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Osteometry of Foramen Magnum by Using Post-Mortem Computed Tomography (PMCT) for Discriminant Analysis of Sex and Population Affinity among Malaysian Population

قياس عظام الثقبة العظمى باستخدام التصوير المقطعي المحوسب بعد الوفاة لإجراء التحليل التمييزي للجنس والتوافق السكاني لدى الماليزيين

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Abstract

Osteometry of the foramen magnum was assessed for discriminant function analysis of biological sex estimation and population affinity estimation based on post-mortem computed tomography (PMCT) images in this Malaysian population-based study.

This retrospective cross-sectional study was performed using convenient sampling of PMCT skull images originating from 300 Malaysian adults. Linear dimensions of the foramen magnum transverse diameter (FMTD) and anterior-posterior diameter (FMAPD) were measured. Statistical analyses were performed via independent t-test, ANOVA, univariate and multivariate analyses concurrently with discriminant function analyses.

There were significant differences in FMTD and FMAPD between males and females within respective population affinities. The foramen magnum dimensions of males were comparably larger than females. There were relatively significant differences in FMTD and generally sufficient significant

المستخلص

أجريت هذه الدراسة المقطعية الاسترجاعية على السكان الماليزيين، حيث تم قياس عظم الثقبة العظمى لإجراء التحليل الوظيفي التمييزي لتقدير الجنس البيولوجي والتوافق السكاني باستخدام صور التصوير المقطعي المحوسب بعد الوفاة.

وذلك من خلال أخذ عينات ملائمة من صورة الجمجمة لدى 300 شخص ماليزي بالغ باستخدام التصوير المقطعي المحوسب بعد الوفاة. حيث تم قياس الأبعاد الخطية للقطر المستعرض والقطر الأمامي الخلفي للثقبة العظمى. كما أُجريت التحليلات الإحصائية من خلال إجراء اختبار -ت- للعينات المستقلة، وتحليل التباين (ANOVA)، والتحليلات أحادية المتغيرات ومتعددة المتغيرات بالإضافة إلى التحليل الوظيفي التمييزي.

تبين وجود فروقات كبيرة في الأبعاد الخطية للقطر المستعرض والقطر الأمامي الخلفي للثقبة العظمى بين الجنسين ضمن مجموعات التوافق السكاني لكل منهما. وكان قياس عظم الثقبة العظمى لدى الذكور أكبر نسبيًا منه لدى الإناث. كما تبين وجود فروقات كبيرة نسبيًا في الأبعاد الخطية للقطر المستعرض وفروقات كبيرة كافية بشكل

Keywords: Forensic science, foramen magnum, forensic anthropology, postmortem computed tomography, discriminant analysis, sex estimation, population affinity estimation.

الكلمات المفتاحية: علوم الأدلة الجنائية، الثقبة العظمى، الأنثروبولوجيا الجنائية، التصوير المقطعي المحوسب بعد الوفاة، التحليل التمييزي، تقدير الجنس، تقدير التوافق السكاني.



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differences in FMAPD among population affinities. The osteometry of foramen magnum dimensions of Chinese were generally larger than Malays and Indians.

This study concluded that the Foramen magnum dimensions have a stronger discriminant function in biological sex estimation with 60% accuracy as compared to population affinity estimation with only 40.7% accuracy. FMTD and FMAPD are the potential sex discriminators among the Malaysian population particularly if other indicators are not available for evaluation.

1. Introduction

The application of virtual anthropology in forensic investigations has been expanding over the past few decades in the advent of postmortem computed tomography (PMCT) and other imaging modalities. Biological profiling is the most basic concept of virtual anthropology examination. Biological profiling which includes biological sex, age at death, population affinity and stature estimations are vital for the identification of unknown skeletonized or severely decomposed human remains particularly in the event of mass disasters and forensic exhumations [1]. Sex estimation mainly depends on the evaluation of the axial skeletons as well as appendicular skeletons available from the scene of death [2]. Sex can be determined with 100% accuracy when the skeleton is complete, at 98% in combination with the pelvis and cranium, at 95% in combination with pelvis and long bones, and at 80% - 90% when only long bones are present [3].

From the previous literature review of the Malaysian population, Ibrahim et al. established sex classification accuracy range of 78.2% to 86.2% based on twenty-two parameters of the Malaysian crania using PMCT images [4]. The foramen magnum has been studied as a potential sex estimator due to its insensitivity to the secondary sexual or hormonal changes, significant robustness,

عام في الأبعاد الخطية للقطر الأمامي الخلفي بين مجموعات التوافق السكاني. في حين كان قياس عظم الثقبية العظمى لدى الصينيين أكبر بشكل عام من نظيره لدى الماليزيين والهنود.

