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Age related morphological variation of the maxilla: a 3-dimensional (3D) geometric morphometrics analysis

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Abstract

Background Age estimation is a critical component of human identification in forensic context. Recent advances in three-dimensional imaging and shape analysis have enabled the assessment of subtle age-related craniofacial variations beyond traditional methods. This study aimed to evaluate age-related maxillary shape variation using a geometric morphometrics (GM) approach based on three-dimensional head and neck computed tomography (CT) scans. A total of 413 adult CT scans with equal distribution of sex and representing three major ethnic groups (Malay, Chinese and Indian) were analysed and categorised into four age groups: 18–30, 31–40, 41–50, and 51–65 years. A total of 45 3D anatomical landmarks were placed on the maxillary bone. The landmarks were analysed using Procrustes ANOVA, canonical variate Analysis (CVA), discriminant function analysis (DFA). Finally, shape variations were visualised using IDAV landmark editor.

Result Procrustes ANOVA showed a statistically significant difference in the maxillary bone shape across different age groups ($p < 0.001$). CVA revealed observable shape differentiation between age groups along the canonical variate axes, while DFA achieved classification accuracies ranging from 70.8% to 78.4% in age group discrimination.

Conclusion There were significant variations in terms of the maxillary bone shape across different age groups with acceptable accuracy values. This population-specific data could be applied as forensic reference that tailored to Malaysian population.

Keywords Age, Geometric morphometrics (GM), CT, Variations

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Background

Age estimation is a fundamental component of the biological profile in forensic context (Ubelaker and Khosrowshahi 2019). This component plays a crucial role in both medicolegal investigations and human identification (Abualhija et al. 2024; Lynnerup 2013). Accurate age assessment assists in narrowing the pool of potential identities, particularly in cases involving unidentified remains, mass disasters, and missing persons (de Boer et al. 2019; Dirkmaat and Cabo 2016; Jayakrishnan et al. 2021). In adult individuals, age estimation remains challenging due to the progressive reduction of distinct developmental markers and highly influenced by degenerative skeletal changes (Zhang 2022).

Craniofacial structures have long been utilised in forensic age estimation, as they undergo gradual morphological modifications throughout adulthood (Desai et al. 2023; Shui et al. 2016). The maxillary bone, in particular, is a structurally complex and functionally significant component of the facial skeleton (Chen et al. 2021; Tolt and Nortje 2003). Various factors such as dental status and masticatory function may influence the morphology which making it a potential anatomical region for investigating age-related morphological variation (Tolt and Nortje 2003). However, traditional age estimation methods based on linear measurements or qualitative assessment are limited in their ability to capture subtle shape changes associated with ageing (Kouchi and Mochimaru 2011; Woon et al. 2019; Zulkifli et al. 2023).

Geometric morphometrics (GM) provides a robust framework for quantifying shape variation while preserving the geometric relationships among anatomical landmarks (McKeown and Schmidt 2013; Slice 2007). By analysing the spatial configuration of landmarks rather than linear distances, GM enables the detection of subtle morphological differences that may not be evident using conventional morphometric approaches (Mitteroecker and Gunz 2009). The integration of GM with three-dimensional (3D) imaging modalities, such as computed tomography (CT), allows for high-resolution, non-destructive analysis of craniofacial structures (Cooke and Terhune 2015; Richtsmeier et al. 2002) and offers improved reproducibility in forensic research (Fruciano 2016; Zelditch and Swiderski 2025).

Previous forensic studies applying GM have primarily focused on sexual dimorphism with relatively fewer investigations addressing age-related craniofacial shape variation (Freudenthaler et al. 2017; Soundarya et al. 2021; Tanikawa et al. 2016), particularly in the maxilla. Moreover, craniofacial morphology is known to exhibit population-specific characteristics, highlighting the importance of developing regionally relevant reference data for forensic applications (Ross et al. 2010; Wang et al. 2024). In the Malaysian context, comprehensive

GM-based studies examining age-related maxillary shape variation remain limited.

Therefore, the present study aimed to evaluate age-related variations in maxillary bone shape using a geometric morphometric approach based on 3D head and neck CT scans of Malaysian adults. By analysing landmark-based shape differences across defined adult age groups, this study could assess the potential of maxillary morphology for age estimation and later contribute to formation of a population-specific forensic reference data that are relevant to human identification.

Methodology

Study design

This cross-sectional study was conducted at the Department of Biomedical Imaging, Pusat Perubatan Universiti Malaya (PPUM). A total of 413 head and neck computed tomography (CT) scans were included, comprising adult individuals aged between 18 and 65 years. The sample consisted of an approximately equal distribution of sex, with 208 males and 205 females, and representation from three major ethnic groups in Malaysia, namely Malay ($n=145$), Chinese ($n=136$), and Indian ($n=132$). The samples were categorised into four adult age groups: 18–30, 31–40, 41–50, and 51–65 years.

Inclusion and exclusion criteria

The study included Malaysian adults aged between 18 and 65 years with available high-resolution head and neck CT scans demonstrating complete visualization of the maxilla and intact craniofacial structures. Only scans with clearly documented demographic information, including sex and ethnicity, were selected. CT images were excluded if they demonstrated motion artefacts, poor image quality, or incomplete anatomical coverage. In addition, individuals with a history of craniofacial trauma, congenital craniofacial anomalies, maxillofacial surgery, reconstructive procedures, or pathological conditions affecting the maxilla (such as tumors, cysts, or severe inflammatory lesions) were excluded to avoid distortion of anatomical landmarks and morphological measurements.

Landmarking

3D landmarking of the maxillary bone was performed using the Stratovan Landmark Editor (Version 2023), with a total of 45 anatomical landmarks digitised on each specimen as adapted from few studies (Radulesco et al. 2018; Schuh et al. 2020). Head and neck CT scans in standard DICOM format were imported into the software. To visualize the bony structures of the maxilla, a 3D volumetric isosurface mesh was generated within the software by adjusting the bone density attenuation threshold. Once the 3D surface mesh was rendered, a template of

Table 1 Landmarks at the maxillary bone

Landmarks (Right)	Landmark (Left)	Definition
Landmark 1	Landmark 24	Superolateral nasion.
Landmark 2	Landmark 25	Dacryon.
Landmark 3	Landmark 26	Medial orbital margin.
Landmark 4	Landmark 27	Proximal point of the infra orbital margin.
Landmark 5	Landmark 28	Lateral point of the infra orbital margin.
Landmark 6	Landmark 29	Zygorbitale.
Landmark 7	Landmark 30	The lowest end of slanted part of zygomaxillary suture.
Landmark 8	Landmark 31	Upper point zygomaxillary suture.
Landmark 9	Landmark 32	Lower point zygomaxillary suture.
Landmark 10	Landmark 33	Zygomaxillare.
Landmark 11	Landmark 34	Lateral zygomaxillary-ectomalare.
Landmark 12	Landmark 35	Medial zygomaxillary-ectomalare.
Landmark 13	Landmark 36	Ectomalare.
Landmark 14	Landmark 37	First molar tooth.
Landmark 15	Landmark 38	First pre-molar tooth.
Landmark 16	Landmark 39	Lateral incisor teeth.
Landmark 20	Landmark 40	Nasolaterale-the most lateral point of nasal aperture.
Landmark 21	Landmark 41	Upper point of nasal aperture.
Landmark 22	Landmark 42	End point of nasomaxillary suture.
Landmark 23	Landmark 43	Upper point of nasomaxillary suture.
Landmark 44	Landmark 45	Infraorbital foramen (IOF).

