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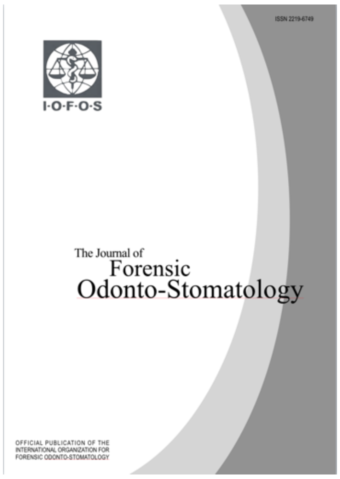

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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Dental ancestry estimation in a 1500 years old human skeleton from Slovenia using a new web-based application rASUDAS

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The authors declare that they have no conflict of interest.

KEYWORDS

Dental morphology, Arizona State University Dental Anthropology System, Forensic anthropology, Cone-beam computed tomography, Huns, Slovenia

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ABSTRACT

The present study was performed on a skeleton excavated from the Late Roman Period necropolis in Ptuj, Eastern Slovenia. Previous anthropological analysis has revealed that the skeleton belongs to a male, who died in his early twenties; however, determination of ancestry was problematic. The skull displays artificial cranial deformation of circular fronto-occipital type and shows some Asiatic anthroposcopic features. However, the inter-orbital projection method of Gill and Hughes has placed him into the Western Eurasian group. The aim of the present investigation was to estimate whether this individual belonged to Western Eurasian or Eastern Asian ancestry group by analyzing his dental morphology. Twenty-one key dental traits for ancestry assessment were scored following the Arizona State University Dental Anthropology System (ASUDAS). Morphology of the roots was scored from cone-beam computed tomography (CBCT) images of the dentition. These scores were input into a web application rASUDAS (beta version) which uses a naive Bayes classifier algorithm to assign an individual to a preselected number (from two to seven) of ancestry groups. The analysis revealed a complex tooth crown morphology (moderate maxillary incisor shoveling, 5-cusped maxillary first and second molars, 6-cusped mandibular first molars, 5-cusped mandibular second molars, multiple molar enamel extensions) and a simplified external root morphology (27 single-rooted teeth out of 32). Both mandibular second molars and the right mandibular third molar possess a C-shaped root canal. In addition, the mandible bilaterally exhibits accessory mental foramina. In a two-group analysis, the application calculated that the probability of assigning the individual from Ptuj to Eastern Asian ancestry group was close to 1.0. The result is in agreement with archaeological evidence which has indicated that this individual was a Hun warrior from the Migration Period of Europe. This study demonstrates a modern approach to estimating ancestry from dental morphology in bioarcheological and forensic contexts.

INTRODUCTION

In 2000, during the excavation of the Late Roman Period necropolis in Ptuj archaeologists discovered an almost complete skeleton with artificially deformed skull (Ptuj-ŠC 2000, grave no. 50). The skeleton was buried in an abandoned Roman lime-kiln together with the following grave-goods: gilded bronze earring, parts of belt set, coin, arrowhead, and iron sword. Archaeological evidence has indicated that the skeleton belongs to a Hun warrior from the 5th century AD.[1] Macroscopic and anthropometric anthropological analysis was done by one of the present authors.[2,3] The results have indicated that the skeleton undoubtedly belongs to a male who died in his early twenties. No clues have been found about the cause or manner of death. Artificial cranial deformation is of a typical circular fronto-occipital type caused by circular head-binding during early childhood (Fig. 1). Findings of artificially deformed skulls are quite rare. Moreover, this grave is considered to be the first material trace of the Huns on the territory of Slovenia. The skeleton is currently housed in the National Museum of Slovenia.

Osteological ancestry assessment has taken into account the artificial cranial deformation and other physical characteristics. The former is, however, not a reliable indicator of ancestry because it has not been observed only in skulls from Hun cemeteries but also in those from Gothic-Alan (Western Eurasian tribes) cemeteries.[4] It is also possible, that in some regions this practice was picked up by the indigenous Romanised population. In Slovenia, deformed crania have been observed in skulls of the Ostrogoths from three Migration Period archaeological sites: Dravlje near Ljubljana,[5] Lajh in Kranj,[6,7] and Miren.[8]

Analysis of physical characteristics has favored Asiatic origin of this individual. Although the inter-orbital projection method of Gill and Hughes has placed him into the Western Eurasian group, the skull also shows some Asiatic anthroposcopic features: unmarked masculine sexual traits, rounded orbits, broad zygomatic bones with inferior zygomatic projection, wide ramus of mandible, elliptic palate with straight suture, and 6-cusped mandibular first molars.[9] Moreover, the individual had a stature of 163 cm, calculated by a variety of different methods (Manouvrier, Dupertius and Hagen, Shitai, Stevenson, and Trotter and Gleser), and a relatively gracile body composition. The aim of the present investigation was to estimate whether this individual belonged to Western Eurasian or Eastern Asian ancestry group by analyzing his dental morphology.

MATERIALS AND METHODS

The dentition is composed of maxillae with 14 permanent teeth (right premolars lost *post mortem*) and a mandible with 16 permanent teeth. The tooth crowns and roots were examined macroscopically (using naked eye and a magnifying glass) and by using cone-beam computed tomography (CBCT).

The CBCT images of the dentition were taken using a Veraviewepocs 3D R100 machine (Morita, Kyoto, Japan) operating at 90.0 kV and 3.0 mA, with exposure time 9.4 s. The isometric voxel size was 0.160 mm and the slice thickness was 0.960 mm. The scans were produced according to the manufacturer's recommended protocol. A field of view of 88.160 mm × 88.160 mm × 80.640 mm was used. The CBCT images were analysed with dedicated software (i-Dixel One Volume Viewer 2.0.0) in a personal computer, with a 19-inch Hewlett Packard LCD screen with a resolution of 1280 × 1024 pixels in a darkroom. The contrast and brightness of the images were adjusted using the image processing tool in the software to ensure optimal visualization of the roots.

Key dental traits for ancestry assessment (14 crown traits, six root traits, and pegged-reduced-missing maxillary third molars) were scored following the Arizona State University Dental Anthropology System (ASUDAS).[10] Two authors (I. Š. and T. H.) scored the traits independently; in the case of variant scoring, a third, joint evaluation was conducted. Scores were input into the beta version of the rASUDAS application (freely available on the link http://apps.osteomics.com/rASUDAS/) and both historically relevant groups (Western Eurasians and Eastern Asians) were selected for analysis.

RESULTS

Excellent preservation of teeth, absence of dental caries and heavy wear, and the use of CBCT for assessment of root morphology have made it possible to score all dental traits included in the rASUDAS except deflecting wrinkle (Table 1). The dentition is characterized by moderate maxillary incisor shoveling, 5-cusped maxillary first and second molars, 6-cusped mandibular first molars, 5-cusped mandibular second molars, molar enamel extensions (Fig. 2) and simplified external root morphology (Fig. 3). In a two-group analysis, the application assigned the individual from Ptuj to Eastern Asian and Western Eurasian ancestry groups with posterior probabilities of 99.97% and 0.03%, respectively. Additionally, the CBCT images of the dentition revealed two distinctive anatomical features that are not included in the ASUDAS system. First, both mandibular second molars and the right mandibular third molar possess a C-shaped root canal (Fig. 4A). Second, the mandible bilaterally exhibits accessory mental foramina (Fig. 4B).

Table 1. Dental morphological traits which were used for ancestry estimation, scored according to the ASUDAS system

Dental trait	Tooth	Range	Grade
Winging	UI1	0, 1	0
Shoveling	UI1	0-7	2
Interruption groove	UI2	0, 1	0
Hypocone	UM2	0-5	3
Carabelli's trait	UM1	0-7	0
Metaconule	UM1	0-5	2
Enamel extension	UM1	0-3	2
Multiple lingual cusps	LP2	0-3	2
Groove pattern	LM2	x, +, y	x
Cusp number	LM2	4, 5	5
Entoconulid (Cusp 6)	LM1	0-5	2
Metaconulid (Cusp 7)	LM1	0-4	0
Protostylid	LM1	0-7	1
Deflecting wrinkle	LM1	0-3	-
Tomes's root	LP1	0-7	3
Root number	UP1	1, 2	1
Root number	UM2	1-3	1
Root number	LC	1, 2	1
Root number	LM1	1-3	2
Root number	LM2	1-3	1
Pegged-reduced-missing	UM3	0, 1	0

Figure 1. The skull of the individual from Ptuj displaying artificial cranial deformation.

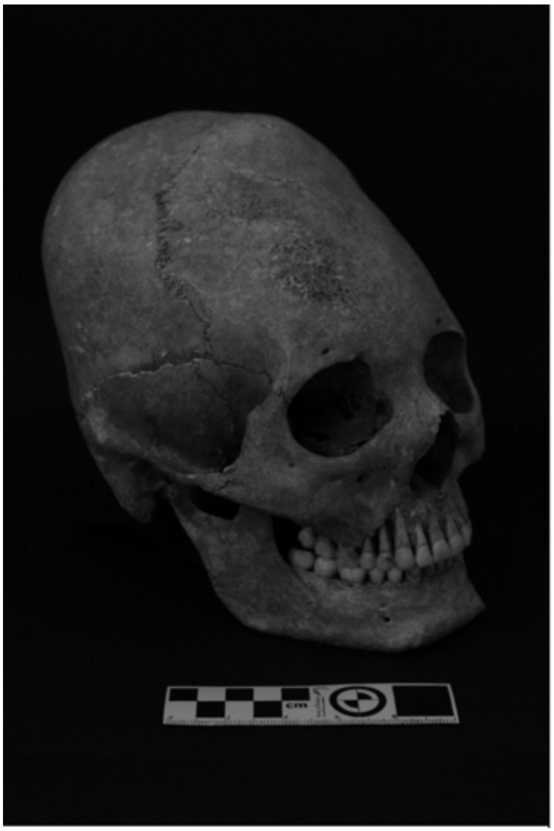


Figure 2. Crown traits of the individual from Ptuj. In maxillary arch (**A**) arrows indicate cusp 5 (metaconule) on occlusal surfaces of both maxillary first molars and the right maxillary second molar. Arrowheads indicate exposed dentine cores of marginal ridges on both maxillary central incisors. In mandibular arch (**B**) arrows indicate cusp 6 (entoconulid) on occlusal surfaces of both mandibular first molars and cusp 5 (hypoconulid) on both mandibular second molars. Lateral view of the jaws in occlusion (**C**). Note enamel extensions (arrows) on buccal side of the right maxillary and mandibular molars.

