



# OPEN Sex prediction from human tooth dimension by ROC curve analysis: a preliminary study

Panjit Chunhabundit, Tawepong Arayapisit & Natchalee Srimaneekarn✉

Though mathematical approaches are effective in developing reliable odontometric equations with high predictive accuracy for sex estimation in forensic odontology, these methods are often complex to apply, making them impractical in certain scenarios. Consequently, efforts continue to refine these models for greater accessibility. This study aims to develop a sex prediction method based on the horizontal width of anterior teeth using ROC analysis and evaluate its accuracy. Maximum horizontal measurements were taken from all anterior teeth on 100 dental casts from male and 100 from female patients. ROC curve analysis, along with Youden's index and Euclidean index, was used to determine cut-off points for sex estimation. An additional set of 50 male and 50 female dental casts was used to assess the method's accuracy. Results showed that only the canines were statistically significant for sex identification. AUC values were 0.634 and 0.626 for maxillary canines ( $p = .001$ ) and 0.724 and 0.727 for mandibular canines ( $p < .001$ ), with accuracy ranging from 69 to 77%. In conclusion, a sex prediction method based on the horizontal width of the canine, using ROC analysis, was developed, demonstrating reasonable accuracy. This methodological improvement is expected to enhance its practical application in forensic investigations.

Sex identification is one of the crucial steps in establishing the biological identity of anonymous human remains. Traditionally, this process relies on the examination of sexual organs, including external genitalia and other sex-related organs such as testes, uterus or ovaries, which generally allows for straightforward and accurate estimation<sup>1–3</sup>. However, challenges arise in cases with ambiguous genitalia due to such conditions as the disorders of sexual development or gender affirmation surgeries. For those instances, alternative approaches, such as genetic testing for sex chromosome analysis, can be helpful<sup>4,5</sup>. Moreover, when severe damage to body tissues makes sex identification impossible through conventional anatomical or genetic methods, supplementary techniques must be explored. Consequently, one reliable alternative involves predicting sex based on skeletal characteristics<sup>6</sup>, including tooth dimensions<sup>7</sup>.

Teeth are extremely durable organs, resistant to bacterial decomposition and high temperatures, often surviving in fire accidents<sup>7</sup>. Owing to their high individual specificity, teeth are highly valuable tools for personal identification<sup>8,9</sup>. Previous studies have shown that tooth size differs between sexes as a result of natural selection processes. From an evolutionary perspective, male human ancestors used their teeth as tools for combating and protecting females, as well as in male-to-male fighting during mating seasons, leading to significantly larger teeth in males compared to females. Nevertheless, over a long period of time, as humans transitioned from using teeth as weapons to using hands to hold weapons, there was a marked reduction in the size of male teeth. This evolutionary shift resulted in male tooth becoming almost indistinguishable from those of females<sup>10–12</sup>. Despite this convergence, sexual dimorphism in tooth size persists, albeit not discernible to the naked eye. Therefore, the development of special methods is needed to detect and analyze these subtle differences.

To address this solution, mathematical and statistical approaches, such as logistic regression<sup>13</sup> and discriminant analysis<sup>14</sup>, are deemed appropriate for developing reliable predictive equations for sex estimation based on tooth size. While these methods can generate highly accurate predictive equations, they are complicated to apply, generally requiring computer or other special assistance for calculations, making them impractical in certain scenarios. Therefore, there is an ongoing effort to refine these models to enhance their accessibility and practicality. Another potential method for simplification lies in the application of Receiver Operating Characteristic (ROC) curve analysis.

ROC curves are well-known, established tools in epidemiology, frequently employed to assess cut-off points between dichotomous datasets, such as the presence or absence of disease<sup>15</sup>. Applying ROC analysis to determine cut-off points for tooth sizing in males and females presents a novel and promising opportunity. This

Department of Anatomy, Faculty of Dentistry, Mahidol University, 6 Yothi Road, Ratchathewi, Bangkok 10400, Thailand. ✉email: natchalee.sri@mahidol.edu

analysis could generate practical decision approaches suitable for actual applications, potentially offering more expedient and relatively accurate sex prediction compared to current methods. Therefore, the primary objective of this study is to develop a human sex prediction method based on horizontal width of anterior teeth using ROC analysis and to assess the accuracy of this newly developed technique. This methodological advancement was expected to enhance the efficiency and effectiveness of forensic sex estimation processes, thus improving practical applications in forensic settings.

