over 4,000 skeletons, only 208 skeletons met the initial criteria of having both the lesser horn of the hyoid bone and the styloid process. After applying the exclusion criteria, 80 skeletons were found to have both structures intact and suitable for study.

Morphometric analyses. The length of the styloid process was measured from the point where the bone protrudes from the temporal bone to its distal end. The length of the lesser horn of the hyoid bone was measured from the point where it emerges from the hyoid bone to its distal end, along the bone's longitudinal axis as shown in Figure 1. Data were collected by measuring the lengths of the styloid process and the lesser horn of the hyoid bone on both left and right sides using a digital vernier caliper.

Additionally, the weight of each hyoid bone was measured using a digital scale (Fig. 2). All measurements were recorded in a data collection form. Each skeleton was measured twice by different researchers to ensure consistency.

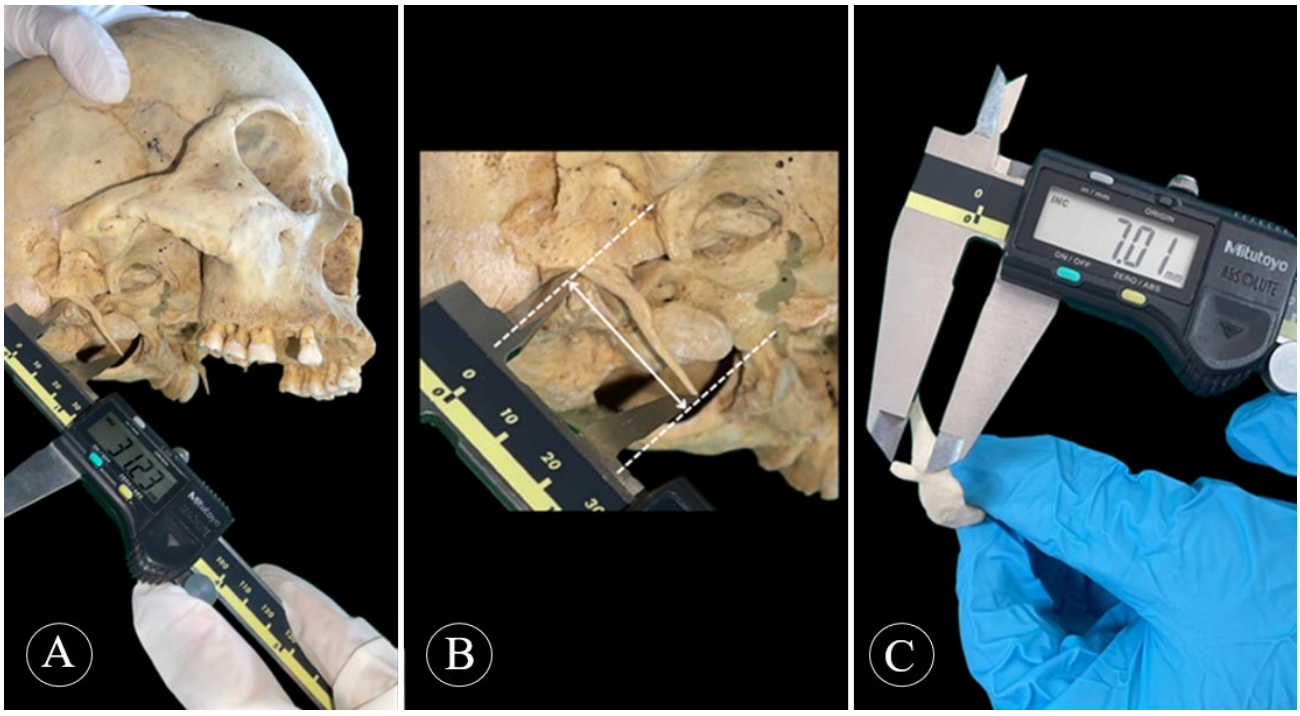

Fig. 1. (A) Measurement of the styloid process using a digital vernier caliper. (B) Measurement points on the styloid process. (C) Measurement of the lesser horn of the hyoid bone using a digital vernier caliper.

