

Evaluation of age variation changes in cervical vertebrae: 2-Dimensional (2D) geometric morphometrics approach

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ABSTRACT

Age estimation in adults is a complicated task because of various external factors occur concurrently with increasing age. The geometric morphometric method (GMM) is an approach that focuses on shape analysis and is widely recognized for its high reliability and reproducibility. The aim of this study was to explore the variation of cervical vertebrae among different age groups of the Malaysian population by GMM. Lateral skull radiographs of 432 subjects comprising four adult age groups; young adult age group (20–30 years old), early middle age group (31–40 years old), late middle age group (41–50 years old) and, elder adult age group (51–60 years old) were selected. Fifty-three 2-dimensional (2D) landmarks were applied to the digitalized radiographs by TPSDig2 (Version 2.31) software. Geometric morphometric analysis was performed by MorphoJ software. Results showed that the first three principal components (PC) contributed to 47.71 % of the cervical vertebrae variation and were shown in both lollipop and wireframe graphs. Procrustes ANOVA indicated that the shape was significantly different among different age groups. Canonical variate analysis revealed significant differences of both mahalanobis and procrustes distances among age groups with substantial individual overlap within groups. Discriminant function analysis (DFA) showed a correct classification rate for 61.5 % of cases respective to age groups. In conclusion, this study found significant differences in the shape of cervical vertebrae among different age groups of the Malaysian population using the GMM.

1. Introduction

Forensic science is the application of empirical expertise and techniques to forensic investigation and legal issues. There are several branches of the field, and forensic anthropology is one of them [1]. Forensic anthropologist deals with human remains skeletons identification and assist in numerous medico-legal cases [2,3]. Their expert opinions on skeletonized subjects lead to frequent invitations by the legal authorities, which will help to establish the identity of those

severely decomposed and degraded human remains [4]. Four biological profiles that are critically required for human identification are sex, ancestry, age, and stature [5].

Age estimation is the most challenging task faced by forensic anthropologists [6] and various methods have been discussed in the last decades to achieve the objective [7]. The skeletal age cannot be assumed equal to chronological age as the former is highly affected by other external factors such as nutrition, exercise, drugs, climates, etc. [8]. Thus, a comprehensive understanding of the relationship between the

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Table 1
Landmark definitions of cervical vertebrae on lateral cervical radiograph.

Cervical Vertebrae	Anatomical landmarks (P)	Definitions
First cervical (C1)	P1	The most anterior point of the vertebra
	P2	The most anterior and superior point of the vertebra
	P3	The most posterior and superior point of the vertebra
	P4	The most posterior point of the vertebra
	P5	The most posterior and inferior point of the vertebra
	P6	The most anterior and inferior point of the vertebra
Second cervical (C2)	P7	The most anterior point of the vertebra
	P8	The most posterior point of the vertebra
	P9	The most posterior and inferior point of the vertebra at the articular surface of the vertebra process
Third cervical (C3)	P10	The most anterior and superior corner of the C3 body
	P11	The most posterior and superior corner of the C3 body
	P12	The most posterior and inferior corner of the C3 body
	P13	The most anterior and inferior corner of the C3 body
	P14	The most anterior and superior articular process
	P15	The most posterior and superior articular process
	P16	The most posterior and inferior articular process
	P17	The most anterior and inferior articular process
	P18	The most superior anterior corner of the spinous process
P19	The most inferior anterior corner of the spinous process	
Forth Cervical (C4)	P20	The tip of the spinous process
	P21	The most anterior and superior corner of C4 body
	P22	The most posterior and superior corner of C4 body
	P23	The most posterior and inferior corner of C4 body
	P24	The most anterior and inferior corner of C4 body
	P25	The most anterior and superior articular process
	P26	The most posterior and superior articular process
	P27	The most posterior and inferior articular process
	P28	The most anterior and inferior articular process
	P29	The most superior anterior corner of spinous process
	P30	The most inferior anterior corner of spinous process
Fifth cervical (C5)	P31	The tip of the spinous process
	P32	The most anterior and superior corner of the C5 body
	P33	The most posterior and superior corner of the C5 body
	P34	The most posterior and inferior corner of the C5 body
	P35	The most anterior and inferior corner of the C5 body
	P36	The most anterior and superior articular process
	P37	The most posterior and superior articular process
	P38	The most posterior and inferior articular process
	P39	The most anterior and inferior articular process

Table 1 (continued)

Cervical Vertebrae	Anatomical landmarks (P)	Definitions
Sixth cervical (C6)	P40	The most superior anterior corner of the spinous process
	P41	The most inferior anterior corner of the spinous process
	P42	The tip of the spinous process
	P43	The most anterior and superior corner of the C6 body
	P44	The most posterior and superior corner of the C6 body
	P45	The most posterior and inferior corner of the C6 body
	P46	The most anterior and inferior corner of the C6 body
	P47	The most anterior and superior articular process
	P48	The most posterior and superior articular process
	P49	The most posterior and inferior articular process
	P50	The most anterior and inferior articular process
	P51	The most superior anterior corner of the spinous process
	P52	The most inferior anterior corner of the spinous process
P53	The tip of the spinous process	