## Methods

The methodology is illustrated in a flow chart presented in Fig. 1. This study was designed in two parts to fulfill the objectives. The first part involved developing a cut-off point for sex prediction using ROC curve analysis, and the second part evaluated the metrics performance of this newly developed sex prediction method.

### Part 1: developing a cut-off point for sex prediction using ROC curve analysis

#### *Cast selection*

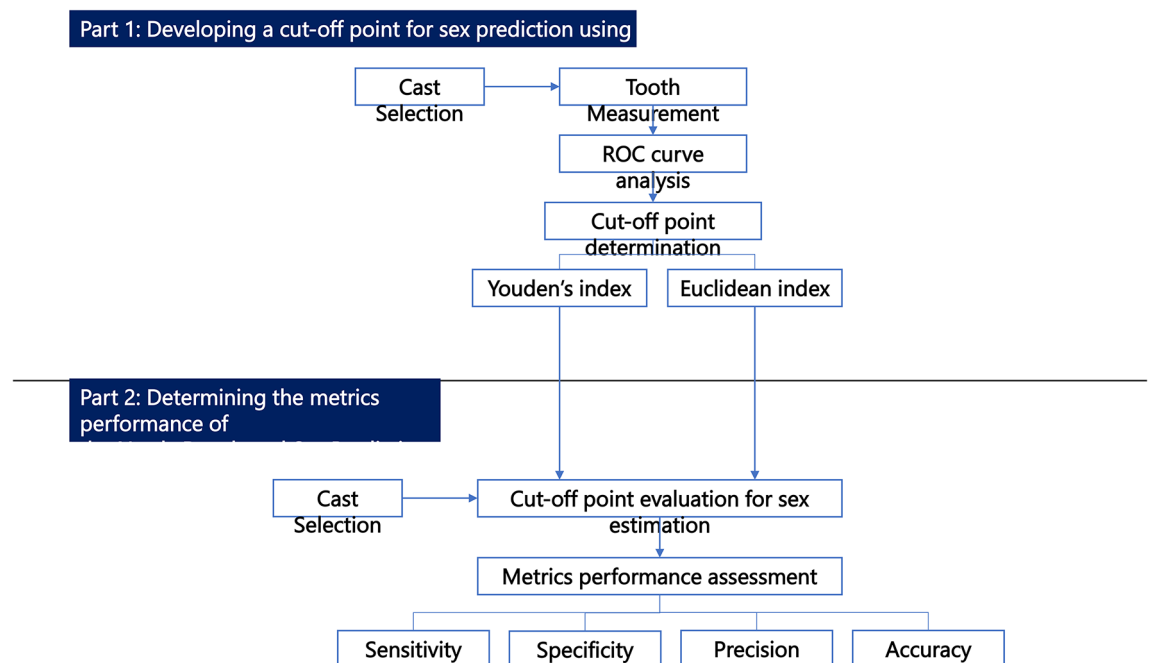
A total of 100 pre-orthodontic treatment dental casts from male patients and 100 casts from female patients were randomly recruited from the Orthodontic Clinic at the Dental Hospital, Faculty of Dentistry, Mahidol University, with approval from the hospital director. All casts were obtained from patients with fully erupted anterior teeth, comprising central incisors, lateral incisors, and canines on both the left and right sides, as well as in both the maxillary and mandibular arches. These teeth were required to have normal morphology without proximal caries, restorations, or any prosthodontic treatments. Additionally, any dental casts with anterior teeth exhibiting crowding, which would impede accurate measurement of the horizontal width of each tooth, were excluded from the study.