استنتجت هذه الدراسة أنه كان لأبعاد الثقبية العظمى وظيفة تمييزية أقوى في تقدير الجنس البيولوجي بدقة تبلغ 60٪ مقارنة بتقدير التوافق السكاني الذي تبلغ دقته 40.7٪ فقط. ويمكن أن يشكل القطر المستعرض والقطر الأمامي الخلفي عوامل محتملة للتمييز الجنسي بين السكان الماليزيين، خاصة إذا لم تكن مؤشرات ازدواج الشكل الجنسي الأخرى متاحة للتقييم.

protected location to withstand external forces and relative morphologic stability after reaching adult size [5]. This is ultimately important when a dead body is discovered in fragmented parts with only cranium available at the scene of death.

Discriminant analysis of sex and population affinity based on the osteometry of foramen magnum using PMCT images has not been endeavored in the Malaysian population. In the first part of our research, we performed a feasibility study that yielded moderate to substantial reliability using PMCT as a measuring tool for foramen magnum dimensions [6]. Subsequently, the general objective of this study was to generate population-based data to assess sexual dimorphism in foramen magnum dimensions based on the three main population affinities in Malaysia. The specific objective of this study was to investigate discriminant analysis of sex and population affinity based on the osteometry of foramen magnum transverse diameter (FMTD) and foramen magnum anterior posterior diameter (FMAPD) using PMCT images.

2. Methods

2.1 Study design and sample size

This was a retrospective cross-sectional study performed at the National Institute of Forensic Medicine, Malaysia. The sample size of 384 was



estimated to be sufficiently powerful based on the confidence level of 95% and response distribution of 50% to represent the population size of 32.7 million in Malaysia by using Raosoft Sample Size Calculator [7]. As described by Wong et al. (2022), population affinity could be a confounder to assess if there was truly a significant difference between males and females of different population affinities [6]. Hence, convenient sampling of 300 anonymized PMCT skulls in equal distribution of both sexes among three main population affinities in Malaysia i.e. Malay, Chinese and Indian were included in this study. The selected PMCT images were of adults above 18 years old and cases performed between 2015 to 2021. Cases with skull base fractures extending to the foramen magnum and congenital skull base abnormalities were excluded from this study.

2.2 PMCT Image acquisition

All PMCT skulls were acquired prior to the autopsy examinations in NIFM with a 64-multislice helical CT scanner obtained at 135 kV (AquilionTM 64 TSX-191A/E, TSX-101, System Software Version: Apps: V4.51, Base V6-50) according to standard operating procedures. The CT scan parameters were auto-modulated MAs based on field of view of 320 mm², pitch of 0.641, slice interval of 0.8 mm and slice thickness of 1.0 mm. The selected samples consisted of 150 males and 150 females. Within each sex group, they were further divided equally to 50 samples each among Malays, Chinese and Indian population affinity groups. All images were extracted in Digital Imaging and Communications in Medicine (DICOM) format from the archived server database.