Fig. 2. Weighing the hyoid bone using a digital scale.

All measurers received training on anatomical reference points and measurement techniques. To control for factors affecting measurements, equipment was calibrated for accuracy and precision, and measurements were spaced at least three days apart. The researchers were blinded to the sex and age of the skeletons during measurements. Data were recorded in Microsoft Excel, and average values were calculated. Finally, the sex and age of each skeleton were recorded in the Excel.

Statistical Analysis. Data analysis was performed using STATA version 18 for Windows. Descriptive statistics, including mean, standard deviation, median, and interquartile range, were used. The relationship between the lengths of the styloid process and the lesser horn of the hyoid bone was analyzed using Pearson correlation and simple linear regression. The association of factors affecting the length of the styloid process and the weight of the hyoid bone was analyzed using Student's t-test or Mann-Whitney U test, depending on the data distribution. Normality of data distribution was tested using the Shapiro–Wilk test.

Ethical Considerations. This research project received ethical approval for human research from Khon Kaen University (project number HE671159)

RESULTS

The distribution of the length of the lesser horn of the hyoid bone was concentrated between 0-3 millimeters, as shown in Figure 3.

The ratio of lengths between the left and right sides did not show similarity. Additionally, the distribution of the length of the styloid process was found to be balanced on both sides, as indicated by the linear trend in the graph shown in Figure 4.

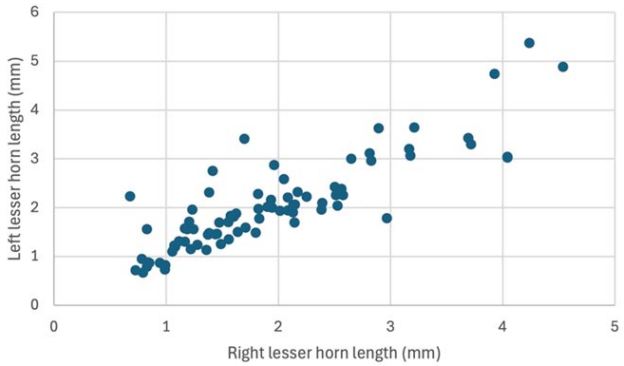

Fig. 3. Scatter plot showing the distribution of the lengths of the lesser horn of the hyoid bone on both the left and right sides, measured from skeletal samples from the northeastern region of Thailand (70 male samples, 10 female samples). Each point represents the lengths of the lesser horn of the hyoid bone on both sides of a single individual.

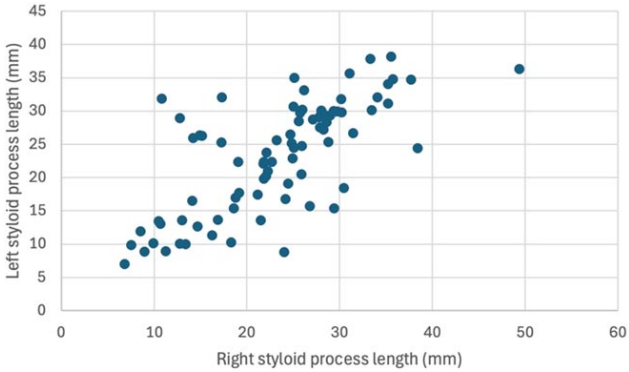

Fig. 4. Scatter plot showing the distribution of the lengths of the styloid process on both the left and right sides, measured from skeletal samples from the northeastern region of Thailand (70 male samples, 10 female samples). Each point represents the lengths of the styloid process on both sides of a single individual.

