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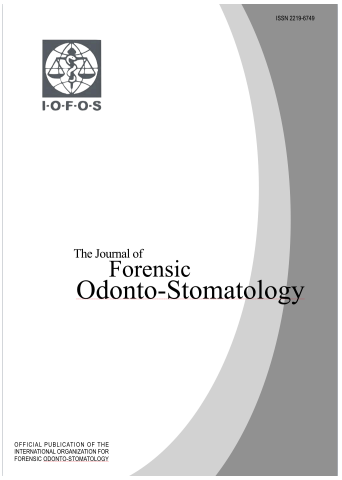

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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CONTENTS

Applicability of London atlas of tooth development and eruption for dental age estimation in children of the Malaysian population using maxillofacial imaging

Radhadevi Kuppusamy[1], Norhayati Jaffar[2], Raja Zarina Raja Shahardin [3], Hemlatha Pandey [1], Scheila Mânica[1]

[1]Centre of Forensic and Legal Medicine and Dentistry, University of Dundee, United Kingdom.
[2]Dental Forensic Unit, Department of Oral Medicine and Maxillofacial, Hospital Kuala Lumpur, Malaysia.
[3]Department of Paediatric Dentistry, Hospital Tunku Azizah, Malaysia

Corresponding author:
radhadevi@moh.gov.my

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KEYWORDS

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ABSTRACT

In forensics, dental age estimation is crucial, and literature has many methods for estimating dental age. London Atlas of Human Tooth Development and eruption method was developed on British and Bangladeshi populations in 2010, and there are likely to be differences between other populations.

Malaysian children have not yet been extensively tested for the method's applicability despite its universal acceptance and reliability. This research aimed to test the applicability of The London Atlas of human tooth development and eruption in children of the Malaysian population aged 4 to 16.99 years old. The study sample included 523 panoramic radiographs of healthy patients who had attended the Dental Paediatrics Department Hospital Tunku Azizah, Malaysia, between May 2019 and December 2019. The intra- and inter-observer errors were analysed by taking 53 radiographs and evaluating them over 14 days using the interclass correlation and Cohen's Kappa index. A paired t-test was used to compare chronological and estimated age ANOVA F-tests were used to establish if the difference is statistically significant between chronological and estimated age. The range of age estimation fell within a year due to the mean absolute difference of 0.60 years. The mean age for estimated age was 9.31±3.18 for the overall sample, 9.06±3.09 for males, and 9.60±3.27 for females. Paired t-test analysis showed the mean difference between chronological and estimated age of -0.0365 and was not statistically significant (p=0.240). The result also showed no statistically significant difference between the sexes. The difference between the chronological and estimated age was underestimated within minimal range, 0.002 years in males and 0.08 years in females. The London Atlas of human tooth development and eruption showed high accuracy in Malaysian children.

INTRODUCTION

Dental age estimation is vital for individuals lacking identification documents, especially in legal contexts involving refugees, asylum seekers, victims of human trafficking, suspects or victims in criminal cases, as well as in forensic identification.[1] Forensic Age Estimation (FAE), an expertise in forensic medicine, aims to estimate the chronological age of individuals involved in judicial and legal proceedings, utilizing various biological methods such as hand-wrist bone ossification,

pubic symphysis changes, dental maturity, cranial suture fusion, dental maturity, and somatic indicators. There is considerable heterogeneity among these measures, often affected by genetic and environmental factors.[2] The incremental formation and periodic mineralization of dental maturation surpasses other techniques in reliability.[3]

Legal documentation solidifies an individual's identity and establishes their rightful place in society. Birth registrations serve as irrefutable proof of one's existence, laying the foundation for all legal rights and responsibilities that accompany citizenship. Without this vital record, individuals are left vulnerable, their very identity called into question, and their access to essential services and opportunities jeopardized. In Malaysia, civil registration is mandatory for births, deaths, adoptions, marriages, and divorces at the National Registration Department under the Ministry of Home Affairs, with penalties for non-compliance.[4] Failure to register a birth can result in statelessness, depriving individuals of citizenship rights such as education and healthcare. However, certain groups of people like refugees and asylum seekers, victims of human trafficking, homeless individuals, undocumented immigrants, marginalized communities, children born in remote areas, and some elderly individuals may not have legal documentation as proof of their age.

Dental age estimation in children and adolescents relies on techniques like radiographic assessment and dental and skeletal development evaluation. The London Atlas of Human Tooth Development and Eruption, published in 2010, includes dental growth and eruption sequences from the 30th week of pregnancy to the age of 23 years.[5-6] The atlas illustrates 31 diagrams of tooth development and eruption at the midpoint of the chronological age and the development stages. It is the only one that has covered the sequence at the midpoint and is well-recognized for being comprehensive, well-developed, and frequently used for dental age estimation.[5-7] It is worthwhile to try this method with non-European groups, including Malaysians, given that research is being conducted using it among European populations, and it is very adaptable and reliable.[8-10] As the original study was centred on the Bangladeshi and British populations, it is anticipated that different patterns of tooth growth and eruption will exist across other demographic groups.[6]

The applicability of this atlas to the diverse Malaysian population still needs to be explored. Given Malaysia's multiethnic population, genetic and dietary variables, and other environmental factors, this study on Malaysian children aged 4 to 16.99 years aims to assess the suitability of the London Atlas for this population, potentially enhancing age estimation practices.

MATERIALS AND METHODS

A total of 1029 orthopantomograms (OPG) retrieved from the Dental Paediatric Department, Hospital Tunku Azizah Malaysia database from May 2019 to December 2021 were analyzed. Ethical approval was received National Medical Research Register (NMRR) Malaysia ethics committee (NMRR ID-22-01089-T58 (IIR)).

The overall sample included images of healthy Malaysian children population aged 4 to 16.99 years.Poor quality images, the presence of artifacts, subjects with any systemic diseases, developmental problems, and abnormal dental development, including the presence of other conditions such as the presence of gross pathology related to the jaw or teeth, gross caries, periapical pathosis, and extensive restorations or crowns, indication of trauma during the tooth development period, impacted or embedded tooth were excluded. A total of 529 radiographs (279 males and 244 females) adhered to the inclusion criteria.

The sample's complete information was anonymized by a code and collated into a Microsoft Excel 2021 (Redmond, WA, USA) with variables such as a)the subject's sexes, b) the date of birth, and c) the date when the radiograph was taken. The chronological age was calculated by subtracting the date of birth (a) from the date when the radiograph was taken (b) using decimal age. Thirteen groups have been created to classify the subjects based on their age range, as shown in Table 1. A single observer (principal author) used the London Atlas of Human Tooth Development and Eruption Software available on the Queen Mary University of London website (https://www.qmul.ac.uk/dentistry/atlas/software-app/) to estimate dental age. Development and eruption stages were assessed on the right mandible and right maxilla of each subject; however, if there is a presence of the exclusion criteria on the right side, the left side of the mandible and maxilla were assessed. There is no statistically significant

difference between dental age assessment on the left and right sides of the mouth. However, the London Atlas of human tooth development and the eruption were typically analyzed from the right.

Table 1. Number of subjects distributed according to age group.

Age group	Female	Male
4.00 - 4.99	12	12
5.00 - 5.99	28	35
6.00-6.99	24	33
7.00 - 7.99	33	40
8.00 -8.99	29	38
9.00 - 9.99	23	31
10.00 - 10.99	17	19
11.00 -11.99	14	14
12.00 - 12.99	12	15
13.00 - 13.99	15	16
14.00 -14.99	14	10
15.00 - 15.99	18	13
16.00 - 16.99	5	3
	244	279

The dental age estimation was defined as how closely chronological age could be predicted, measured as the difference between chronological age and the estimated age for each subject. A paired subject t-test was used to study the variation between the chronological age and estimated age. The chronological age was subtracted from the dental age and a positive result shows an overestimation and a negative result shows an underestimation. One-way ANOVA's F-tests were performed to establish whether the discrepancy between chronological age and estimated age was statistically significant. A significance level of 5% ($p < 0.001$) was used, and all statistical tests were performed with IBM Statistical Package of the Social Sciences (SPSS) Statistics software v29 (SPSS Inc., Chicago IL). A re-evaluation of 10% of the overall sample was carried out 14 days after the first author's original evaluation and a certified forensic odontologist evaluated the same 10% of the data for inter-rater reliability. An intraclass correlation (ICC) and Cohen's Kappa were performed to assess the variances between and among examiners.

RESULTS

Results showed that the intraclass correlation coefficient (ICC 2.1, absolute) produced a reasonable degree of agreement, with 0.992 and 0.976, respectively, from the intra-examiner and inter-examiner. A significant agreement is shown by Cohen's Kappa scores of 0.769 for intra-examiner and 0.693 for inter-examiner.

The mean for chronological age was 9.27 for the overall sample, 9.05 and 9.53 for males and females, respectively. Meanwhile, the mean age for estimated age was 9.31 for both sexes, 9.06 for males, and 9. 60 for females (Table 2). The paired t-test revealed that -0.0365 was not statistically significant ($p=0.240$), showing a clear distinction between chronological age and estimated age. (Table 3). It can be seen in Table 2 of the results that the range of age estimations fell within a year because of the mean absolute difference of 0.60 years of estimations.

Table 2. The mean for CA and EA and mean absolute error between CA and EA

	CA		EA		MAE	
	MEAN	SD	MEAN	SD	MAE	SD
Male	9.05	3.08	9.06	3.09	0.5821	0.3931
Female	9.53	3,41	9.60	3.27	0.6030	0.3889
Overall sample	9.27	3.24	9.31	3.18	0.5937	0.3909

Table 3. Comparison of CA and EA using paired t-test.

Age	Sex	N	CA		EA		CA-EA		95% CI	
			Mean	SD	Mean	SD	Mean	SD	Lower	Upper
4 – 4.99	M	12	4.53	0.28	4.33	0.39	0.20	0.38	0.44	0.44
	F	12	4.53	0.28	5.17	0.49	-0.63	0.31	-0.43	-0.83
5 – 5.99	M	35	5.45	0.28	5.64	0.65	-0.20	0.51	-0.37	-0.02
	F	28	5.50	0.30	5.96	0.51	-0.46	0.48	-0.65	-0.27
6 – 6.99	M	33	6.5	0.27	6.74	0.56	-0.25	0.48	-0.42	-0.08
	F	24	6.38	0.31	6.5	0.51	-0.12	0.10	-.033	0.09
7 – 7.99	M	40	7.45	0.29	7.45	0.55	0.01	0.52	-0.16	0.17
	F	33	7.46	0.27	7.43	0.70	0.02	0.64	-0.20	0.23
8 – 8.99	M	38	8.45	0.28	8.42	0.82	0.03	0.69	-0.19	0.25
	F	29	8.46	0.28	8.60	0.86	-0.15	0.80	-0.45	0.16
9 – 9.99	M	31	9.41	0.28	9.24	0.89	0.17	0.74	-0.10	0.44
	F	23	9.38	0.30	9.54	0.88	-0.16	0.86	-0.54	0.21
10 – 10.99	M	19	10.47	0.25	10.08	0.61	0.39	0.58	0.11	0.68
	F	17	10.53	0.26	10.21	0.85	0.32	0.83	-0.10	0.75
11 – 11.99	M	14	11.56	0.25	11.42	0.73	0.14	0.70	-0.27	0.54
	F	14	11.53	0.30	11.79	0.73	-0.26	0.64	-0.62	0.11
12 – 12.99	M	15	12.38	0.21	12.97	0.83	-0.59	0.90	-1.09	-0.09
	F	12	12.40	0.34	12.58	0.90	-0.18	0.69	-0.62	0.25
13 – 13.99	M	16	13.42	0.30	13.5	1.03	-0.08	0.88	-0.55	0.39
	F	15	13.46	0.28	13.63	0.64	-0.17	0.58	-0.49	0.15
14 – 14.99	M	10	14.42	0.33	15.00	0.85	-0.58	0.87	-1.21	0.05
	F	14	14.53	0.29	14.29	0.70	0.24	0.66	-0.14	0.63
15 – 15.99	M	13	15.43	0.28	14.73	0.73	0.70	0.75	0.24	1.16
	F	18	15.47	0.28	14.72	0.55	0.75	0.14	0.45	1.04
16 – 16.99	M	3	16.43	0.32	15.50	0.00	0.93	0.32	0.13	1.73
	F	5	16.54	0.18	16.9	0.55	-0.36	0.42	-0.88	0.16

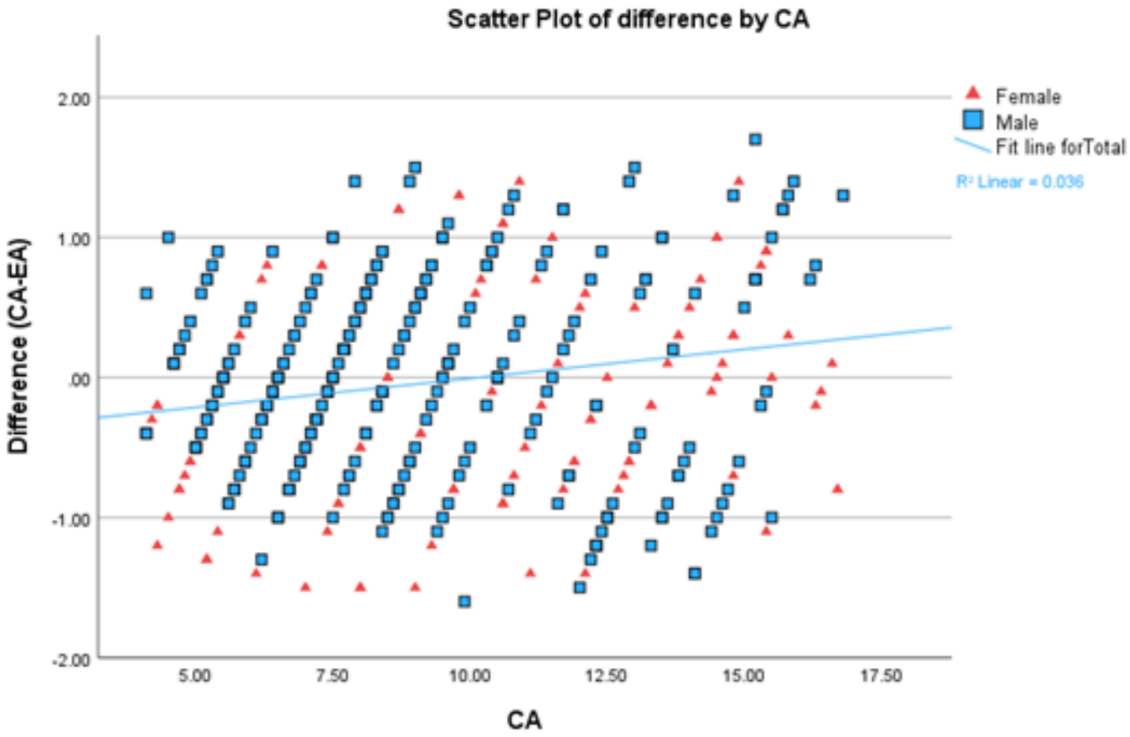
A significant difference in chronological age and estimated age appears between sexes in the age groups of 12-12.99 and 15-15.99 for males and 5-5.99, 10-10.99, and 15-15.99 for females. Similarities were found in both sexes in the age group of 15-15.99. Comparisons among the chronological age and estimated age were insignificant in most age groups, with the p-value less than 0.001 ($p<0.0001$). The most significant difference was seen in the female age group of 4-4.99 years, showing disparities of-0.63 ± 0.31, and the minor disparities were seen in the male age group of 16-16.99 with a difference of -0.08±0.88. The estimated age is remarkably close compared to chronological age in the age group of 7-7.99 for both sexes, with 0.01±0.52 and 0.02±0.69 in males and females, respectively. Age estimation reliability varies by sexes and age group, with some groups showing more excellent reliability than others, as shown in Table 4.