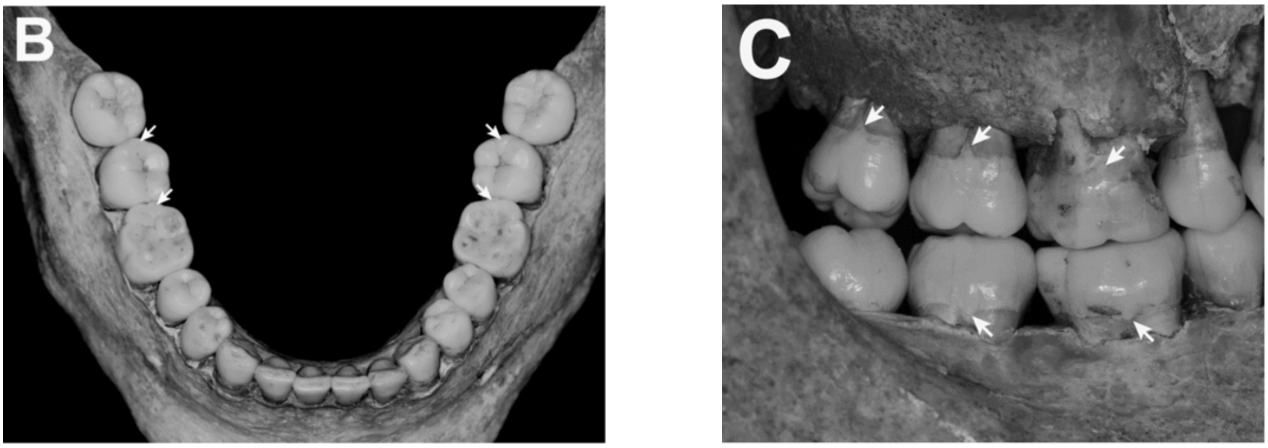


Figure 3. Panoramic CBCT reconstructions showing maxillary (**A**) and mandibular (**B**) teeth. There are five teeth with more than one root: RM1 – right maxillary and mandibular first molars, LM1 – left maxillary and mandibular first molars, LM3 – left mandibular third molar. Arrowheads indicate endosseous canals in the symphysis region of the mandible.

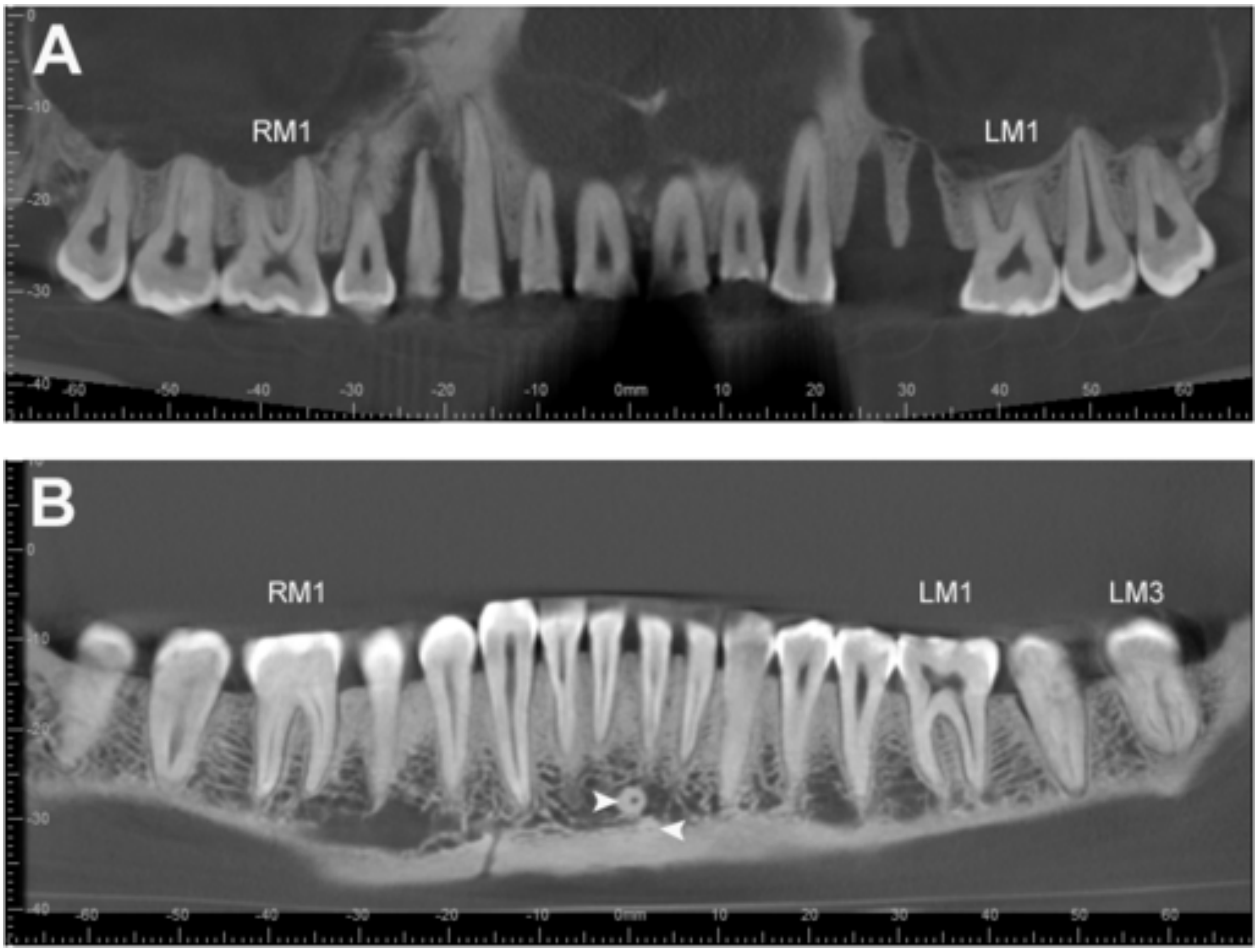
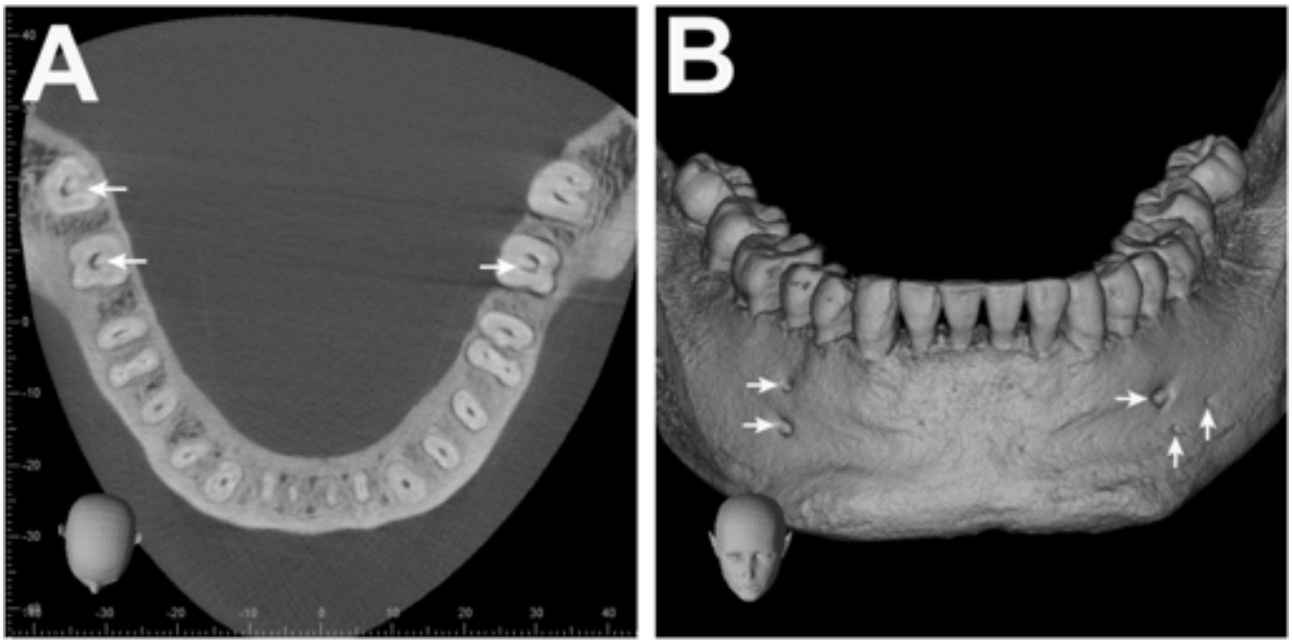


Figure 4. CBCT axial section of the mandible (A) shows a C-shaped canal system (arrows) in pyramidal roots of both mandibular second molars and the right mandibular third molar. 3D reconstructed CBCT image of the mandible (B) displaying multiple mental foramina bilaterally (arrows).