From Table I, it is evident that the mean length of the styloid process in males (23.59 ± 8.31 mm) was greater than that in females (20.70 ± 8.28 mm) on both the right and left sides. Similarly, the mean length of the lesser horn of the hyoid bone was found to be greater in males (2.01 ± 0.90 mm.) than in females (1.83 ± 1.12 mm) on both sides. However, the differences in the lengths of these bones between sexes were not statistically significant (P-value > 0.05), as shown in Table II.

When testing the correlation between the lengths of the styloid process and the lesser horn of the hyoid bone on each side, as shown in Table III, the statistical results

Table I. Mean values (mean ± SD) of the lengths of the styloid process and lesser horn of the hyoid bone on each side in the Northeastern Thai population.

Sex	Length of styloid process (mm)			Length of lessor horn of hyoid bone (mm)		
	Overall	Right	Left	Overall	Right	Left
Overall (N=80)	23.23±8.34	23.30±8.37	23.16±8.35	1.99±0.93	1.93±0.92	2.03 ±0.95
Male (N=70)	23.59±8.31	23.82±8.39	23.37±8.29	2.01±0.90	1.96±0.88	2.06±0.92
Female (N=10)	20.70±8.28	19.73±7.73	21.67±9.10	1.83±1.12	1.78±1.17	1.87±1.13

Table II. Correlation between the lengths of the styloid process and Lesser horn of the hyoid bone on each side in the Northeastern Thai population.

Parameter	Pearson cor. (r)	P-value
Rt. Styloid – Rt. lessor	0.912	0.421
Lt. Styloid - Lt. lessor	0.039	0.728

indicated that the lengths of these two bone types on the same side were not significantly correlated (P-value > 0.05). This means that in a single skeleton, the length of one bone type could not predict the length of the other bone type on the same side. For example, a longer right-side styloid process did not indicate a corresponding variation in the length of the right-side lesser horn of the hyoid bone.

When comparing the mean lengths of the lesser horn of the hyoid bone on the right and left sides, as shown in Table IV, it was found that the mean lengths on both sides did not differ significantly (P-value > 0.05).

Similarly, the comparison of the mean lengths of the styloid process between the right and left sides, as shown in Table V, revealed no statistically significant difference between the sides (P-value > 0.05).

Table III. Comparison of the mean lengths of the lesser horn between the right and left sides in the Northeastern Thai population.

Parameter	Length	Mean	P-value
Rt. Lesser horn	1.93 ± 0.92	-0.10	0.393
Lt. Lesser horn	2.03 ± 0.95		

Table IV. Comparison of the mean lengths of the styloid process between the right and left sides in the Northeastern Thai population.

Parameter	Length	Mean	P-value
Rt. Styloid process	23.30 ± 8.37	0.14	0.825
Lt. Styloid process	23.16 ± 8.35		

Furthermore, when comparing the mean lengths of the styloid process and the lesser horn of the hyoid bone between males and females, as shown in Table II, the researchers found that the mean lengths of both bone types did not differ significantly between males and females on either side (P-value > 0.05).

From Table VI, the test results showed that the weight of the hyoid bone in males was significantly greater than in females (P-value < 0.001).

When examining the relationship between age and the weight of the hyoid bone in both males and females, as shown in Table VII, it was found that age and hyoid bone weight were not significantly correlated in either sex (P-value > 0.05).

However, when comparing the weight of the hyoid bone in males across five age groups, as shown in Figure 5, it was observed that the weight of the hyoid bone tended to decrease with increasing age.

Table VI. Comparison of the weight of the Hyoid bone between males and females.

Sex	N	Average (g)	S.D.	P-value
Male	70	1.13	0.33	<0.001
Female	10	0.66	0.15	

Table VII. Correlation between age and the weight of the Hyoid bone in both males and females.

Sex	R weight-age	P-value
Overall	0.0479	0.673
Male	-0.0270	0.825
Female	-0.1223	0.736

Table V. Comparison of the mean lengths of the styloid process and lesser horn of the hyoid bone on each side between males and females.