Table 4. Frequency of estimated age from real age deviation range by sex and age group

Age group	Sex	Age deviation range			total
		>-1	-1 to +1	>1	
4 – 4.99	M	0	12	0	12
	F	1	11	0	12
5 – 5.99	M	0	35	0	35
	F	F	24	0	28
6 – 6.99	M	1	32	0	33
	F	1	23	0	24
7 – 7.99	M	1	38	1	40
	F	2	31	0	33
8 – 8.99	M	1	36	1	38
	F	2	25	2	29
9 – 9.99	M	2	27	2	31
	F	2	18	3	23
10 – 10.99	M	0	17	2	19
	F	0	12	5	17
11 – 11.99	M	0	12	2	14
	F	1	13	0	14
12 – 12.99	M	6	8	1	15
	F	1	11	0	12
13 – 13.99	M	1	14	1	16
	F	0	15	0	15
14 – 14.99	M	3	6	1	10
	F	0	13	1	14
15 – 15.99	M	0	8	5	13
	F	1	12	5	18
16 – 16.99	M	0	2	1	3
	F	0	5	0	5

From the overall sample size, 460 subjects (87.95%) were estimated within -1 to +1 years, signifying a greater degree of reliability. Meanwhile, 30 subjects (5.73%) were overestimated, indicating age estimated more than one year, and 33 subjects (6.31%) were underestimated below one year from the chronological age. The male group had a more significant percentage of 53.7% age estimated between -1 and +1 compared to the female group with 46.3%. Meanwhile, the percentage of underestimation was slightly higher in the male group, and the distribution for overestimation was equal for both sexes. The percentage of prediction accuracy is shown in Figure 1, where equal distribution is seen in both sexes for overestimation of more than one year and underestimation of less than one year. Overall, the outcome shows very equivalent performance. The comparison pattern between chronological age and estimated age with chronological ageis demonstrated in Fig.1.

Figure 1.



The paired sample t-test results show no significant difference between chronological age and estimatedage; hence, the estimation based on the London Atlas of Human Tooth Development and Eruption reflects a precise estimation of the subjects. Table 5 shows that the mean difference between the chronological age and estimated age for both male and female groups was underestimated within an exceedingly small range of 0.002 years for males and 0.08 years for females. It can be concluded that the London Atlas has a low margin of error and provides a consistent estimation for both male and female subjects.

Table 5. Summary of comparison of CA and EA

Sex	N	CA	DA	CA-EA	T	One-sided P	Two-sided P
M	279	9.05±3.08	9.06±3.09	-0.002±0.70	-0.05	0.48	0.96
F	244	9.53±3.41	9.60±3.27	-0.08±0.72	-1.65	0.50	0.10

DISCUSSION

Despite improved dental age estimation techniques, a significant gap remains in population-specific studies for Malaysian children aged 4 to 16.99 years. Accurate age estimation is vital for immigration and medico-legal matters in this age group. Current dental age estimate techniques often exhibit specificity to the Malaysian population, limiting their applicability and accuracy.[11] This study aims to assess the suitability of the London Atlas Human Tooth Development and Eruption method for Malaysian children aged 4 to 16.99 years, utilizing the London Atlas' online software program, which provides graphical and textual information on tooth growth and eruption.[7]

The intra-observer value in this study was more significant than the original study by AlQahtani (2010), which has an ICC of 0.879;[6] however, it has similar ICCs in the study on the Chinese population by Jianxin et al.(2023), which has an ICC of 0.98,[12] and the study by Pavloic *et al.* (2017), on Portuguese population which has an ICC of 0.988[8] and study by Sharma and Wadhwan on Indian children which has an ICC of 0.997.[13]

A primary factor in deciding if the method is applicable is the mean difference between estimated and chronological ages. This study showed that the difference between estimated and chronological ages differs statistically significantly when estimating Malaysian children's ages using the London Atas of Tooth Development and Eruption. In this population, the mean difference was 0.365 years, indicating a propensity for overestimation by 3.7 months so this information should be factored in any report. The mean absolute error between them represents accuracy independent of bias.[6] The mean absolute error for the overall group, male group, and female group were lower, with 0.59, 0.58, and 0.60, respectively.

It has been reported that a difference of one year between chronological age and estimated age has been found in several studies assessing the applicability of London Atlas of Tooth Development and Eruption.[8,14] However, this study found a discrepancy of 0.93 years, indicating better accuracy when applied to Malaysian children. Approximately 87% of estimates were within one year of chronological age. The highest discrepancies for underestimation were in the male group aged 16-19.99, with a mean difference of 0.93, followed by the male and female groups aged 15-15.99, with 1.70 and 1.75, respectively. Overestimation discrepancies were more prominent in the female group aged 4-4.99, with a mean difference of -0.63. Similar trends were observed in Saudi Arabian studies by Alshiri *et al.*(2015), where 65% were estimated within a year of chronological age, with a discrepancy of 7 months at 13-15 years of age.[14] Ghafari *et al.* (2019), compared the London Atlas with the Smith method in 339 Iranian children, reporting similar accuracy and practical applicability of the London Atlas on the population. They found that 77.6% of subjects were underestimated within one year, 16.8% were overestimated over one year, and 6.8% were underestimated over one year.[15] The original study by AlQahtani *et al.* (2012), reported 53% estimated accurately, with 23% and 24% overestimation and underestimation, respectively, based on Bangladeshi and British Caucasian populations.[7]

This study revealed significant differences in chronological age and estimated age between males and females in the age groups 15-15.99 and 16-16.99, with differences of 0.70, 0.75, and 0.93, respectively, indicating overestimation. This contrasts with Ismail et al.'s study, which found an underestimation in the 15-year-old age group. Ismail *et al.* (2018) study only examined Malay ethnicity.[5] Estimating age for this group was solely based on third molar development, which reaches complete root formation at about 25 years old.[16-18] Age estimation based on a single tooth may result in underestimation or overestimation due to variations in angles, formation, and morphology.

This study found no significant sex difference in third molar development, consistent with findings in the Bangladeshi population.[19] However, some studies have reported that males' third molar develop earlier than females.[20] Both males and females in this study had advanced stages of third molar development compared to the London Atlas of Human Tooth Development and Eruption software.[16-17] Dhamo et al. found that a higher percentage of the Asian population had faster dental development in contrast to the European population, which exhibited a delayed development.[21]

Using the London Atlas software, this study showed no significant difference between males and females in most age groups, which was

similar with the Hispanic population,[9] the Saudi Arabian population,[22] and the American population.[23] The London Atlas software did not reveal significant sexes differences in most age groups in the Malaysian population. However, it's essential to remain cautious about sexes differences, as they may result from variations in sample size or the small number of subjects since orthopantomograms (OPGs) are less common with young children.

Various methods have been tested in Malaysian children, including Demerjian's,[11,24-25] Willem's,[11,24,26] Nolla's,[11] Haavikko's,[11] and Cameriere's,[11] with varying results, but Willem's method was deemed to be most suitable.[23,26] In each study, there was a level of advancement in females than males at a certain age; however, there was no significant difference in overestimation or underestimation between the age groups. Mani et al. *(2008)*, reported overestimation in males was seen in 10-12 years and 9-11 years in the female group,[24] and Nik-Hussein *et al.* (2011) reported a more significant difference was identified in the age group of 5-6.9 and underestimation in the age group of 14-15.9 in both sexes. In this study, the trend for overestimation shows a higher number than underestimation in the older age group, which is from 10 years old in both sexes. The study demonstrates heightened age overestimation almost before pubertal changes, likely due to a rapid surge in dental tissue and overall growth. This was ascribed by Mani *et al.* (2008) to the irregular and uneven dental development process, which is related to changes in para-pubertal pace.[24]

Malaysian neighboring countries have tested the applicability of the London Atlas of Human Tooth Development and Eruption.Using the Thai population, Namwong and Manica (2022) found a maximum discrepancy of 1.3 years without a significant sexes effect. Thailand's population aged 7 to 15 years was also tested by P. Duangto *et al.* (2022) who reported the mean absolute error consistent with the current study. A comparison of the London Atlas of Human Tooth Development and Eruption with the Demerjian method was conducted on the East China population, and the mean error was less than one year for both methods.[8,27]

In cases of dental profiling for unidentified remains, the London Atlas of Human Tooth Development and Eruption method can be used confidently, as the study did not analyze different ethnicities separately.[28] Ethnicity may not be a reliable alternative to genetic heritage, given substantial genetic diversity within and among ethnic groups.[19]

However, it's important to acknowledge the limitations of atlas-based methods, as they may not account for all variations in tooth development and emergence stages. Additionally, estimating dental age based on eruption or emergence is influenced by various factors, including missing teeth, misalignment, impacted teeth, and early extraction, which can affect the eruption phase.[29-30] Recommendations have been made to address these issues by identifying which teeth tend to be more stable during development.[31]

CONCLUSION

The London Atlas method produced excellent results for age estimation in Malaysian children aged 4 to 16.99, with most age groups showing disparities of less than two years. Caution is advised for age groups with developing third molars, therefore, multiple methods are recommended for age estimation. Sexes had no significant impact, making this method universally applicable. This marks the first large-scale study on Malaysian children, indicating strong reliability as a reference method.

Future research should include larger, standardized sample sizes for improved accuracy and comparability. Even though sex differences were not significant in this study, large samples may provide more insight. In addition, it would be valuable to assess accuracy and reliability across Malaysia's three major ethnic groups (Malays, Chinese, and Indians).

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Development and validation of salivary analysis for forensic evidence (SAFE) scale

Fida Mansha Ottapurakkal Thazath[1], Ram Sabarish[2], Akila Ganesh[3]

[1] Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India. [2] Department of Periodontology, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India. [3] Department of Public Health Dentistry, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India

Corresponding author:
akilaganesh@sriramachandra.edu.in

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ABSTRACT

Background: Despite the surging crime rates and diverse operating methods, identifying the perpetrators and suspects is challenging. Incorporating forensic odontology has expanded the scope of forensics. Over the years, saliva has become the hallmark of forensics. This study aimed to develop a scale for assessing the knowledge and awareness about the role of saliva in forensic odontology.

The scale was developed sequentially according to the qualitative research methodology: formation of a conceptual framework, systematic development of an item pool and refinement of an item pool (focus group discussion, cognitive interviewing and pretesting). Then, the validity and reliability of the scale were tested.

Results: A conceptual framework was developed based on five constructs, which included forensic sciences, forensic odontology, crime investigations, salivary analysis and forensic genetics. A pool of 28 items was designed based on existing literature and refined through a focus group discussion involving the target audience and expert panellists. After validation by cognitive interview and pretesting, the scale was condensed into a pool of 23 items. Statistical analysis revealed a Cronbach's alpha of 0.8 (good reliability) and kappa value of 0.79, indicating a strong level of agreement.

Conclusion: The Salivary Analysis for Forensic Evidence scale is a valid and reliable tool that consolidates all possible constructs involved in assessing the role of saliva in forensic odontology.

INTRODUCTION

With the surging crime rate and diverse operating methods, identifying the perpetrators and suspects has become increasingly challenging. Thus, the field has expanded into forensic odontology – a branch of forensic medicine that deals with proper handling, examination and presentation of dental evidence for appropriate justice. Forensic odontologists assist the legal authorities through dental evidence in various situations.[1] This includes management, examination, evaluation and presentation of dental evidence in civil or criminal proceedings along with background research.[2] In addition, saliva has become the mainstay in forensics and is now used as a biological sample like blood or urine.[3] Currently, it is an indispensable fluid for various genomic analysis needed for

forensic applications. Identifying age and gender narrows down the profiles and restricts the suspects.[4] Importantly, several advancements have been made globally in the field of forensic and crime investigations.[2]
Despite the efforts to raise awareness and apply forensic odontology, the potential of dental surgeons is yet underutilized. According to Milcah Roy et al.,[5] the scope of forensic odontology is well-established among dentists; however, its practical application is limited. The end effects of abuse, assault or any other crime-related injuries that cause trauma to teeth, jaws or oral tissues should be appropriately evaluated, examined, recorded and documented at the Primary Health Centre for forensics.
Hitherto, the indispensable role of salivary analysis in crime scenes has been neglected, thereby necessitating the need to spread knowledge about the implications of forensic odontology. This lacunae led to the development and validation of a Salivary Analysis for Forensic Evidence (SAFE) scale to assess saliva's role in forensic odontology.

MATERIALS AND METHODS

The SAFE scale was developed using a valid and recognized methodology proposed by Vaughn et al.[6] as follows: (i) Formation of a conceptual framework, (ii) Systematic development of the item pool, (iii) Refinement of the item pool, (iv) Validity testing and (v) Reliability testing (Figure 1).
The ethics approval was obtained from the Institutional Ethics Committee [Reference Number: CSP/22/MAR /106/74]. Informed consent and non-disclosure consent were obtained from all participants involved in the various stages of the scale development.

Formation of a conceptual framework
PubMed, Google Scholar and Scopus were used to retrieve complete, thorough and comprehensive literature on the concepts of forensic odontology[7–9]. A conceptual framework was designed to include the key components that defined the constructs intended to evaluate the role of saliva in forensic odontology.[10] Each of the constructs was multidimensional and viewed as an individual component[7] (Figure 1).

Systematic development of an item pool
An initial pool of items was generated for each construct extracted from literature and available instructional resources, such that they represented the whole attribute.[11] This perspective should be consistent across items,[12] and each item layout should be short, close-ended questions in simple language avoiding double-barrelled items, leading questions, double negatives, slangs and abbreviations, and based on knowledge and awareness (Figure 1).[7,8,9,10] The items drafted should represent the construct of interest by ensuring the theoretical concept of the construct.[8]

Refinement of the item pool
The initial pool of items was refined via semi-structured and structured discussions through a sequence of processes: (a) Focus group discussion (FGD), (b) Cognitive interviewing and (c) Pretesting. The item pool was further refined based on the participants' feedback to ensure construct's conceptualization (Figure 1).[8]

Focus group discussion (FGD)
During the COVID outbreak, the FGD was conducted via video conferencing through Google Meet. It was moderated by the Principal Investigator. Two expert panellists from the Indian Academy for Clinical and Dental Genetics were part of the FGD, and the participants included undergraduate clinical dental students (Table 2). The ultimate goal of the FGD was to initiate interactions and structured discussions between researchers, expert panellists and the participants, as well as among the participants.[10] The participants were informed of the FGD's objectives before volunteering, and consent was obtained to record the whole conversation. Each item was displayed and questions like *'Do you find this item misleading? Do you want to modify this item in any simpler form? Do you want to retain this item or exclude it?'* were put forth, and participants were urged to voice their opinions. Also, new items generated from the participants were included. The elicited responses were analysed, and the items' language and phrasing were amended. The participants were awarded an incentive for their time and effort (Figure 1).

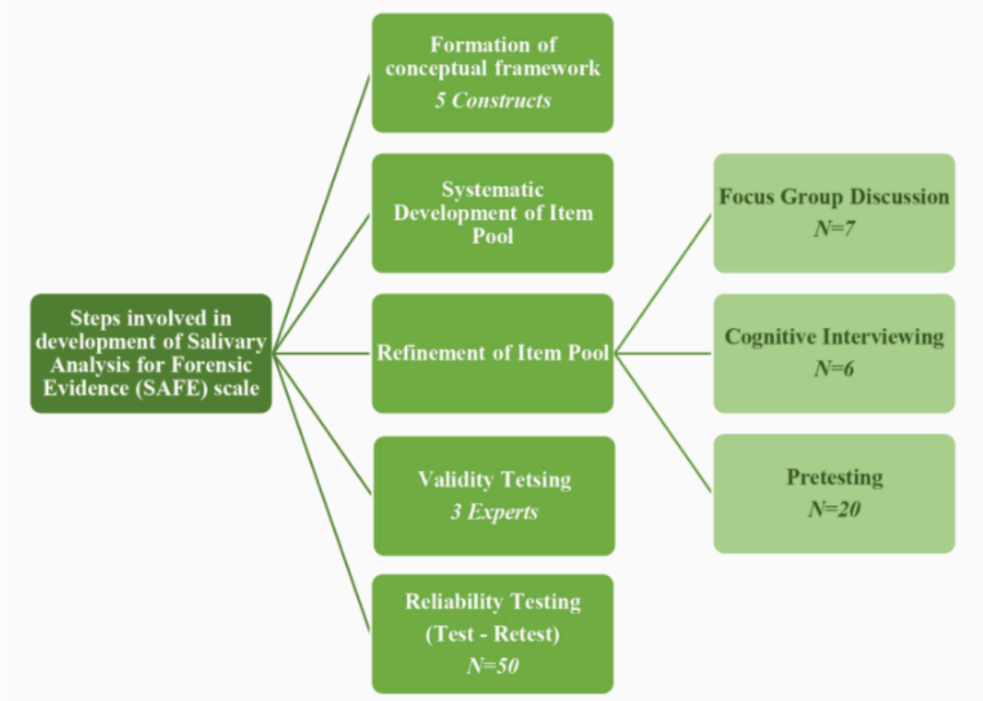
Cognitive interviewing
Cognitive interviewing was carried out as a face-to-face interview with six clinical undergraduate

dental students using the *'think aloud protocol'* (Table 2)[10] in order to test the participants' understanding of terms, clarity of words, items relevance and response option of the developed scale and to address the related.[8] The respondent's cognition of questions and response options were evaluated.[10] Each item was read aloud to the participants with verbal probing questions: *'Was the question easy/hard to answer? Does this question make sense to you? Will this question make sense to people like you? Is there a better way to ask this?'* The answers were tabulated. Confusing, reductant and overlooking items were identified and modified accordingly following several discussions (Figure 1).[10]

Pretesting

Cognitive interviewing was followed by a qualitative evaluation.[8] The revised items were pretested among volunteers to ensure accurate rendering of their vernacular usage[10] and identify difficulties related to data collection and find solutions. The questionnaire was pretested on 25 clinical undergraduate dental students through face-to-face interviews (Table 2). The items were tested in terms of understanding, and adequate responses were documented with skipped patterns.[10] The primary rationale was to eliminate items that participants perceived as relatively unimportant (Figure 1).[13]

Figure 1. Steps involved in the development of Salivary Analysis for Forensic Evidence (SAFE) scale. This figure depicts the five major steps involved in the development of a scale and the number of participants involved at each stage.