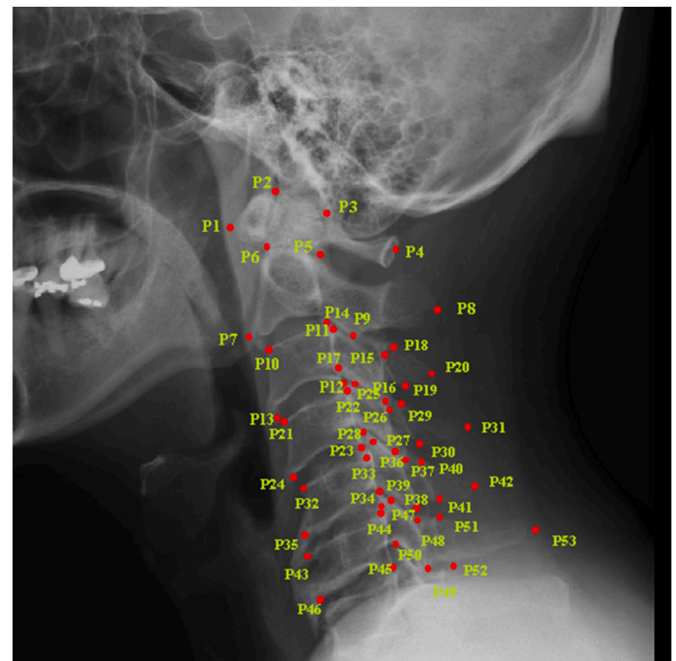


Fig. 1. Landmarks on the representative lateral cervical radiograph.

Table 2
Demographic profile of the subjects.

Demographic criteria	Incidence (N-432)	Percentages (%)	Cumulative Percentages (%)
Age Group			
20–30 years old	137	31.7	31.7
31–40 years old	101	23.4	55.1
41–50 years old	85	19.7	74.8
51–60 years old	109	25.2	100.0

Table 3
Reliability tests (Intra and inter-observer errors).

Landmarks	Intra-observer		Inter-observer	
	p-value	SEM	p-value	SEM
1	1.000	0.025	0.765	0.023
2	1.000	0.014	0.656	0.020
3	1.000	0.031	0.889	0.029
4	1.000	0.042	0.756	0.034
5	1.000	0.030	0.696	0.028
6	0.988	0.026	0.744	0.019
7	1.000	0.029	0.690	0.035
8	1.000	0.014	0.432	0.022
9	1.000	0.011	0.873	0.019
10	1.000	0.025	0.800	0.019
11	1.000	0.028	0.723	0.022
12	1.000	0.023	0.239	0.035
13	1.000	0.021	0.321	0.048
14	1.000	0.049	0.085	0.025
15	1.000	0.026	0.999	0.031
16	0.949	0.023	0.873	0.014
17	1.000	0.030	0.811	0.053
18	1.000	0.030	0.760	0.010
19	1.000	0.028	0.543	0.017
20	1.000	0.047	0.891	0.013
21	1.000	0.020	0.732	0.023
22	1.000	0.024	0.233	0.017
23	1.000	0.050	0.543	0.032
24	1.000	0.025	0.734	0.043
25	1.000	0.024	0.987	0.030
26	1.000	0.015	0.764	0.024
27	1.000	0.022	0.717	0.018
28	1.000	0.031	0.342	0.026
29	0.965	0.045	0.873	0.032
30	1.000	0.031	0.675	0.012
31	1.000	0.031	0.564	0.034
32	1.000	0.021	0.887	0.020
33	0.976	0.025	0.402	0.031
34	1.000	0.047	0.519	0.036
35	1.000	0.032	0.872	0.023
36	1.000	0.045	0.765	0.037
37	1.000	0.045	0.781	0.032
38	1.000	0.034	0.543	0.013
39	1.000	0.028	0.453	0.028
40	1.000	0.023	0.736	0.023
41	1.000	0.021	0.712	0.021
42	1.000	0.049	0.651	0.032
43	1.000	0.026	0.710	0.026
44	1.000	0.014	0.176	0.014
45	1.000	0.011	0.328	0.031
46	1.000	0.025	0.533	0.025
47	1.000	0.028	0.267	0.028
48	1.000	0.023	0.167	0.025
49	1.000	0.021	0.650	0.013
50	1.000	0.049	0.652	0.034
51	0.976	0.026	0.145	0.022
52	1.000	0.013	0.967	0.015
53	0.875	0.043	0.285	0.047

SEM-Standard Error Mean.

p-value <0.05 is a significant level.

factors and how they affect the skeletons are crucial for accurate age estimation [9]. Age estimation of the juvenile groups is easier as the bone is actively growing, therefore it provides remarkable skeletal structural changes [10]. Meanwhile, adults are more susceptible to external environmental changes and prolonged usage i.e. degenerative changes due to excessive wear and tear [7]. Apart from that, there is a lack of methods to produce a clear-cut age range when assessing the skeletal parts due to a vast number of classifications [8].