DISCUSSION

The primary role of dental morphology in forensic anthropology is in discerning the ethnic identity or ancestry of an individual when only skeletal-dental remains are available.[11] It has long been acknowledged that morphological dental traits show significant differences in frequency and grade of expression among populations around the world. When viewed as trait constellations, rather than isolated traits, they can provide clues about the individual's ancestry. Nevertheless, this research area has not received much attention until recently. Scott and Turner[12] listed a suite of 12 crown and root traits that could help establish the least and the most likely ethnic affiliation of an individual. Irish[13] developed a method that assigns an individual to one of five groups (East Asian, American Indian, White, Polynesian, and Black) and involves scoring a suite of ten crown traits. The main disadvantage of both methods is that they do not produce associated probabilities. From forensic perspective, such a result could be of little value in court procedures. Edgar[14] presented a method which uses ten crown traits in conjunction with logistic regression to assign individuals to one of four groups (African American, European American, Hispanic from New Mexico, and Hispanic from South Florida). This method produces probabilities but the reference sample is limited for use in the United States.

In 2015, David Navega and João Coelho developed a web application rASUDAS which was based on naive Bayes classification algorithm and generated posterior probability of an individual being assigned to one or more ancestry groups.[15] The application's label refers to the ASUDAS system and R programming language. This alpha-version involved crown and root trait frequencies for 21 ancestry groups taken directly from the appendix of the textbook *The Anthropology of Modern Human Teeth* by Scott and Turner.[12] These trait frequencies are predominantly based on archaeological samples. With further development, the number of ancestry groups was reduced, some traits were added and some removed, some traits were divided into more categories than presence or absence, and the reference sample was expanded with new and improved trait frequencies. This resulted in a beta-version of rASUDAS which involves 21 traits (14 crown traits, six root traits, and pegged-reduced-missing maxillary third molars) and seven ancestry groups (American Arctic and Northeast Asia, Australo-Melanesia and Micronesia, East Asia, American Indian, Southeast Asia and Polynesia, Sub-Saharan Africa, Western Eurasia). The authors tested the application on 150 individuals (with data for a minimum of 12 traits) from the reference sample. The overall accuracy of the application in predicting group assignment was 51.8%; however, it improved with a decrease in the number of ancestry groups to four (66.7%) or three (Western Eurasia, Sub-Saharan Africa, and East Asia; 72.7%).[15] Classification accuracy attained its highest level in pairwise comparisons: East Asians and East Asian–derived populations (Northeast Asian, Arctic, and Native American) could be distinguished from Western Eurasians and Sub-Saharan Africans roughly 90% of the time.[15]

The present study demonstrates a modern approach to estimating ancestry from dental morphology in bioarcheological and forensic contexts. A CBCT was used to evaluate root morphology without damaging the skeletal material. In the presented case, the skeleton under consideration was excavated from the archaeological site in Ptuj, Slovenia and according to the results of archaeological and osteological analyses belongs to a young Hun warrior from the Migration Period of Europe.[1] Literature data indicate that the Huns were a heterogeneous mixture of nomadic tribes who had their origins in China and Inner Mongolia.[16] Scott et al.[17] pointed out that North and East Asians and Native Americans, in contrast to Western Eurasians, exhibit rich crown morphology coupled with the relatively common occurrence of unseparated roots. The dentition of the individual from Ptuj matches to this pattern: the tooth crown morphology is complex (moderate maxillary incisor shoveling, 5-cusped maxillary first and second molars, 6-cusped mandibular first molars, 5-cusped mandibular second molars, molar enamel extensions); however, the external root morphology is simplified (27 single-rooted teeth out of 32). As pointed out by Scott and Turner,[12] moderate incisor shoveling, molar enamel extensions, 1-rooted maxillary first premolars, and cusp 6 on mandibular first molars would form a set of dental traits rarely found in a Western Eurasian. Posterior probabilities calculated with rASUDAS do not allow any doubt about his Eastern Asian descent (Eastern Asia 99.97%, Western Eurasia 0.03%). Moreover, both mandibular second molars of this individual possess a C-shaped root canal configuration, which is according to a global survey of recent populations significantly more prevalent in China than in any other geographic region.[18] In addition, his mandible bilaterally exhibits accessory mental foramina, which are according to another global survey of recent populations more prevalent in East and Northeast Asians (15%-25%) than in Europeans (5%-15%).[19]The estimation of ancestry is an important part of individual's biological profile in bioarcheological and forensic investigations. There are many methods to estimate ancestry but the most common are based on craniometrics and cranial morphoscopics. Recently, several methods employing dental morphology proved effective at differentiating ancestral groups.[13-15] When a cranium is damaged (e.g. trauma, burning, weathering, soil acidity) or artificially deformed, as in the presented case, the analysis of dental morphology can be the primary method for ancestry estimation. In the present study, we decided to use the rASUDAS application for several reasons. First, the beta version of rASUDAS has a reference sample that is primarily archaeological (approximately 30,000 individuals from seven biogeographic regions with a time span of several thousand years) which makes it particularly useful in bioarcheological contexts. Secondly, the application employs crown and root traits and therefore has the ability to incorporate more information from the dentition compared to methods which employ only crown traits. Nevertheless, this potential can be fully exploited only when the application is used in conjunction with 3D imaging technologies like CBCT which enable evaluation of root morphology without damaging the skeletal material. Thirdly, the application calculates probabilities and has been validated on archaeological material. Moreover, the application is user-friendly an accessible online. Despite these advantages, it should be emphasized that the current version of rASUDAS should not be used as the sole method for ancestry estimation in modern forensic casework; it is still necessary to validate it using forensic cases and expand its reference sample with data on living populations.[15]

To conclude, the dentition of the skeleton from Ptuj is characterized by a suite of specific traits which correspond to the Sinodont dental complex found in East Asia and East Asian–derived populations. This is in agreement with archaeological evidence which indicates that this individual was a Hun warrior from the Migration Period of Europe.

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Cheiloscopy in individuals with Down syndrome and their nonsyndromic biological siblings

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ABSTRACT
The study aimed to carry out a comparative analysis between the lip print patterns in individuals with Down Syndrome and their nonsyndromic biological siblings. This was a cross-sectional blind study using an inductive approach and extensive direct observation procedures. A total of 68 cheiloscopic charts, named cheilograms, were divided into two groups (n=34), as follows: G1, including Down Syndrome individuals; and G2, including their nonsyndromic siblinggs. The convenience sample was selected in the city of João Pessoa, PB, Brazil. The following features were evaluated in eight labial regions called sub-quadrants: oral commissures (downturned, horizontal and upturned); lip thickness (thin, medium, thick and mixed); and labial grooves (I - complete vertical; I '- incomplete vertical; II – bifurcated; III – criss-cross; IV – reticular; or V - undefined). The data were analyzed by paired Student's t test and McNemar's Chi-square, with a 5% significance level. Most Down Syndrome individuals were found to have downturned oral commissures in 73.5% of cases, while their siblings showed a predominance of horizontal commissures in 73.5% of cases (p=0.009). There was no statistically significant difference for lip thickness between groups. In the analysis of labial groove patterns, Down Syndrome individuals (G1) showed a significant prevalence of the type I pattern (52.2%) as compared to their nonsyndromic siblings (30.1%) (p<0.001). Due to the tendency of having vertical labial groove patterns and downturned commissures, Down Syndrome individuals present cheiloscopic differences in relation to their nonsyndromic biological siblings, which suggests that syndromic genetics influences the development of these features. However, this may imply in a reduced potential of cheiloscopic identification due to the low divergence of labial phenotypes among Down Syndrome individuals.

INTRODUCTION
Down Syndrome (DS) was first described by John Langdon Down in 1866 as a chromosomal disorder resulting from the trisomy of the 21st pair. It is considered a natural and universal genetic event affecting 0.6-1/1,000 live births, and this proportion increases with maternal age. [1,2] DS is the most prevalent and studied congenital disease affecting the individual's intellectual capacity. [3] DS individuals may have changes in their physical, behavioral and cognitive

development, [1,3-5] as well as a number of rare and/ or anomalous patterns in their digital, palmar and plantar dermatoglyphics. These peculiar markers have been frequently used as an aid in diagnosis of this genetic alteration in 80% of cases. [6-8]
Cheiloscopy (from the Greek "*Cheilos*", lips; "Skopein", marks) refers to the registration and classification of the lip as to thickness, commissural arrangement and groove impressions, printed by an individual onto a given substrate. The study of cheiloscopy is based on the fact that the mucosal lip is covered with small grooves that reflect individual differences from a genetic basis and ethnic influence, thus providing specificity and variability to this method. [9-12]
Given their peculiar pattern – which is comparable to that of fingerprints – labial marks constitute a feasible identification technique applicable to forensic sciences. They possess the technical (classificability and practicability) and biological (uniqueness and immutability) requirements recommended by the literature and are hardly changeable, even in cases of trauma and inflammation. [11-16]
Due to their genetic background, DS individuals are more alike among themselves than when compared to their nonsyndromic biological siblings. As labial grooves have a genetic basis, it is still questionable whether such background influences the development of labial features and groove patterns in DS individuals. Thus, this study investigated the cheiloscopic features of DS individuals and their nonsyndromic siblings, in order to verify whether such chromosomal alterations may influence labial phenotypes and thus be used as a novel forensic tool for individual identification.