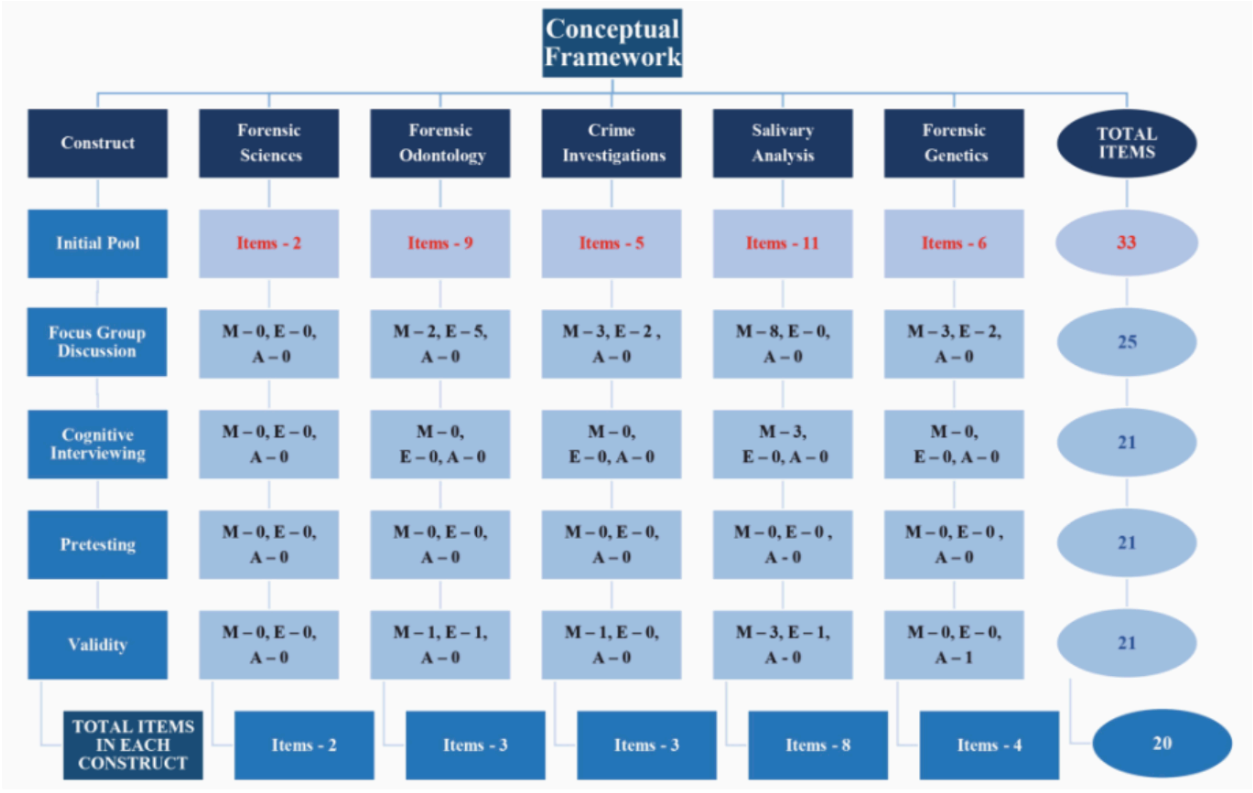
Validity Testing

The subject experts reviewed the scale to assess the relevance, clarity and comprehension of the item.[11] A three-membered expert team, including an oral and maxillofacial pathologist, an oral medicine and radiology specialist and a senior forensic expert, validated the items' accuracy and representativeness. Each item was scrutinised, accepted and validated as excluded or to be modified, respectively. The recommendations for each item were recorded eventually and modified accordingly (Figure 1).[8,10]

Reliability Testing

The consistency over time was assessed through internal consistency (Cronbach's alpha) and test-retest reliability (kappa). The reliability of the questionnaire was evaluated through face-to-face interviews conducted with 50 undergraduate clinical dental students (Table 2) who were unaware of the planned second test beforehand. About 10–14 days interval was planned between the baseline and the experts' second test (Figure 1).[10]

Figure 2. Conceptual Framework and items developed
This figure illustrates the number of items developed at each stage followed by the number of items modified (M), retained (R), added (A) and eliminated (E) at each stage.



RESULTS

Phase 1: Formation of a conceptual framework
Five main constructs, forensic sciences, forensic odontology, crime investigations, salivary analysis and forensic genetics, comprised the conceptual framework (Figure 2).

Phase 2: Systematic development of an item pool
The initial pool consisted of 33 items under the five broad constructs from the strongest to the weakest. The forensic sciences construct had two items related to basic knowledge of forensic science. The second construct had nine items on the basic knowledge of forensic odontology. The third construct, crime investigation, comprised five items related to the investigation of the crime scene. A maximum of 11 items were a part of the salivary analysis construct, which assessed the knowledge of the role of saliva in forensic odontology. Finally, the forensic genetics construct consisted of six items on the role of genetics in forensics (Figure 2).

Phase 3: Refinement of the item pool
Focus Group Discussion
Questions were discussed for each of the 33 items, and options were framed under the five constructs. Some items were eliminated, whereas some others were modified. At the end of FGD, 25 items were retained (Figure 2, Table 1).

Cognitive Interviewing
Undergraduate clinical dental students from various colleges were involved in cognitive interviewing. Each item was refined using the 'Think Aloud Protocol' and verbal probing. Although none were removed, the items were slightly modified by rephrasing the options for close-ended questions. For example, an option of 'don't know' was added to some of the 'yes/no questions'. Three items were modified, and the total number decreased to 21 items (Figure 2, Table 1).

Pretesting
A sample of 20 clinical undergraduates who volunteered to participate in the study were pretested, and the items remained unchanged (Figure 2, Table 1).

Table 1. Items involved in Salivary Analysis in Forensic Evidence (SAFE) scale
This table shows the additions, modifications and eliminations of items involved during the development of this scale

	Added	Modified	Eliminated
Focus Group Discussion		- Dentists have role in person/victim identification in the event of mass disaster - Forensic odontology helps in identifying victims - Forensic odontology can help in person/victim identification (options) - Saliva can be used as an evidence (and its options) - Salivary analysis is a method to narrow down the suspects - In a crime scene where all could saliva usually be deposited (options) - Where all saliva could be identified as evidence (and its options) - Saliva can be recovered from skin - Saliva to detect recent drug abuse - Sex determination in identification using saliva - Types of DNA that can be isolated from Saliva - Salivary biomarkers play role in forensic genetics	- Any postgraduate program in India offering Forensic Odontology - Dentists eligible to appear in court to present as evidence following the principle of forensic odontology - IPC section under which this fall - Maintaining dental record in your clinic - Duration of the records to be maintained - Maintain dental records at institutional level - Maintain dental records as a private practitioner - RNA markers in identified in saliva
Cognitive Interviewing	-	- Salivary analysis is a method to narrow down the suspects (Options – Crime detection, Drug abuse, Sexual Assault, Genocide, Suicide) - Where all saliva could be identified as evidence (Options – On the victim, crime scene evidence, sewer) - In a crime scene where all could saliva usually be deposited (options – Lip prints, Bite Marks, Chewing Gum/left over food, Clothes and surfaces.)	-
Pretesting	-	-	-
Validity Testing	- It is possible to identify RNA markers from Saliva	- Do you think forensic odontology has any role in dentistry? If yes, are you aware of a sub speciality as forensic odontology? -Do you think dentists can help in analysis of crime (in place of victim identification)? - In your opinions can individuals (in place of victims) be identified with forensic odontology? - Salivary analysis is a method to narrow down the suspects (Option - Child abuse in place of genocide) - Can saliva be collected from skin (Option - PHADEBAS test)? - Do you know the mechanism by which drugs end up in saliva?	- Duration of records to be maintained - Where all saliva could be identified as evidence (Options – On the victim, crime scene evidence, sewer)

Phase 4: Validity

The scale was validated by three experts, whose comments were consolidated, discussed and duly incorporated into the scale. None of the items were stated as 'inappropriate', and an item was added (Figure 2, Table 1).

Phase 5: Reliability

The test-retest reliability analysis showed a substantial level of agreement (kappa 0.79) and a statistical significance at $p \leq 0.05$. The internal consistency, measured with Cronbach's alpha, showed an agreement value of 0.816, indicating a good internal consistency (Figure 2).

DISCUSSION

Nowadays, the rising occurrence of both man-made and natural disasters highlights the critical need for accurate identification of individuals, particularly when bodies are severely decomposed or deliberately dismembered. Thus, comparing post-mortem and ante-mortem records is essential to determining a person's identity.[14] A strong knowledge of this field is important for dental professionals.[15] Recently, saliva analysis has emerged as a valuable diagnostic tool, offering an alternative to blood and urine and plays a significant role in victim identification during mass disasters.[3,16] Saliva consists of 99% water and 1% solids, which include inorganic substances such as chloride, calcium and potassium, as well as organic compounds such as proteins, vitamins, hormones and amino acids. Saliva is common at crime scenes on various surfaces, including skin, food, clothing and other objects and is essential for linking individuals to criminal activities such as homicide, assault, abuse and substance misuse.[16]

SAFE is a new scale developed and validated to evaluate the knowledge and awareness of saliva's role in forensic odontology. The steps recommended by Vaughn et al.[6] are crucial in directing the development process. Hitherto, studies have assessed knowledge and awareness using local inventory questionnaires. The major focus of the present study is on dental surgeons and undergraduate students' knowledge.

Two expert panellists from the Indian Academy for Clinical and Dental Genetics participated in the FGD. These experts were selected based on their extensive experience of a decade, which equipped them with in-depth knowledge of the role of saliva in forensic odontology.

The FGD, cognitive interviewing and pretesting was conducted on undergraduate clinical dental students selected for their limited knowledge of the role of saliva in forensic odontology, thereby deeming them an ideal group to gain valuable insights about the study.

A Senior Forensic Assistant from the Crime Branch with over six years of experience in forensic odontology was involved during the validity testing phase. Additionally, the expert served as a former senior resident in the Department of Forensic Odontology. The other two experts, each with over 15 years of experience, were from the Department of Oral and Maxillofacial Pathology and Microbiology and the Department of Oral Medicine and Radiology, respectively (Table 2).

The concept validity of the role of saliva in forensic odontology has been created and assessed in this study. The existing literature was beneficial during the development of an item pool that was further improved via FGDs. Although time-consuming, engaging diverse viewpoints from different groups is essential during the development process. This increases the scale's content validity by ensuring the consistent features of the trait. A panel of specialists evaluated each item according to its significance and implications, thereby improving the scale's comprehensiveness and removing any potential bias.

The test-retest reproducibility was used to gauge reliability, and most of the items' showed a kappa value of 0.8, indicating a substantial level of agreement. The internal consistency measured using Cronbach's alpha showed an agreement value of 0.816, indicating good internal consistency.

Importantly, the scale can be used for large-scale data collection. Experts from various fields participated at each stage of development, which strengthened the scale. The verbal probing in cognitive interviewing helped the participants to clarify the intent of the item without being presumptuous. The varied FGDs provided an in-depth perspective of the target audience.

Nevertheless, the scale has some limitations. It might be unable to capture nonverbal cues. The lack of context could lead to assumptions and misinterpretations. Also, due to the COVID pandemic, eliciting clarifications from online FGD participants would have been challenging.

To the best of our knowledge, this is the first scale developed to assess the knowledge and awareness about the application of saliva in forensic odontology. This scale can be further modified for medical and allied health science students and forensic experts, emphasizing the role of saliva in various forensic applications.

However, the potential role of saliva in forensic application is yet to be considered. Thus, further studies are required to assess the awareness and knowledge of dental undergraduate students.

Table 2. Data of participants involved in Salivary Analysis in Forensic Evidence (SAFE) scale
This table displays information about the participants involved in the development of this scale, including their designations and the number of individuals in each group.

S. no	Participant	Designation	No of Participants
FOCUS GROUP DISCUSSION			
1	Expert Panelist	Founder, Indian Academy for Clinical and Dental Genetics and Founder and Chairman, International Saliva Summit of India (SALSI)	1
2	Expert Panelist	Assistant Editor, Genomeden e-magazine, Indian Academy for Clinical and Dental Genetics and Founder	1
3	Target Audience	Undergraduate Clinical Dental Students	4
COGNITIVE INTERVIEWING			
4	Target Audience	Undergraduate Clinical Dental Students	6
PRETESTING			
5	Target Audience	Undergraduate Clinical Dental Students	25
VALIDITY TESTING			
6	Expert Panelist	Ex. Senior Resident, Dept of Forensic Odontology, Current Designation: Senior Forensic Assistant, Crime Branch	1
7	Expert Panelist	Associate Professor, Department of Oral and Maxillofacial Pathology and Microbiology	1
8	Expert Panellist	Associate Professor, Department of Oral Medicine and Radiology	1
RELIABILITY TESTING			
9	Target Audience	Undergraduate Clinical Dental Students	50

CONCLUSION

The SAFE scale is a valid and reliable scale that involves all the possible constructs to assess the knowledge and awareness of the role of saliva in forensic odontology.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for the survey was obtained from the Institutional Ethics Committee (Ref. No. CSP/22/MAR /106/74).

Participants was informed about the purpose and benefits of the survey and were invited to participate voluntarily. Informed consent was obtained for each participant who was assured of data confidentiality.

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AUTHORS' CONTRIBUTIONS

All authors have made substantial contribution in the conception of the scale. FM was involved in conceptualisation, methodology, data collection, analysis and drafting of the manuscript. RS was

involved in conceptualisation, review of the study, revising of the manuscript critically and given final approval of the version to be published. AG was involved in conceptualisation, methodology, revising of the manuscript critically and given final approval.

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Discriminant function analysis of maxillary bone measurements for sex estimation in a Colombian population by using cone-beam computed tomography

Sergio Iván Tobón-Arroyave [1,2], Santiago Palacio-Gutiérrez [1], Sara Morales-Galeano [1], Clara Inés Saldarriaga-Naranjo [3], Carlos Alberto Tangarife-Villa[4]

[1] Oral and Maxillofacial Surgery Program, Faculty of Dentistry, University of Antioquia. Medellín, Colombia. [2] Laboratory of Immunodetection and Bioanalysis, Faculty of Dentistry, University of Antioquia. Medellín, Colombia. [3] Department of Radiology, CES University and RADEX 3D Specialized Radiology Center. Medellín, Colombia. [4] Department of Basic Sciences, National Faculty of Public Health, University of Antioquia. Medellín, Colombia

Corresponding author:
sergio.tobon@udea.edu.co

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KEYWORDS

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ABSTRACT

Sexual dimorphism is an important biological factor for sex estimation from skeletal remains in medicolegal identification. This study aimed to determine using a discriminant function analysis, whether specific maxillary linear and angular measurements performed in cone-beam computed tomography (CBCT) images may be useful to determine the sex in a Colombian population. The sample consisted of 212 CBCT scans acquired from 86 males and 126 females. The protocol included the assessment of 23 parameters, of which 16 were bilateral and seven were non-bilateral. An intra-observer variability test was performed to ensure data reliability and unpaired t tests were applied to determine between-group differences. Significant predictor variables were subjected to univariate and multivariate discriminant function analyses. A total of five non-bilateral and 14 bilateral measures were statistically significant. Univariate discriminant analyses produced a mean percentage of correct prediction after cross-validation ranging from 55.20% to 72.60% for non-bilateral and from 58.70% to 73.10% for bilateral maxillary variables. The association of variables in the multivariate models increased the percentages of correct sex prediction even after cross-validation up to 77.80% for non-bilateral and up to 77.40% for bilateral maxillary measurements. It was concluded that CBCT measurement of maxillary bone parameters may be applied as a complementary technique to discriminate the sex from human remains through discriminant function analysis methods in the Colombian population.