Parameters	Male	Female	Mean	P-value
Rt. Lesser horn	1.96 ± 0.88	1.78 ± 1.17	0.18	0.224
Lt. Lesser horn	2.27 ± 1.18	1.98 ± 1.33	0.29	0.344
Rt. Styloid process	23.82 ± 8.37	19.73 ± 7.78	4.09	0.149
Lt. Styloid process	23.37 ± 8.29	21.67 ± 9.10	1.70	0.631

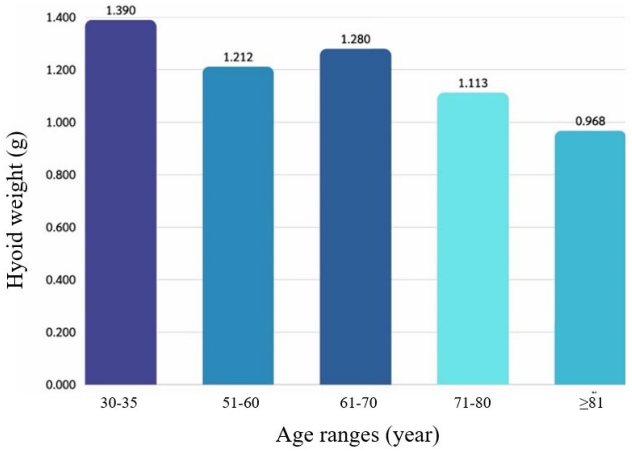

Fig. 5. Bar graph showing the mean weight of the Hyoid bone in males across different age groups, categorized as follows: Group 1 (35-50 years), Group 2 (51-60 years), Group 3 (61-70 years), Group 4 (71-80 years), and Group 5 (81 years and older).

DISCUSSION

The distribution of the lengths of the lesser horn of the hyoid bone on the left and right sides was imbalanced, with most lengths on both sides clustering below 3 centimeters. The length of this bone type has not been extensively studied, making it difficult to compare with other populations. The distribution of the lengths of the styloid process on the left and right sides was found to be balanced in the same individual. The average lengths in this study were 23.16 and 23.30 mm on the left and right sides, respectively. A previous study using Multidetector computed tomography scan (MCTS) on a sample population in Iran found the lengths of the styloid process to be 25.4 and 25.2 millimeters on the left and right sides, respectively (Shayganfar *et al*., 2018). This discrepancy can be explained by the fact that other research compared data from actual patients, eliminating the need to consider the preservation of dry bones, which might alter bone quality. Additionally, genetic differences in populations may also affect bone lengths (Ari & Kafa, 2009; Sládek *et al*., 2015; Hou *et al*., 2021).

This study found that the average lengths of both bone types were greater in males than in females, which can be explained by various physiological and evolutionary factors related to human physiology and evolution. For instance, the testosterone hormone, which is more prevalent in males, plays a crucial role in stimulating bone growth and physical structure development (Tracz *et al*., 2006). In contrast, females have higher levels of the hormone estrogen, which accelerates the closure of the epiphyseal plate, leading to shorter bone growth periods and smaller bone sizes on average. Additionally, evolutionary factors suggest that males historically required more physical strength for hunting or heavy labor, resulting in overall stronger and longer bone structures to support muscle use and tension.

The study also found that the lengths of the styloid process and the lesser horn of the hyoid bone on the same side were not significantly correlated. This can be explained by the ligament (stylohyoid ligament) connecting these two bone types, which does not significantly influence their lengths. The average length of the stylohyoid ligament is 2-3 centimeters, and its delicate structure may not be strong enough to affect the lengths of these bones. Therefore, it can be inferred that the lengths of these two bone types are not correlated.

Furthermore, no significant differences were found between the lengths of the styloid process on the left and right sides, similar to the lengths of the lesser horn of the hyoid bone on both sides. This indicates that the stylohyoid ligament does not influence the lengths of these bones, allowing them to develop independently on both sides. Therefore, the lengths of these bones on both sides are not correlated.