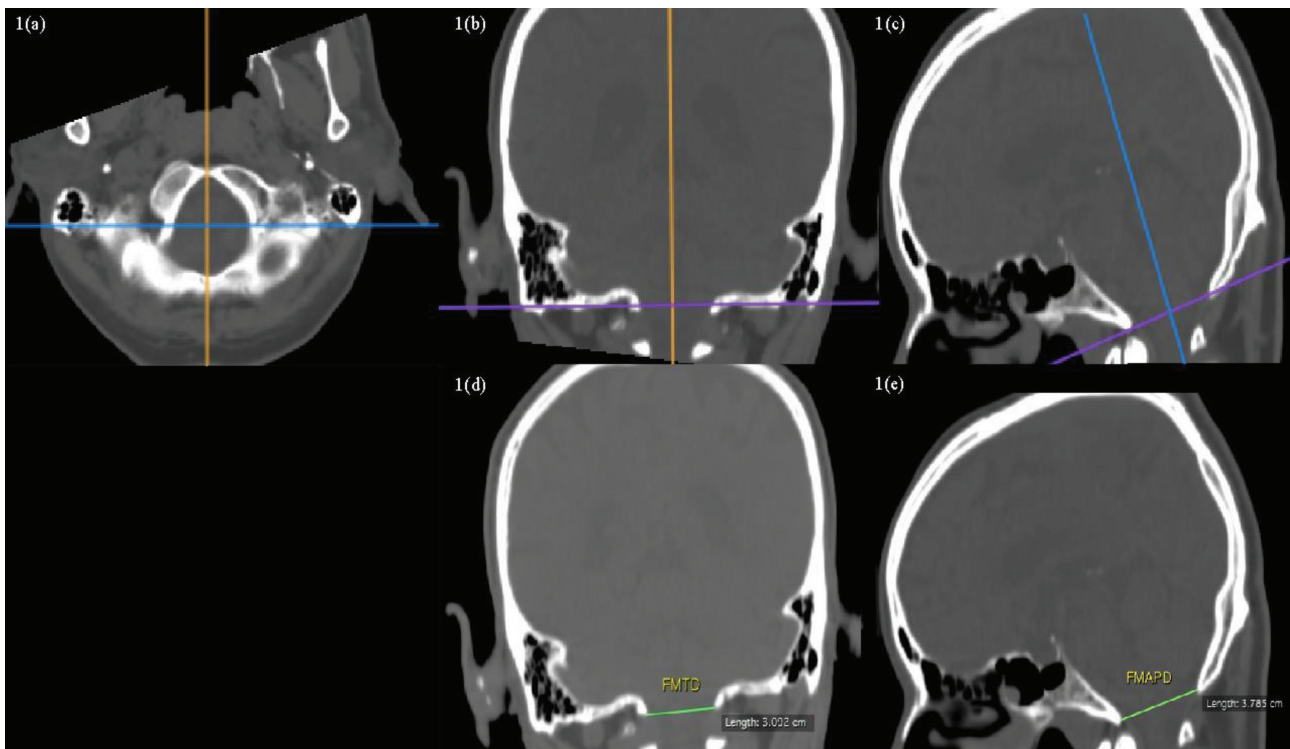


Figure 3- PMCT Brain 3D-MPR bone reconstruction in (a) axial, (b) coronal and (c) sagittal views adjusted according to the plane of foramen magnum before evaluation of parameters. Measurements are taken in a standardized manner (d) FMTD (width) in coronal Plane & FMAPD is measured in sagittal Plane after angle adjustment. Source: Adopted from Wong et al. (2022) [6].



2.3 Data collection and measurements

The images were anonymized with a unique identification numbers and viewed using the INFINITT system in bone windows after the 3D-multi-planar (MPR) bone reconstructions of the skull and adjusted parallel to the plane of the foramen magnum according to our feasibility study as portrayed in Figure 1 [6]. The foramen magnum measurements were taken with FMAPD measured in sagittal plane and FMTD measured in coronal plane. FMAPD was defined as maximum antero-posterior length of foramen magnum measured from basion to opisthion at mid sagittal plane whilst FMTD was defined as maximum width between the lateral margins of foramen magnum perpendicular to mid sagittal plane [6]. The measurements were made in the unit of millimeter (mm) in two decimal points. Single reading was performed by a forensic scientist with 9 years of experience in PMCT research at the institute blinded to sex, age and population affinity of the samples. Observer training was given by experienced forensic radiologists prior to data collection for appropriate angle adjustments prior to measuring dimensions as suggested in our feasibility study [6].

2.4 Statistical analysis

IBM SPSS (Statistical Package for Social Sciences) Version 20.0 software was used for data analysis. The level of significance was set as 0.05 for all statistical tests. Significance of differences between biological sex and population affinities were determined using independent t-test and analysis of variance (ANOVA) respectively. The multivariate analysis was performed to study the confounding effects of sex and population affinity by including them as covariates that could contribute to the significant differences in dimensions of the foramen magnum. Discriminant function analyses of sex and population affinity were conducted to determine the relative discriminator strength based on the osteometrics of the foramen magnum. Left-one-out cross validation was done in the analysis and considered as the correct classification rate based on the discriminant function.

3. Results

3.1 Descriptive Analysis

Based on the total of 300 selected foramen magnums, the mean age and its standard deviation

Table 1- Summary of FMTD and FMAPD measurements

Population affinity	Foramen Dimensions	Male Mean \pm SD* (mm)	Female Mean \pm SD* (mm)	Overall Mean \pm SD* (mm)
Malay	FMTD	28.85 \pm 1.91	27.70 \pm 2.10	28.27 \pm 2.08
	FMAPD	33.62 \pm 2.75	32.09 \pm 1.90	32.86 \pm 2.48
Chinese	FMTD	29.47 \pm 2.03	28.47 \pm 2.13	28.97 \pm 2.13
	FMAPD	34.59 \pm 2.33	32.71 \pm 2.16	33.65 \pm 2.43
Indian	FMTD	28.57 \pm 1.81	27.63 \pm 1.98	28.10 \pm 1.95
	FMAPD	33.32 \pm 2.69	32.64 \pm 2.62	32.98 \pm 2.66
Overall	FMTD	28.96 \pm 1.95	27.93 \pm 2.09	28.45 \pm 2.08
	FMAPD	33.84 \pm 2.64	32.48 \pm 2.25	33.16 \pm 2.54

SD*: Standard deviation; FMTD: Foramen magnum transverse diameter; FMAPD: Foramen Magnum Anterior-Posterior diameter.