INTRODUCTION

Sexual dimorphism has been defined as the systematic difference in size, shape, or color among males and females of the same species.[1] Although this phenomenon in humans is lesser than in nonhuman primates, allows a great discrimination of the individuals according to its chromosomal sex.[2] In this line, it has been acknowledged that skeletal characteristics are determined by an individual genetic background, its environment, and the functional demand.[3] Due to hormone-dependent variations,[4] men have developed greater muscularity in comparison to women, which has led to a larger stature as well as more robust and demarcated cranial and facial traits.[5,6] Sex estimation from skeletal remains is a procedure that has been used in the identification process

during medico-legal examination of decomposed, fragmented, burned, or skeletonized bodies[2,6,7] as bones are considered one of the most stable tissues of the human being.[8] In parallel, craniometric analysis has demonstrated to be an outstanding method for anthropological examination, given its objectivity, reproducibility and statistical value,[9] though its applicability for determining the sex of a cranium is only feasible in adults, when the development of secondary sexual characteristics has been completed.[10] Under this perspective, while some researchers have demonstrated that a number of isolated cranial anatomical structures may be useful in the estimation of sex,[6,8,10] maxillary bone, a fundamental structure for the facial formation and stability, has shown to have many morphological variations and dimensional differences that might be strongly liaised to sex.[8,11-13]

Various techniques have been used in forensic sciences to assess the dimensions of anatomical structures in order to establish the sex of cranial skeletal remains including measurements on dry skulls, conventional radiography, computed tomography, and cone-beam computed tomography (CBCT).[7] Among these, convincing evidence has demonstrated the ability of CBCT images to characterize the morphology of different structures of the maxillary bone and to determine the variations in the morphometric characteristic with high-dimensional accuracy.[8,12-17] Nevertheless, research data in this field have been divergent and may differ according to the populations studied, the methodological approaches, and diagnostic tools used.

Additionally, anthropometric studies have used different statistical models for sex identification,[5] among which discriminant function analysis has been widely employed with excellent results.[6] This method uses linear combinations of quantitative variables to determine and predict group membership (i.e., male or female) within the sample, seeking the best combination that maximizes inter-class difference and minimizes intraclass variance. Even so, it has been described that discriminant functions are sensitive to population variations, so that these formulas should be locally settled and validated for individual populations considering the variations in ethnic parameters affecting the phenotypic characteristics.[6,10] Considering that maxillary bone dimensions have shown great inter- and intra-population variability,[13,18] this study aimed to determine using a discriminant function analysis, whether specific maxillary linear and angular measurements performed in CBCT images may be useful to determine the sex in a Colombian population.

MATERIALS AND METHODS

Study design, sample population, and setting:

This cross-sectional study was evaluated and approved by the Ethical Research Board (Approval N° 62-2020) and the Technical Research Council (Code 2021-40871) of the Faculty of Dentistry of the University of Antioquia. All aspects of the research were performed in compliance with the ethical standards outlined in the Declaration of Helsinki. The sample size was estimated based on the patients' population referred for CBCT imaging of the maxillary bone, during the period from June 2020 to December 2022, to a private imaging specialized center (RADEX 3D Specialized Radiology Center) in Medellín, Colombia. Taking into account a total of 467 referred patients, an online calculator tool (Raosoft® Inc., Seattle, WA, USA) indicated a sample size requirement of at least 212 digital imaging and communications of medicine (DICOM) files to identify significant between-group differences in the bivariate comparisons with a 95% confidence level and 5% margin of error. Eligible cases were those aged 18 years or older with no congenital, pathological, or traumatic lesions of the maxillary bone. Exclusion criteria applied were evidence of ongoing orthodontic treatment or maxillofacial surgery, limited field of view hampering the visualization or location of the maxillary landmarks, and poor quality of CBCT images. Written informed consent was obtained from each patient for the use the scans for research purposes prior to CBCT imaging and data were used anonymously.

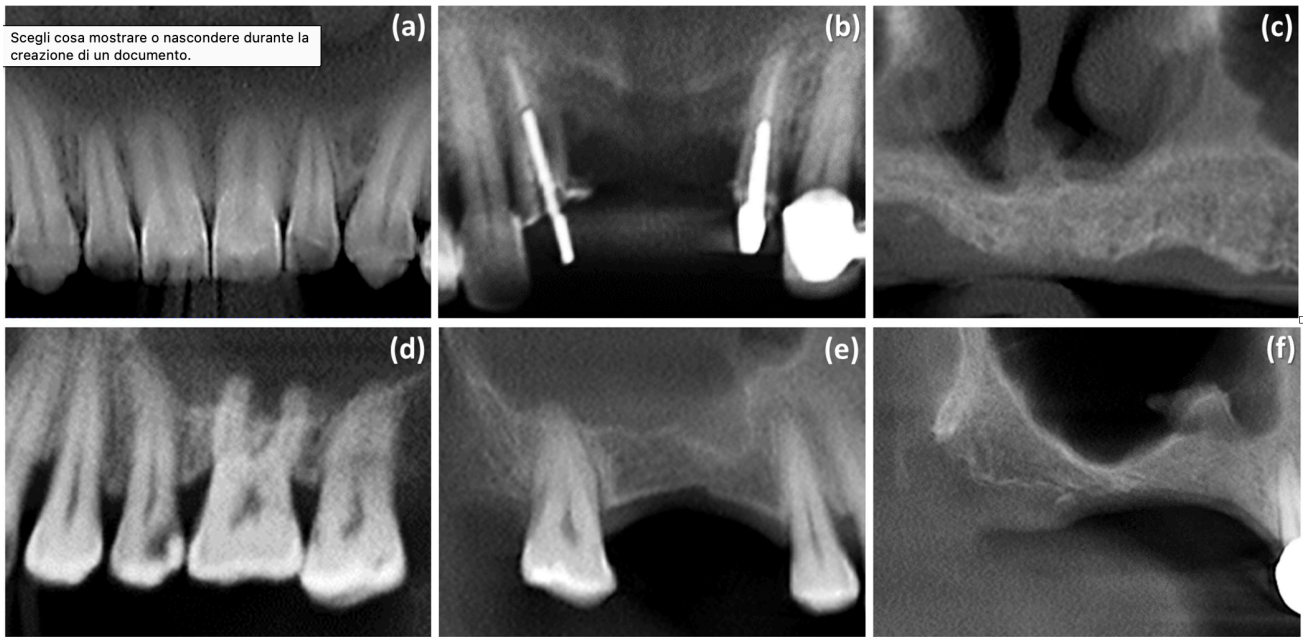
All of the cases underwent CBCT scanning with an i-CAT® 17-19 system (Imaging Sciences International, Inc., Hatfield, Pennsylvania, USA) at 120 kVp, 37.07 mA, 16 cm x 13 cm of field vision, 0.20 mm voxel size, and acquisition time of 26.9 seconds. Both the Frankfort horizontal and the midfacial planes were used to construct the horizontal and vertical axes of the reference

frame during the scanning process to avoid measurement pitfalls induced by the head position. Using the i-CATVision 1.9® software (Imaging Sciences International), CBCT scans were examined synchronously by two experienced Maxillofacial Surgeons (S.P.-G. and S.M.-G.) calibrated by a single "gold-standard" Maxillofacial Radiologist (C.I.S.-N.). The calibration was accomplished using illustrative images and written descriptions of the morphometric features to be measured. Any discrepancy between raters was arbitrated by using the Maxillofacial Radiologist judgment. Measurements were conducted on a computer screen in a darkened room by using the maximum intensity projection mode of the software to reach superior image contrast and the magnification function to amplify the images.

Patient-related data acquisition

Before performing the measurements, study sample characteristics such as age of the patient at the time of CBCT acquisition and sex (i.e., male vs female) were documented for each CBCT included in the study. Likewise, the alveolar process status was documented separately for each maxillary segment (i.e., right posterior, left posterior, and anterior maxilla) and categorized based on the absence of teeth (Fig. 1a-f) as fully dentate or completely/partially edentulous (with totally edentulous maxillary segments or at least one extracted tooth, except third molars).[19]

Figure 1. Panoramic views of a panel of CBCT scans showing representative features of the alveolar process status in different maxillary segments. (a) Dentate anterior alveolar process showing the presence of the six anterior superior teeth. (b) Partially edentulous anterior alveolar process displaying the loss of both maxillary central incisors. (c) Completely edentulous maxillary alveolar process as defined by the loss of all anterosuperior teeth. (d) Dentate right posterior alveolar process containing the two premolars and molars (except the third molars) within the alveolar bone. (e) Partially edentulous posterior alveolar process illustrated by the loss of the second premolar and first molar teeth in the left maxillary side. (f) Completely edentulous left posterior maxillary alveolar process showing the total absence of teeth.



Landmarks selection for morphometric assessment

Using multiplanar reconstructions, the measurement protocol was composed of two sets of measurements. The first set included seven non-bilateral maxillary parameters and the second set of measurements was composed by 16 bilateral maxillary morphometric data. Linear measurements were obtained in millimeters (mm) using the distance measuring tool of i-CATVision® software, whereas angular measurements were taken in degrees (°) with the aid of an image analyzer system (AxioVision 3.1®, Carl Zeiss®, Oberkochen, Germany) as follows:

- Non-bilateral maxillary variables included the length of the maxillary complex, determined in

the axial plane viewing simultaneously a line drawn through the palatal plane in the sagittal image, as the greatest distance between tip of the anterior nasal spine (ANS) of the maxilla and the tip of the posterior nasal spine (PNS) of the palatal bone; as well as the maximum inter-maxillary width, measured as the greatest distance between the right and left maxilla taken between lateral walls of the two maxillary sinuses (MS) (Fig. 2a). Furthermore, in the sagittal plane, several morphometric parameters were measured on the maxillary midline according to earlier descriptions (Fig. 2b and c),[13,16,17] including: the nasopalatine canal (NPC) length, measured as the distance among the midpoints of incisive foramen (IF) and nasopalatine foramen (NPF); the NPC angle, located anteriorly among the axis of NPC and the palatal plane; the minimum anterosuperior buccal bone thickness, measured as the shortest distance from the NPC to the buccal cortical border; the anteroposterior width of IF, assessed at the level of the hard palate; and the anteroposterior width of NPF, assessed at the nasal fossae level. When the NPC had two or more nasal and/or palatal openings, all the anteroposterior widths of visible foramina were summed.

- Bilateral maxillary morphometric data included the measurement of maximum craniocaudal, anteroposterior, and transverse dimensions of the MS (Fig. 3a-c). The maximum craniocaudal dimension (height) of the MS was measured on coronal reconstructions as the distance between the most superior and inferior points, while the anteroposterior (depth) and transverse (width) dimensions were determined on axial sections measuring the distances between the most anterior and posterior walls and among the most lateral and medial points of the MS, respectively. Also, the minimum distance from the MS floor to the alveolar crest (AC) was estimated by tracing a perpendicular line from the deepest point of the MS floor within the alveolar process to the surface of the alveolar crest viewing simultaneously the panoramic and coronal images (Fig. 3d and e).[13] Furthermore, in the infraorbital region, the greater width of infraorbital foramen (IOF), measured in the anterior wall of MS in the three orthogonal planes, and the vertical distance from the IOF to the AC[20,21] were included in the analysis (Fig. 3f-h). Finally, the morphometric assessment of the greater palatine canal (GPC) included the length of the GPC in the sagittal plane, measured from the central point of the pterygopalatine fossa to the central point of the greater palatine foramen (GPF)[22] taking into account the sum of the length of two lines intersecting in the center of the canal (Fig. 3i); the maximum anteroposterior width of the GPC in the axial plane and its distance to the pterygoid hamulus, NPC, and posterior nasal spine (PNS)[15,23] measured with the sagittal palatal plane positioned through the center of the supero-inferior dimension of the hard palate (Fig. 3j); the distance from the medial wall of GPF to the midline maxillary suture (Fig. 3k)[15,24] the distance from the center of GPF opening to the AC (Fig. 3k); and the angle between the vertical axis of the GPC and the horizontal plane of the palatine bone measured in the coronal view (Fig. 3l).[11,15]

Data management and statistical analysis

All data were statistically analyzed using standard statistical software (SPSS, v.29.0, IBM, Armonk, NY). Immediately after a twelve-month study period, which focused on completing all of the required measurements, 10% of the sample (21 CBCT scans) selected following a simple random sampling procedure, was reassessed simultaneously by the same examiners and the intraclass correlation coefficient (ICC) was calculated to determine intra-observer variability. Interpretation of ICC was based on the following categorization system: values <0.40 indicated poor reproducibility, 0.40-0.59 indicated fair reproducibility, 0.60-0.74 good reproducibility, and values ≥0.75 indicated excellent reproducibility.[25]

The distribution pattern of quantitative variables was analyzed using the Kolmogorov-Smirnov (K-S) test. Considering that the data showed a normal distribution pattern (P-values ranging from 0.062 to 0.200, K-S test), they were analyzed using the parametric t-test for independent samples to identify differences between the sexes. Homoscedasticity was verified through Levene's test for equality of variances. Also, Pearson's chi-square test (χ^2) was used to compare categorical variables. All significant predictor variables identified in the bivariate analyses were subjected to univariate discriminant analyses to calculate the mean percentage of correct prediction after cross-

validation of each of them. Cross-validation was used to adjust for the percentage of misclassified observations.[10] Afterward, a multivariate discriminant function analysis was conducted using the variables that presented a mean correct prediction percentage above 60% to estimate the sexual dimorphism of the sample. Wilks' lambda (λ) and Box's M tests were used to examine the statistical significance of the discriminant functions and the homogeneity of covariance matrices, respectively. Furthermore, eigenvalues and canonical correlations were also included to analyze the relationships among predictor variables and the sex categories. Fisher's linear discriminant function coefficients of the selected variables, with their respective constants, yielded to the development of the following equation as previously suggested:[1,6,10] $N = b1^* x1 + b2 * x2 + bm * xm + a$, where b1 through bm represent the discriminating coefficients, x1 through xm are discriminating variables, and a is the discriminant function constant. To assign the sex, the values of measurements must be separately substituted in the equation for male and female, so that the greater value of N indicates the sex.[6,10] The level of significance was set at 5% ($\alpha = 0.05$).

Figure 2. CBCT images showing morphometric evaluations of different non-bilateral maxillary landmarks. All of the lines were heightened using the AxioVision® software to illustrate the path by which the measurements were performed: (a) Axial section displaying the measurement of *(1)* maxillary complex length (distance ANS-PNS) and *(2)* maximum inter-maxillary width. (b) Sagittal section showing the measurement of *(1)* length of the NPC, *(2)* width of NPF and *(3)* width of IF. (c) Sagittal section illustrating the assessment of *(1)* the minimum anterosuperior buccal bone thickness and *(2)* the NPC angle with reference to the palatal plane.