Table 2 Landmarks at the inter-maxillary suture

Landmarks	Definition
Landmark 17	Prosthion
Landmark 18	Mid-philtrum
Landmark 19	Anterior nasal spine

45 pre-defined anatomical landmarks was applied. The 3D landmarks were manually digitized on each specimen using a point-and-click interface directly onto the surface mesh according to strict anatomical definitions (Tables 1 and 2). To ensure high spatial accuracy and avoid errors, the 3D model was dynamically rotated, zoomed, and viewed from multiple anatomical planes (anterior, lateral, and inferior views) during the placement of each coordinate. The resulting raw 3D coordinate data (x, y, z) for all 45 landmarks across all 413 specimens were exported into the MorphoJ (Version 1.08.02) for subsequent GM analysis. The landmarks were showed in Fig. 1. To assess landmarking reliability, intra-observer and inter-observer error analyses were conducted, and no statistically significant differences were observed, indicating high measurement consistency. For inter-observer reliability, specimens were randomly selected and independently landmarked by two observers. Both observers are experienced anatomists with professional backgrounds as dental and medical lecturers at the university. The inter-observer agreement was evaluated to ensure consistency in landmark identification and reliability of the GM measurements.

Geometric morphometrics analysis

Geometric morphometric analysis was performed using MorphoJ (Version 1.08.02) software to analyse the shape variations (Klingenberg 2011). Generalised Procrustes Analysis (GPA) was done to remove non-shape variation related to size, orientation, and position (McKeown and Schmidt 2013; Polly 2018). Canonical variate analysis (CVA) was subsequently applied to evaluate shape differentiation among the defined age groups (Campbell and Atchley 1981), followed by discriminant function analysis (DFA) to assess age group classification performance (Friedman 1989; Ramos and Rickard Liow 2012). Age-related shape variations of the maxilla were visualised using the IDAV Landmark Editor.

Results

Generalised Procrustes Analysis (GPA) was performed to remove non-shape variation related to rotation, translation, and scaling, allowing assessment of true maxillary shape differences (Gunz 2019; Mitteroecker and Gunz 2009; Polly 2018; Slice 2007). Most landmarks showed consistent deviations around the mean configuration, with an even distribution of individual landmarks. Greater dispersion was observed at landmarks 6–10 and

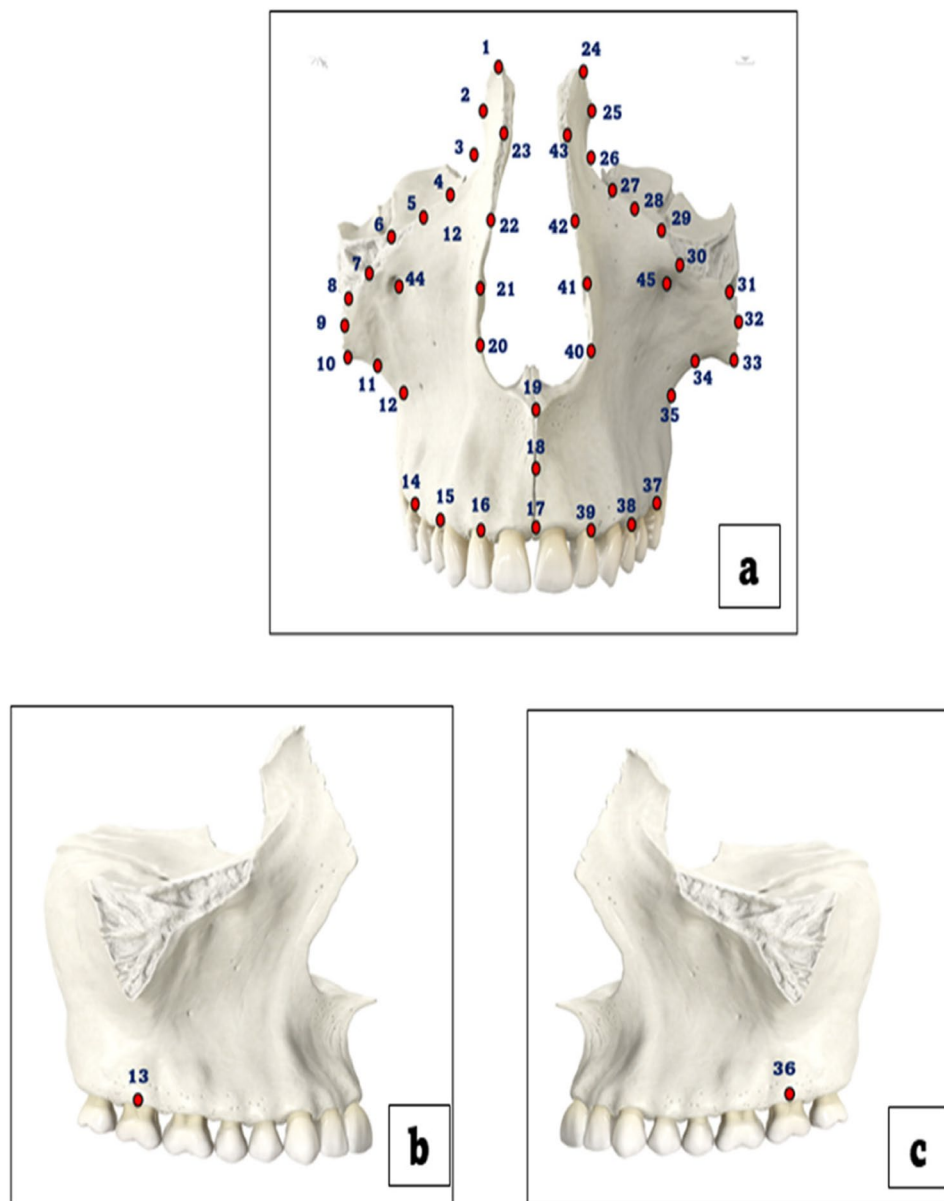


Fig. 1 Landmarks on: (a) Right and left anterior maxillary bone and intermaxillary suture; (b) Right and left ectomalare landmark from lateral view

29–33, indicating increased anatomical variability in the lateral maxillary region (Fig. 2).