#### *Tooth measurement*

Tooth measurements were taken using a digital vernier caliper (Mitutoyo, Japan) with a resolution of 0.01 mm. The mesiodistal width of each anterior maxillary and mandibular tooth was measured from the selected casts. The caliper was positioned parallel to the long axis of each tooth, with the mesiodistal width defined as the greatest distance between the mesial and distal contact points on the proximal surfaces of the tooth crown. Two experienced operators (TA and NS), each with over 10 years of expertise in Forensic Anthropology and Forensic Odontology, measured all the dental casts independently using a blind technique. The average of their measurements was used as the final tooth size. Then, a third operator (PC) compiled all the data for analysis. To assess inter-observer reliability, an Intraclass Correlation Coefficient (ICC) analysis was performed, demonstrating good reliability with values ranging from 0.850 to 0.943.

#### *Sexual dimorphism assessment<sup>8</sup>*

The mean mesiodistal tooth width of both males and females was calculated to assess the degree of sexual dimorphism for each tooth using the following formula:



**Fig. 1.** The methodology flow chart.

$$\text{Sexual dimorphism (\%)} = \left( \frac{\text{Mean of male mesiodistal tooth width}}{\text{Mean of female mesiodistal tooth width}} - 1 \right) * 100$$

A positive value from this calculation indicates that, on average, male teeth are larger than female teeth, whereas a negative value suggests that female teeth are larger than male teeth.

#### ROC curve analysis and cut-off point estimation

The mesiodistal width of each tooth was analyzed to determine metrics such as sensitivity and specificity for distinguishing between male and female patients. Subsequently, Receiver Operating Characteristic (ROC) analysis, using the Area Under the Curve (AUC) as an inferential statistical method, was employed to evaluate the classification power of tooth size for sex estimation in IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). The optimal cut-off points for each tooth, distinguishing between male and female patients, were calculated from the ROC curve using both Youden's index and the Euclidean index.

#### Youden's Index<sup>16</sup>

Youden's index represents the vertical distance from each point on the ROC curve to a diagonal line with a slope of 1 passing from the coordinate (0, 0) through the coordinate (1, 1). This index is calculated using the following formula:

$$\text{Youden's index} = \text{Sensitivity} + \text{Specificity} - 1$$

The point where this value index reaches its maximum is identified as the optimal cut-off point.

#### Euclidean Index<sup>17</sup>

The Euclidean index is the straight-line distance from each point on the ROC curve to the coordinate (0, 1). The Euclidean index is evaluated as follows:

$$\text{Euclidean index} = \sqrt{(1 - \text{Sensitivity})^2 + (1 - \text{Specificity})^2}$$

The value where this index reaches its minimum is regarded as the optimal cut-off point.

## Part 2: determining the metrics performance of the newly developed sex prediction method

#### Cast selection

An additional set of 50 pre-orthodontic treatment dental casts from male patients and 50 casts from female patients were randomly selected from the Orthodontic Clinic at the Dental Hospital, Faculty of Dentistry, Mahidol University with approval from the hospital director. All casts met the same inclusion criteria described in Part 1.

#### Tooth measurement

The mesiodistal width of each anterior maxillary and mandibular tooth from the selected casts was measured, following the procedure described in Part 1, using a digital vernier caliper (Mitutoyo, Japan) with a resolution of 0.01 mm.

#### Metrics performance determination

The mesiodistal widths measured from the new set of dental casts were compared to the cut-off points derived from the Youden's index and the Euclidean index from Part 1.

Sensitivity for the cut-off point was determined using the following formula:

$$\text{Sensitivity} = \frac{\text{Number of truly male casts correctly predicted as male}}{\text{Total number of Male casts}}$$

Specificity for the cut-off point was determined using the following formula:

$$\text{Specificity} = \frac{\text{Number of truly female casts correctly predicted as female}}{\text{Total number of Female casts}}$$

Precision for the cut-off point was determined using the following formula:

$$\text{Precision} = \frac{\text{Number of truly male casts correctly predicted as male}}{\text{Total number of casts predicted as Male}}$$