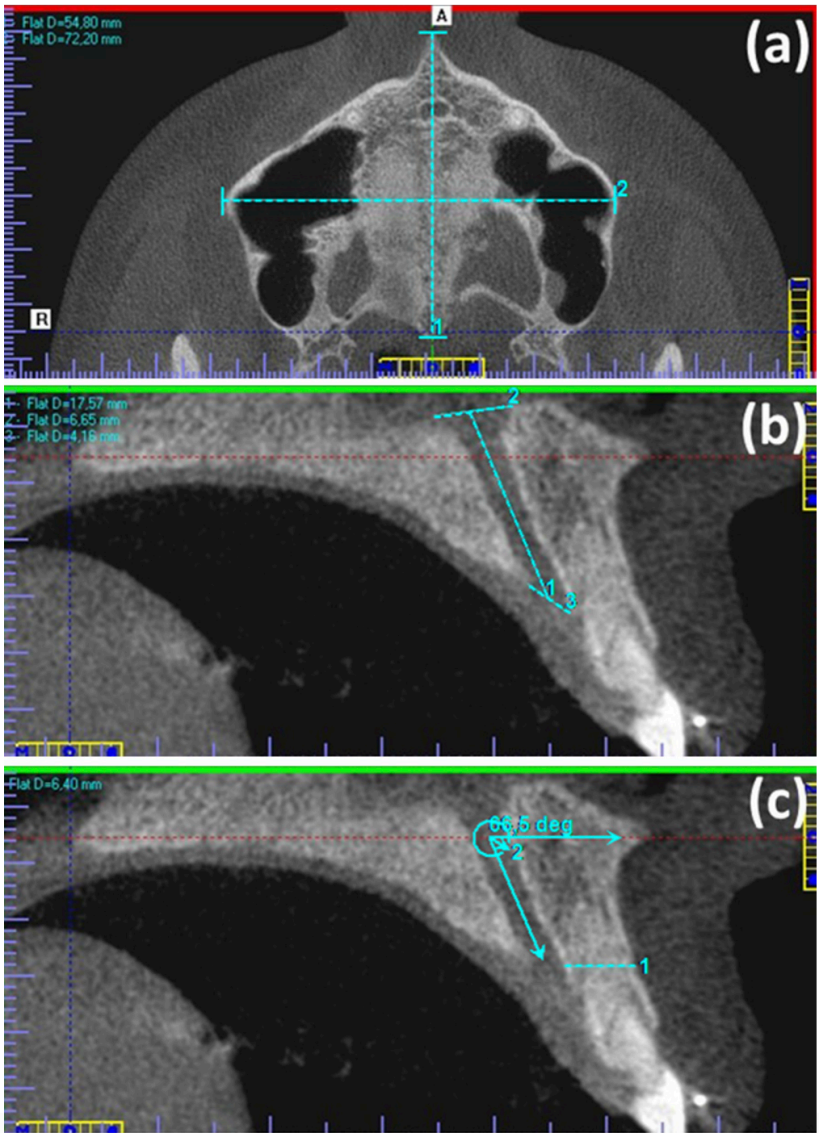
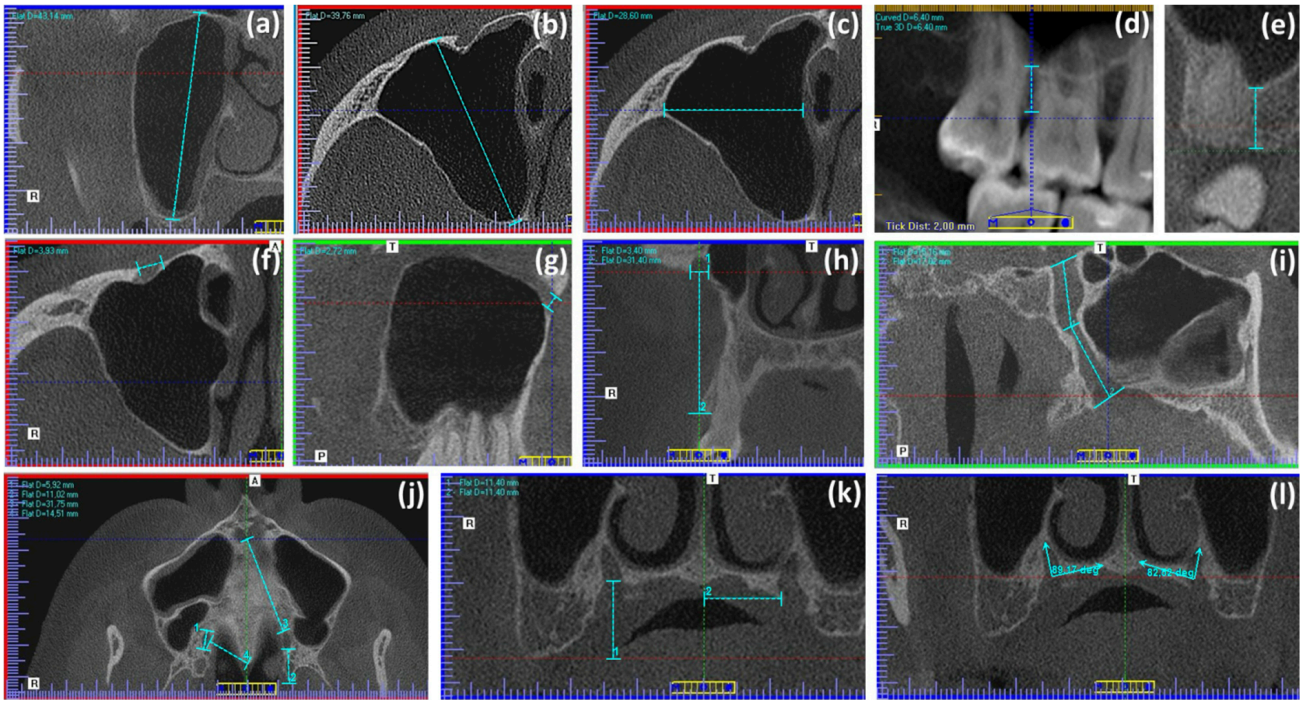


Figure 3. Representative CBCT scans of different sections of maxillary bone illustrating the way of measurement (green lines) of some variables as follows: (a) maximum MS height, (b) maximum MS depth, (c) maximum MS width; (d and e) minimum distance from the MS floor to the AC, (f) greater transverse width of IOF, (g) greater sagittal width of IOF, (h1) greater coronal width of IOF, (h2) distance from the mid-point of the IOF to the AC, (i) length of GPC in the sagittal plane, (j1) maximum anteroposterior width of the GPC in the axial plane, (j2) distance between GPC and pterygoid hamulus, (j3) distance between GPC and NPC, (j4) distance between GPC and PNS, (k1) distance from the center of GPF to the AC, (k2) distance between the medial wall of GPF and the midline maxillary suture, (l) angle formed by the horizontal plane of the palatine bone and the vertical axis of the GPC.



RESULTS

Study sample and reliability analysis:
Among DICOM files analyzed, 86 corresponded to males with an age range from 18 to 87 years (mean 46.99 ± 15.86) and 126 to females with an age range from 18 to 69 years (mean 50.46 ± 12.97). For each DICOM file, regardless of the sex, age, and alveolar process status, seven non-bilateral and 16 bilateral parameters were measured. The anterior maxillary alveolar process status was categorized as completely edentulous in 23 (10.80%) out of 212 cases, partially edentulous in 46 (21.70%) cases, and dentate in 143 (67.50%) cases. Likewise, 50 (11.8%) out of 424 posterior maxillary segments were classified as completely edentulous, 136 (32.10%) as partially edentulous, and 238 (56.10%) as dentate. No significant differences could be established for age and anterior/posterior alveolar process status with respect to sex nor for posterior alveolar process status between the left and right sides (all P-values >0.05, unpaired t or $\chi 2$ tests, data not shown), indicating an optimal comparability of data between the sexes in terms of age and alveolar process status. Likewise, there were no significant differences for bilateral measurements between maxillary sides (all P-values >0.05, unpaired t test, data not shown). The reproducibility analysis showed an excellent agreement among the two series of data obtained by the examiners for any of the quantitative parameters tested, with ICC values ranging between 0.893 and 0.999 (all P <0.001).

Between-group comparisons of tomographic measurements obtained from the sample
Tables 1 and 2 depict the bivariate comparisons of the non-bilateral or bilateral maxillary variables regarding to the sex distribution. From Table 1 can be seen that while no significant differences (all P >0.05 unpaired t test) were evidenced between sex categories regarding the NPC angle at sagittal plane and anteroposterior width of NPF, the mean values of the maxillary complex length, the maximum inter-maxillary width, the

length of NPC at sagittal plane, the minimum anterior-superior buccal bone thickness, and the anteroposterior width of IF were significantly higher in the male group (P <0.01) in comparison with those of the female group. In addition, Table 2 outlines how the dimensions related to the maximum height, depth, and width of MS; the axial, sagittal, and coronal measurements of IOF; the distance from the mid-point of IOF to the AC; length of GPC in the sagittal plane; the anteroposterior width of the GPC in the axial plane; the distance from the GPC to the pterygoid hamulus, NPC, and to PNS; along with the distance of GPF to the MMS and to AC were significantly higher (all P <0.05) in the male group in comparison with those calculated for the female group, whereas no significant differences (all P >0.05) were found among males and females in regards to the minimum distance from the sinus floor to the AC or the angle among the horizontal plane of PB and the GPC.

Table 1. Comparison of measurements related to specific non-bilateral maxillary variables with reference to the sex categories

Parameter[a]	Sex		*P*-value[b]
	Male (♂) (*n* = 86)	Female (♀) (*n* = 126)	
Maxillary complex length (mm)	53.81 ± 3.45	50.27 ± 3.14	<0.001
Maximum inter-maxillary width (mm)	67.67 ± 6.23	63.99 ± 5.51	<0.001
Length of NPC at sagittal plane (mm)	12.92 ± 2.72	11.08 ± 2.44	<0.001
NPC angle at sagittal plane (degrees)	69.48 ± 8.18	67.42 ± 9.58	0.104
Minimum anterosuperior buccal bone thickness (mm)	6.78 ± 1.68	5.91 ± 1.66	<0.001
Anteroposterior width of IF (mm)	3.92 ± 0.70	3.63 ± 0.85	0.009
Anteroposterior width of NPF (mm)	3.70 ± 1.85	3.45 ± 1.63	0.314

Abbreviations: NPC, nasopalatine canal; IF, incisive foramen; NPF, nasopalatine foramen

[a]Values are given as mean ± SD

[b]Two-sided unpaired *t*-test

Table 2. Comparison of measurements related to bilateral maxillary variables according to the sex category

Parameter[a,b]	Sex[a]		*P*-value[c]
	Male (♂) (*n* = 172)	Female (♀) (*n* = 252)	
Maximum maxillary sinus height (mm)	39.85 ± 3.87	35.27 ± 3.89	<0.001
Maximum maxillary sinus depth (mm)	37.59 ± 2.96	34.86 ± 3.10	<0.001
Maximum maxillary sinus width (mm)	28.98 ± 3.98	26.11 ± 4.21	<0.001
Minimum distance from the sinus floor to the AC (mm)	5.78 ± 3.44	6.08 ± 2.91	0.332
Greater width of IOF in the axial plane (mm)	4.70 ± 0.90	4.15 ± 0.94	<0.001
Greater width of IOF in the sagittal plane (mm)	4.14 ± 0.83	3.54 ± 0.87	<0.001
Greater width of IOF in the coronal plane (mm)	3.92 ± 0.68	3.56 ± 0.75	<0.001
Distance from the mid-point of the IOF to the AC (mm)	30.93 ± 2.95	29.73 ± 2.66	<0.001

Length of GPC in the sagittal plane (mm)	37.47 ± 3.17	35.11 ± 2.93	<0.001
Anteroposterior width of the GPC in the axial plane (mm)	6.28 ± 1.30	5.31 ± 1.27	<0.001
Distance from the GPC to the pterygoid hamulus (mm)	9.68 ± 1.45	8.93 ± 1.64	<0.001
Distance from the GPC to the NPC (mm)	33.20 ± 3.82	32.12 ± 3.00	0.001
Distance from the GPC to the PNS (mm)	15.97 ± 1.16	15.22 ± 1.28	<0.001
Distance from the medial wall of GPF to the MMS (mm)	13.77 ± 1.44	13.45 ± 1.38	0.021
Distance from the center of GPF to the AC (mm)	10.89 ± 2.30	9.45 ± 2.33	<0.001
Angle among the horizontal plane of PB and the GPC (degrees)	93.83 ± 8.02	93.82 ± 10.80	0.990

Abbreviations: AC, alveolar crest; IOF, infraorbital foramen; GPC, greater palatine canal; PNS, posterior nasal spine; NPC, nasopalatine canal; GPF, greater palatine foramen; MMS, midline maxillary suture; PB, palatine bone
[a]Data based on the sum of right- and left-maxillary posterior sides
[b]Values are given as mean ± SD
[c]Two-sided unpaired *t*-test

Findings from univariate and multivariate discriminant analysis.

To find the best axial, sagittal, and coronal maxillary predictors, all statistically significant variables identified in the bivariate analyses were included in univariate discriminant analyses, which are presented in Tables 3 and 4. From these tables is evident that Wilks' λ values varied from moderately high to very high in all of the constructs (0.748 to 0.987), thus indicating that there is considerable overlapping between the two groups. However, all χ^2 critical probability values were <0.05, demonstrating significant differences among men and women maxillary morphometric data. Furthermore, the results of the Box's M tests did not show statistically significant differences in the covariance matrices between sexes (all P >0.05) suggesting equality of covariance matrices between both groups. The univariate discriminant analysis of non-bilateral maxillary variables (Table 3) revealed a mean percentage of correct prediction after cross-validation ranging from 55.20% to 72.60%. The maxillary complex length showed the greatest accuracy (72.60%), and the anteroposterior width of IF exhibited the lowest value (55.20%). Similarly, the univariate discriminant analysis of bilateral maxillary variables (Table 4) showed mean percentages of correct prediction after cross-validation ranging from 58.70% to 73.10%. The maximum maxillary sinus height displayed the greatest accuracy (73.10%) whereas the distance from the medial wall of GPF to the MMS had the lowest value (58.70%). It is important to highlight that, cross-validation analysis demonstrated reduced accuracy percentages for 9 out of 19 variables included in the univariate discriminant analyses, while 10 variables showed the same mean correct prediction values, indicating that these methods produce acceptable results and are not overconfident. Considering that only the measurements obtained for maxillary complex length and maximum maxillary sinus height corresponded to average percentages above 70% in the univariate discriminant analyses, just the variables that presented correct prediction percentages above 60% were included in the multivariate discriminant analyses.

The results obtained from multivariate discriminant analysis of non-bilateral and bilateral maxillary variables for correct assignment by sex are presented in Tables 5 and 6, respectively. From these tables it can be seen the Fisher's linear discriminant function coefficients and the constants necessary to construct the discriminant equations, thus providing a direct method of classification for clinical application. It may be also noted in both predictive constructs that the Box's M covariance tests revealed equality between the matrices of male and female groups (P >0.05) thus suggesting a limited discrepancy in the predictor variables. Furthermore, Wilk's λ values decreased in the two multivariate models in comparison with those of the univariate counterparts, indicating that the groups of predictor measurements allow making statistically significant predictions in their outcomes (P <0.05). Moreover, the eigenvalues were far from zero and the canonical correlation

were moderate, so it is possible to assume that the variables used in each of the constructs led a moderately accurate distinction between the male and female groups. It was also noteworthy that the association of the variables in the multivariate models increased the percentages of correct sex prediction, even after cross-validation, up to 77.80% for non-bilateral and up to 77.40% for bilateral maxillary measurements.