Procrustes ANOVA was performed to assess shape variations of the maxillary bone based on the procrustes coordinates (Daboul et al. 2018). There was a significant shape variation in the maxillary bone across different age groups (Table 3).

CVA was also applied to four age groups: Group 1 (18–30 years old), Group 2 (31–40 years old), Group 3 (41–50 years old), and Group 4 (51–65 years old). The samples in each group were divided equally in terms of proportion. There were 104 samples in group 1, 103 samples in group 2, 103 samples in group 3, and 103 samples in group 4. CVA generated three CVs: CV1, CV2 and CV3

with their respective eigenvalues. CV1 accounted for the greatest shape variation of the maxillary bone within the age groups, which contributed to 41.11% of the total variations. The remaining CV2 and CV3 contributed to 34.26% and 24.63% of the total variations respectively (Table 4).

CV1 showed a high degree of variations at landmarks 29–34 and 37–36. This indicated that these variations occurred in the left maxillary bone, including the zygomatic maxillary suture and the left lateral alveolar process (Fig. 3).

For CV2, the variations were observed at landmarks 6–10 and 29–33 (Fig. 4). This CV indicated that the symmetrical shape variation involved both lateral sides of

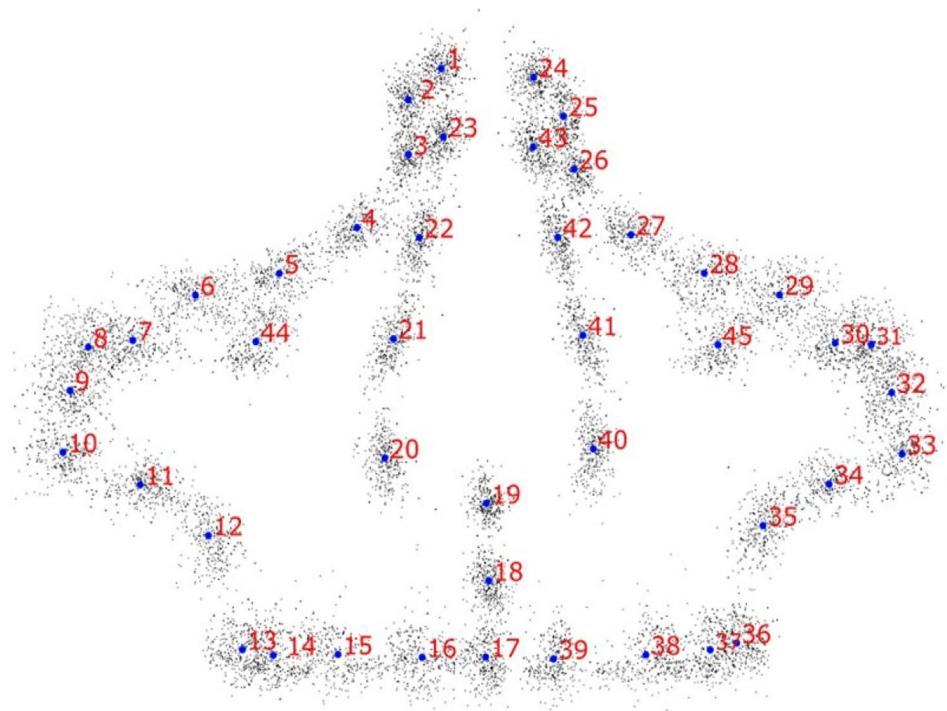


Fig. 2 Generalised Procrustes Analysis (GPA). Blue dots represent the mean landmark positions, while black dots indicate individual landmark coordinates. Increased dispersion is observed at landmarks 6–10 and 29–33

Table 3 Procrustes ANOVA of the maxillary shape

Effect	Sum of squares (SS)	Sum of Squares percentage (SS%)	Mean squares (MS)	df	F	p-value
Age Group	0.067	1.59	0.0001	384	1.65	<0.001*

$p < 0.05$, significant value

Table 4 Eigenvalues and percentage of variance of the age groups

CV	Eigenvalues	Variance (%)	Cumulative percentage (%)
CV1	0.8891	41.11	41.11
CV2	0.7410	34.26	75.37
CV3	0.5325	24.63	100.00

the maxillary body (right and left). From the wireframe graph, the lateral bodies were displaced outward relative to the average bone shape.

For CV3, the variations were also observed at landmarks 6–10 and 29–33 (Fig. 5). However, the wireframe graph revealed that the lateral bodies were displaced inward relative to the average bone shape.

For the age groups, the highest Mahalanobis distance ($D^2 = 2.597$) was observed between group 1 (18–30 years old) and group 4 (50–65 years old). It was followed by the distances between group 1 and group 3 (2.521), group 2 and group 4 (2.451), group 1 and 2 (2.332), group 2 and group 3 (2.204) and group 3 and group 4 (2.130). The permutation tests (10000 permutation rounds) were significant among all age groups (Table 5).

Similarly, the highest Procrustes distance was observed between group 1 (18–30 years old) and group 4 (50–65

years old) with a Procrustes distance's value of 0.027. It was followed by the distances between group 1 and group 3 (0.024), group 1 and group 2 (0.020), group 2 and group 4 (0.017), group 2 and group 3 (0.015), and group 2 and group 4 (0.013). The permutation tests (10000 permutation rounds) for Procrustes distances were significant among all age groups (Table 6).

Figure 6 showed the shape distribution of the maxillary bone along the CV axes. Group 1 (18–30 years old) was distributed toward the positive direction along the CV1 axis. The group 4 (51–65 years old) was distributed more on the negative direction along the same CV axis. Meanwhile, group 2 (31–40 years old) and group 3 (41–50 years old) occupied intermediate positions between them. The variations among the age groups were also captured along the CV2 axis. Both group 2 and group