(SD) of this study was 48.55 ± 14.56 years, with age range between 18 to 92 years. However, there was no significant Spearman's correlation between foramen magnum dimensions and age at $p > 0.05$. The descriptive analysis of the foramen magnum dimensions based on the sex and population affinity was displayed in the Table 1. The parameters of FMTD and FMAPD were generally in a normal distribution throughout the population sample chosen in this study as assumed through both Kolmogorov-Smirnov and Shapiro-Wilk tests at $p > 0.05$ (Figure 2). Hence, parametric statistical analysis was applicable in this study.

3.2 Differences of foramen magnum dimensions between sexes and its discriminant function analysis

The foramen magnum dimensions of males (FMTD 28.96 ± 1.95 mm, FMAPD 33.84 ± 2.64 mm) were larger than females (FMTD 27.93 ± 2.09 mm, FMAPD 33.84 ± 2.64 mm, Table 1). These differences were significant at $p < 0.001$ based on the independent t-test as well as multivariate analysis, considering population affinity as a potential covariance. A prediction analysis for sex showed that FMAPD was a stronger predictor than FMTD.

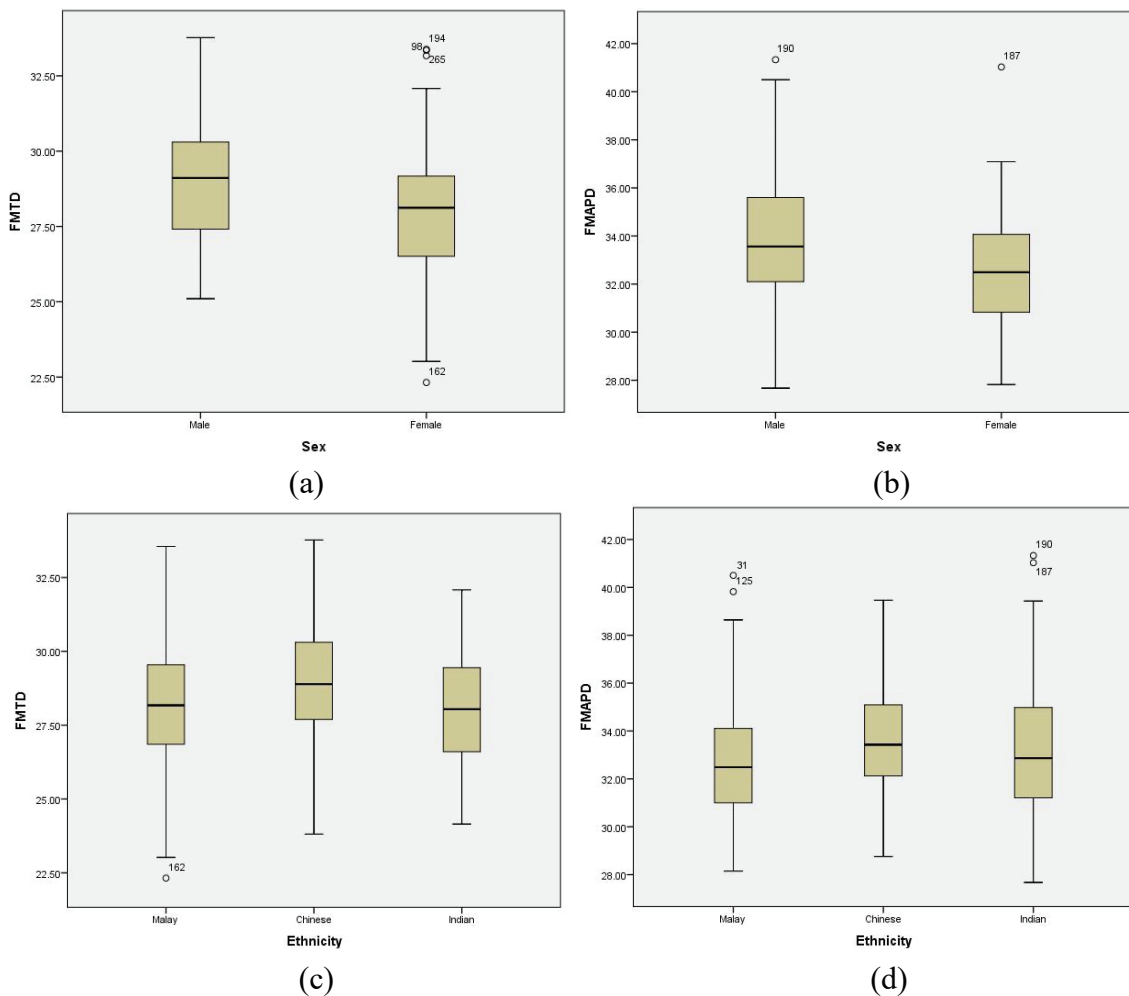


Figure 2- Box plots for the measurements of FMPD and FMAPD according to sex and population affinity
Note: x-axis represents sex (a,b) and population affinity (c,d); y-axis represents foramen magnum dimensions (mm)



Furthermore, the discriminant function analysis for predicting sex consisting of both parameters ($-15.717 + 0.244 \cdot \text{FMTD} + 0.264 \cdot \text{FMAPD}$) showed a higher classification accuracy (60%) than the models consisting of only one parameter (Table 2 and Figure 3). In each population sample, sex could be predicted with similar accuracy (Malays 58% ~ 62%, Chinese 63%, Indians 50% ~ 60%) using the two-parameter model. However, FMAPD and combination of both parameters among Indians did not show significant discriminant function between sexes.