Table 3. Univariate discriminant analysis and cross-validation for measurements related to specific non-bilateral maxillary variables

Parameter	Wilks' λ		Box's M		Correct prediction % ♂	Correct prediction % after cross-validation ♂	Correct prediction % ♀	Correct prediction % after cross-validation ♀	Mean correct prediction %	Mean correct prediction % after cross-validation
	Value	Significance	Value	Significance						
Maxillary complex length	0.778	<0.001	0.890	0.347	54.70	52.30	86.50	86.50	73.60	72.60
Maximum inter-maxillary width	0.911	<0.001	1.556	0.213	36.00	36.00	84.90	84.90	65.10	65.10
Length of NPC at sagittal plane	0.888	<0.001	1.133	0.288	44.20	44.20	83.30	82.50	67.50	67.00
Minimum anterior-superior buccal bone thickness	0.939	<0.001	0.016	0.898	33.70	33.70	84.10	84.10	63.70	63.70
Anteroposterior width of IF	0.968	0.009	3.660	0.056	18.60	16.30	81.70	81.70	56.10	55.20

Abbreviations: NPC, nasopalatine canal; IF, incisive foramen

Table 4. Univariate discriminant analysis and cross-validation for measurements related to bilateral maxillary variables

Parameter	Wilks' λ		Box's M		Correct prediction % ♂	Correct prediction % after cross-validation ♂	Correct prediction % ♀	Correct prediction % after cross-validation ♀	Mean correct prediction %	Mean correct prediction % after cross-validation
	Value	Significance	Value	Significance						
Maximum maxillary sinus height	0.748	<0.001	0.007	0.934	60.50	60.50	81.70	81.70	73.10	73.10
Maximum maxillary sinus depth	0.837	<0.001	0.450	0.503	49.40	49.40	81.30	81.30	68.40	68.40
Maximum maxillary sinus width	0.895	<0.001	0.633	0.427	44.20	44.20	80.60	79.80	65.80	65.30
Greater diameter of IOF at axial plane	0.920	<0.001	0.336	0.563	32.60	32.60	80.60	80.60	61.10	61.10
Greater diameter of IOF at sagittal plane	0.894	<0.001	0.527	0.468	39.00	39.00	82.10	81.30	64.60	64.20

Greater diameter of IOF at coronal plane	0.945	<0.001	1.480	0.224	25.00	25.00	83.30	83.30	59.70	59.70
Distance from the mid-point of the IOF to the AC	0.956	<0.001	2.239	0.135	27.30	27.30	88.10	88.10	63.40	63.40
Length of GPC at sagittal plane	0.872	<0.001	1.269	0.261	41.90	41.90	82.10	82.10	65.80	65.80
Anteroposterior diameter of the GPC at axial plane	0.878	<0.001	0.067	0.796	38.40	38.40	81.00	81.00	63.70	63.70
Distance from the GPC to the pterygoid hamulus	0.948	<0.001	2.883	0.090	24.40	24.40	82.90	82.50	59.20	59.00
Distance from the GPC to the NPC	0.973	0.001	2.984	0.084	19.80	19.20	91.30	90.90	62.30	61.80
Distance from the GPC to the PNS	0.918	<0.001	2.218	0.137	34.90	34.90	79.80	79.80	61.60	61.60
Distance from the medial wall of GPF to the MMS	0.987	0.021	0.468	0.495	7.00	4.70	95.60	95.60	59.70	58.70
Distance from the center of GPF to the AC	0.915	<0.001	0.027	0.871	34.90	34.90	85.30	83.70	64.90	63.90

Abbreviations: IOF, infraorbital foramen; AC, alveolar crest; GPC, greater palatine canal; PNS, posterior nasal spine; NPC, nasopalatine canal; GPF, greater palatine foramen; MMS, midline maxillary suture

Table 5. Multivariate discriminant analysis of measurements related to specific non-bilateral maxillary variables for correct assignment by sex

Measurements, Fisher's linear discriminant function coefficients, and equations	**Fisher Coefficient ♂**	**Fisher Coefficient ♀**	**Canonical discriminant functions and classification results**
Maxillary complex length	4.707	4.401	*n* = 212
Maximum inter-maxillary width	1.600	1.533	Box's M value = 7.651; significance = 0.679
Length of NPC at sagittal plane	1.382	1.130	Wilks' λ = 0.680; significance = <0.001
Minimum anterior-superior buccal bone thickness	0.337	0.170	Eigenvalue = 0.471; canonical correlation = 0.566
Constant	-191.746	-166.948	Correct prediction % ♂ = 67.40
Equations ♂ = 4.707*Maxillary complex length + 1.600*Maximum maxillary transverse width + 1.382*Length of NPC at sagittal plane + 0.337*Minimum anterior-superior buccal bone thickness – 191.746 ♀ = 4.401*Maxillary complex length + 1.533*Maximum maxillary transverse width + 1.130*Length of NPC at sagittal plane + 0.170*Minimum anterior-superior buccal bone thickness – 166.948			Correct prediction % after cross-validation ♂ = 66.30 Correct prediction % ♀ = 87.30 Correct prediction % after cross-validation ♀ = 85.70 Mean correct prediction % = 79.20 Mean correct prediction % after cross-validation = 77.80

Abbreviations: NPC, nasopalatine canal; IF, incisive foramen

Table 6. Multivariate discriminant analysis of measurements related to bilateral maxillary variables for correct assignment by sex regardless of the side

Measurements, Fisher's linear discriminant function coefficients, and equations	Fisher Coefficient ♂	Fisher Coefficient ♀	Canonical discriminant functions and classification results
Maximum maxillary sinus height	0.358	0.179	*n* = 424
Maximum maxillary sinus depth	2.354	2.258	Box's M value = 84.401; significance = 0.089
Maximum maxillary sinus width	-0.330	-0.356	Wilks' λ = 0.605; significance = <0.001
Greater diameter of IOF at axial plane	2.812	2.454	Eigenvalue = 0.652; canonical correlation = 0.628
Greater diameter of IOF at sagittal plane	-0.668	-0.971	Correct prediction % ♂ = 69.20
Distance from the mid-point of the IOF to the AC	3.243	3.231	Correct prediction % after cross-validation ♂ = 68.00
Length of GPC at sagittal plane	2.176	2.123	Correct prediction % ♀ = 84.10
Anteroposterior diameter of the GPC at axial plane	3.728	3.259	Correct prediction % after cross-validation ♀ = 83.70
Distance from the GPC to the NPC	2.466	2.407	Mean correct prediction % = 78.10
Distance from the GPC to the PNS	9.498	9.039	Mean correct prediction % after cross-validation = 77.40
Distance from the center of GPF to the AC	-0.324	-0.429	
Constant	-270.389	-241.129	

Equations:

♂ = 0.358*Maximum maxillary sinus height + 2.354*Maximum maxillary sinus depth - 0.330*Maximum maxillary sinus width + 2.812*Greater diameter of IOF in the axial plane - 0.668*Greater diameter of IOF in the sagittal plane + 3.243*Distance from the mid-point of the IOF to the AC + 2.176*Length of GPC in the sagittal plane + 3.728*Anteroposterior diameter of the GPC in the axial plane + 2.466*Distance from the GPC to the NPC + 9.498*Distance from the GPC to the PNS - 0.324*Distance from the center of GPF to the AC - 270.389

♀ = 0.179*Maximum maxillary sinus height + 2.258*Maximum maxillary sinus depth - -0.356*Maximum maxillary sinus width + 2.454*Greater diameter of IOF in the axial plane - 0.971*Greater diameter of IOF in the sagittal plane + 3.231*Distance from the mid-point of the IOF to the AC + 2.123*Length of GPC in the sagittal plane + 3.259*Anteroposterior diameter of the GPC in the axial plane + 2.407*Distance from the GPC to the NP+ 9.039*Distance from the GPC to the PNS - 0.429*Distance from the center of GPF to the AC – 241.129

Abbreviations: IOF, infraorbital foramen; AC, alveolar crest; GPC, greater palatine canal; PNS, posterior nasal spine; NPC, nasopalatine canal; GPF, greater palatine foramen; MMS, midline maxillary sutur

DISCUSSION

It is accepted that skeletal sex estimation in unknown human remains is based on the sexually dimorphic expression of bony features that occur through different patterns, rates and growth periods.[26] Given that mandible and maxilla exhibit different morphological variations between males and females,[6,27-29] these bones may be suitable for sex estimation. However, although compelling evidence concerning sexual dimorphism has emphasized the need for determining anthropometric standards for

different populations,[2,6,8,10] to the best of the authors knowledge, this is the first study to demonstrate sex dimorphism of adult humans based on the discriminant function analysis of morphometric parameters of various maxillary structures analyzed using CBCT scans. In a previous study, the authors demonstrated, using similar imaging methods that maxillary bone can present several dimensional differences that may be strongly liaised to sex, but are independent of age, side, and of the alveolar process status[13]. In the present study, the authors used a different method to compare the maxillary dimensions with the majority of the DICOM files previously analyzed, but excluding of all cases with outlier data,[30] and including new DICOM files in order to uphold the estimates at an optimal level of accuracy against the effect of decreased sample size caused by the exclusions. It is worth noting that intra-observer repeatability was significantly excellent for the measurements evaluated in all multiplanar reconstructions, so this study supports the view that CBCT imaging constitutes a simple, reliable, and valid technique for the assessment of morphometric characteristics of the maxillary bone.

The bivariate analyses of this study demonstrated higher mean values for several non-bilateral and bilateral maxillary measurements in males compared to female data, with the exception of the NPC angle at sagittal plane, the anteroposterior width of NPF, the minimum distance from the sinus floor to the AC, and the angle between the horizontal plane of PB and the GPC. Among the analyzed variables, 7 out of 9 (77.77%) non-bilateral and 14 out of 16 (87.5%) bilateral maxillary parameters showed sexual dimorphism. Altogether, these results confirm previous findings demonstrating that maxillary bone can present multiple morphological and dimensional differences toughly liaised to sex.[7,8,12,13,23,27] Alternatively, the present findings might parallel, at least partially, those of previous anthropometric studies that have applied diverse statistical protocols for sex prediction from cranial metric traits using limiting points, sectioning points, demarking points, logistic regression analysis, discriminant function analysis, support vector machine modeling, machine learning algorithms, and deep learning artificial neural network models.[5,31-35] Although the exact explanation for sex differences has not been fully clarified, it has been argued that these variations might be caused by several factors influencing the bone remodelling process including genetic background, ethnicity, sex hormone actions, muscle mass, masticatory muscle activities, and socio-economy environment.[27]

Discriminant function analysis has become a broadly used and accepted approach for sex estimation from human skeletal remains. In the current study, several univariate discriminant analyses were conducted to evaluate the usefulness of individual predictor variables to discriminate the sexes from each other. It should be noted that this stage focused on the inclusion of variables that showed significant differences in the bivariate comparisons, while non-significant variables were excluded due to the lack of discriminatory capacity. For the included variables, Wilks' λ values ranged from 0.987 to 0.748 across the analyses, representing a range from 1.3% to 25.2% of variance explained. Given that all χ^2 critical probability values were <0.05, these results suggest that, when considered individually, all morphometric variables included in the analyses contributed to the sex group separation. Among these variables, both the maxillary complex length linked to specific non-bilateral maxillary variables (mean correct prediction % after cross-validation = 72.60%) and the maximum maxillary sinus height linked to bilateral maxillary variables (mean correct prediction % after cross-validation = 73.10%) presented the greatest sexual dimorphism confirming the results of other researchers.[7,8] Notwithstanding the aforementioned, just the variables showing correct prediction percentages above 60% were merged in the multivariate analyses.

The stepwise multivariate discriminant function analysis was conducted to determine the optimal combination of variables for discriminant functions and their weighting to reflect the contribution to sex estimation.[1] It is important to point out that, in agreeance with previous results,[10] although Wilks' λ values revealed a low discriminant power of the variables included in the constructs, their values improved as more variables were included in each discriminant model. In consonance with the former, it has been recognized that the increasing number of variables might affect the results and enhance the accuracy of measurements.[36] The discriminant functions obtained from this study were tested

for accuracy using leave-one-out cross-validation methods to evaluate such classifications.[5] It has been accepted that, since sex constitutes a discrete dichotomous variable, the probability of correctly estimating the sex at random would be 50%.[10] In the present study using a series of maxillary bone measurements, the mean correct percentage reached after cross-validation to estimate sex was statistically significant and 27.40% to 27.80% higher using either bilateral or non-bilateral maxillary variables than that yielded at random, thus suggesting a good level of sex identification. Moreover, in view of that sex may be determined from the complete skull with 80% accuracy,[37] the findings herein presented indicate that maxillary bone is a reliable sex predictor.

Finally, several limitations were identified in this study. First, acquisition of CBCT images was based on parameters used for live adult individuals, so these could be adapted to preserve the image quality and make the measurements reproducible in case of evaluating deceased individuals and bone remnants.[8] Second, Colombian population possesses a heterogeneous ethnographic profile. This circumstance may impede the generalizability of the findings to other ethnic groups with dissimilar maxillary morphological characteristics.[13] Consequently, it is necessary to design discriminant functions for different populations in order to evaluate the effectiveness of this sex identification method. Third, considering that some researchers argue that sexual dimorphism is not manifest at an appreciable level until after pubertal modifications have taken place,[38,39] mainly due to the different growth and development patterns between males and females,[27] the functions presented can be applied to maxillary bones of adult individuals but have limited value before puberty. Hence, additional studies using different approaches, such as discriminant analyses based on permanent tooth dimensions, are required for subadult sex estimation. Fourth, although the increased values of 19 variables were significantly associated with the male sex in the bivariate comparisons, additional factors, comprising age, extent of tooth loss, and wearing prosthesis might have an important influence on the reported data. Nevertheless, according to the findings presented, it seems that, irrespective of age, dental, and prosthetic conditions, all of these parameters are robustly liaised to the male sex.

CONCLUSION

Within the limitations of the study, it can be concluded that, CBCT measurement of maxillary bone parameters may be applied as a complementary technique to discriminate the sex from human remains through discriminant function analysis methods in the Colombian population.

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Human identification through 3D images: a comparative method based on common dental morphological traits

Ana Catarina Silva[1,2], Alexandra Teixeira[1,2], Teresa Pinho[3], Daniel Pérez-Mongiovi[1,2]

[1]*Associate Laboratory i4HB - Institute for Health and Bioeconomy, University Institute of Health Sciences - CESPU, Gandra, Portugal.* [2]*UCIBIO - Applied Molecular Biosciences Unit, Forensics and Biomedical Sciences Research Laboratory, University Institute of Health Sciences, (1H-TOXRUN, IUCS-CESPU), Gandra, Portugal.* [3]*UNIPRO – Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal*

Corresponding author:
daniel.mongiovi@iucs.cespu.pt

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KEYWORDS

Three-dimensional images, Dental traits, Disaster victim identification, Forensic dentistry, Forensic anthropology

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ABSTRACT

New complementary methods to aid in the rapid identification of human remains, particularly in cases involving multiple victims and heavily degraded bodies, are valued in the forensic field. In these circumstances, the properties and characteristics of teeth make them incredibly useful for human identification. Dental morphological traits, commonly studied in anthropology, exist in the population with varying frequencies, which makes them very informative. Thus, everyone has their own dental trait profile, that may manifest with greater or lesser frequency in the population. We propose a discriminative method by exclusion for the identification of individuals, based on the evaluation of dental traits, using intraoral 3D images. For this purpose, a group of 10 dental traits was chosen to obtain a personal profile in a population group of 76 individuals. Various scenarios were created to analyze different problem cases, by using matching-exclusion tables. Three individuals (problem cases) with dental trait profiles of very low (1/27703 individuals), medium (1/136 individuals), or high frequency in the population (1/42 individuals) were randomly chosen for the experiment. Using a conservative approach, the most likely candidate for positive identification was determined in eight steps for problem case 1, five steps for problem case 2, and two final candidates were identified in problem case 3, with only one achieving 100% similarity. In contrast, the less conservative approach identified the most likely candidate in four steps for case 1, three steps for case 2, and four steps for case 3. The rarest dental profile did not necessarily require fewer steps. Our work suggests that using the well-known dental morphological traits and recent 3D imaging technology could significantly improve the efficient triage and identification of victims, especially in cases of closed mass disasters.

INTRODUCTION

Forensic identification of victims is of extreme importance, not only for humanitarian reasons but also in civil or criminal investigation cases. Identifying human remains frequently requires a multidisciplinary approach, and techniques that best adapt to the circumstances are recommended.[1]

Identification with an odontological approach has proven to be very effective, relatively quick, and cost-effective. Forensic odontological methods follow an evaluation process that

considers characteristics of dental morphology, the number and position of teeth, dental pathologies and treatments, and other maxillofacial identifiers.[2-4] Whenever possible, dental evaluation is the preferred approach for many mass disaster casualties. In such situations, a quick triage (through the biological profile) for later application of comparative techniques will lead to a shortening of the delivery time of bodies to their families.[1,5,6]

The search for identity through teeth begins by comparison of postmortem (PM) and antemortem (AM) records.[6] These include dental pictures, clinical notes, x-rays, and three-dimensional (3D) models, among others.[3-6] The greater the number of physiological, pathological, or therapeutic modifications, and the less frequent the morphological characteristics are in the general population, the easier the identification will be.[4]

Currently, computerized tomography (CT) scans and intraoral 3D images are also recorded, especially in dental clinics with more advanced equipment. [7-10] Although radiographs continue to be the gold standard as an imaging support for forensic identification, these new techniques are beginning to be, at the very least, a relevant complement to forensic investigation.[6,9,11,12]

In mass disaster events, shortening the delivery time of mortal remains is very important, but the high number of victims and the conditions found by the Disaster Victim Identification (DVI) teams on the ground can generate complex situations, and all care must be taken to avoid making mistakes.[1,3,5,6,11] This is why additional methods that aid in quickly triaging candidates and confirming their identification are desirable.