Some bones are frequently studied for adult age estimation. The pubic symphysis is the most common bone selected due to its aging-associated structure variation [11]. The Suchey-Brooks method [12] which classified pubic symphyseal aging into six phases has been applied until today by forensic anthropologists. In addition, the auricular surface of the ilium is also assessed for age estimation in adults due

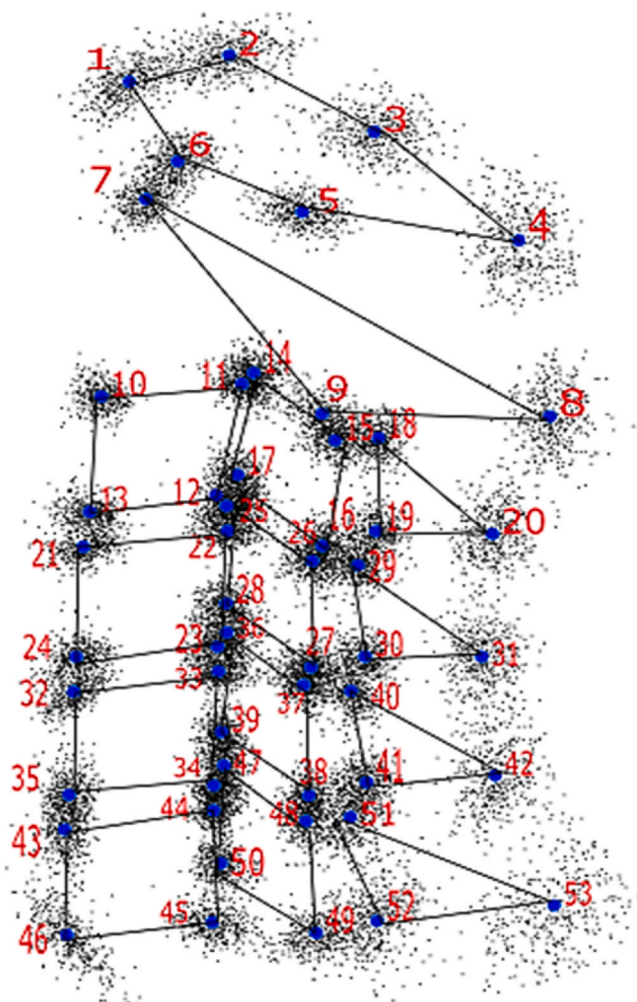
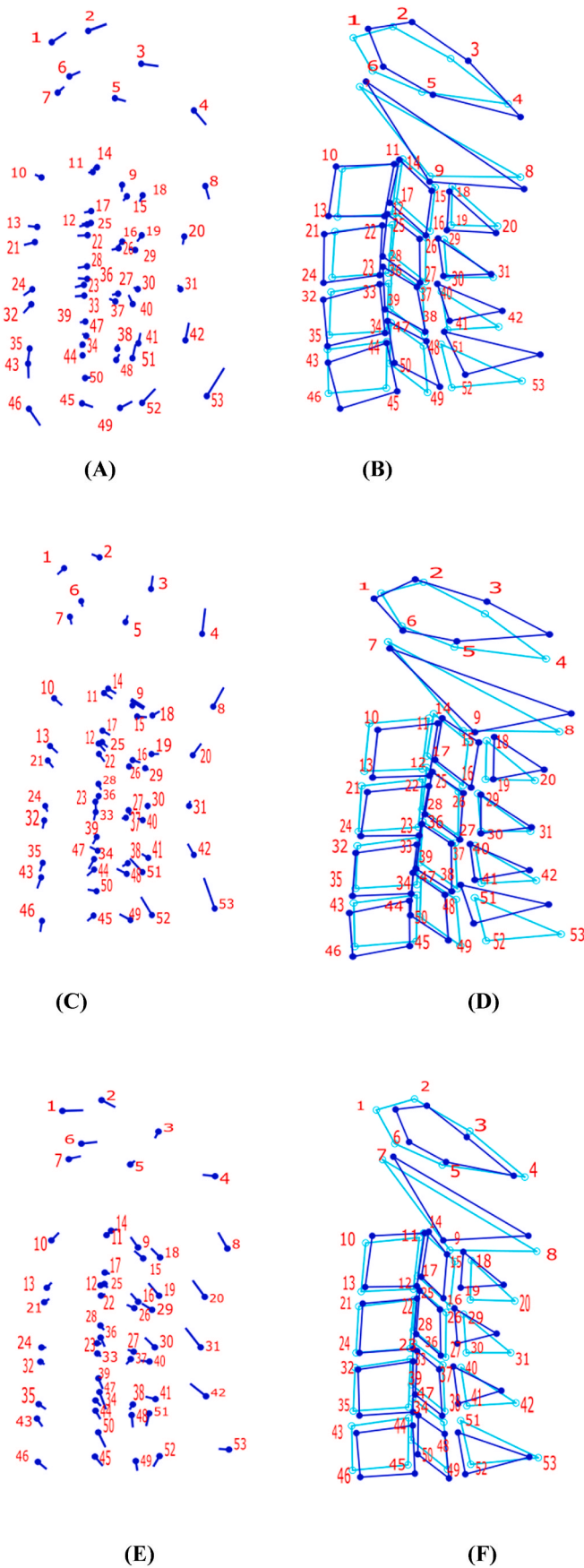


Fig. 2. Generalize procrustes analysis of cervical vertebrae from lateral view; Blue dot indicates mean landmark, small dot indicates individuals' landmark, and dark line indicates the outline of the vertebrae. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to its well-capsulated location [9]. Apart from osteological analysis, other methods such as dentition, histological, biochemical and, taphonomic assessment are increasingly employed for age estimation [13].