3.3 Differences of foramen magnum dimensions between population affinities and its discriminant function analysis

Foramen magnum dimensions were generally larger in Chinese compared to those in Malays and Indians (Table 1). There were significant differences in FMTD between population affinities, especially between Chinese and Indians ($p = 0.008$ and $p = 0.006$) followed by Chinese and Malays ($p = 0.045$ and $p = 0.038$) through the respective post-hoc analysis (Table 3). In addition, the multivariate analysis showed significant differences of FMAPD between population affinities at $p = 0.047$ (Table 3). This was reflected in the population affinity

Table 2- Discriminant function analysis of sex based on population affinity in measurements of foramen magnum dimensions

Foramen Dimensions	FMTD (X)	FMAPD (Y)	FMTD (X) & FMAPD (Y)
Sex discriminant within Malay			
Chi-square value	7.905	9.921	11.350
df	1	1	2
p-value	0.005	0.002	0.003
Discriminant function	$0.498 \cdot X - 14.071$	$0.423 \cdot X - 13.895$	$0.222 \cdot X + 0.288 \cdot Y - 15.731$
Correct classification	58.0%	62.0%	60.0%
Sex discriminant within Chinese			
Chi-square value	5.565	15.961	15.901
df	1	1	2
p-value	0.003	<0.001	<0.001
Discriminant function	$0.480 \cdot X - 13.914$	$0.445 \cdot X - 14.973$	$0.022 \cdot X + 0.433 \cdot Y - 15.230$
Correct classification	63.0%	63.0%	63.0%
Sex discriminant within Indian			
Chi-square value	5.933	1.590	5.917
df	1	1	2
p-value	0.015	0.207	0.052
Discriminant function	$0.527 \cdot X - 14.811$	$0.377 \cdot X - 12.432$	$0.512 \cdot X + 0.021 \cdot Y - 15.105$
Correct classification	60.0%	50.0%	60.0%
Overall sex discriminant			
Chi-square value	18.923	22.249	26.745
df	1	1	2
p-value	<0.001	<0.001	<0.001
Discriminant function	$0.495 \cdot X - 14.081$	$0.408 \cdot X - 13.537$	$0.244 \cdot X + 0.264 \cdot Y - 15.771$
Correct classification	56.3%	58.7%	60.0%

FMTD: Foramen magnum transverse diameter; **FMAPD:** Foramen Magnum Anterior-Posterior diameter.

Note: Discriminant value > 0 represents male; Discriminant value < 0 represents female