Ancestry (or population affinity) is one of the parameters that help reconstruct the biological profile of victims' remains through their teeth, together with the sex, age, and height estimation; this is especially important when there are no records available for comparison of AM and PM data.[5,13,14] This allows us to reconstruct a profile in cases of severely degraded human remains. Anthropological studies have thoroughly researched and classified dental traits that are more prevalent in certain populations.[15,16] For instance, Europeans are more likely to have high degrees of the Carabelli's cusp of the first upper molar, Asians and Indo-Americans often have shovel-shaped incisors, while sub-Saharan Africans have a higher prevalence of Bushman's canine. [15,16] However, other dental traits are present in all populations with minor variations, including the number of cusps on lower premolars or the pattern of grooves on lower molars. [17] Thus, everyone has a particular profile of dental traits, which may be very common in certain individuals but rare in others. [18]

There are scenarios of closed mass disasters with a high number of victims, such as plane crashes, fires, or shipwrecks, in which the severely affected bodies need to be identified.[5] The use of intraoral scanners would allow almost instant 3D images of the victims' dentition (complete or partial) that could even be obtained on the ground with a mobile scanning station. The availability of AM intraoral 3D recordings allows for the acquisition of dental traits' profiles, which could be quickly compared with PM profiles to speed up triage, or even clearly indicate a probable identification.

In this study, we propose a new exclusion comparative method based on dental morphological traits to assist in the triage and identification of victims in closed mass disasters.

MATERIALS AND METHODS

Selection of dental traits and classification

The selection of traits was based on their varying frequencies in northern Portugal, using a study conducted on a collection of crania and mandibles approximately one century old as a reference.[19] A representative mixture of easily observable traits was chosen, with variable frequencies, on different types of teeth and upper and lower jaws. The ten chosen traits were: upper central incisive shoveling, upper lateral incisive interruption groove, upper canine distal accessory ridge, lower 2nd premolar cusp number, Carabelli's cusp, hypocone of the upper second molar, lower 1st molar cusp number, lower 2nd molar cusp number, lower 1st molar groove pattern, lower 2nd molar groove pattern.

Table 1 summarizes the frequencies of selected dental traits based on anthropological studies of the Northern Portuguese population.[19] The breakpoint indicates the extent to which the presence of the trait is considered unequivocally present; for lower grades than the breakpoint, the trait is considered absent. For some traits, there are no grades; the breakpoint consists only of the presence or absence. Each observed trait was classified based on the ASUDAS classification (Arizona State University Dental Anthropology System).[16]

To classify the different dental traits, two intra-observer assessments were carried out over several days for all the individuals. In case of non-coincidence between assessments, a third final assessment was carried out.

Table 1. Frequencies of the chosen traits in northern Portugal after Marado and Silva.[18] The breakpoint indicates the grade from which the presence of the trait is considered unequivocally present.

Trait:	Marado & Silva		Breakpoint
Shovel shaped upper central incisor	Presence	Absence	Presence- Grade 3-6
	3.5%	96.5%	
Interruption grooves in the upper lateral incisor	Presence	Absence	Presence/Absence
	12.3%	87.7%	
Distal accessory ridge in the upper canine	Presence	Absence	Presence- Grade 2-5
	21.5%	78.5%	
Number of lingual cusps of the lower second premolar	2 or 3 lingual cusps	1 lingual cusp	Presence- Grade 2-9
	21.7%	78.3%	
Carabelli´s Cusp	Presence	Absence	Presence- Grade 5-7
	12.8%	87.2%	
Hypocone	Presence	Absence	Presence- Grade 2-6
	49.8%	50.2%	
Cusp number of the lower first molar	Presence	Absence	Presence of cusp number 5
	72.2%	27.8%	
Cusp number of the lower second molar	Presence	Absence	Presence of cusp number 5
	12.6%	87.4%	
Groove pattern of the lower first molar	Y pattern	Another pattern	Y pattern
	87.8%	12.2%	
Groove pattern of the lower second molar	Y pattern	Another pattern	Y pattern
	83.5%	16.5%	

Sample and registration of 3D images

Intraoral images of 76 patients from the Clínica Médico Dentária de São João da Madeira, Lda., (North of Portugal) were used in this retrospective study. Informed consent for the scientific use of the data was obtained at the time of collection, and the images were subsequently pseudo-anonymized (approval from the Institutional Ethics Committee, approval statement number 19/CE-IUCS/2021).

The individuals were of both sexes, aged between 18 and 52 years, circumscribing an adult age and a provable low number of missing teeth. The inclusion criteria were individuals with permanent dentition of both sexes and from the North of Portugal. The exclusion criteria were individuals with deciduous dentition, foreign or with dental treatment that severely affects the morphology or visibility of most traits.

The 3D images were obtained using the iTero® intraoral 3D scanner from Align Technology, Inc. MeshLab software (MeshLab_64_fp v2023.12) and Blender software (www.blender.org) were also used to observe and analyze 3D images and to obtain two-dimensional (2D) photos.

Calculation of probabilities

The probability of an individual having a certain trait profile in the North of Portugal was calculated by multiplying the frequency ratio of each trait, shown in Table 1 of Supplementary Information, considering the data of Table 1. To simplify the method, only the left teeth were considered. For example, we consider an individual from the northern region of Portugal who matches the following profile: absence of upper central incisive shoveling, presence of upper lateral incisive interruption groove,

absence of upper canine distal accessory ridge, presence of lower 2nd premolar cusp number, presence of Carabelli's cusp, absence of hypocone of the upper second molar, presence of lower 1st molar cusp number, presence of lower 2nd molar cusp number, Y lower 1st molar groove pattern, X lower 2nd molar groove pattern. Based on the information provided, the probability of this person having the described traits would be calculated as follows: 0.965 x 0.123 x 0.785 x 0.9 x 0.217 x 0.217 x 0.502 x 0.722 x 0.126 x 0.835 x 0.165 = 1.145 x 10^-5. This is equivalent to 1 in 8,733 persons.

Matching-exclusion comparative table and terminology used

A matching-exclusion table was created integrating all candidates for identification and the trait profile of the problem case. The sequence analysis of the traits was ordered from a more evenly distributed (Hypocone: 49.8% present – 50.2 % absent) to a less balanced distribution (Upper Central Incisor Shoveling: 3.5% present – 96.5 % absent) within the population.

To fill in the table, a color code is suggested to facilitate visualization, which is explained in Table 2. In each box, similarities are indicated during the analysis, but the filling stops its progression when there is no coincidence (considered "unexplainable discrepancy"). The operator did not know the reference number of the problem cases. Some factors were taken into consideration to reflect real scenarios (see Table 2). When the operator labels the trait as "explainable discrepancy" or "Insufficient evidence", the classification continues without exclusion. This procedure is very conservative because it does not exclude the candidate when considered "Insufficient evidence", despite the strong indications that do not point to him as the problem case.

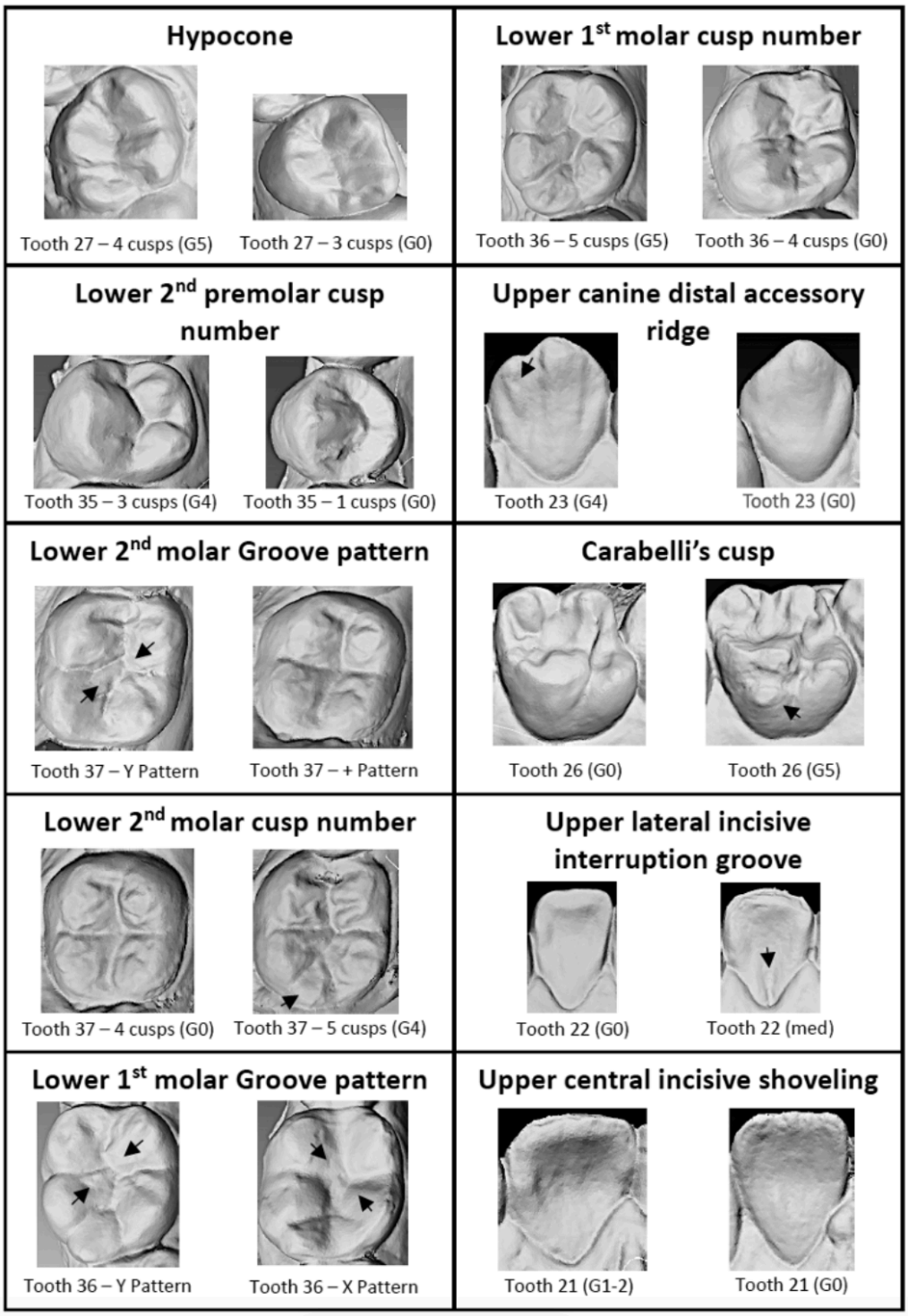
Table 2. Color code for the matching-exclusion comparative method, and terminology used.

Color Code	Relevance	Explanation
	SIMILARITY	When both classifications coincide.
	EXPLAINABLE DISCREPANCY	When the trait is visible in the candidates but not visible in the problem case (in a real scenario, it could correspond to a trauma at the time of the accident or dental treatment at a later date, after the provided images); or when the trait is not observable either in the candidate or in the problem case due to dental treatment; or when the difference between a present trait is up to two grades, considering ASUDAS classification (a safety inter-observer margin of two grades is applied when the trait is present).
	INSUFFICIENT EVIDENCE	When the trait is visible in the problem case but not in the candidates. It is more likely a case of unexplainable discrepancy due to dental treatment or missing teeth in the candidates, which is not observable in the problem case. However, the current analysis approach is very conservative, so this trait will not be considered for analysis because it cannot be compared. Also, in a real scenario, a poor image quality of a candidate's dental trait could lead to it not being considered for analysis.
	UNEXPLAINABLE DISCREPANCY	When the trait is present in one individual and absent in the other; when there is a difference in the trait classification of more than two grades. The discrepancies are irreconcilable. The candidate is excluded. When the colors green, yellow, or orange are assigned, the classification continues. With the red color, the classification stops, and the individual is excluded.

RESULTS

The analysis of 3D intraoral images allows the observation and classification of dental traits. Some images of traits chosen for this study are shown in Figure 1. The profile of the 76 individuals for the 10 dental traits used in this exercise is presented in Table 2 of the Supplementary Information.

Figure 1. Examples of intraoral 3D images focused on certain dental traits used in this study



Three individuals were randomly chosen as problem cases for a simulation. Profiles with different probabilities of being present in our studied population were selected (problem case 1: 1/27703 individuals; problem case 2: 1/136 individuals; problem case 3: 1/42 individuals).

The dental traits profile of the problem case 1 is shown in Figure 2. The exclusion comparative

method was then applied, coming up with a single candidate (candidate 47F) who turned out to be the correct individual (Figure 3). For problem case 2, the correct individual came up after covering half of the dental traits (candidate 59M) (Figures 4 and 5).

Figure 2. Dental traits profile of the problem case 1. Abbreviatures of the traits are shown at the bottom lane. G: Grade, NV: Not visible, P: Present (Grade), Pa: Pattern, DT: Dental Treatment.

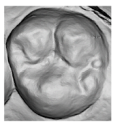
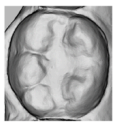
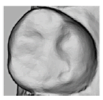
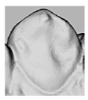
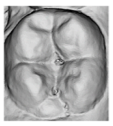
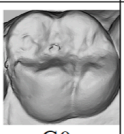
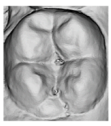
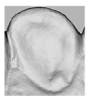
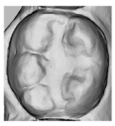
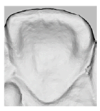
Hypocone	Lower 1st Molar cusp number	Lower 2nd Premolar cusp number	Upper canine distal accessory ridge	Lower 2nd molar Groove pattern	Carabelli's cusp	Lower 2nd molar cusp number	Upper lateral incisive interruption groove	Lower 1st molar Groove pattern	Upper central incisive shoveling
 G2	 P(5) DT	 G2	 G2	 Pa +	 G0	 P(5)	 M	 NV DT	 G1
Hyp	L1MCN	M2MCN	UCDAR	L2MGP	Cbll	L2MCN	ULIIG	L1MGP	UCIS

Figure 3. Matching-exclusion table integrating all candidate individuals for identification. The references of the candidates are listed in the first column.

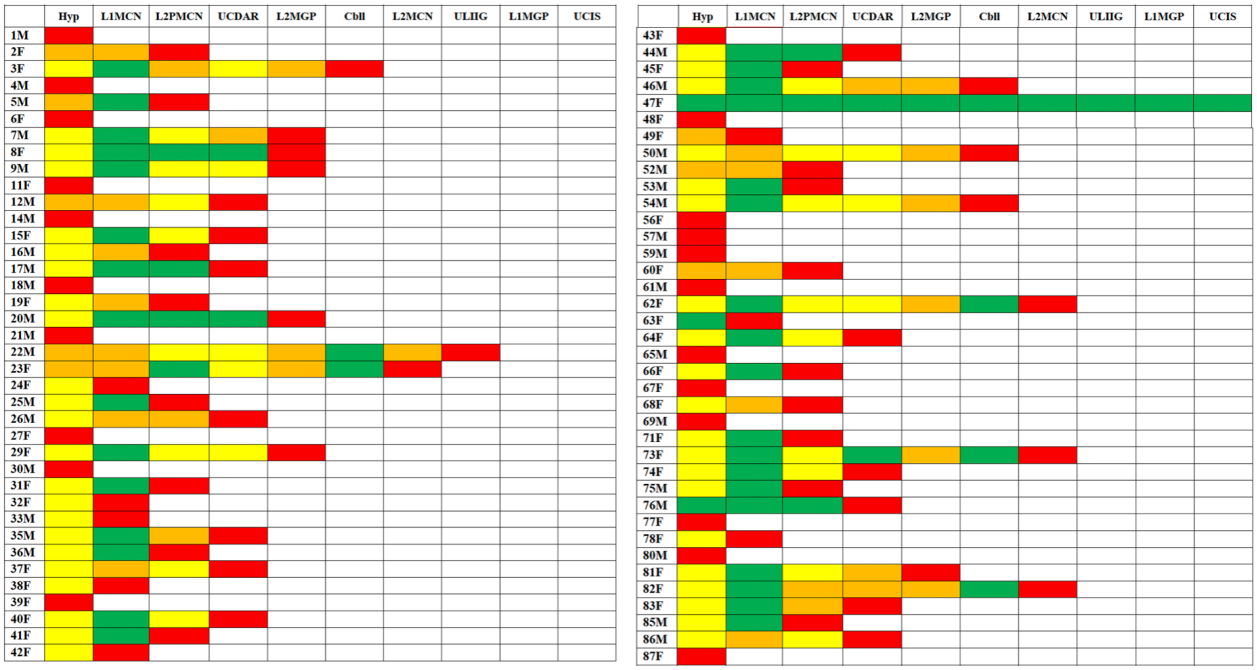


Figure 4. Dental traits profile of the problem case 2. Abbreviatures of the traits are shown at the bottom lane. G: Grade, NV: Not visible, P: Present (Grade), A: Absent (Grade), Pa: Pattern, DT: Dental Treatment.