Percentage of accuracy for the cut-off point was determined using the following formula:

$$\text{Percentage of accuracy} = \frac{\text{Number of the truly - sex predicted casts}}{\text{Total number of casts}} * 100$$

Results

Part 1: developing a cut-off point for sex prediction using ROC curve analysis

A total of 100 male dental casts and 100 female dental casts were used to develop the cut-off point for sex prediction. The average age of the males was  $21.25 \pm 5.42$  years ranging from 14.08 to 35.17, while the average age of the females was  $20.58 \pm 5.32$  years ranging from 13.08 to 34.08. The mean and standard deviation of the mesiodistal width of all maxillary and mandibular anterior teeth for both males and females are presented in Table 1, along with the degree of sexual dimorphism. The data revealed that the sexual dimorphism for canines was substantial, ranging from 3.22 to 5.51%, whereas the values for incisors were below 2%.

ROC analysis was conducted to evaluate the diagnostic accuracy of estimating sex based on the mesiodistal width of each anterior tooth. The results indicated that all canine teeth were statistically significant as diagnostic tools for sex identification, with AUC values of 0.634 and 0.626 for the maxillary right and left canines, respectively ( $p = .001$ ), and 0.724 and 0.727 for the mandibular left and right canines, respectively ( $p < .001$ ), as shown in Table 1. In contrast, the central and lateral incisors were not found to be statistically significant for sex identification in either the maxilla or the mandible ( $p > .05$ ). The ROC curves for all canines were presented in Fig. 2.

The results indicated that all canines demonstrate statistically significant diagnostic potential for sex identification. Based on these findings, all canine measurements were further used to develop a statistical index and establish cut-off points for sex estimation. The cut-off point for each canine was established using the maximum of Youden's index and the minimum of the Euclidean index. Notably, only the maxillary right canine had different values for Youden's index and the Euclidean index. All other canines shared the same cut-off points from both indices, as shown in Table 2.

Part 2: determining the metrics performance of the newly developed sex prediction method

A total of 50 male dental casts and 50 female dental casts were used to assess the accuracy of the newly developed sex prediction method. The average age of the males was  $20.69 \pm 4.61$  years ranging from 13.58 to 32.00, while the average age of the females was  $20.43 \pm 4.28$  years ranging from 13.25 to 30.33.

The cut-off points determined by Youden's index and the Euclidean index, along with the sensitivity, specificity, precision, and accuracy of the newly developed index for the test dataset, were displayed in Table 2. The results for the maxillary right canine indicated that Youden's index provided high specificity (0.74), whereas the Euclidean index demonstrated high sensitivity (0.80).

The analysis revealed that if the size of the mandibular right canine was equal to or greater than 6.77 millimeters, or 6.95 millimeters for the mandibular left canine, the predicted sex was male. The accuracy results indicated that the mandibular canines were more reliable than the maxillary canines, with the mandibular right canine achieving an accuracy of 77%.

Discussion

The prediction of sex based on tooth size remains a critical issue of ongoing development in forensic research, given its potential to contribute to the identification of unknown individuals. Over time, mathematical models have been employed to enhance the accuracy of sex prediction using quantitative tooth measurements. Logistic regression, for example, creates exponential multivariate functions by incorporating dimensions of multiple teeth, while discriminant function analysis relies on linear multivariate functions for similar purposes. However, the growing complexity of these models has introduced challenges in practical applications. Because these models require precise measurements from multiple specific teeth for accurate calculations, the frequent absence of even