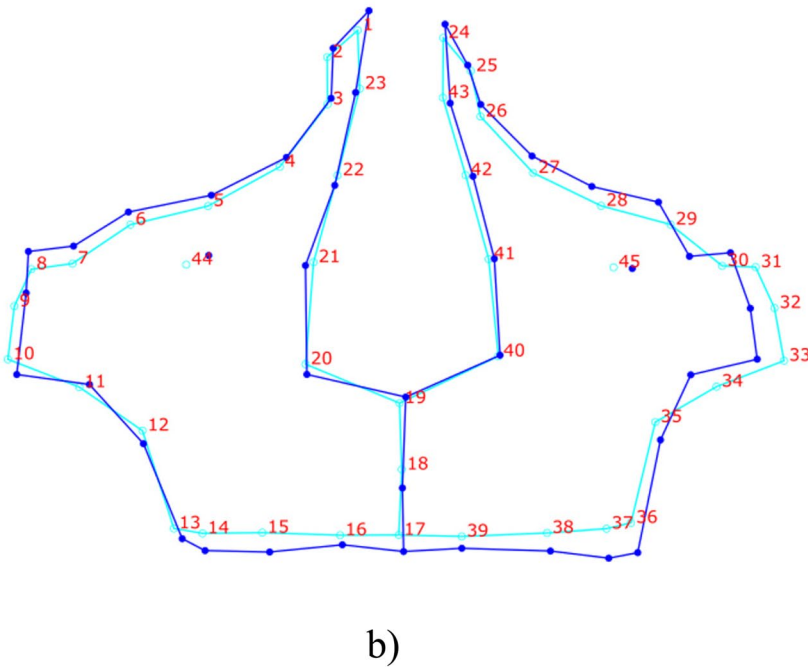
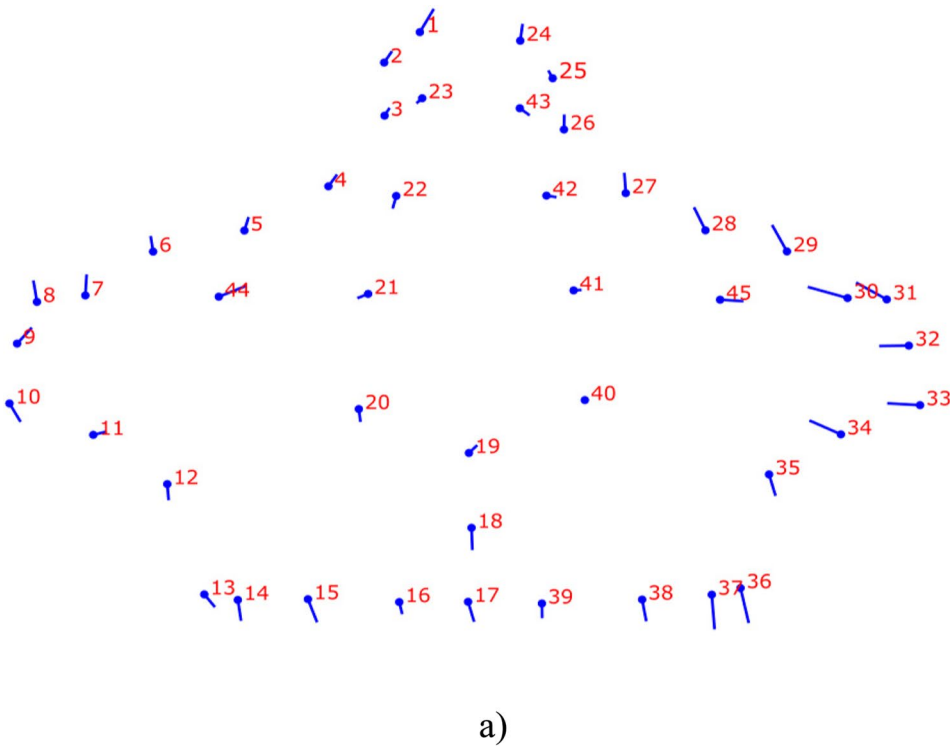