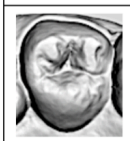
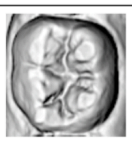
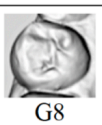
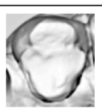
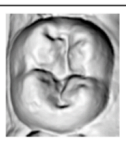
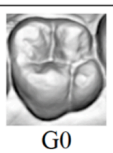
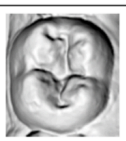
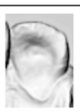
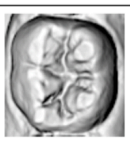
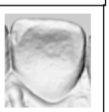
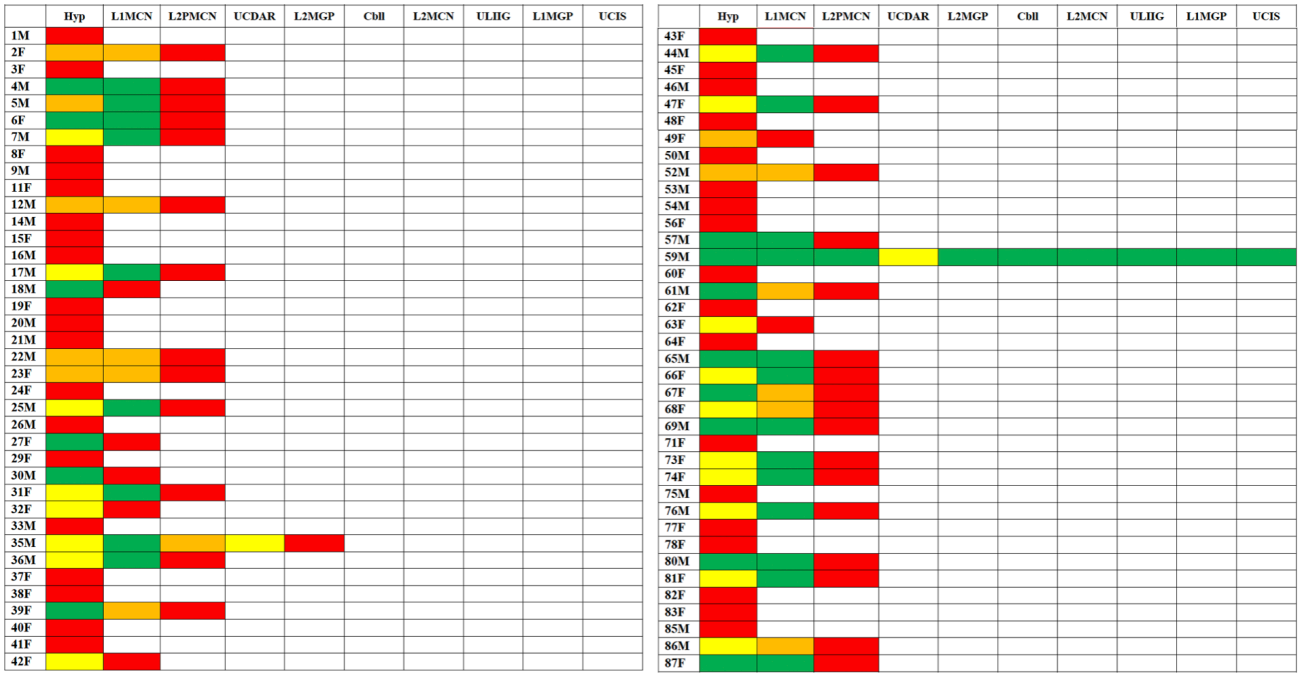
Hypocone	Lower 1st Molar cusp number	Lower 2nd Premolar cusp number	Upper canine distal accessory ridge	Lower 2nd molar Groove pattern	Carabelli's cusp	Lower 2nd molar cusp number	Upper lateral incisive interruption groove	Lower 1st molar Groove pattern	Upper central incisive shoveling
 G0	 P(5)	 G8	 NV DT	 Pa X	 G0	 A(4)	 A	 Pa Y	 G0
Hyp	L1MCN	M2MCN	UCDAR	L2MGP	Cbll	L2MCN	ULIIG	L1MGP	UCIS

Figure 5. Matching-exclusion table integrating all candidate individuals for identification. The references of the candidates are listed in the first column.



Finally, two candidates came up for problem case 3 (candidates 11F and 48F), but only one presented 100% similarities (candidate 11F), and for the other one, more than half of the traits were not visible due to dental treatments (Figures 6 and 7).

Of the three examples of problem cases shown, problem case 2 was the one in which an identification was reached the fastest (5 steps). In this case, if we reverse the order in which the traits are compared, from the most unequally distributed in the population to the most balanced, an identification would also be reached, however, with more steps, since it would be necessary to analyze the 10 traits (data not shown).

In summary, using a conservative approach ("explainable discrepancy" or "Insufficient evidence" results were considered without exclusion), the most likely candidate for positive identification was determined in eight steps for problem case 1, five steps for problem case 2, and two final candidates were identified in problem case 3, with only one achieving 100% similarity. In contrast, the less conservative approach ("explainable discrepancy" or "Insufficient evidence" results excluded the candidate) identified the most likely candidate in four steps for case 1, three steps for case 2, and four steps for case 3.

Figure 6. Dental traits profile of the problem case 3. Abbreviatures of the traits are shown at the bottom lane. G: Grade, NV: Not visible, P: Present (Grade), Pa: Pattern, DT: Dental Treatment.

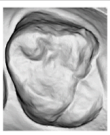
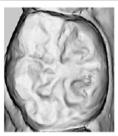
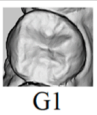
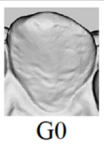
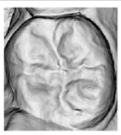
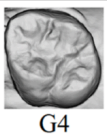
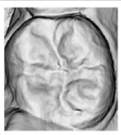
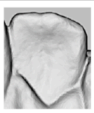
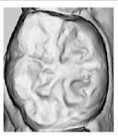
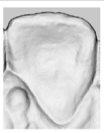
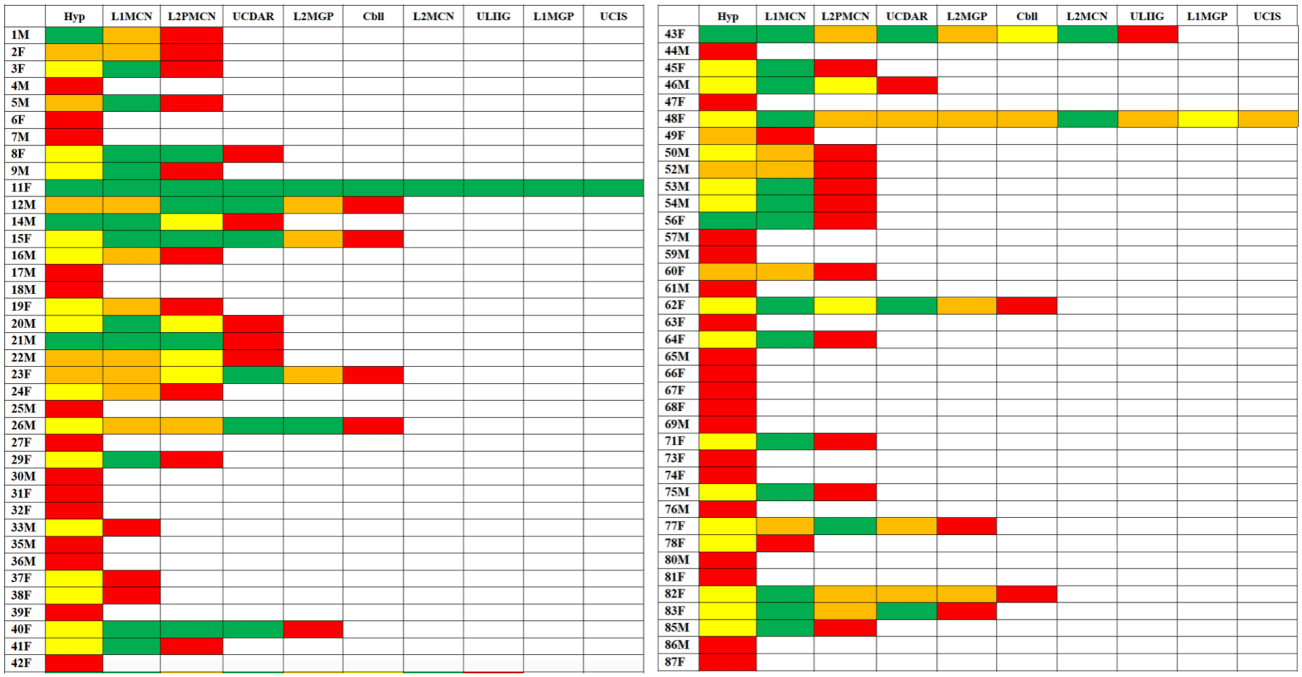
Hypocone	Lower 1st Molar cusp number	Lower 2nd Premolar cusp number	Upper canine distal accessory ridge	Lower 2nd molar Groove pattern	Carabelli's cusp	Lower 2nd molar cusp number	Upper lateral incisive interruption groove	Lower 1st molar Groove pattern	Upper central incisive shoveling
 G5	 P(5)	 G1	 G0	 Pa +	 G4	 A(4)	 A	 NV DT	 G0
Hyp	L1MCN	M2MCN	UCDAR	L2MGP	Cbll	L2MCN	ULIIG	L1MGP	UCIS

Figure 7. Matching-exclusion table integrating all candidate individuals for identification. The references of the candidates are listed in the first column.



DISCUSSION

In this study, we show that using a comparative approach, dental traits (typically linked to anthropological research on population affinities) can also serve as a methodical forensic tool for human identification.

The frequencies of many traits vary among different populations; what is uncommon in one population may be very present in another. However, these differences tend to diminish due to increasingly widespread migration phenomena. In this study, we used the frequencies of these traits in a population from the north of Portugal for guidance purposes only.[19] The intention was to demonstrate the variability in the frequencies of dental trait profiles within the population. This variability in trait frequencies will, a priori, help decrease the number of candidates at each step of the matching-exclusion process. On one hand, more evenly distributed traits allow for discarding approximately half of the candidates (present or absent). In contrast, a more extreme unequal distribution favors, in theory, discarding many candidates at once when the trait is infrequent in the problem case. The chosen traits in this study are relatively common in different populations.[16] The very rare traits, despite their undeniable value for identification,[20] would not apply to this approach.

In the three problem cases used as examples of how the methodology works, the correct identification of the problem individual was always achieved, despite very conservative rules being applied. When the trait cannot be observed in the problem case or the candidate (masked by dental treatment or missing tooth), the procedure continues. However, in a normal comparative identification process by the forensic odontologist, dental treatment or antemortem tooth loss in the candidate, not confirmed in the problem case, would be sufficient for unexplainable discrepancy and exclusion.

To simplify the procedure, only the left side of the jaw was used. The truth is that the method's effectiveness can be increased by using more information. Nevertheless, in real-life scenarios, the trend will be loss of information, with situations such as fragmented jaws or missing teeth. In such situations, we believe that this comparative exclusion method would still be useful. It is important to note that due to better oral health care among young people, fewer treatments are performed that alter dental morphology, which would help in identifying individuals through dental traits.

This comparative exclusion methodology could be ideally applied in various forensic scenarios involving mass casualties: accidents, natural disasters, terrorist acts, and historical or clandestine mass graves. For example, dental traits could be used to identify individuals from the same family in a mass grave.[21] This technique

preserves the remains without causing damage, and the images accurately show all the essential morphological details, keeping the original dimensions.[7,22,23] Yet, there may be challenges in capturing 3D images on the ground, at the site of the disaster. These challenges include limited access to the oral cavity in cases of burns or rigor mortis; the need to clean debris that could potentially affect the interpretation of morphological traits in 3D images; and finally, collecting images from fragile and partially fragmented jaws. Other limitations may arise at the time of analysis: training in observing and classifying dental traits is necessary; some training in dental medicine, particularly in dental anatomy, would be desirable to identify therapeutic modifications (the use of dental materials or dental devices is quite common, and can hide traits such as the groove pattern of the mandibular molars or accessory ridges on the canine, among others) (see images in Figures 2, 4, and 6); and when classifying certain dental traits, there is a level of subjectivity that should be considered.

What we present here is a first proposal for a methodology that has room to evolve: selection of other traits, selection of traits less subject to dental treatments, and application in other populations is recommended. Another possibility to enhance the discriminative power of this method could be to combine dental trait profiles with the presence and types of dental treatments carried out, and the presence-absence of teeth. Steps may also be taken in the direction of using artificial intelligence and deep learning. Automated comparative analysis, and comparison between different records (x-rays, tomography, and intraoral scans) is developing rapidly, and could help to grow this methodology.[12,24-29] In this context, several computer software programs already utilize dental coding systems to assist in the automated comparison of AM and PM data in mass disaster scenarios. DVI System International is the official software endorsed by INTERPOL and is among the most widely utilized tools for disaster victim identification.[30] Odontosearch, UDIM, and WinID are also software tools designed to assist forensic odontologists in human identification by utilizing dental records.[31-33] They primarily rely on radiographs and photographs to analyze dental characteristics, including missing teeth, restorations, and anomalies. These software applications are continuously updated with more efficient versions and by integrating new tools. OdontoSearch 3.2 provides statistical values and an objective method for quantifying the relative frequency of dental patterns in the general population. It is based on individual patterns of missing, filled, and unrestored teeth on a large scale. Interestingly, it can statistically assess whether a specific dental pattern is rare or more prevalent within the studied population.[31,34] The study of dental trait patterns proposed in this work aligns perfectly with this approach. Integrating these traits into dental records would significantly enhance the database and improve the effectiveness of automated victim identification analysis.

However, the mentioned software applications do not rely on 3D images obtained through intraoral scanning. It is only recently that these images have started gaining wider use in dental clinics, and the potential for conducting intraoral scans on disaster victims is now being explored.[6,35] Promising software tools have been proposed for the comparative analysis of 3D-3D and 3D-2D images in the identification process.[36] Interestingly, other authors propose a Digital Dental Biometrics framework for human identification. Utilizing 3D dental point clouds with machine learning algorithms, they achieve a recognition rate of 100% using complete tooth crown contour samples.[37] This highlights the increasing adoption of new technologies for analyzing 3D images from intraoral scanners. The use of these images is now a reality, and their extensive application, particularly with the integration of Artificial Intelligence in the forensic field, is highly likely. Incorporating visible dental trait patterns present in 3D images could be a valuable advancement in the natural evolution of analytical methods for identification in forensic contexts.

CONCLUSION

Using the matching-exclusion tables, the problem individuals appear to be reliably identified, despite the application of very conservative rules. Our research indicates that utilizing well-established dental morphological characteristics and modern 3D imaging technology could significantly enhance the efficient triage and identification of victims.

The key points of this study are:

1. The proposed exclusion comparative method based on dental morphological traits may be valuable for the triage and identification of victims.
2. Matching-exclusion tables integrate all candidate individuals for identification, and their dental trait profile can progressively be compared to a problem case.
3. AM and PM dental trait profiles are necessary for comparison.
4. 3D image quality allows dental traits comparison.
5. A simple collection of 3D images with an intraoral scanner of disaster casualty victims could speed up the identification process.

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