Tooth	Mesiodistal width		Sexual dimorphism (%)	AUC	95% CI of AUC		p-value*
	Male	Female			Lower bound	Upper Bound	
Maxillary							
Right canine	8.07 ± 0.49	7.81 ± 0.46	3.23	0.634	0.557	0.710	0.001
Right lateral incisor	7.10 ± 0.58	7.01 ± 0.56	1.32	0.561	0.481	0.642	0.136
Right central incisor	8.56 ± 0.54	8.51 ± 0.52	0.55	0.542	0.461	0.623	0.310
Left central incisor	8.59 ± 0.51	8.51 ± 0.55	0.96	0.557	0.477	0.637	0.159
Left lateral incisor	7.22 ± 0.58	7.09 ± 0.60	1.80	0.576	0.497	0.656	0.061
Left canine	8.05 ± 0.51	7.80 ± 0.46	3.22	0.626	0.549	0.703	0.001
Mandibular							
Left canine	7.11 ± 0.49	6.74 ± 0.42	5.40	0.724	0.653	0.794	<0.001
Left lateral incisor	6.00 ± 0.45	5.99 ± 0.44	0.28	0.532	0.452	0.613	0.434
Left central incisor	5.44 ± 0.40	5.40 ± 0.41	0.80	0.527	0.447	0.608	0.507
Right central incisor	5.41 ± 0.41	5.36 ± 0.41	1.08	0.547	0.466	0.627	0.255
Right lateral incisor	5.98 ± 0.41	5.96 ± 0.45	0.38	0.525	0.444	0.605	0.547
Right canine	7.13 ± 0.46	6.76 ± 0.42	5.51	0.727	0.658	0.796	<0.001

**Table 1.** Mesiodistal width, sexual dimorphism and area under the curve (AUC) in ROC analysis of anterior teeth. p-value\* from Nonparametric ROC analysis.

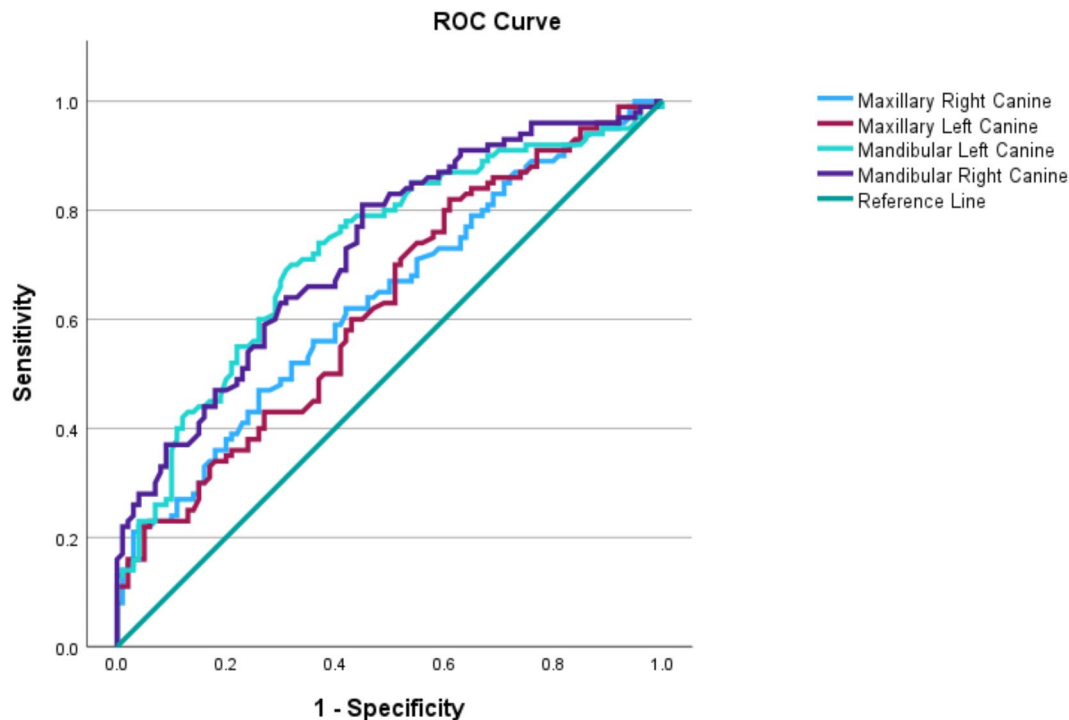


Fig. 2. The ROC curve for all canines.