However, for accurate age estimation, a well-described morphological feature of the bones is required quantitatively and qualitatively [14–16]. Geometric morphometrics (GM) is an approach that meets the criteria as it is able to preserve the shape of the bones before analysis [17]. This is implemented by utilizing Cartesian coordinates data of the anatomical landmarks from respective bones [15]. In comparison with traditional morphometrics, GM is known for lower measurement errors and consistent homologous landmarks due to size standardization by rotation, scaling, and rotation [16,18,19]. Cervical vertebrae are useful in forensic anthropology when human remains are fragmented and impossible for morphological assessment [20,21]. Apart from bones present in large numbers, the exposed surface area of the cervical vertebra is relatively small compared to other bones such as hip bones. Thus, their surfaces are more well preserved in the degeneration process [22,23]. Cervical vertebrae age is also highly correlated with skeletal age of hand-wrist radiographs, manifesting the reliability of its usage for age estimation [24].

To date, few morphometrics studies have been conducted to estimate age using skeletal parts such as mandibular central incisors [25], hip bones [26], acetabulum [27], mandible [28], and cervical vertebrae



(caption on next column)

Fig. 3. First three PCs; (A) Lollipop graph of PC1 with blue dots represented the mean shape of the vertebrae and the line demonstrated how far the variation of the PC1 with the mean shape, (B) Wireframe graph of PC1 with the light blue represented mean shape of the vertebrae and the dark blue represented the shape of the PC1, (C) Lollipop graph of PC2 with blue dots represented the mean shape of the vertebrae and the line demonstrated how far the variation of the PC2 with the mean shape, (D) Wireframe graph of PC2 the light blue represented mean shape of the vertebrae and the dark blue represented the shape of the PC2, (E) Lollipop graph of PC3 with blue dots represented the mean shape of the vertebrae and the line demonstrated how far the variation of the PC3 with the mean shape, and (F) Wireframe graph of PC3 the light blue represented mean shape of the vertebrae and the dark blue represented the shape of the PC3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[29]. However, there are limited GM studies performed on the Malaysian population that specifically aimed for age estimation and variation of vertebrae. Therefore, the objective of this study was to explore the variation of cervical vertebrae morphology among four adult age groups of the Malaysian population using the 2-dimensional (2D) GM method. This present study hopes to compile an extensive database of 2D cervical vertebrae morphology from lateral cervical radiographs, that can assist in forensic investigations in the near future.

2. Materials and methods

2.1. Study design

This is a retrospective observational study. Ethical approval was obtained from the Medical Research Ethics Committee, Pusat Perubatan University Malaya (PPUM) with reference number (MREC ID NO: 201944-7288). A total of 432 lateral skull radiographs of the adult Malaysian population were taken retrospectively from the PPUM database via Picture Archiving and Communication System (PACS) dated from July 2017–July 2019. Inclusion criteria consist of good quality lateral cephalograph radiographs of adult Malaysian population aged equal and more than 20 years old of both male and female patients of three main ancestries (Malay, Chinese, and Indian). Patients who were non-Malaysian or had cervical bones abnormalities, fractures, or a history of cervical surgery were excluded. The samples were divided into four age groups: young adults (20–30 years old), early middle-aged adults (31–40 years old), late middle-aged adults (41–50 years old), and elder adults (51–60 years old).

2.2. Materials

The TPSdig2 (Version 2.31 software) was used for landmarks application on respective digitalized radiographs meanwhile MorphoJ (version 1.06d) software was applied for data analysis [30,31]. Other software namely, Excel, Inscape, and Notepad ++ were used for managing the data.

2.3. Landmark application

This study examined the first cervical vertebra (C1) to the sixth cervical vertebra (C6) as the seventh cervical vertebra (C7) was mostly obscured by shoulder shadow from lateral skull radiograph images and might not appear clearly on the radiograph [32]. There were fifty-three 2D landmarks identified on the digitalized radiograph adapted from the previous study [33–35]. The definition of every landmark is presented in Table 1 and Fig. 1.

2.4. Reliability tests

To confirm the reliability of this method, both intra-observer and inter-observer errors were analyzed. The intra-observer error rates were

Table 4

Procrustes ANOVA of centroid size and shape of age groups.

Effect	SS	MS	df	F	p-value
Shape	0.0695859	0.000227405	306	2.60	<0.001*
Centroid size	73171.48	24390.49	3	1.44	0.230

SS-Sum of Squares, MS-Mean Squares, df-Degree of freedom, F-Goodall's value of statistics.

*p-value<0.05 is significant level.

Table 5

CV's Eigenvalues and percentage of the variance of age groups.

CV	Eigenvalues	Variance (%)	Cumulative percentage (%)
CV1	1.232195	65.87	65.87
CV2	0.374148	20.00	85.87
CV3	0.264367	14.13	100.00

calculated by re-measuring ten radiographs one week after the initial measurements were recorded. Meanwhile, the inter-observer error rates were calculated with the aid of another research assistant. The measurements were analyzed using paired T-test and independent T-test respectively.