Fig. 3 CV1 shape changes showed in **a)** lollipop and **b)** wireframe graphs

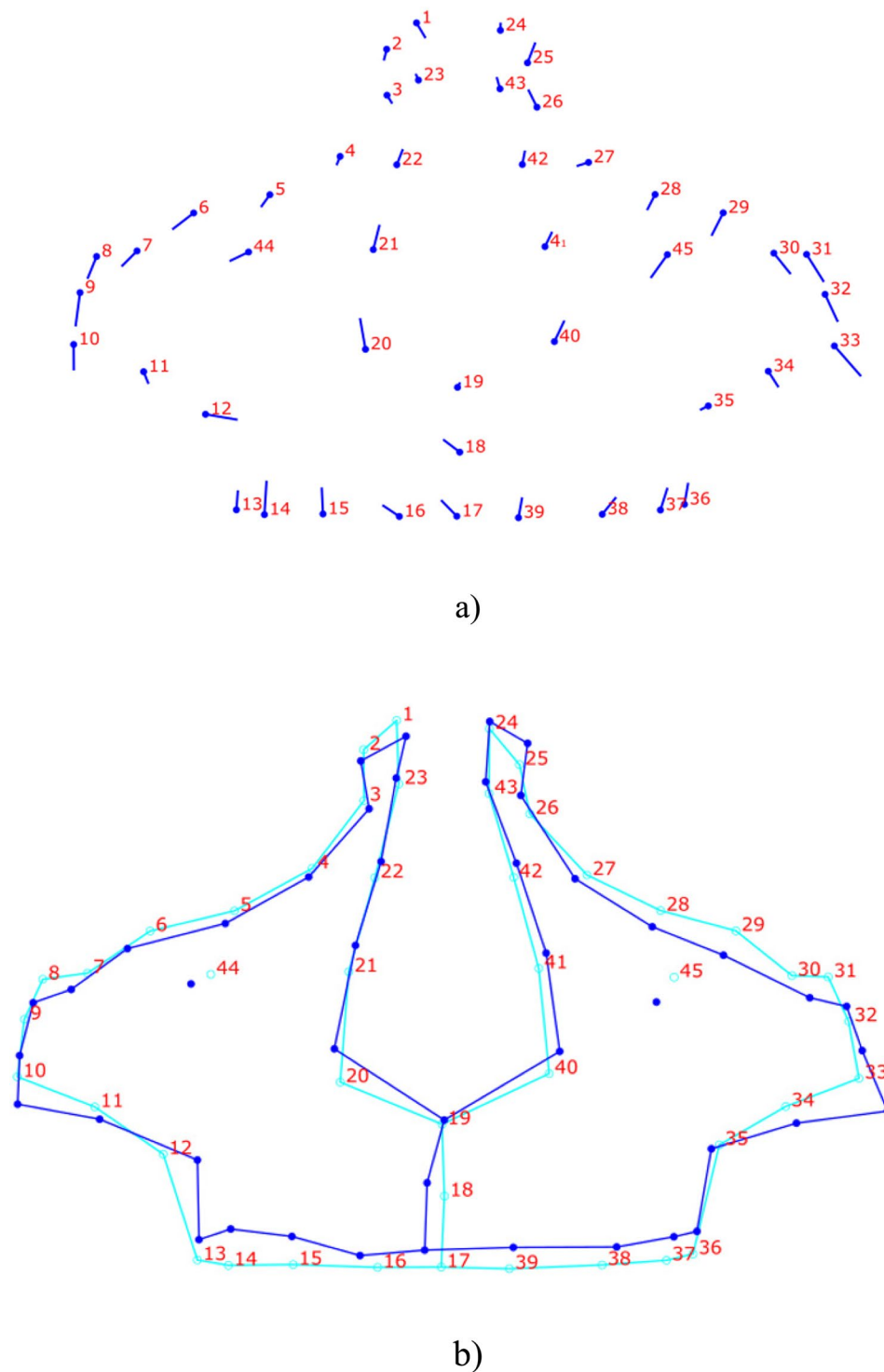


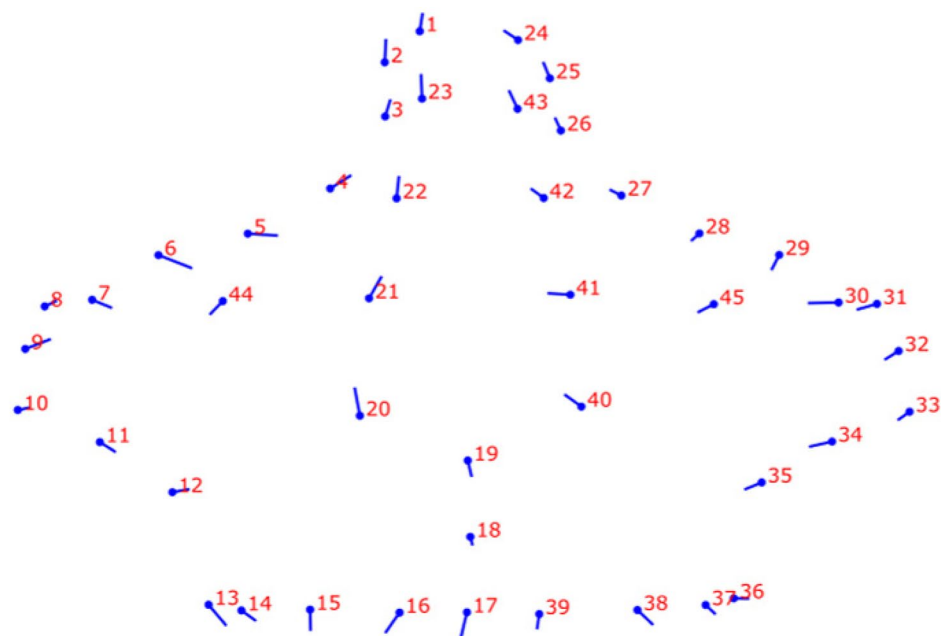
Fig. 4 CV2 shape changes showed in **a)** lollipop and **b)** wireframe graphs

3 were distributed more toward the positive direction along the CV2 compared to groups 1 and 4.

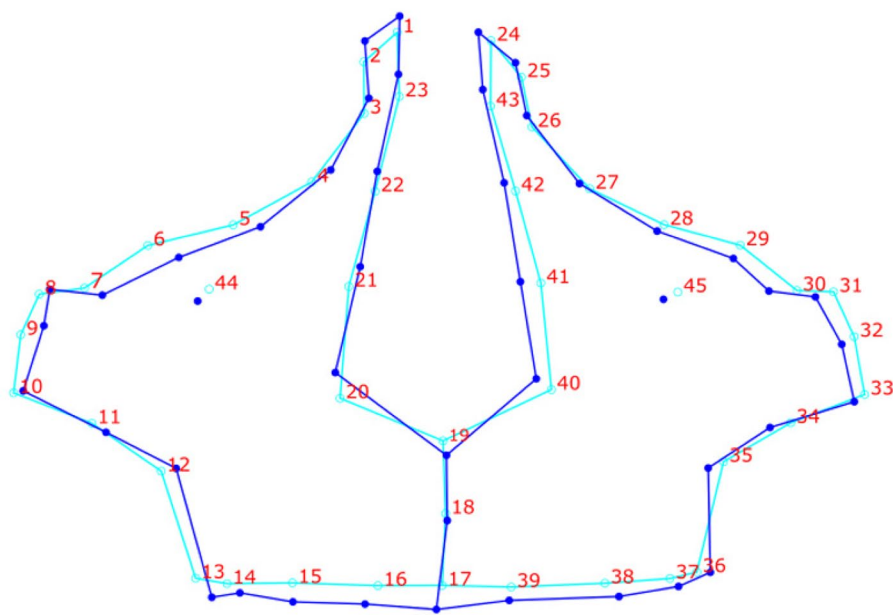
Morphing of maxillary bone for age group

The visualization of the maxillary bone shape according to different age groups was performed using IDAV

landmark editor (Stadt et al. 2007). The mean shape of each age group was integrated with the CV1 coordinates as this CV represented the greatest variation among age groups (Campbell and Atchley 1981; Gardner et al. 2006).



a)



b)

Fig. 5 CV3 shape changes showed in **a)** lollipop and **b)** wireframe graphs

Figure 7 showed the maxillary bone shape of each age groups. For the youngest age group (18–30 years old), the lateral margins of the bone exhibited a more pronounced curvature than in the other groups.

The alveolar process appeared broader compared to other groups. The surface showed rougher surface as the age increases, with the eldest group (51–65 years old) demonstrated the most prominent surface

Table 5 Mahalanobis distance of the age group

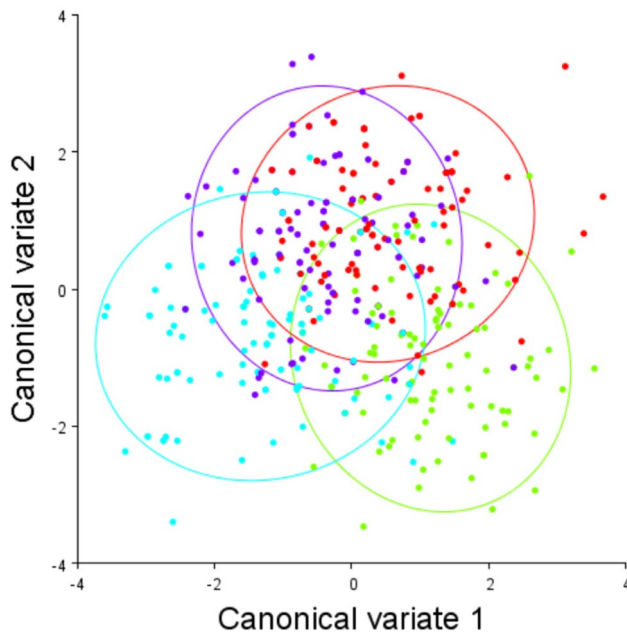
Age group	Group 1(18–30 years old)	Group 2 (31–40 years old)	Group 3 (41–50 years old)
Group 2 (31–40 years old)	2.332 $p < 0.001^*$	-	2.204 $p < 0.001^*$
Group 3 (41–50 years old)	2.521 $p < 0.001^*$	2.204 $p < 0.001^*$	-
Group 4 (51–65 years old)	2.597 $p < 0.001^*$	2.451 $p < 0.001^*$	2.130 $p < 0.001^*$