Tooth	Cut-off point	Sensitivity	Specificity	Precision	Accuracy (%)
Youden Index					
Maxillary right canine	8.11	0.64	0.74	0.71	69.00
Maxillary left canine	7.80	0.82	0.52	0.63	67.00
Mandibular left canine	6.95	0.72	0.76	0.75	74.00
Mandibular right canine	6.77	0.88	0.66	0.72	77.00
Euclidean Index					
Maxillary right canine	7.94	0.80	0.58	0.66	69.00
Maxillary left canine	7.80	0.82	0.52	0.63	67.00
Mandibular left canine	6.95	0.72	0.76	0.75	74.00
Mandibular right canine	6.77	0.88	0.66	0.72	77.00

Table 2. Cut-off points (millimeters) and their metrics performance from Youden index and euclidian index for each tooth.

a single required tooth in forensic contexts often renders them ineffective. Therefore, there is a need for simpler methods that utilize fewer teeth while still achieving acceptable accuracy in sex prediction.

Numerous studies<sup>18,19</sup>, including the present research work, have consistently demonstrated that canines are more reliable indicators of sex estimation compared to other type of teeth. This has led to the development of the “canine index,” a method that calculates from the ratio between the width of the canine tooth and the intercanine distance, which is then evaluated against a standard cut-off point for sex estimation<sup>20</sup>. The primary advantage of this method is its simplicity, as it does not require complex calculations or expert interpretation, making it accessible for broader forensic applications. However, many studies reported that although the method is convenient, its predictive power is often suboptimal<sup>21,22</sup>. To improve upon these limitations of canine index, researchers have explored the application of ROC curve analysis to refine cut-off points used for sex prediction. ROC curve analysis allows for the identification of optimal thresholds that maximize both sensitivity and specificity, thereby improving the accuracy of the canine index. By moving away from static cut-off points to more dynamic, data-driven thresholds, the application of ROC curve analysis enhances the reliability of sex prediction using canine measurements<sup>23</sup>. Nevertheless, some practical challenges remain. For instance, the measurement of intercanine width requires the presence of both left and right canines. In cases where one canine is missing due to tooth loss or anomalies, the measurement becomes impossible, limiting the method’s applicability in certain forensic contexts. Another challenge to the canine index approach is the displacement of the canine tooth from its original position. Displacement may occur due to the extraction of adjacent teeth, orthodontic

treatments, or natural shifting over time<sup>24</sup>. This movement affects the intercanine width measurement, which in turn compromises the accuracy of the canine index. Such challenges highlight the need for continued research and refinement of methods that can adapt to these variables, ensuring more robust sex prediction models across diverse forensic scenarios.

This study addresses some of these limitations by proposing an alternative approach to sex prediction using ROC curve analysis. Instead of relying on intercanine width or complex multivariate functions, this method focuses on identifying optimal cut-off points for individual teeth. By examining each tooth independently, particularly canines, which exhibit greater sexual dimorphism, this approach avoids the need for combining measurements across multiple teeth. This individualized method is more resilient in cases where teeth are missing or displaced, offering a viable alternative for forensic application.

The finding of this study was consistent to previous research showing that central and lateral incisors exhibit minimal sexual dimorphism, making them less reliable for sex prediction. In contrast, canine teeth, especially mandibular canines, exhibited significantly greater sexual dimorphism. This pattern has been observed across various ethnic groups and populations<sup>18</sup>, suggesting a strong evolutionary basis for the sex difference. The hypothesis proposed that in male terrestrial primates, canines developed as weapons for combating enemies and protecting their families, leading to more robust canines in males compared to females. This trait has persisted in modern humans, with male canines remaining notably larger than female canines, despite the overall reduction in tooth size through the evolutionary process<sup>10–12</sup>. As a result, canine teeth continue to be among the most reliable indicators of sex in forensic anthropology.

Beyond evolutionary factors, hormonal influences also play a critical role in the development of sexual dimorphism in teeth. Growth hormone is responsible for overall body growth, including tooth development<sup>25,26</sup>, but its effects are modulated by sex hormones. Increase estrogen concentration, the female sex hormone, inhibits growth hormone activity<sup>27</sup>. Testosterone, the dominant male sex hormone, enhances growth hormone effects<sup>28</sup>. This hormonal regulation underlies the persistent size difference between male and female teeth, even in modern human populations.