2.5. Statistical analysis

Shape analysis was performed mainly by Morpho J (version 1.06d). The initial step involved size standardization of the samples by generalized procrustes analysis (GPA). Non-size factors such as size, orientation, and rotation were discarded [17]. The procrustes superimposition yielded the centroid size representing the biological expression of the object [36] which is defined as the square root of the sum of the squared distance of the landmarks involved from their centroid [16]. Principal component analysis (PCA) was used to assess the shape variance percentages in the total samples. Size and shape differences were assessed by Procrustes Anova. Canonical variate analysis (CVA) was applied to assess shape changes within the groups according to positive and negative directions. Discriminant function analysis (DFA) was used for accurate classification percentages with validation of procrustes distances using a repeated permutation test (10000x) [30].

3. Results

3.1. Demographic data

A total of 432 lateral cervical radiographs were retrieved from the database. The data comprised Malay (240; 55.6%), followed by Indian (108; 25.0%) and Chinese (84; 19.4%) of both males and females of the Malaysian population aged between 20 and 60 years old (mean, 39.1 years; median, 38.0 years) (Table 2).

3.2. Reliability tests

There were no significant differences ($p > 0.05$) in term of procrustes coordinates of all fifty landmarks in both intra and inter-observer analysis manifested the consistency of landmarking application (Table 3). The method is considered to be reliable and reproducible.

3.3. Generalized procrustes analysis (GPA)

Fig. 2 shows a scatterplot of the superimposed landmarks configurations, indicating the general cervical vertebrae (C1 to C6) morphology from fifty-three landmarks of 432 cervical vertebrae radiographs.

3.4. Principal component analysis (PCA)

There were 102 principal components (PCs) responsible for 100% variation exhibited by cervical vertebrae. The first three principal components contributed to 47.71% of the cervical vertebrae variation and were shown in both lollipop and wireframe graphs (Fig. 3). In lollipop graphs, the PC landmarks variation from the average cervical vertebra's landmarks (represented by the blue dots) were displayed. In addition, the shape changes were also presented in the form of wireframe graphs. The light blue line was the average cervical vertebra shape whereas the dark blue line represented the PCs involved. The high amount of variation was exhibited at landmarks 4 and 53. The posterior tubercle of C1 (L4) protruded inferiorly in PC1 and superiorly in PC2, as compared to the mean cervical shape. In PC3, the protrusion of the posterior tubercle was aligned with the mean shape. Meanwhile, the spinous process of C6 (L53) was elevated and tapered upwards in both PC1 and PC2 as compared to the mean cervical shape.

3.5. Procrustes ANOVA

There was a significant difference in the shape ($p < 0.001$) of cervical vertebrae among different age groups with Goodall's F statistic (F) was 2.60. However, centroid size showed no significant difference in cervical vertebrae among age groups ($p = 0.230$, $F = 1.44$) (Table 4).

3.6. Canonical variate analysis (CVA)

Three canonical variates (CVs) were produced from the CVA. The CV1 represented 65.87% of shape variation, CV2 represented 20.0% of shape variation and CV3 represented 14.13% of shape variation among age groups (Table 5).

Fig. 4 showed the wireframe graphs of both CV1, CV2, and CV3. The dark blue line represented the CVs and the light blue line was the average shape of cervical vertebrae. The wire-frame graphs showed the comparison of mean shape in positive and negative directions with a scaling factor of -4 to 4 for both CV1 and CV2.

There was substantial individual overlap between age groups (Fig. 5). Shape changes corresponding to scores of -4 and 4 for CV1 and -4 and 4 for CV2 displayed the least individual overlap between 20 and 30 years old (youngest adult group) and 51–60 years old (eldest adult group). In term of cervical shape changes, the age group of 20–30 years old (youngest adult group) was distributed more on the positive direction whereas the age group of 51–60 years old (eldest age group) was distributed more on the negative direction of the CV1-axis. Both were distributed in the positive direction of the CV2-axis. The age group of 31–40 years old (early middle age) was distributed in the positive direction of the CV1-axis whereas the age group of 41–50 years old (late middle age) was distributed in the negative direction of the CV1-axis.

The highest mahalanobis distance (D^2) was exhibited between the age group of 20–30 years old and the age group of 51–60 years old which was 2.80 ($p < 0.001$). It showed that the cervical vertebrae size was highly different between the youngest age group with the eldest age group. Meanwhile, the highest procrustes distance (d) was exhibited between the age group of 20–30 years old and the age group of 41–50 years old which was 0.030 ($p < 0.001$) and it showed the cervical vertebrae shape was highly different between the youngest age group with the late middle age group (Tables 6 and 7).