MATERIALS AND METHODS:

This study was carry out in accordance with the Resolution no. 466/12 of the National Health Council, Ministry of Health, which regulates research involving human beings. This study had prior approval of the Research Ethics Committee at the Center for Health Sciences, Federal University of Paraíba (CAAE no. 02955912.0.0000.5188).
This was a cross-sectional blind study using an inductive approach and extensive direct observation procedures.[17] A pilot study including 20 cheiloscopic records was previously performed to train the examiners. For the numerical variable (lip thickness), the intraclass correlation coefficient ranged from 0.922 to 0.984, while the Kappa statistics (labial grooves and commissures) showed agreement values between 0.767 and 1. Both analyses indicated satisfactory agreement between examiners with regard to the criteria used in our cheiloscopic study.
The sample was composed of 68 individuals divided into two groups (n=34): G1, consisting of DS individuals; and G2, consisting of their nonsyndromic biological siblings. The convenience sample was selected from a reference center for disabled individuals in the city of João Pessoa, PB, Brazil.
The volunteers presenting inflammation, trauma, malformation or other condition in the oral or perioral regions, were excluded from the sample. The data were collected based on three analyses, as follows: [9]

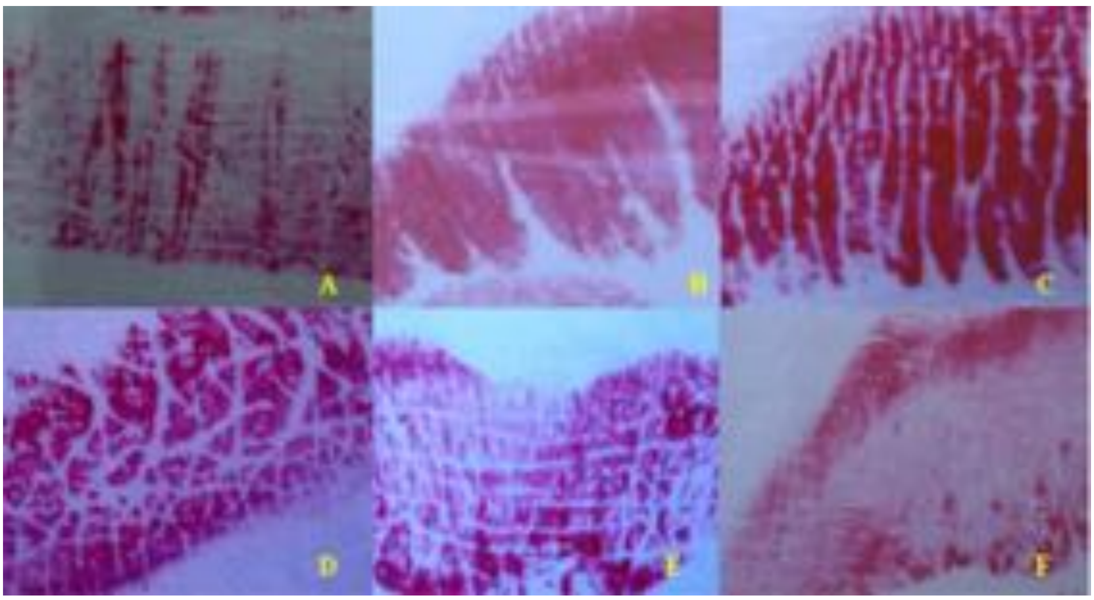
1. Lip thickness: After making sure that lips were free from cosmetics and/or other impurities, we used a needle point to measure the upper and lower lip thickness individually, taking as anatomical references the upper edge of the lip cord to the oral rhyme (for the upper lip); and the oral rhyme to the lower edge of the lip cord (for the lower lip). All measurements were made at the level of the median sagittal line. The record of the numerical value corresponding to the measurements was made with the use of a millimeter ruler (Hyzer-Krauss scale, adopted by the American Board of Forensic Odontology, ABFO, no. 2). The lips were analyzed and classified as: [18] thin (<8 mm), medium (8-10 mm), thick or very thick (>10 mm) and mixed (lips which denoted different categories for the upper and lower components).

2. Oral commissures: A high-resolution digital camera (DSLR Nikon D3200 24.2 Mega Pixels, São Paulo, SP, Brazil) with no flash was used to obtain two photographs (one more general and another with a close-up) for the analysis of the disposition of the oral commissures. The participant was positioned with their Frankfurt plane parallel to the ground and lips at rest. The oral commissures were categorized [9] as downturned, horizontal and upturned based on their disposition in relation to a virtual line perpendicular to the labial median line (which was tangent to the labial tubercle).

3. Labial groove pattern: an individual sample containing 0.8 g of lipstick (Avon Renew Renovador Intensivo Batom Cor Intensa® - red color, long-wearing, São Paulo, SP, Brazil) was applied onto the lips of the subjects, which should be dry, static, closed and relaxed. Then the lips were pressed and slightly scrolled from left to right against a white cardstock supported on a glass plate. The lip print was secured with an adhesive tape (3M®, transparent color, 45 mm, Sumaré, SP, Brazil). For the analysis of labial grooves, the collected lip print was divided into eight sub-quadrants (four upper and four lower sub-quadrants) (Fig. 1), which were numbered clockwise starting in the upper right sub-quadrant. The identification of groove types was carried out with the aid of a magnifying glass, in which the predominant type in each sulcular sub-quadrant was noted in cheilograms according to the previously described method: [19] Type I - complete vertical line; Type I' – incomplete vertical line; Type II - bifurcated lines; Type III – criss-crossed lines; Type IV - reticular lines; and Type V – undefined pattern not applicable to the previous classification (Fig. 2).

Figure 1: Schematic division of the lips into eight sub-quadrants, as follows: 1 and 2 – upper right sub-quadrants; 3 and 4 – upper left sub-quadrants; 5 and 6 – lower left sub-quadrants; and 7 and 8 – lower right sub-quadrants.

Figure 2: Labial groove types: Type I (A); I' (B); II (C); III (D); IV (E) and V (F). Source: Oliveira; Rabello; Fernandes (2012).



The data were processed on the Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive and inferential statistical techniques were used. Student's *t* test was used for pair-wise comparisons between numerical variables, and McNemar's Chi-square test was used to compare categorical variables, in addition to Chi-square test of equal proportions in a single group. A 5% significance level ($p \leq 0.05$) was adopted.

RESULTS

Of the total sample, 63.24% were females. Individually, the groups had similar proportions of subjects regarding sex: G1 (70.58% females) and G2 (63.24% females). The age of DS individuals in G1 ranged from 3 to 42 years, with a mean age of 13.88 years, while their nonsyndromic siblings (G2) were aged between 3 and 39 years, with a mean age of 17.35 years (p=0.004).

As for lip thickness (Table 1), G1 individuals showed mean thickness of 8.88 mm and 9.62 mm for the upper and lower lips, respectively. G2 individuals

showed mean lip thickness of 9.84 mm and 11.07 mm, respectively, with a statistically significant difference between groups for both the upper (p=0.002) and lower (p=0.001) lips. Furthermore, we observed that individuals in G1 had a higher percentage of medium lips (50.0%), followed by mixed (35.3%), thick/very thick (11.8%), and thin lips (2.9%). Among the individuals with mixed lips, their upper lips showed predominance of the medium thickness (23.5%), in contrast with their lower lips showing thick or very thick patterns (20.6%). Individuals in G2 were found to be the ones with the highest prevalence of thick or very thick lips (44.1%), followed by medium (38.2%) and thin (17.6%) types, with no mixed lips identified in the sample.

The analysis of oral commissures (Table 1) revealed that DS individuals tend to have downturned commissures (73.5%). No upturned commissure was identified in DS individuals (G1). Nevertheless, their siblings (G2) showed a high percentage of the horizontal pattern (73.5%), followed by the downturned (20.6%) and upturned (5.9%) patterns (p=0.009).

As shown in Table 2, the most prevalent type of labial groove was type I in all sub-quadrants analyzed in G1 individuals (1- 44.1%; 2- 64.7%; 3- 41.2%; 4- 32.4%; 5-50.0%; 6- 70.6%; 7- 70.6%; 8- 44.1%) (Fig. 3). In G2, type I was most prevalent pattern in sub-quadrants 2 (32.4%), 3 (26.5%), 6 (55.9%) and 7 (52.9%). There was a predominance of type III in sub-quadrants 1 (29.4%) and 4 (32.4%), and of type II in sub-quadrants 5 (44.1%) and 8 (32.4%)" (Fig. 4)".

Type I labial groove was the most prevalent pattern in all sub-quadrants of G1 individuals (52.2%), followed by types I' and II (both 16.9%). Likewise, type I was the most prevalent pattern (30.1%) in G2 as well, followed by types II (23.9%) and III (19.9%), although with a significant difference between groups G1 and G2 ($p<0.001$) (Table 3).

DISCUSSION

Down syndrome is currently the most prevalent human trisomy worldwide, which is responsible for intellectual, physical and cognitive changes. [1-5] Since ancient times it has been known that singularities present in an individual may facilitate the task of their forensic identification. [20-23]

DS individuals have highly typical dermal ridge patterns, making it possible to diagnose this syndrome just by dermatoglyphic examination. Examples of irregularity in the palmprint pattern of DS individuals include the presence of a single palmar transverse crease or "simian crease"; prevalence of ulnar loop; and of a t-like axial palmar triradius displaced distally (which is present in about 70% individuals), usually associated with some patterns in the hypothenar area. [6-8]

This cheiloscopic study revealed common features shared by DS individuals (G1), such as the prevalence of downturned commissures and complete vertical lines (type I) in all labial sub-quadrants analyzed. These findings disagree with those found for their nonsyndromic biological siblings (G2), thus indicating that the trisomy of the chromosome 21 influences the lip phenotype. As for the grooves, such a finding is confirmed mainly in the central sub-quadrants of the lips. Studies have shown that marks on the lip corners are not clearly captured in the printing process, even those of great quality, which makes the central portion of the lip a decisive area for human identification procedures. [22,23]

Labial phenotypes are transmitted on the basis of heredity precepts, e.g., thin and thick lips are more typically found in white and black individuals, respectively. The results observed in the present study are in disagreement with those

Table 1: Description of the variables lip thickness and oral commissure according to the study group.