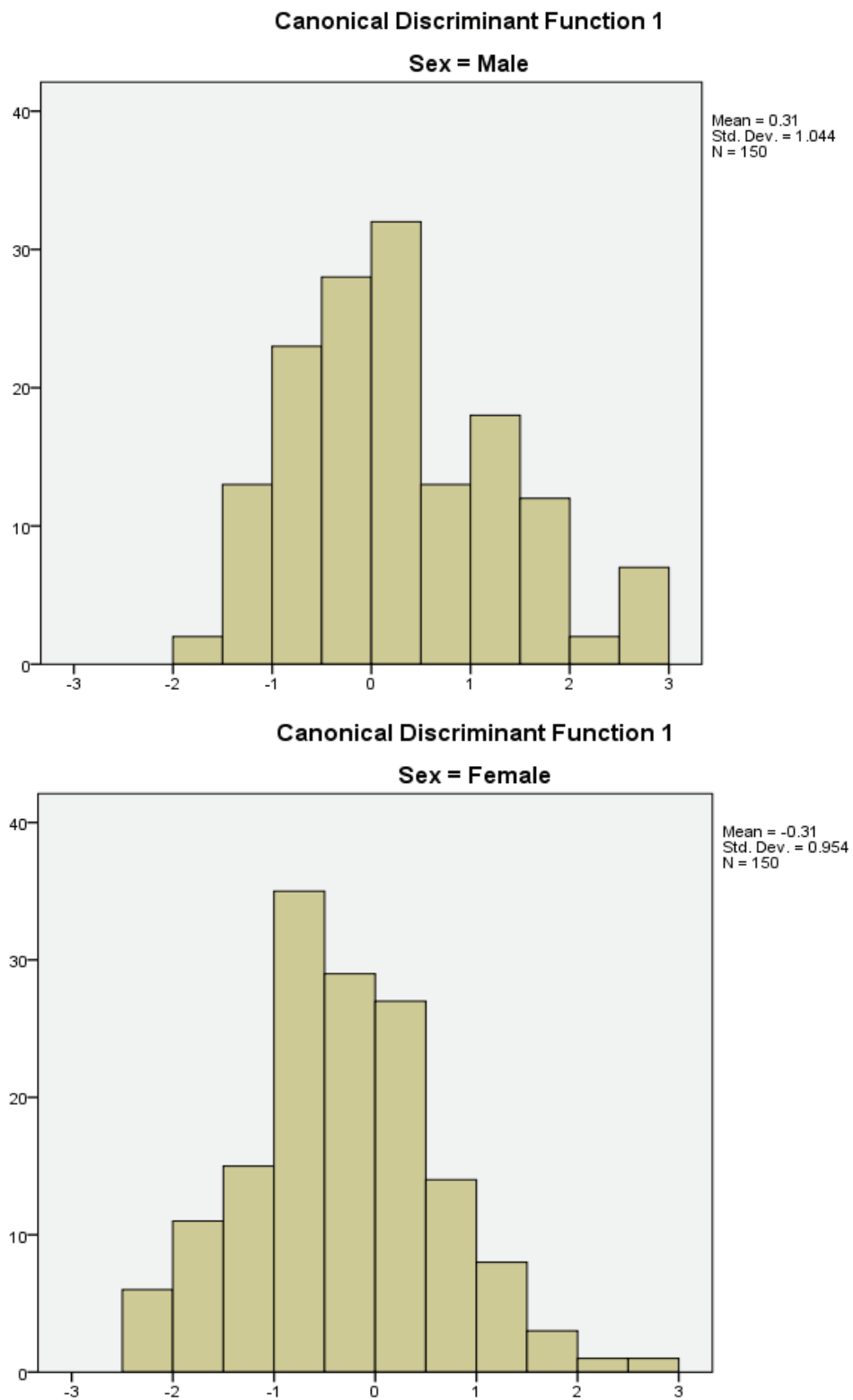


Figure 3- Overall discriminant function analysis of sex in both measurements of FMPD and FMAPD

Note: x-axis represents discriminant score; y-axis represents number of samples



Table 3- Statistical differences between sex and population affinity in the measurements of foramen magnum dimensions

Statistical Differences	FMTD	FMAPD
Sex: Independent t-test	4.424	4.811
t value	298	298
df	<0.001	<0.001
p-value		
Sex: Variate Analysis	20.017	23.565
F value	1, 294	1, 294
df	<0.001	<0.001
p-value		
Population affinity: ANOVA	5.027	2.869
F value	2, 297	2, 297
df	0.007	0.058
p-value		
Population affinity: Variate Analysis	5.318	3.099
F value	2, 294	2, 294
df	0.005	0.047
p-value		
Sex*Population affinity: Variate Analysis	0.078	1.629
F value	2, 294	2, 294
df	0.925	0.198
p-value		

FMTD: Foramen magnum transverse diameter; FMAPD: Foramen Magnum Anterior-Posterior diameter.

discriminant function analysis that FMTD, even in separate sex groups, became the stronger discriminator because of FMAPD was only significant within the male group. Moreover, combination of both parameters has revealed an improved population affinity discriminant function (Function 1: $-14.928 + 0.409 \cdot \text{FMTD} + 0.099 \cdot \text{FMAPD}$) at relatively low accuracy of 40.7% (Table 4 and Figure 4). All in all, statistical analyses showed that both FMTD and FMAPD were stronger predictors of biological sex compared to population affinity based on discriminant function analysis. However, multivariate analysis demonstrated no statistically significant interaction between sex and population affinity as confounders.

4. Discussion

4.1 Discriminant analysis of sex and population affinity

Differences in foramen magnum dimensions have been found to be useful for sexing in different populations, including Malaysian. This study has shown that the foramen magnum dimensions could be one of the discriminators in sex estimation in the Malaysian population. We concur with Santhosh et al. (2013) whereby they had found statistically significant differences of foramen magnum dimensions on dry skulls sample between both sexes within the South Indian population [8]. Galdemas et al. (2009) also found differences between males and females, but with a relatively higher discriminating power of 66.5% accuracy compared to ours with 60.0% accuracy [9]. Summary of the percentages of correct sex classification were observed in other studies as portrayed in Table 5. This indicates that sexing based on only one trait is insufficient for highly accurate estimation of biological sex.