3.7. Discriminant function analysis

A total of 89.7% of the samples were correctly classified into their age groups and 61.5% of the samples were correctly classified into their age groups after cross-validation. Specifically, 91.3% of the age group of 20–30 years old were correctly classified into their age group while after cross-validation the classification accuracy was 68.1%, 86.8% of the age group of 31–40 years old were correctly classified into their age

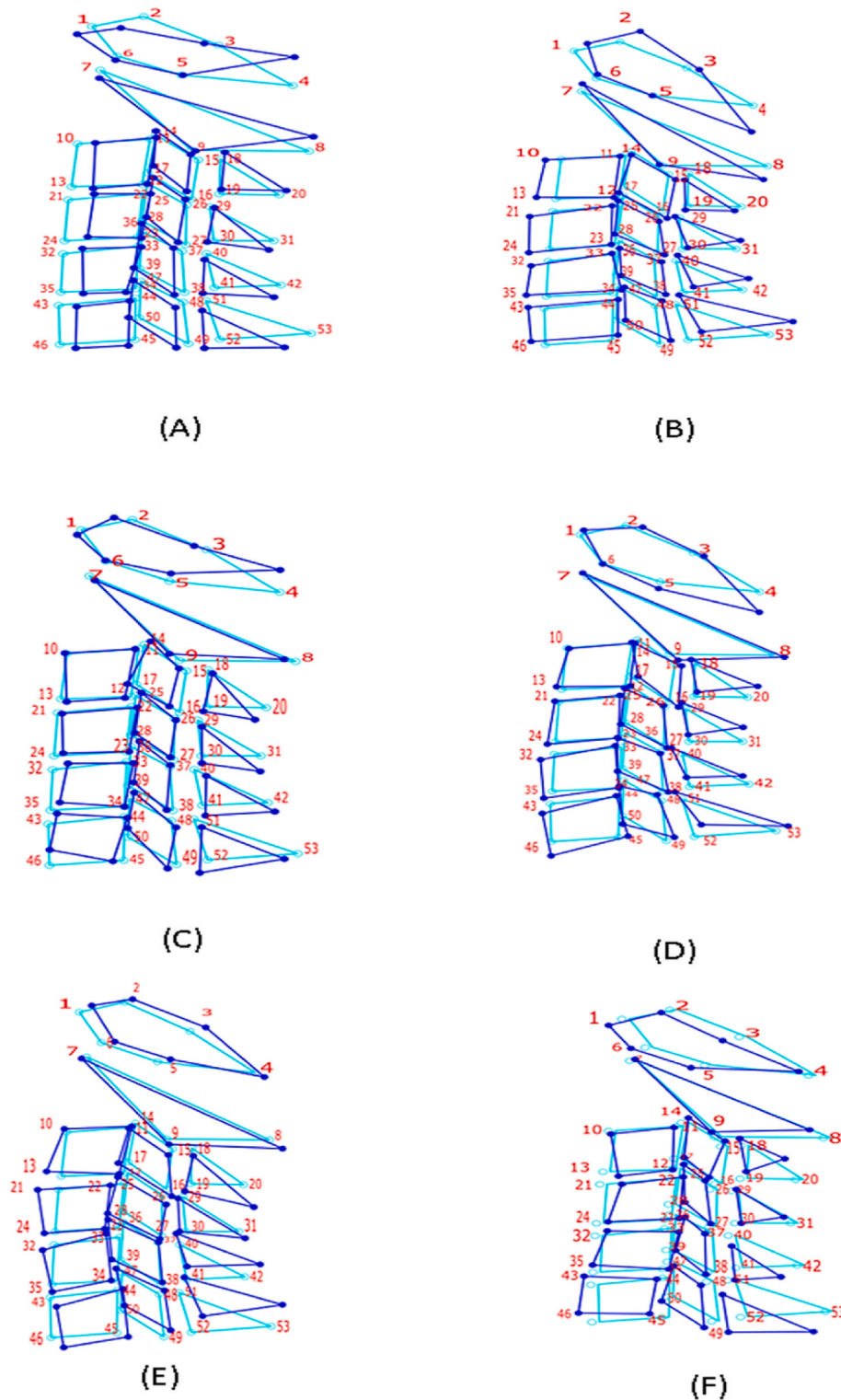


Fig. 4. Wireframe graphs of CVs with scaling factor -4 to 4 ; (A) CV1 in the positive direction, (B) CV1 in the negative direction, (C) CV2 in the positive direction, (D) CV2 in the negative direction, (E) CV3 in the positive direction and (F) CV3 in the negative direction.

group while the classification accuracy was 59.4 % after cross-validation, 87.5 % of the age group of 41–50 years old were correctly classified into their age group while the classification accuracy was 54.1 % after cross-validation and lastly 93.0 % of the age group of 51–60 years old were correctly classified into their age group while the classification accuracy was 64.5 % after cross-validation. (Table 8).