Variable	DS (G1)		Nonsyndromic sibling (G2)		p-value
	n	%	n	%	
TOTAL	34	100,0	34	100,0	
Maximum thickness of the upper lip (average)	8.88 mm		9.84 mm		$p^{(1)} = 0.002$*
Maximum thickness of the lower lip (average)	9.62 mm		11.07 mm		$p^{(1)} < 0.001$*
Lip thickness					
Thin	1	2.9	6	17.6	
Medium	17	50.0	13	38.2	**
Thick/Very thick	4	11.8	15	44.1	
Mixed	12	35.3	-	-	
Mixed upper lips					
Thin	4	11.8	1	2.9	
Medium	8	23.5	13	38.2	**
Thick/Very thick	-	-	1	2.9	
Not mixed	22	64.7	19	55.9	
Mixed lower lips					
Thin	1	2.9	-	-	
Medium	4	11.8	2	5.9	**
Thick/Very thick	7	20.6	13	38.2	
Not mixed	22	64.7	19	55.9	
Oral commissure					
Horizontal	9	26.5	25	73.5	
Upturned	-	-	2	5.9	**
Downturned	25	73.5	7	20.6	

(*): Non-significant difference at a 5.0% level.
(**): It was not determined due to the difference in the number of categories.
(1): Paired Student's *t* test.

Table 2: Description of the variable type of labial groove according to the study group and the sub-quadrant.

Type	Sub-quadrant 1				Sub-quadrant 2				Sub-quadrant 3				Sub-quadrant 4			
	G1		G2		G1		G2		G1		G2		G1		G2	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
I	15	44.1	7	20.6	22	64.7	11	32.4	14	41.2	9	26.5	11	32.4	4	11.8
I'	8	23.5	4	11.8	6	17.6	5	14.7	8	23.5	5	14.7	9	26.5	8	23.5
II	4	11.8	5	14.7	5	14.7	6	17.6	6	17.6	8	23.5	7	20.6	5	14.7
III	1	2.9	10	29.4	-	-	5	14.7	1	2.9	5	14.7	1	2.9	11	32.4
IV	-	-	6	17.6	-	-	-	-	-	-	7	20.6	-	-	6	17.6
V	6	17.6	2	5.9	1	2.9	7	20.6	5	14.7	-	-	6	17.6	-	-
Type	**Sub-quadrant 5**				**Sub-quadrant 6**				**Sub-quadrant 7**				**Sub-quadrant 8**			
	G1		G2		G1		G2		G1		G2		G1		G2	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
I	17	50.0	7	20.6	24	70.6	19	55.9	24	70.6	18	52.9	15	44.1	7	20.6
I'	3	8.8	3	8.8	4	11.8	2	5.9	3	8.8	3	8.8	5	14.7	4	11.8
II	7	20.6	15	44.1	4	11.8	8	23.5	4	11.8	7	20.6	9	26.5	11	32.4
III	3	8.8	7	20.6	1	2.9	3	8.8	-	-	3	8.8	1	2.9	10	29.4
IV	-	-	1	2.9	1	2.9	1	2.9	1	2.9	2	5.9	1	2.9	-	-
V	4	11.8	1	2.9	-	-	1	2.9	2	5.9	1	2.9	3	8.8	2	5.9

Table 3: Description of the variable *type of prevailing labial groove* according to the study group.

Variable	DS (G1) n	DS (G1) %	Nonsyndromic sibling (G2) n	Nonsyndromic sibling (G2) %	p-value
TOTAL	272	100.0	272	100.0	
Total of sub-quadrants					
I	142	52.2	82	30.1	
I'	46	16.9	34	12.5	
II	46	16.9	65	23.9	$p^{(1)} < 0.001$*
III	8	2.9	54	19.9	
IV	3	1.1	32	11.8	
V	27	9.9	5	1.8	

(*): Significant difference at a 5.0% level.
(1): According to McNemmar's test.

Figure 3: Distribution of the most prevalent type of labial groove in G1 individuals.

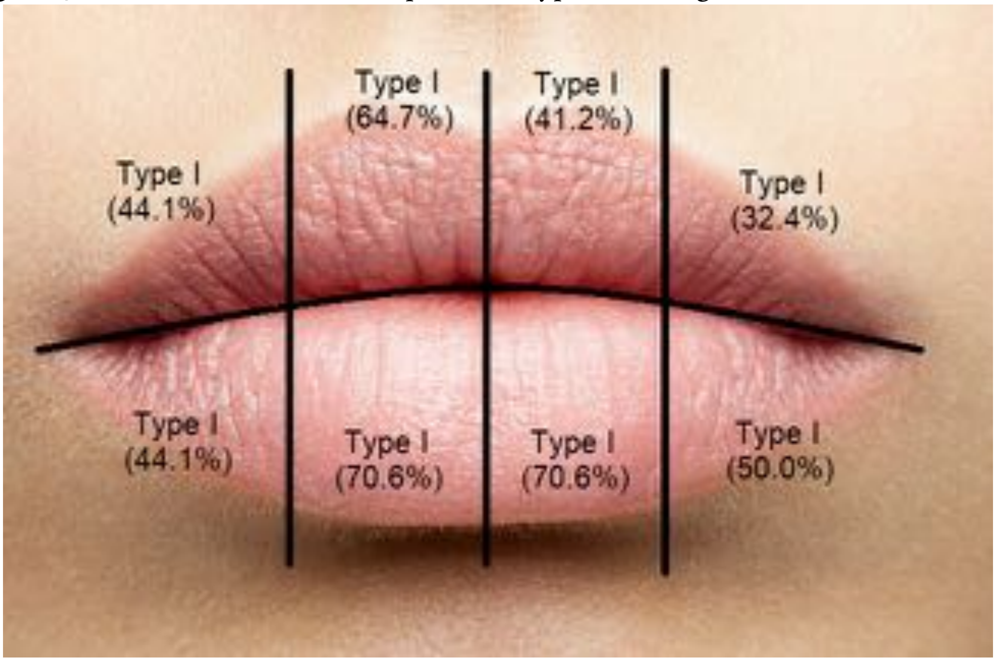
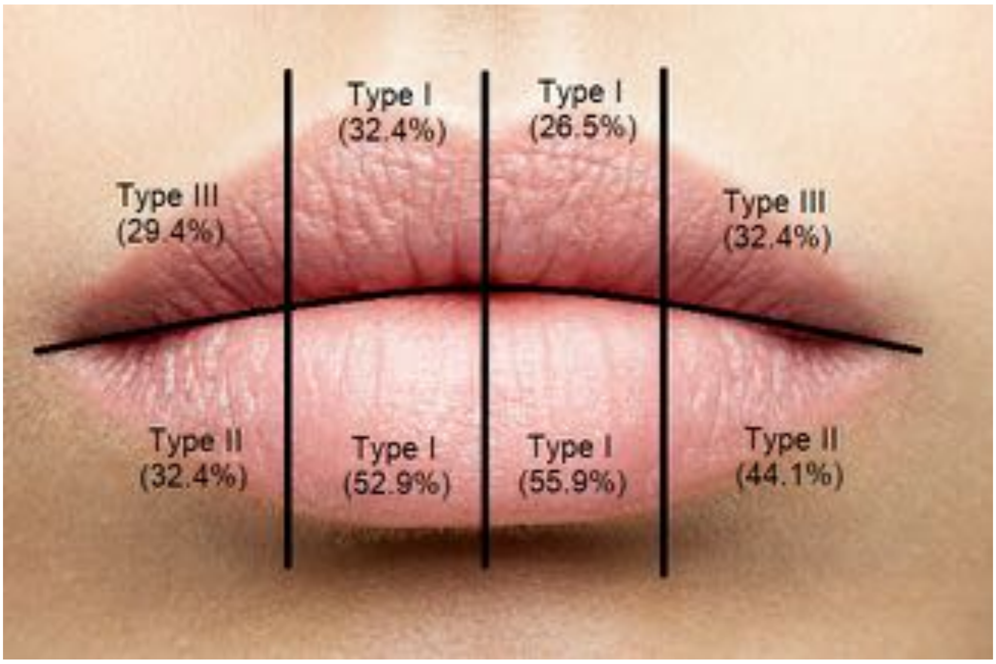


Figure 4: Distribution of the most prevalent type of labial groove in G2 individuals.



found in a previous similar study[9] including individuals from northeastern Brazil. The authors pointed out that the upper lips appeared more often with the thin type and that, contrary to this trend, the lower lips predominated as the thick type. The variation of thickness patterns in both groups can be attributed to ethnical miscegenation as a result of the historical and heterogenic Brazilian colonization, especially in northeast where africans, Indians and Europeans settled down. [9,16,24]

Other studies [9,16] have shown the predominance of horizontal commissures in their sample subjects, followed by downturned and upturned commissures. These results corroborate our findings concerning the group of nonsyndromic siblings, but diverge from those of the DS group, which showed a high percentage of the downturned commissural standard. This could be explained by the position and arrangement of the muscle fibers of perioral muscles in this group of individuals.