*p-value < 0.05 is significant

Table 6 Procrustes distance of the age group

Age group	Group 1(18–30 years old)	Group 2 (31–40 years old)	Group 3 (41–50 years old)
Group 2 (31–40 years old)	0.020 $p < 0.001^*$	-	0.015 $p < 0.001^*$
Group 3 (41–50 years old)	0.024 $p < 0.001^*$	0.015 $p < 0.001^*$	-
Group 4 (51–65 years old)	0.027 $p < 0.001^*$	0.017 $p < 0.001^*$	0.013 $p < 0.001^*$

*p-value < 0.05 is significant

**Fig. 6** Shape distribution along CV1 and CV2 axis showing a substantial overlap among all age groups (Green-Group 1; Blue-Group 4; Purple-Group 2; Red-Group 3)

irregularities. The IOF exhibited a smaller aperture in the eldest group (51–65 years old) compared to other age groups.

From lateral views, alveolar process showed a smoother surface in the youngest age group compared to the other groups. Otherwise, the variations captured from these views were subtle (Figs. 8 and 9).

DFA with cross-validation yielded classification accuracies of 78.4%, 71.9%, 70.8%, and 76.3% for Group 1, Group 2, Group 3, and Group 4, respectively (Table 7).

Discussion

Malaysia is a multi-ethnic country comprises various facial features (Alazzawi et al. 2012; Chan et al. 2020; Tan et al. 2021). Although previous studies have investigated on the skeletal age variations, most were conducted on populations outside Malaysia. Due to that, a population-specific data is required to facilitate the identification process of the remains within Malaysian context (Chan et al. 2020; Chin et al. 2013; Miller 2018). Hence, the method is more reliable and reproducible as the differences within the population are addressed (Agbolade et al. 2020; Chin et al. 2013).

This study applied several analyses to investigate maxillary bone variations across four adult age groups (18–30, 31–40, 41–50, 51–65 years) of the adult Malaysians. Procrustes ANOVA revealed significant differences in the maxillary bone shape among age groups. CVA identified three canonical variates (CV) representing the primary shape differences, with the greatest Mahalanobis and Procrustes distances observed between the youngest (18–30 years) and eldest (51–65 years) groups, indicating maximal morphological variation between these two age groups.

Visualization of the maxilla showed subtle age-related differences. In the youngest group, the lateral margins exhibited increased curvature and the alveolar process appeared broader. Surface roughness increased with age, with the eldest group displaying the most pronounced irregularities. Additionally, the infraorbital foramen (IOF) aperture was smaller in the eldest group compared to younger individuals.

To date, no GM study has assessed the entire maxillary shape across age. Previous studies focused on partial structures such as the maxillary sinus (Al-Zahrani et al. 2020); Guo et al. 2025; Velasco-Torres et

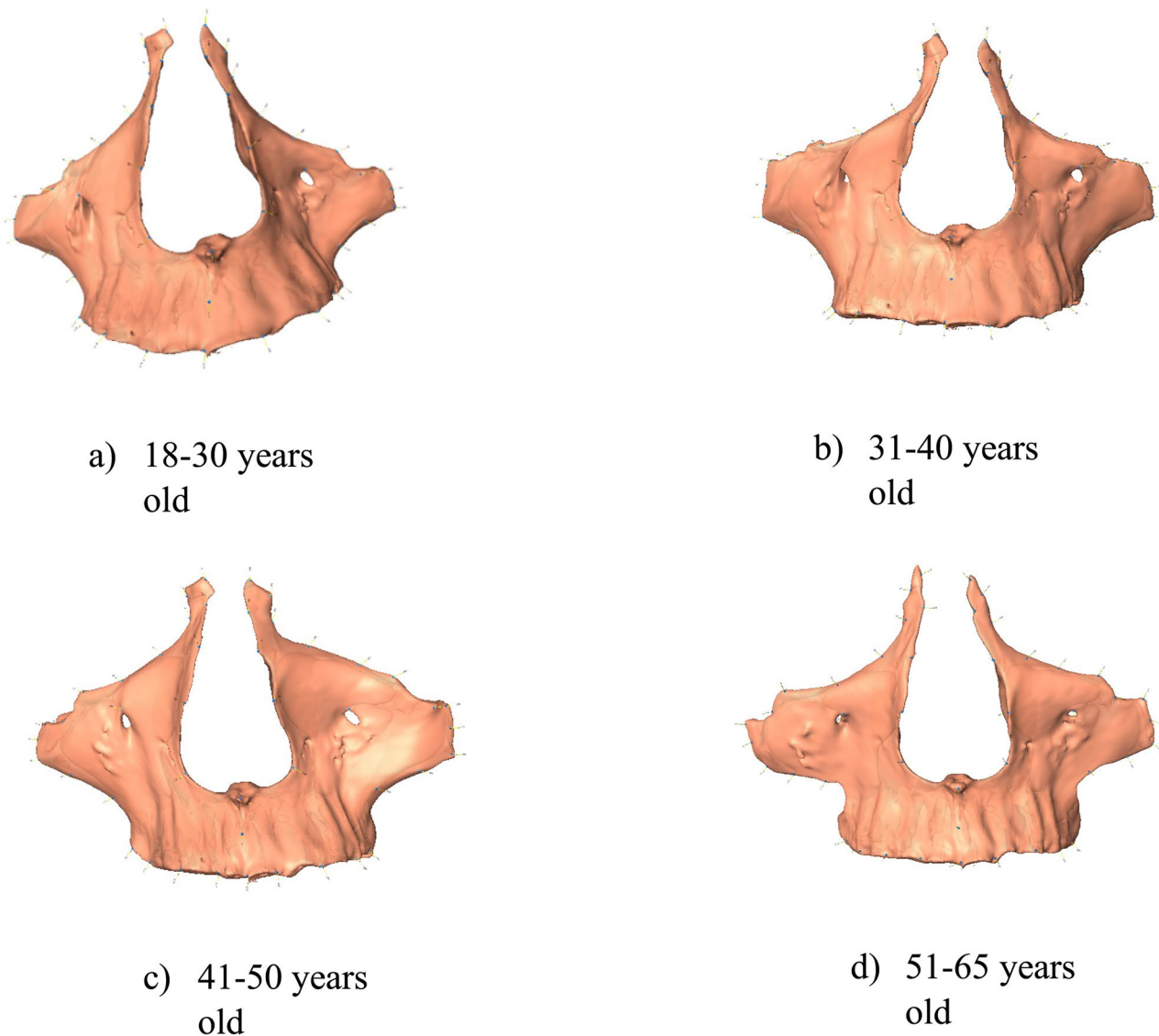


Fig. 7 Maxillary shape changes based on age group (anterior) showing subtle differences

al. 2017; Wu et al. 2022), dental structures (Karobari et al. 2024); Neves et al. 2024), and sutures (Kobayashi et al. 2023); Sinthubua et al. 2016). For example, cone beam computed tomography (GM.