4. Discussion

Geometric morphometrics method (GMM) is an advanced statistical analysis of shape variation and its covariation with other variables [16, 17]. However, GMM has a few potential limitations and sources of error, such as the subjective identification of anatomical landmarks. The method requires the identification of anatomical landmarks, which can

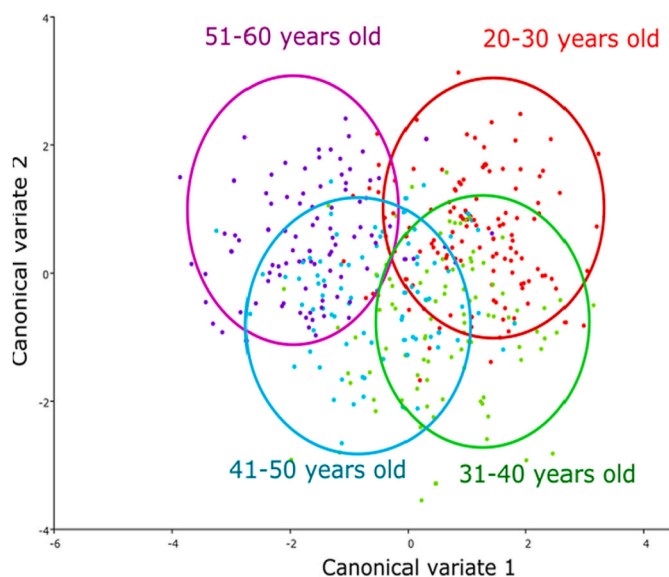


Fig. 5. Shape differences of age groups associated with canonical variate axes; There was substantial individual overlap between the age groups with the greatest overlap between age group of 31–40 years old (Green) and 41–50 years old (Blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Mahalanobis distance of age groups.

Age group	20–30	31–40	41–50
31–40	1.68 <i>p</i> < 0.001 ^a	–	1.90 <i>p</i> < 0.001 ^a
41–50	2.18 <i>p</i> < 0.001 ^a	1.90 <i>p</i> < 0.001 ^a	–
51–60	2.80 <i>p</i> < 0.001 ^a	2.55 <i>p</i> < 0.001 ^a	1.90 <i>p</i> < 0.001 ^a

^a *p*-value < 0.05 is significant level.

Table 7
Procrustes distance of age groups.

Age group	20–30	31–40	41–50
31–40	0.0145 <i>p</i> - 0.036*	–	0.0222 <i>p</i> - 0.015*
41–50	0.0300 <i>p</i> < 0.001*	0.0222 <i>p</i> - 0.015*	–
51–60	0.0287 <i>p</i> < 0.001*	0.0208 <i>p</i> - 0.022*	0.0141 <i>p</i> - 0.953

**p*-value < 0.05 is significant level.

be subjective and prone to error [17].

Radiography plays a pivotal role in the field of forensic anthropology, and its relevance endures to this day. In this context, conventional radiography has become a straightforward and economically feasible radiological method, offering a rapid and cost-effective approach compared to alternative modalities [32]. The application of X-rays in forensic anthropology serves as a fundamental tool for establishing the identity of unknown skeletal remains [24,28,29]. As forensic anthropology continues to advance, there is a growing recognition of the need for 3D imaging techniques. While conventional radiography has been invaluable, it is important to acknowledge the limitations that could be encountered in the 2D representations of complex anatomical structures [11,32]. The 3D imaging technologies, such as computed tomography (CT) scans and three-dimensional reconstructions, offer a more comprehensive and detailed view of skeletal remains. These techniques

allow for a thorough examination of anatomical features and can provide crucial insights into the identification process [9,10,37].

The present study has found significant differences in cervical vertebrae shape (*p* < 0.001) with substantial individual overlap within the groups. A total of 61.5 % of the samples were correctly classified into respective age groups.

There was a significant difference in cervical vertebrae shape among different age groups. This is in agreement with a previous study conducted among the Greek population which showed the vertebral shape of cervical vertebrae from lateral cephalograms was significantly associated with skeletal age by the GM analysis method [33]. Another study conducted on the Saudi’s population revealed a high correlation between cervical vertebral maturation stages of three cervical vertebrae (C1, C2, and C3) with chronological age [29]. In addition, the vertebral bodies of C3 and C4 demonstrated few spectrum of changes as the age increased such as from trapezoid to rectangular from metric evaluations of lateral cephalograms of German population [38]. Cervical vertebrae volumetric measurements of the Japanese population were also found to be strongly correlated with increasing age [39] indicating cervical vertebrae morphology is associated with age changes to an acceptable degree.

Our study revealed no significant difference in the centroid size of cervical vertebrae. A similar finding was found in a GM study conducted on acetabulum skeletal parts derived from the Iberian peninsula, which showed no differences in the centroid size among adult age groups [27]. Apart from that, centroid size was also not significantly different among adult age groups in a GM study performed on computed tomography (CT) scans of the rib cage bones [40].

DFA revealed that 61.5 % of the samples were correctly classified into their age groups after cross-validation. It was higher compared to the classification accuracy of age using teeth structures among Kosovo samples which was 60 % of accuracy [41]. In addition, a previous study that utilized patella bones of the Japanese population demonstrated lower classification accuracy of age ranging from 31.8 % to 60.7 % [42]. Different percentages of classification might be due to the unstandardized number of variables and equations applied for percentage calculation [23,43,44].

Dynamically, the vertebrae are one of the trabecular bones that undergo a remodeling process which consists of bone resorption and bone formation. As age increases, the balance of these two processes is disrupted, in which the latter will start to diminish. Therefore, the bone’s structures are affected geometrically such as thinning of trabeculae and enlargement of vascular canals [45]. For example, elderly people have profound degenerative changes in their cervical vertebrae as compared to the younger age groups i.e. loss of cervical spine curvature and osteophytic changes [37]. The mechanical properties of collagen network and bone microstructures are also reduced with increasing age [46] which could contribute to these changes.