Despite lip thickness and commissural disposition are easily obtainable, such variables are considered essential for the initial acceptance or refusal of printings that are compared in a pairwise manner. [16,25] Only the detailed analysis of labial grooves by experienced examiners confronting the lip prints questioned with those of a possible suspect, can confirm or not the identification of the given evidence.

A study investigating lip prints in a sample from Lebanon [26] showed a predominance of groove types I, II and III, in that order, which was also observed herein in the group of nonsyndromic siblings (G2).

The cheiloscopy is considered a new method of human identification – including monozygotic individuals. The biological properties (uniqueness, permanence and immutability) of labial grooves are comparable to that of fingerprinting methods. [27,28] The latter are adopted worldwide as important resources for human identification, because although there is a resemblance between parents' and their children's features, their lines and groove patterns are not identical among them. [29-32] However the queiloscopy still needs of more accurate methods, since the labial anatomy can be altered according to the age, sex and ethnicity of the individual. [12,28,33]

CONCLUSION

It may be concluded that DS individuals present cheiloscopic differences in relation to their nonsyndromic biological siblings as the former have vertical groove patterns and downturned commissures. The findings reported herein suggest that chromosomal trisomy influences the development of such labial phenotypes. This fact minimizes the potential of cheiloscopic identification, since its technical foundations of classificability and practicability would be compromised due to the low divergence of labial phenotypes among DS individuals.

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The use of panoramic images for identification of edentulous persons

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KEYWORDS

Forensic odontology,
Panoramic radiography,
Identification,
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ABSTRACT

The aim of this study was to determine if edentulous persons could be identified using panoramic images by: I) investigating the possibility of matching two panoramic radiographs of the same person obtained on two different occasions, II) determining what anatomical features are used as the base for matching, III) investigating if oral and maxillofacial radiologists (OMR) and dentists who were not oral and maxillofacial radiologists (NOMR) differed in their ability to match the images, and IV) determining if the time elapsed between the images affected the results or the confidence of the match. Panoramic image pairs from 19 patients obtained on two different occasions were included, plus 10 images from other edentulous patients. The time elapsed between the image pairs varied between 4 months and 6 years. Four OMR and four NOMR were asked to match the image pairs depicting the same patient. The participants marked each match as "certain", "likely", or "possible" and what anatomical structure they used for matching. The OMR group correctly matched 100% of the images and the NOMR group correctly matched 96%. The anatomy of the mandible was most often used for matching. The OMR group was more certain in their decisions than the NOMR group. The time elapsed between the examinations did not affect the result. In conclusion, panoramic images can be used to identify edentulous patients. Both OMR and NOMR could identify edentulous individuals when only panoramic radiographic images were available and the OMR were especially confident in the identification process.

INTRODUCTION

Several studies have demonstrated the possibilities and importance of forensic odontology in human identification.[1-3] Intra-oral radiographic images including teeth are particularly helpful in the identification process due to large variations in the number of teeth, teeth anatomy and dental treatment. After the tsunami disaster in Asia in 2004, 79% of the deceased were identified using forensic odontology based on intra-oral radiography alone and 87% were identified when it was used in combination with other methods.[4] Also, panoramic radiographs have been used as a base for identification.[5-7] In this single image, a large number of characteristic features can appear.[8] Computer programs have been developed for automatic matching of panoramic images. However, this

automatic matching is highly dependent on sufficient dental characteristics in the images.[9]
The 2010 global prevalence of edentulism was 2.3% (158 million people). This is a large group of individuals; thus, studies of the possibilities to identify deceased edentulous people are of importance.[10] Panoramic radiographic examinations are often performed on edentulous patients. These images often disclose retained roots and/or pathological lesions. In a study by Serman and Nortje, pathological lesions were seen in 47% of the 539 studied panoramic radiographs. In the same material, 412 retained roots were found. The authors concluded that the combination of pathology and the varying anatomy have important forensic implications.[11] The study also showed that a large number of edentulous people do not have retained roots and/or pathological lesions.
The bone resorption that occurs after tooth loss can impede the ability to identify edentulous people by means of radiographs. In a study of a group of patients wearing dentures, the use of panoramic images > 3 years old in the identification process was questioned. The reason for this was that changes in bony anatomy over time can affect the ability to make a correct match between ante-mortem and post-mortem images taken > 3 years apart.[12]
The panoramic technique is sensitive to misalignments of the patient and this can lead to image distortion. However, it has been shown that the inner bony structures in the study, represented by mathematical anatomical models, can be recognised in panoramic images in spite of the distortion due to different patient positioning.[13]
It is known that dentists' qualifications, with respect to experience and training, affect the accuracy of radiographic identification.[14,15] It has also been shown that oral and maxillofacial radiologists (OMR) have better accuracy than dentists who are not oral and maxillofacial radiologists (NOMR) when studying radiographs in cases with reduced dental characteristics. This was seen in a study that aimed to match bitewing examinations performed within 1-3 years on 6-13 year-old children with un-restored dentitions.[16] The OMR have also shown higher success rates than NOMR when using intra-oral radiographs to identify edentulous individuals[17] and edentulous individuals treated with implants.[18] However, it is unclear if it is possible to match panoramic examinations obtained on different occasions of edentulous individuals without distinctive pathological lesions or retained teeth and/or roots and if there is a difference between OMR and NOMR in the success rate when performing such matches.
The aims of this study were: I) investigate the possibility of matching two panoramic radiographs of the same person obtained on two different occasions, where the patient was edentulous on at least at one occasion, II) determine what anatomical features are important when identifying edentulous individuals using panoramic images, III) investigate if there is a difference between OMR and NOMR in their abilities to match these types of images, and IV) determine if the time elapsed between the images affected the results or the confidence of the match.

MATERIAL AND METHODS:

The study materials were collected from the archives at the Department of Oral Prosthodontics, University Hospital in Umeå (Norrlands Universitetssjukhus, NUS), Sweden. Inclusion criteria were two panoramic images obtained on different occasions of the same patient, where on at least one occasion the patient was edentulous and without implants, impacted teeth, pathological lesions, or foreign bodies. There were 217 patient records screened with 19 presenting two panoramic images that fulfilled the inclusion criteria. The panoramic examinations had been performed by different examiners. This resembles a realistic scenario where the ante-mortem panoramic images are compared with panoramic images taken as part of the post-mortem examination. The elapsed time between the two images from the same patient ranged from 4 months to 6 years. Fourteen cases had images taken with 3 years or less between the images and five cases had an elapsed time >3 years.
Ten extra panoramic images of edentulous patients were added to complicate the task so that not all images had a match. All images were anonymised. In the ante-mortem images, the patients had teeth (8 cases) or were edentulous (11 cases). In the post-mortem images, the patients were either edentulous (20 cases) or edentulous and treated with implants (9 cases). The images were placed in two PowerPoint presentations. One presentation represented ante-mortem images marked with the numbers from 1-19 and the patient's gender and age. The other

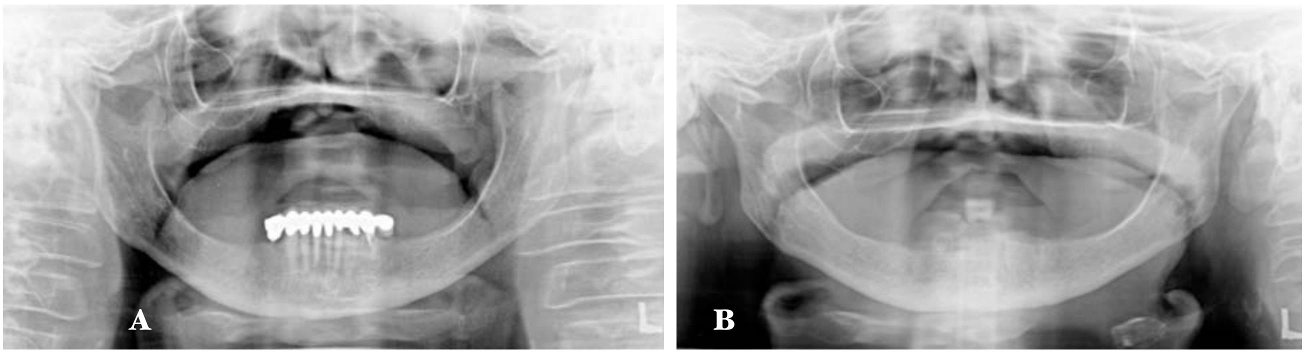
presentation (29 images) represented post-mortem images that were marked with letters and the patient's gender. It was possible to look at the two PowerPoint presentations at the same time and independently of which presentation, to scroll among the images and to zoom in and out. The presentations were installed on a computer assigned for the test. The task was performed by four OMR and four NOMR all working at the University Hospital of Umeå, Sweden. There were three general practitioners and one oral and maxillofacial surgeon in the NOMR group. The material was available for one month on the assigned computer at the department of Oral and Maxillofacial Radiology, Umeå University and the participants were free to choose when, during this period, to complete the task. Two participants were not able to use the assigned computer. They were given the material on an USB-drive to use on their own computers.

All participants received a form in which to fill in their assessments. They were asked to evaluate each match with respect to their confidence in their decision using one of the following words: "certain", "likely", or "possible". In each case, they also filled in which anatomical structures or other features they used to match the images. Other features could be, e.g. deviant anatomy or any other feature they noticed in the images. The forms were anonymous except for their declaration of being an OMR or NOMR. All assessors provided their informed consent to participate in the study. The study was performed in accordance with the principles of the Helsinki Declaration. Fisher's exact test and Pearson Chi-square test were used for statistical analyses.

RESULTS

All participants completed the study. The OMR group had 100% correct matches, while the NOMR group had a mean of 96% correct matches (min: 16, max: 19). This difference was not statistically significant, (p=0.1). In total, three cases were not correctly matched by one participant in the NOMR group. Figure 1 shows one of these cases.