Table 4- Discriminant function analysis of population affinity based on sex in measurements of foramen magnum dimensions

Foramen Dimensions	FMTD (X)	FMAPD (Y)	FMTD (X) & FMAPD (Y)
Population affinity discriminant within Male	5.630 2	6.402 2	7.730 4
Chi-square value	0.060	0.041	0.102
df	0.521*X-15.076	0.385*X-13.036	0.261*X+0.243*Y-15.790
p-value	38.7%	39.3%	40.0%
Discriminant function			
Correct classification			
Population affinity discriminant within Female	5.014 2	2.285 2	7.274 4
Chi-square value	0.082	0.319	0.122
df	0.483*X-13.485	0.445*X-14.458	0.535*X-0.129*Y-10.764
p-value	42.0%	36.7%	43.3%
Discriminant function			
Correct classification			
Overall population affinity discriminant	9.888	5.683	11.136
Chi-square value	2	2	4
df	0.007	0.058	0.025
p-value	0.487*X-13.846	0.396*X-13.143	0.409*X+0.099*Y-14.928
Discriminant function	38.7%	39.3%	40.7%
Correct classification			

FMTD: Foramen magnum transverse diameter; FMAPD: Foramen Magnum Anterior-Posterior diameter.

Morphological and dimensional differences between sexes may be present with geographical variations in different populations [10]. It is ideal to provide up-to-date population specific reference ranges for foramen magnum dimensions which have not been attempted before in Malaysia to justify this study. We have discovered that the sex differences in both parameters were consistent in each of the population affinity selected in this study. Our findings concurred with the literatures that found significant differences in the foramen magnum dimensions between both sexes in other populations [11-15].

In this study, the Chinese had significantly larger foramen magnum dimensions compared to the Indians and Malays. These variations could be due to the different ancestral origins and migrations of three main population affinities into Malaysia. Population variability was also supported by

Galdames et al. (2009) who reported that foramen magnum dimensions of central Europeans was larger than Middle Eastern and South American [9]. Nevertheless, genetic convergence effect may also contribute to the challenges in differentiating population affinities especially in mixed marriages where estimation accuracy was reported to be as low as only 40.7%.

4.2 Confounder effects

In general, maximum foramen magnum length can be reached by three to four years of age in childhood and is relatively stable after reaching the adult size [5]. There was no correlation between foramen magnum dimensions and age in this study as supported by Amir et al. (2021) hence, foramen magnum is not suitable for age determination [16]. Phillip et al. (2009) also reported that neither individual age-dependency,



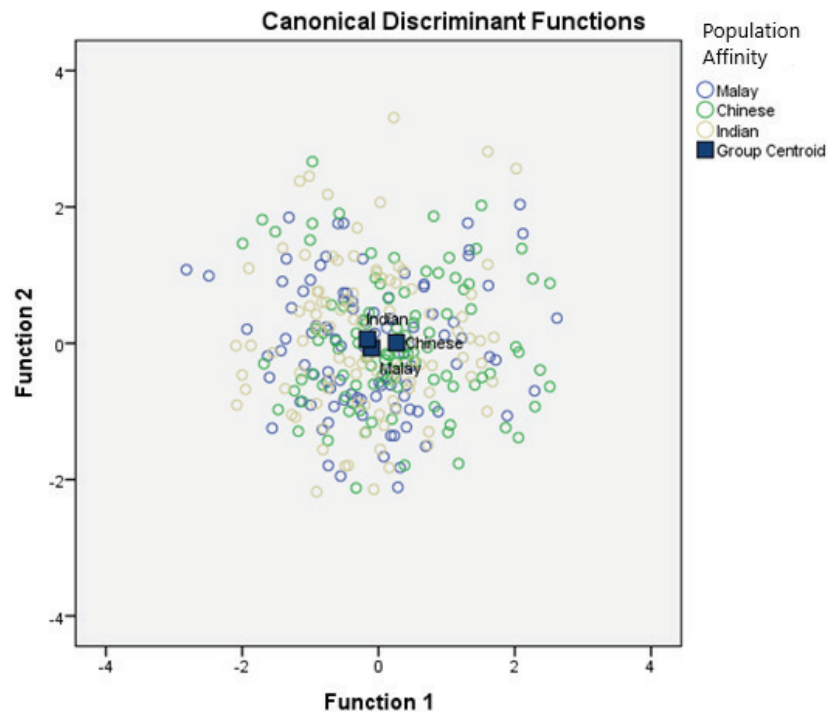


Figure 4 - Overall discriminant function analysis of population affinity in both measurements of FMPD and FMAPD

Note: x-axis represents discriminant scores of Function 1; y-axis represents discriminant scores of Function 2

nor secular trend was found for either foramen magnum dimensions [17]. As of individual stature, the relationship with foramen magnum dimensions was weak [17]. Hence, the authors did not consider these confounder effects in this study.