) studies of Chinese and Spanish populations reported reduced maxillary sinus size in older individuals, partly due to decreased pneumatization (Guo et al. 2025; Velasco-Torres et al. 2017); Wu et al. 2022). Septa prevalence within the sinus was also higher in elderly individuals, often involving multiple sites (Al-Zahrani et al. 2020).

Similarly, age-related changes were observed in maxillary dental structures. In Arab patients, the position of premolar root canals correlated with age (Karobari et al. 2024). In Iraqi subpopulations, enamel thickness decreased and pulp volume reduced with age in central and lateral incisors (Al-Zahawi et al. 2023; Kazzaz et

al. 2025). These findings highlight that both the skeletal and dental components of the maxilla undergo measurable morphological changes with ageing.

In the present study, DFA was used to assess age classification based on maxillary bone morphology, yielding cross-validated accuracies of 78.4% (Group 1), 71.9% (Group 2), 70.8% (Group 3), and 76.3% (Group 4). These results are higher than those reported in a Greek skull study, which applied landmarks including the maxillary region and achieved age classification accuracies of 41.2–62.8% (Chovalopoulou et al. 2017). Comparable or higher accuracies have been observed in studies using maxillary sinuses of Korean patients (86.8–90.4%) and premolar teeth from μ CT imaging (82%) for age estimation (Kim et al. 2021; Nudel et al. 2020). Other GM studies in the Malaysian

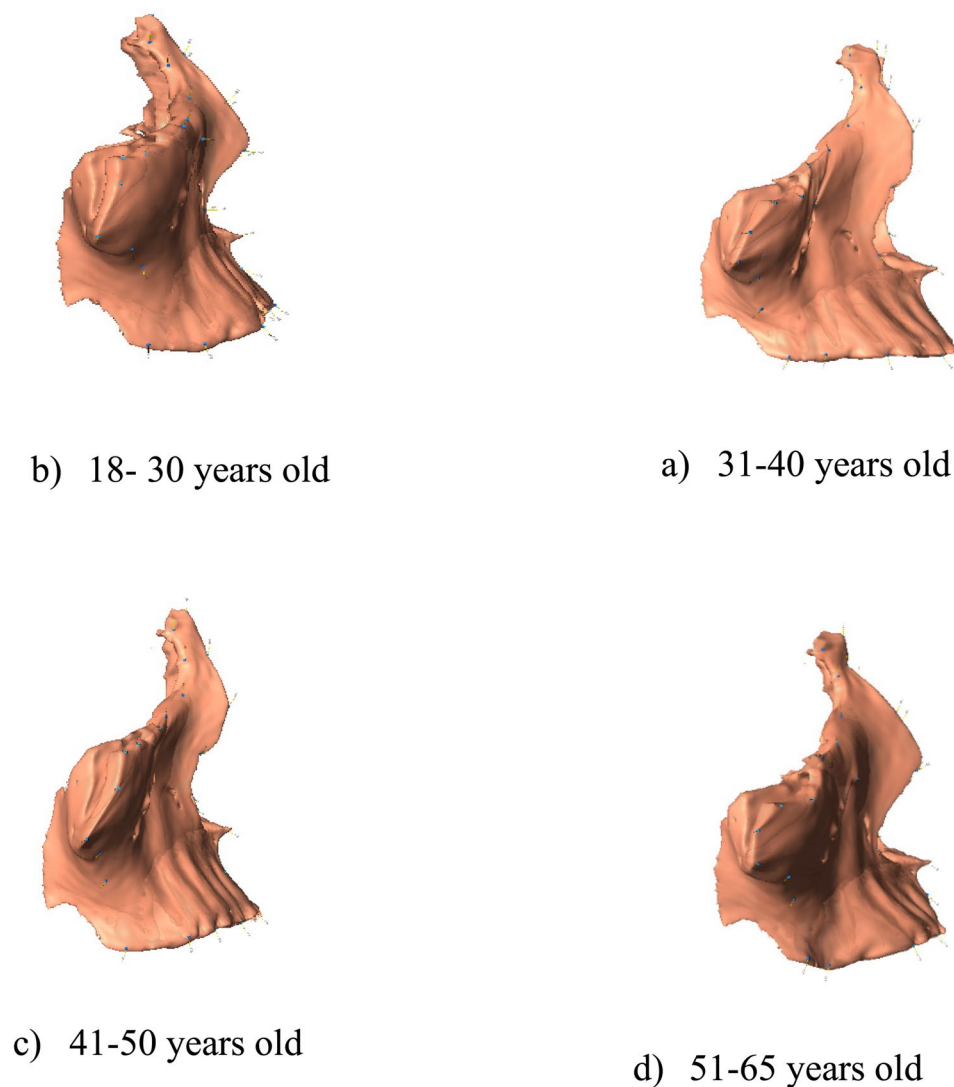


Fig. 8 Maxillary shape changes based on age group (right lateral) showing subtle differences

population showed age classification accuracies of 53–79% for mandibles and 61.5% for cervical vertebrae (Mohd Fauad et al. 2023; Zulkifli et al. 2023). Overall, these findings indicate that maxillary morphology provides reliable information for adult age estimation.

The structure of the maxillary bone is influenced by a complex interplay of biological, environmental, and mechanical factors that change with age. Bone remodelling and mineral density decrease over time, leading to thinning, increased porosity, and weakening of the spongy layer of the skull (Schini et al. 2023; Urban et al. 2016; Skrzat et al. 2004; Zhang et al. 2024; Shaikh et al. 2022). Age-related changes in intracranial volume and cerebrospinal fluid pressure also affect skull morphology, while histological transformations in cranial sinuses—including mucosal thickening, cysts, septa, and ostium obstruction—are more prevalent in older individuals (Bailes et al. 2025; Fleischman

et al. 2012; Mathew et al. 2009; Yamada et al. 2023; Dogan et al. 2024; Ren et al. 2015). Additionally, tooth loss reduces mechanical stimulation of the alveolar bone, accelerating bone resorption and volume reduction in the maxilla, which contributes to gradual mid-facial shape alterations in the elderly (Bergmann et al. 2023; Daluz et al. 2024; Sathapana et al. 2013; Zhang et al. 2024).