Figure 1. "Ante-mortem" (a) and "post-mortem" (b) image of one of the cases that was not correctly matched by all of the dentists who were not oral and maxillofacial radiologists (NOMR).



The mandible information was most often used (95% of matches) in the matching for all participants (Fig. 2). The OMR used the mandible in 100% of their matches, and the NOMR group used it in 89% of their matches. The maxilla was used by the OMR group in 76% and the maxillary sinus in 88% of the matches compared with 59% and 53% respectively in the NOMR group. A variety of other anatomical features, such as soft tissue; bone patterns in the nasal region, orbit, and ears; and carotid calcification, were also used in the matching process predominantly in the OMR group. (Fig. 2).

The participants in the OMR group were "certain" in 92% of the matches; while, in 7% of matches they considered it "likely" that the images depicted the same person and in 1% they considered it "possible". All their matches were correct. The NOMR group was "certain" in 42% of the matches and all were correct. In 46%, they considered it "likely" that the images depicted the same person and 94% were correct. They considered it "possible" in 12% of the matches and 89% were correct. There was a statistically significant difference between the groups regarding the confidence in their matches, ($p \leq 0.008$) (Table 1). In 74% of the cases, the time between the images to be matched was ≤ 3 years. In the remaining 26%, the time was > 3 years. This time limit was used because of an earlier study questioning post-mortem panoramic images older than 3 years.[12]

Figure 2. Proportion of cases (%) in which various anatomical structures or other details were used by oral and maxillofacial radiologists (OMR) and dentists who were not oral and maxillofacial radiologists (NOMR) when matching panoramic images of edentulous individuals obtained on different occasions

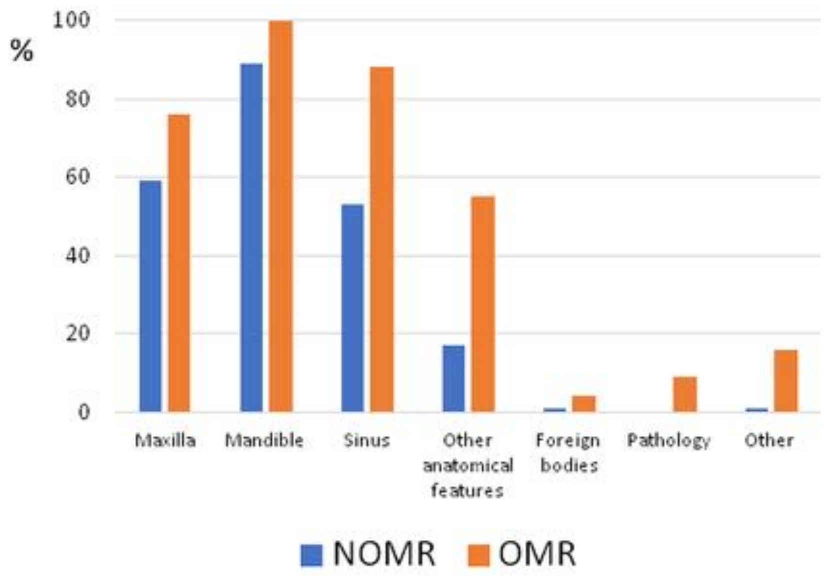


Table 1. Proportion of matches evaluated as "certain" by the oral and maxillofacial radiologists (OMR) and the dentists who were not oral and maxillofacial radiologists (NOMR) for all cases and separated by the time elapsed: ≤3 years and >3 years.

Time elapse between panoramic examinations	Proportion of matches evaluated as "certain"		P-value*
	by OMR	by NOMR	
0-6 years (n=19)	92%	42%	0.001
≤3 years (n=14)	91%	38%	0.001
>3 years (n=5)	95%	55%	0.008

*Pearson Chi Square

The participants in the OMR group were "certain" in 92% of the matches; while, in 7% of matches they considered it "likely" that the images depicted the same person and in 1% they considered it "possible". All their matches were correct. The NOMR group was "certain" in 42% of the matches and all were correct. In 46%, they considered it "likely" that the images depicted the same person and 94% were correct. They considered it "possible" in 12% of the matches and 89% were correct. There was a statistically significant difference between the groups regarding the confidence in their matches, (p≤0.008) (Table 1). In 74% of the cases, the time between the images to be matched was ≤ 3 years. In the remaining 26%, the time was > 3 years. This time limit was used because of an earlier study questioning post-mortem panoramic images older than 3 years.[12]

When assessing image pairs obtained within a short time period (≤ 3 years), the OMR group thought the match was "certain" in 91% of the cases, "likely" in 7%, and "possible" in 2%. These answers were all 100% correct. For the NOMR group, the match was "certain" in 38% of cases and all were correct, "likely" in 48% (93% correct), and "possible" in 14% (90% correct). When assessing cases with a long time-period (>3 years) between the images, the OMR group thought the match was "certain" in 95%, "likely" in 5%, and "possible" in 0%. The NOMR group thought the match was "certain" in 55%, "likely" in 40%, and "possible" in 5%. All of the matches of these cases were correct in both groups. The OMR group was statistically significantly more often certain than the NOMR group, irrespective of the time elapsed between the examinations (Table 1).

DISCUSSION

The results of this study show that it is possible to use panoramic radiographic images to identify edentulous individuals. There were four OMR and four NOMR who conducted the matching. The OMR group had more correct answers and was more certain than the NOMR group in their matches. The difference between the groups could indicate that the OMR group, based on their experience, has an advantage in analysing panoramic images. This is in accordance with earlier studies in which OMR had a higher success rate and greater certainty than NOMR when radiographs were compared.[16-18] There were three different scenarios in this study and correct matching was possible in all of them. In the ante-mortem images, the patients had teeth or were edentulous. In the post-mortem images, the patients were edentulous or edentulous and treated with implants. At least one of the two images in a correct match was edentulous. Most of the post-mortem images contained implants, which is now a common treatment.

It has already been shown that it is possible to use intra-oral radiographic examinations when identifying edentulous patients treated with implants.[8] The findings in this study show that it is also possible to use panoramic images to identify patients treated with implants or a full removable denture (FDR), irrespective of whether the ante-mortem and/or the post-mortem panoramic image shows an edentulous patient without pathological lesions and/or retained teeth or roots. In some countries, FDRs are marked with an ID number that can be used to identify the patient. This could be of value in an identification process of edentulous patients with a FDR.[19] Although, since this method is not yet applied in every country, panoramic examinations can be of great value for identifying edentulous individuals. When using panoramic images in the identification processes, one has to consider many aspects of the technique used to obtain the images. For example, the person should be positioned correctly in the machine and stay still during the exposure. These factors may vary in the ante-mortem images. In post-mortem images, the risk for movement artifacts is negligible. The positioning of the head can, however, be different in the ante-mortem image. It has been shown that two panoramic images taken with different alignments of the examined object present inner structures that are recognisable in spite of the distortion.[13] Therefore, a correct match can be made using two panoramic images with different distortions.

In most cases, both OMR and NOMR used the mandible to make correct matches. This may be explained by the fact that the middle face is more difficult to depict because of the complex anatomy combined with the relatively complicated technique that is the basis for creating panoramic radiographic images. The mandible does not have the same complex anatomy and is not surrounded by other bony details that can be superimposed on the image of the mandible. Therefore, the mandible appears more clear in the images.[20] In a few cases, minor foreign bodies or a pathology, not noticed when selecting the cases, was detected and used by the OMR. An advantage of panoramic images is that they depict anatomical structures outside the tooth bearing areas, revealing e.g. bone patterns in the nasal region, orbit, ears and the region for carotid arteries with possible calcifications, all of which would not appear in intra-oral radiographs. Another advantage is that many anatomical structures are visible in one image and this can facilitate the identification process.

The usefulness of ante-mortem panoramic images older than 3 years in the identification process has been questioned.[12] In the present study, there were five ante-mortem panoramic images that were at least 3 years older than the matching post-mortem image. In one case, the elapsed time between the matched images was 6 years. The OMR group was more certain than the NOMR group when matching images irrespective of the time elapsed between the images and this could probably be explained by their experience in analysing panoramic images. On the other hand, in both the OMR and NOMR groups, neither the confidence nor the correct matches were affected by the time elapsed between the ante-mortem and post-mortem images in this study. This means that in the cases containing panoramic images taken > 3 years apart, the remodeling of the bone and other changes that could have occurred over time did not affect the ability to correctly match. Based on these results, there is no reason to exclude panoramic images taken more than 3 years before the post-mortem image. However, the low number of cases with ante-mortem images older than 3 years in this study may affect the reliability of this result. However, the current results do contradict the conclusions drawn in the study by Richmond et al. in 2007 about not using ante-mortem and post-mortem images taken > 3 years apart.[12]

This study indicates that the identification of edentulous patients with panoramic images is reliable. However, panoramic examinations are normally performed with the person sitting or in a standing position. Since the production of the Zonarc® (Palomex Oy, Helsinki, Finland) and LPX7007 (ASAHI ROENTGEN, Japan) ceased, to our knowledge no panoramic equipment allows examinations with the patient in the supine position. This may be a practical problem when performing post-mortem panoramic examinations. In cases with loose body parts, e.g. the mandible and/or cranium, post-mortem panoramic examinations can be performed in conventional panoramic machines using suitable stands, support and if necessary, tissue equivalent material compensating for missing tissue, such as the cervical spine.[21] In cases with intact bodies, an alternative method for radiographic examination could be the use of cone beam computed tomography (CBCT) or conventional computed tomography (CT). In conventional CT and in some CBCT machines, patients are examined in a supine position. Some software allows the creation of images similar to panoramic examinations based on the three-dimensional data sets created in CT.[22-25] When examining edentulous patients, the problem with metal artifacts appearing in CT images is generally not a problem. However, it is unclear to what extent the reformatted images created from CT data are comparable to panoramic images created using conventional panoramic equipment with an entirely different technique.