Other factors such as nutritional status and dietary lifestyles are known to affect the age estimation of the bones [47]. The remodeling process observed histologically in the bones is influenced by nutritional status as well as physical activity [9]. Chronic diseases that associated with malnutrition such as lack of vitamin D could lead to abnormal growth patterns of the bones. Thus, the errors in age estimation are encountered [10,48]. This was supported by a previous study conducted on starved individuals which had found an extreme disruption of the mineralization process of the bones at the cortical parts [49]. In addition, the callus density and area were smaller in osteoporotic subjects compared to the control groups [50] manifesting the effect malnutrition on the geometric aspect of the bone.

5. Study limitations

Our study encountered limitations in analyzing the full view of cervical vertebrae. This is due to the inability of 2D radiographs to display the full orientation of the cervical vertebrae, which may lead to

Table 8
Discriminant function analysis of age groups.

Classification Result		Age Group (Years)	Predicted Group Membership				Total
			20–30	31–40	41–50	51–60	
Original	Count	20–30	375	19	12	5	411
		31–40	20	263	13	7	303
		41–50	7	13	223	12	255
		51–60	6	7	10	304	327
	%	20–30	91.3	4.6	2.9	1.2	100.0
		31–40	6.6	86.8	4.3	2.3	100.0
		41–50	2.7	5.1	87.5	4.7	100.0
		51–60	1.8	2.1	3.1	93.0	100.0
Cross Validated	Count	20–30	280	52	46	33	411
		31–40	47	180	50	26	303
		41–50	36	44	138	37	255
		51–60	33	35	48	211	327
	%	20–30	68.1	12.7	11.2	8.0	100.0
		31–40	15.5	59.4	16.5	8.6	100.0
		41–50	14.1	17.3	54.1	14.5	100.0
		51–60	10.1	10.7	14.7	64.5	100.0

unexposed certain anatomical landmarks. This might omit the important variations associated with age changes. In this case, 3D radiological imaging such as CT scans and Magnetic Resonance Imaging (MRI) are more appropriate to be used. Besides, there were no juvenile age group data i.e., children and adolescents for comparison. Therefore, it is suggested for future studies to include these age groups for a comprehensive assessment.

6. Conclusion

In conclusion, the study utilized geometric morphometric analysis to examine the variation in cervical vertebrae shapes among different age groups of the Malaysian population. The analysis revealed notable shape variations in the cervical vertebrae among diverse age groups, as ascertained through Procrustes ANOVA. Conversely, the centroid size did not exhibit any statistically significant differences. The principal component analysis (PCA) effectively encapsulated the most prevalent cervical shapes within the population, concisely represented by the initial three principal components (PC). Canonical variate analysis (CVA) underscored the most substantial distinctions, primarily observed between the youngest and eldest age groups, signified by the minimal group overlap. Lastly, discriminant function analysis (DFA) demonstrated a commendable degree of accuracy in classifying samples into their respective age groups based on cervical vertebrae morphology. This study could facilitate forensic anthropology investigation for biological profiling in the future.

Ethical statement

This is a retrospective observational study. Ethical approval was obtained from the Medical Research Ethics Committee, Pusat Perubatan University Malaya (PPUM) with reference number (MREC ID NO: 201944-7288).

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CRediT authorship contribution statement

Muhammad Faiz Mohd Fauad: contributed to the conceptualization, Methodology, and writing of the manuscript, contributed to data

collection, analysis, and interpretation, contributed to the literature review and editing of the manuscript. **Ku Mastura Ku Mohd Noor:** contributed to the conceptualization, Methodology, and writing of the manuscript, contributed to data collection, analysis, and interpretation, contributed to the literature review and editing of the manuscript, provided critical feedback and guidance throughout the research process, supervised the project and secured funding. **Aspalilah Alias:** contributed to the literature review and editing of the manuscript, provided critical feedback and guidance throughout the research process, supervised the project and secured funding. **Ker Woon Choy:** contributed to the conceptualization, Methodology, and writing of the manuscript, contributed to data collection, analysis, and interpretation, contributed to the literature review and editing of the manuscript, provided critical feedback and guidance throughout the research process, supervised the project and secured funding. **Wei Lin Ng:** contributed to the conceptualization, Methodology, and writing of the manuscript, contributed to data collection, analysis, and interpretation, contributed to the literature review and editing of the manuscript, provided critical feedback and guidance throughout the research process. **Eric Chung:** contributed to the conceptualization, Methodology, and writing of the manuscript, contributed to data collection, analysis, and interpretation, contributed to the literature review and editing of the manuscript, provided critical feedback and guidance throughout the research process. **Yuan Seng Wu:** contributed to the conceptualization, Methodology, and writing of the manuscript, contributed to data collection, analysis, and interpretation, contributed to the literature review and editing of the manuscript, provided critical feedback and guidance throughout the research process. **Noraina Hafizan Norman:** contributed to the literature review and editing of the manuscript, provided critical feedback and guidance throughout the research process.

Declaration of competing interest

No conflict of interest.

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Appendix A. Supplementary data

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