Despite the robust sample size and population-specific sampling, several limitations should be acknowledged. First, while the sample includes an equal distribution of individuals across four adult age cohorts up to 65 years, it lacks representation of the elderly population over 65. Craniofacial and skeletal degenerative changes often accelerate significantly in later stages of life and omitting these demographic limits the model's forensic applicability to older seniors. Second, while factors like dental status and

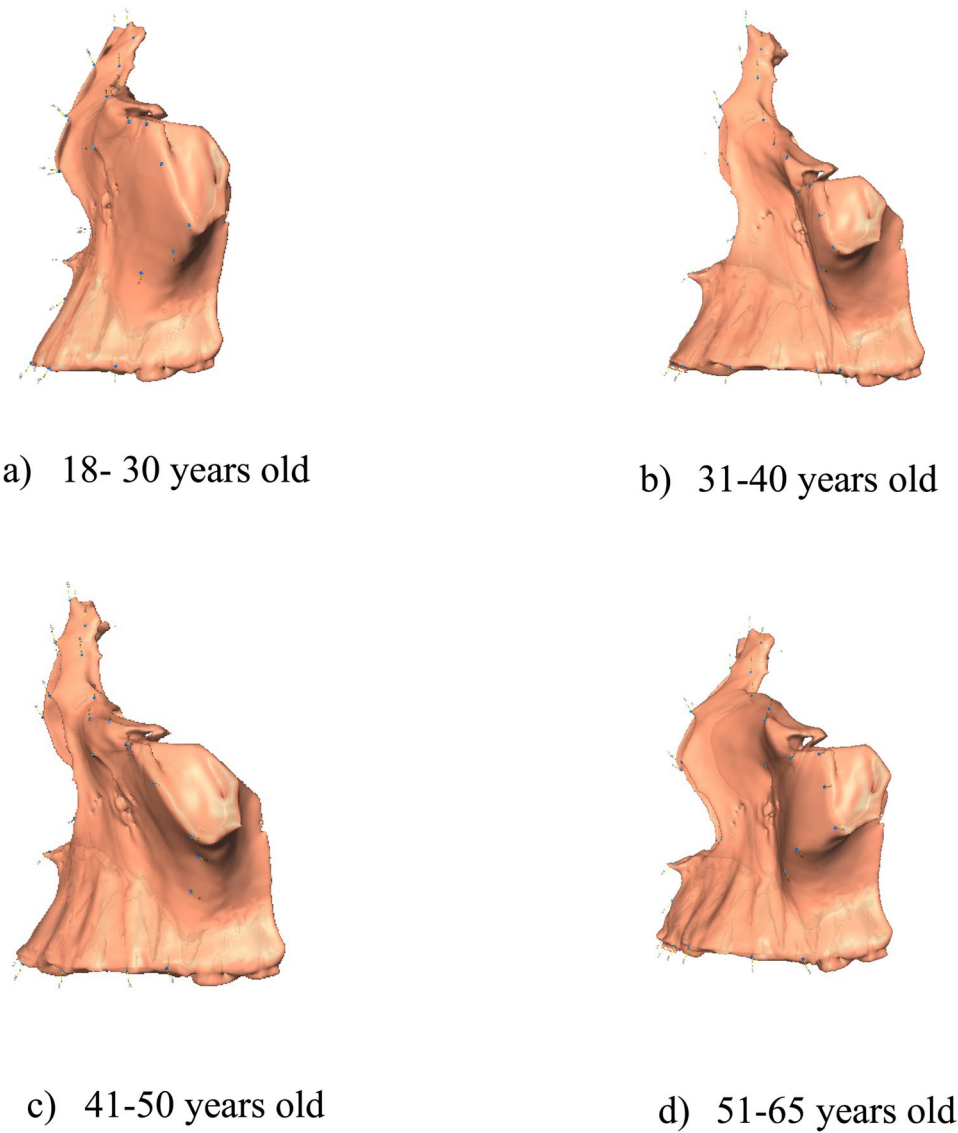


Fig. 9 Maxillary shape changes based on age group (left lateral) showing subtle differences

Table 7 Discriminant function analysis of age group

Classification Result							
		Age Group (Years)	Predicted Group Membership				Total
Cross Validated		18–30	247	25	26	17	315
		31–40	21	223	35	31	310
		41–50	29	38	218	23	308
		51–65	19	33	24	244	320
%		18–30	78.4	7.9	8.3	5.4	100.0
		31–40	6.8	71.9	11.3	10.0	100.0
		41–50	9.4	12.3	70.8	7.5	100.0
		51–65	5.9	10.3	7.5	76.3	100.0

masticatory function were noted as potential morphological influences, the present study did not strictly categorize or control for varying degrees of tooth loss (edentulism) and subsequent alveolar bone resorption

among the subjects. Alveolar resorption significantly alters the lower mid-facial skeleton, which may confound purely age-related skeletal modifications. Finally, this study is inherently limited to the major

ethnic populations residing in Malaysia. Because craniofacial structures display high geographic and population-specific variation, these reference values and classification accuracies cannot be directly extrapolated to global or non-Southeast Asian populations without further comparative research. Future studies will aim to include geriatric cohorts above 65 years and explicitly control for edentulous status to isolate pure age-related remodeling from dental-induced bone resorption.

Conclusion

The present study examined age-related variations in maxillary bone morphology among Malaysian adults using geometric morphometric analysis of CT scans. The results demonstrated significant differences in bone shape across age groups, with the greatest variation observed between the youngest (18–30 years) and eldest (51–65 years) groups. These findings provide insight into how maxillary morphology changes with age and highlight its potential application in forensic age estimation. The data could serve as a foundation for developing population-specific forensic tools that integrate skeletal shape analysis for more accurate adult age assessment.

Abbreviations

3D	Three-Dimensional
CBCT	Cone-Beam Computed Tomography
CT	Computed Tomography
GM	Geometric Morphometrics
PPUM	Pusat Perubatan Universiti Malaya

Authors' contributions

MF, AA and KW conceived and designed the study, conducted research, provided research materials, and collected and organized data. MF, AA, KW, HM, EC, NW, NAS, MA analyzed and interpreted data. MF, AA, KW wrote the initial and final drafts of the article and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Funding

The project received no funding.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Medical Research Ethics Committee, Pusat Perubatan Universiti Malaya (MREC ID No: 201944-7288). The use of computed tomography (CT) scans was authorized by the Committee, and all procedures were conducted in accordance with the approved ethical guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 13 December 2025 / Accepted: 4 August 2026

Published online: 25 August 2026

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