The average lengths of the styloid process and the lesser horn of the hyoid bone between males and females did not show statistically significant differences on either side, consistent with previous studies in Saudi Arabia and Turkey (Magat & Ozcan, 2017; Kaaki *et al*., 2024). However, this finding differs from studies in Bosnia and Herzegovina and India, which found that males had significantly longer styloid processes than females (More & Asrani, 2010; Kapur *et al*., 2022).

The weight of the hyoid bone in males was significantly greater than in females, which can be explained by the overall higher bone density and mass in males at the same age. Additionally, the sample group consisted of bone donors mostly over 50 years old, so the difference in hyoid bone weight may be related to osteoporosis, which is more common in the elderly. Primary osteoporosis is more prevalent in females due to age-related bone loss, while secondary osteoporosis is more common in males due to other medical conditions. This suggests that the hyoid bone in males has greater mass and density than in females, which may have implications for Eagle's syndrome but is useful in forensic science for sex determination from the weight of the hyoid bone (De Martinis *et al*., 2021).

This study found no significant correlation between age and the weight of the hyoid bone in both males and females. However, when divided into five age groups (35-

50, 51-60, 61-70, 71-80, and over 81 years), the weight of the hyoid bone in males tended to decrease with increasing age, particularly in those over 71 years old. This can be explained by the hormone testosterone, which peaks at 20-25 years old and then rapidly declines, affecting bone mass and density.

LIMITATIONS AND CONCLUSION

The study may have limitations due to the collection of bones, as the styloid process and the lesser horn of the hyoid bone are small and fragile, potentially leading to loss or incomplete preservation. Therefore, the sample size was small. Future studies could use radiographic techniques such as Computerized tomography scans or panoramic radiographs to measure bone lengths in living patients, providing more accurate measurements and correlating findings with patient symptoms and presentations. This study found no correlation between the lengths of the styloid process and the lesser horn of the hyoid bone, either between sexes or sides. However, the weight of the hyoid bone was significantly greater in males than in females and decreased with advancing age. The basic knowledge gained from this study can be applied to sex determination in forensic science .

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RESUMEN: El proceso estiloides se conecta al asta menor del hueso hioides a través del ligamento estilohioideo, desempeñando un papel en el control de los músculos vocales. Sin embargo, la relación entre las longitudes de estos huesos no se ha estudiado previamente. Además, el peso del hueso hioides ha sido útil en antropología forense. Por lo tanto, este estudio tuvo como objetivo investigar la relación entre las longitudes del proceso estiloides y el asta menor del hueso hioides y explorar el uso del peso del hueso hioides para determinar el sexo en la población del noreste de Tailandia. El estudio utilizó 80 esqueletos secos de la Unidad de Almacén de Huesos Humanos para Investigación, Universidad de Khon Kaen. Las longitudes de los huesos se midieron utilizando un calibrador vernier digital, y los huesos hioides se pesaron utilizando una báscula digital. Los datos se analizaron para las relaciones de longitud y las diferencias de sexo. Los resultados mostraron que no se encontró relación entre las longitudes del proceso estiloides y el asta menor del hueso hioides en ambos sexos. El peso del hueso hioides puede ayudar a estimar el sexo, ya que los hombres tienen huesos hioides significativamente más pesados que las mujeres. El peso del hueso hioides en los hombres tiende a disminuir significativamente con la edad. En conclusión, si bien el proceso estiloides y el asta menor del hueso hioides están conectados, no hay relación entre sus longitudes. Sin embargo, el peso del hueso hioides puede ser un parámetro útil para la determinación básica del sexo en la ciencia forense de la población del noreste de Tailandia.

PALABRAS CLAVE: Proceso estiloides; Hueso hioides; Asta menor; Determinación del sexo.

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