Among the anterior teeth analyzed in this study, only the four canines, of both maxilla and mandible, were statistically significant for ROC curve analysis to determine cut-off points for sex prediction. The cut-off points were calculated using both the Youden index and the Euclidean index. Interestingly, for all canines except the maxillary right canine, the cut-off points derived from both indices were identical. The cut-off points for the maxillary right canine, however, demonstrated significantly higher specificity than sensitivity when calculated using the Youden index, while the Euclidean index produced a cut-off point with higher sensitivity than specificity. These findings underscore the importance of selecting appropriate cut-off points depending on the forensic application.

Although the cut-off points derived from canines, particularly mandibular canines, in the present study achieve sex estimation accuracies of 74–77%, this may seem lower compared to more complex methods, such as logistic regression, which yields accuracies ranging from 71–100%<sup>13</sup>, or discriminant analysis, which has reported accuracies of up to 90% in Indian populations<sup>14</sup>. However, when considering the ethnic factor, the accuracy of these methods can vary significantly across different populations. For instance, logistic regression achieves 70.5% accuracy in Brazilians<sup>29</sup> but only 69% in Egyptians<sup>30</sup>. Similarly, discriminant analysis shows accuracies ranging from 67.9 to 92.5% in Nepalese populations<sup>31</sup>, but decreases to 69% in Egyptians<sup>30</sup> and 59.7–66.7% in Chileans<sup>32</sup>. From a practical perspective, using ROC curve analysis based on a single canine tooth offers a more accessible and relatively efficient approach to sex estimation. Despite its simplicity and adaptability to population-specific variations, this method demonstrates sufficient accuracy for reliable sex estimation, particularly in preliminary screening contexts. However, more complex methods are required to confirm these results for broader forensic or diagnostic applications.

Canines are also among the most durable teeth in the oral cavity, as they are less prone to periodontal disease<sup>33,34</sup> and are usually the last teeth to be extracted<sup>35</sup>. As a result, canines are frequently found in unidentified remains long after other body parts have decayed. These qualities make canine teeth particularly valuable not only in forensic odontology but also in physical anthropology and archaeology<sup>19,36</sup>. Sex prediction based solely on the size of a single canine tooth is therefore a viable and practical approach, offering a simpler alternative to more complex methods.

The use of digitally developed dental casts or 3D digital models obtained through intraoral scans should be considered in future studies, reflecting advancements in technology. The findings from this study can be applied to dental casts, 3D digital models, or direct measurements from teeth, as the focus was on tooth size regardless of the method used. However, caution is advised in applying and interpreting the results, as the sample size was relatively small and limited to Thai individuals. Further research with larger, more diverse populations is necessary to generalize these findings for broader forensic applications.

## Conclusion

The canine width measurement provides a reliable and straightforward method for sex estimation without the need for complex tools or software. Our human sex prediction method was developed based on the mesiodistal width of the canine using ROC analysis. When the width of the mandibular canine is 6.77 mm or greater on the right side and 6.95 mm or greater on the left side, the individual is likely to be male. The accuracy of this technique is acceptable, ranging from 74 to 77%, with sexual dimorphism values between 5.40% and 5.51%. This methodological improvement is proposed to enhance both the efficiency and accuracy of forensic sex estimation, increasing its practical application in forensic investigations.



## Data availability

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

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## Author contributions

P.C. and T.A. Conceived and designed the experiments. T.A. and N.S. Performed the experiments. P.C. Compiled all the data for analysis. N.S. Analyzed and interpreted the data. All authors Wrote the main manuscript text and reviewed the manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

### Ethical approval

The study received ethical approval from the Institutional Review Board of the Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University, with reference number COA.No.MU-DT/PY-IRB 2024/027.0706. All experiments were conducted in accordance with the ethical principles of the Declaration of Helsinki. The requirement for patient informed consent was waived by the Institutional Review Board due to the retrospective nature of the study.

### Additional information

**Correspondence** and requests for materials should be addressed to N.S.

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