4.3 Virtual measurement and conventional direct measurement

Directing towards the digitalization in forensic application, PMCT plays an important role as data collection tool for the development of virtual anthropology. In order to establish its reliability, Normaizatul et al. (2019) reported that bone measurements by virtual method was highly accurate and as reliable as conventional dry bone method [18]. Stull et al. (2014) had found minimal differences between anthropometric variables obtained with PMCT and dry skulls with high accuracy between observers [19]. Comparison with dry skulls,

however, was not feasible in this retrospective study. Hence, whether PMCT can represent dry skull metric measurements was not investigated. Most importantly, PMCT method offers an alternative non-invasive means of osteometric assessment for identification and biological profiling without the need to remove soft tissue during autopsy, particularly when involving large scale of mass disaster.

4.4 Other limitations

The sample size was less than the targeted 384 skulls that would represent the Malaysian population. This was to allow the equal selection and division of the sexes and main three population affinities, which were not equally distributed within the available database. The measurements were taken once by a single observer, hence results were subject to the observer's training, experience and technical ability to use the software for accurate



Table 5 (continued).

Authors (year) Study Design	Location of study	FMTD & FMAPD (Mean± SD) (mm)	Significance of differences (p values)	Sex classification ac- curacy /Discriminating power
Bahsi et al. (2021) [26] N = 200 Males 200 Females	Gaziantep Univer- sity Faculty of Den- tistry, Turkey	Female: FMAPD 35.84 ± 2.26 FMTD 31.72 ± 2.62	0.001	N/A
Age range: 18-65 years		Male: FMAPD 37.66 ± 2.40 FMTD 33.39 ± 2.99	0.001	
Abbo El-Atta et al. (2020) [11] N = 163 Males 204 Females	Mansoura Universi- ty, Egypt	Female: FMAPD 3.57 ± 0.33 FMTD 2.99 ± 0.27	FMW: < 0.001 FML: 0.002	Female: 69.0%
Age range: 18-75 years		Male: FMAPD 3.68 ± 0.33 FMTD 3.15 ± 0.26	FMW: < 0.001 FML: 0.002	Male: 70.9%
Edwards et al. (2013) [27] N = 144 Males 106 Females	University of Zurich, Switzerland	Female: FMAPD 36.66 ± 2.26 FMTD 31.34 ± 2.19	< 0.00001	FMAPD: Male: 78.5% Female: 49.1% Mean: 66.0%
Age range: Adults		Male: FMAPD 38.17 ± 2.70 FMTD 33.05 ± 2.61	< 0.00001	FMTD: Male: 79.9% Female: 49.1% Mean: 66.8%

landmark identification in accordance with our feasibility study by Wong et al. (2022). In our pilot study, there were non-significant intra-observer errors of double readings with RTEM ranged from 3.65% to 5.00% at reliability of 0.57 - 0.75, but relatively low significant inter-observer errors of three observers were perceived with RTEM ranged from 4.12% to 6.38% at reliability of 0.48 - 0.68 based on the guidelines from the literatures [6, 20-22]. Measurement errors were attributed to variability in the slice, angle and landmark

selections [23]. As such, the experienced observer was further trained to adopt the standardized PMCT foramen magnum measurement techniques used in the feasibility study [6].

5. Conclusions

This study has shown that FMTD and FMAPD are the potential predictors in sex estimation in the Malaysian population with moderate accuracy of 60% especially if there were no other more established sexually dimorphic indicators available.



It is likely that accuracy will increase if these dimensions are applied for sexing in combination with other more established sexually dimorphic indicators whenever available for anthropological analysis. This study also revealed that osteometry of the foramen magnum is insufficient as a population affinity discriminator with relatively low accuracy of 40.7%. All in all, osteometry of foramen magnum has stronger discriminant function in sex estimation compared to population affinity estimation.

List of Abbreviations

FMTD : Foramen Magnum Transverse Diameter
 FMAPD : Foramen Magnum Anterior-Posterior Diameter
 PMCT : Postmortem Computed Tomography
 SD : Standard deviation
 R : Coefficient of Reliability
 RTEM : Relative technical error of measurement

Declarations

Ethics approval and consent to participate

No consent to participate was required as only decedent's secondary data and images were retrospectively utilized without clinical intervention. The manuscript has been sufficiently anonymized and will not cause harm to the patient or their next of kin. Ethical approval was granted by the Medical Research & Ethics Committee under Malaysian Ministry of Health (MOH) [Reference number: KKM/NIHSEC/ P20-2232 (4)].

Availability of data and material

The data obtained from this study will not be shared with anyone except registered co-investigators for result discussion purposes either

for submitting a final report and publications purposes. No personal information will be disclosed, and subjects will not be identified.

Conflict of interest

The authors declare no conflicts of interest.

Source of funding

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Authors' contributions

1. LPS contributed in data collection, review of results, interpretation, statistical analysis and submission for publication. 2. WYL contributed to conceptualization of study design and methodology, preparation of original draft. 3. MHMN and MABI contributed in approval, resources, data collection, editing and revision of the manuscript. All authors approved the final manuscript.

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