CONCLUSION

Panoramic images can be used to identify edentulous persons. Both OMR and NOMR could successfully identify edentulous individuals in cases where only panoramic radiographic images were available. As the OMR were 100% correct in their matches and were more confident in their decisions, their knowledge could be especially valuable in the reconciliation process.

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Morphologic alterations ear, nose and lip detected with aging through facial photoanthropometric analysis

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ABSTRACT

Background: Understanding the morphologic alterations of the human face over the time is an essential step towards optimal simulations of facial age progression. In practice, these simulations contribute to the forensic routine by tracking and recognizing missing persons.

Objective: This study aimed to assess the morphometric facial alterations with aging – concerning specifically the development of ear, nose and lip in relation to other facial structures.

Material and methods: The sample consisted of 700 photographs taken standardly in frontal view from male and female Caucasian subjects aged from 20 to 80 years old. The sample was divided in 7 age groups (20, 30, 40, 50, 60, 70 and 80 years old), in which 50 males and 50 females were distributed homogenously. Photoanthropometric analysis was applied with SAFF 2D® 2.0.05 (SEPAEL/INC, Federal Police, Brazil) software package considering 36 facial landmarks. A single examiner analyzed the sample aided by two other examiners for the assessment of intra-/inter-rater reliability.

Results: The most evident alterations found in the facial structures consisted of the enlargement of the nose and ears with aging, as well the reduction in size of the lip thickness. These alterations varied considerably in the specific age ranges studied in this research being different between males and females.

Conclusion: The present outcomes indicated the possibility of quantifying morphometric alterations observed in the human face with aging. These alterations may support the forensic practice providing tools for age estimation based on photographic analyses.

INTRODUCTION

Missing persons reached alarming rates worldwide. Trafficking, smuggling, illegal immigration, urban and domestic violence, delinquency, armed conflicts and accidents figure among the main causes behind this scenario.[1,2] Urgent improvements in searching systems emerge as a contemporary need. Currently, the search for missing persons is hampered by the lack of facial images registered in national and international databases. Even when registered, facial images may become obsolete by aging modifications over the time,[3] as well by intentional modifications, such as plastic surgeries.[4]

The concept of "updating" was used in the last decades as a tool for the investigation of missing persons. In general, this concept is founded on the projection of a potential contemporary resemblance of the face. Projecting facial traits and estimating the facial modification with aging is a challenging process.[5] Automated algorithms based on photographic registration arose for the simulation of facial aging through digital and artistic representations.[5-7] The demand for facial analysis increased exponentially in the last decades not only because of the increasing rates of missing persons but also because of the massification of imaging devices for social networking.[8] Digital inclusion also contributed to this panorama with the inclusion of closed-circuit television (CCTV) in surveillance systems.[9]

Once registered with photographic or video cameras, facial images undergo a qualitative analysis supported by information provided by the relatives of the alleged missing person. In this process, aging traits that could indicate how facial aspects were modified with time may be simulated in the facial images.[10] In general, this approach considers bone and cartilage grow and skin wrinkles that may contribute to recognition. More specifically, nose cartilage and fat tissue is degenerated gradually with age while the columella retracts.[11] Consequently, the nose is elongated and rotated vertically downwards.[11] In parallel, the ear lobes grow[12] followed by lip thinning and loss of mouth muscle tone.[11] Skin wrinkles also become more evident with facial muscle tone loss and constant influence of gravity.[11,13] Additional aspects figure contributing to the projection of facial aging, such as hair loss, tooth loss, and weight changes.[6]

Despite the need and importance of age projections for the recognition of missing persons, there is no standard method able to reproduce accurately the facial changes that occur during life.[14] Moreover, the scarce scientific literature and the lack of validation studies on image analysis of facial age progression hamper the routine of forensic services dedicated to the search of missing persons. Guided by the hypothesis that ear, nose and lips change considerably over the time, the present study aimed to bridge a gap in the scientific literature testing the application of image analysis for the morphometric investigation of facial aging in subjects aged from 20 to 80 years.

MATERIAL AND METHODS

The present study was conducted with the approval of the local Committee of Ethics in Research (protocol number: 1484305).

The sample consisted of 700 facial photographs taken in frontal view from Caucasian male (n=350) and female (n=350) subjects aged between 20 and 80 years old. The photographs were searched electronically from a civil database of the Brazilian Federal Police using FACE® software package (SEPAEL/INC®, Federal Police, Brazil). The ensure anonymity, the database was searched considering only sex and age information. The sample was divided in 7 age groups (20, 30, 40, 50, 60, 70, 80 years old), in which male (n=50) and female (n=50) subjects were distributed homogeneously. These subjects were aged exactly according to the group in which they were distributed (with a tolerance of +/-1 year, e.g. age group 20 consisted of patients ages 19, 20 or 21 years old).

The photographs were taken by following standards of the International Civil Aviation Organization (ICAO) and included the use of a digital camera with focal length of 35mm, positioned in the level of the eyes with a distance of 1.5 meters for facial photograph acquisition; and the use of dual source frontal flash with light diffusion film. The inclusion criteria consisted of photographs taken with the face centralized vertically and horizontally considering the symmetry between ears and the symmetry between eye corners, respectively; neutral facial expression, looking toward the camera; and image storage in .PNG format with resolution of 480x640 pixels 24-bit RGB. The exclusion criteria consisted of subjects with evident facial deformities or strabismus; facial hair; make up; and jewelry in the ear, nose and lips.

The photographs underwent photoanthropometry using SAFF 2D® 2.0.05 software package (SEPAEL/INC, Federal Police, Brazil). Fourteen examiners performed the analysis placing 36 landmarks on each of the 700 photographs individually. Twelve median uneven landmarks and 14 bilateral even landmarks were selected – 32 landmarks were placed manually and 4 landmarks were automated (Fig. 1). The software package registered the Cartesian coordinates of each landmark to enable morphometric investigations. For intra- and inter-rater reliability, an external examiner selected randomly 10 faces for duplication. The duplicates were included in the

sample and were analyzed 3 times by each examiner (unaware of the duplicates). The software package controlled the image analysis and did not provide the same photograph for analysis in less than 15 days.
Following the literature, the data analysis consisted of quantifying the distances between landmarks and calculating their ratio with the diameter of iris.[3,13] The ratios (n=13) between landmarks of the ear, nose, lips and face were considered the study variables (Table 1) and were tested statistically for their association with age. The statistical tests consisted of Kolmogorov-Smirnov with correction of Lilliefors for assessing the normality of variables; Pearson's correlation coefficient for associations between variables and age; and Intraclass Correlation Coefficient (ICC) for assessing the intra- and inter-rater reliability. All the test were performed with SPSS® 23.0 (International Business Machines®, Armonk, NY, EUA).

Figure 1 – Photoanthropometric landmarks used in the present study
Bilateral manual landmarks: 1 (ectocanthion); 2 (endocanthion); 3 (iris lateral); 4 (iris medial); 5 (upper eyelid sulcus); 6 (upper eyelid); 7 (lower eyelid); 8 (eyebrow medial); 9 (eyebrow lateral); 10 (frontal-temporal); 11 (upper eyebrow); 12 (lower eyebrow); 16 (alar); 17 (upper nose); 18 (nose lateral); 19 (sub-alar); 21 (lower philtrum crest); 22 (cheilion); 27 (gonion); 28 (zygion); 29 (supra-auricular); 30 (post-auricular); 31 (sub-auricular); 32 (ear lobe upper); Median manual landmarks: 13 (trichion); 14 (pro-nasal); 15 (sub-nasal); 20 (upper lip); 23 (stomion); 24 (lower lip); 25 (labiomental); 26 (gnation); Bilateral automated landmarks: a2 (pupils); Median automated landmarks: a1 (midnasal); a3 (glabella); a4 (nasion).

Table 1 - Variables used in the present study and their respective ratio and anatomic structure

Variable	Ratio	Anatomic structure
v01	n-sn / iris	Nose
v02	al-al / iris	Nose
v03	n-prn / iris	Nose
v04	prn-sto / iris	Nose
v05	sn-sto / iris	Lips
v06	ls-sto / iris	Lips
v07	li-sto / iris	Lips
v08	ch-ch / iris	Lips
v09	ls-li / iris	Lips
v10	sa-sba / iris	Ears
v11	slb-sba / iris	Ears
v12	n-gn/iris	Face
v13	sn-gn/iris	Face

n: nasion; sn: subnasal; al: alar; prn: pronasal; sto: stomion; sn: subnasal; ls: upper lip; li: lower lip; ch: chelion; sa: supra-auricular; sba: sub-auricular; slb: upper ear lobe; gn: gnation; iris: diameter of the iris (distance between iris lateral and medial).

RESULTS

Komolgorov-Smirnov test revealed normal distribution in 92% of the variables in male sand 95% in females.
The mean and standard deviation of each variable distributed according to sex and age range are expressed in Table 2, while the variations of accumulated means are reported in Table 3. The means related to the variables "alal_íris" and "prnsto_iris" reached the highest variations, and were especially more evident in subjects aged >50 years old.
In females (Fig.3), strong correlations were observed for the variables listo_iris and lsli_iris, while moderate correlations were observed for the variables sasba_iris, alal_iris and slbsba_iris.
In males (Fig.4), strong correlations was observed for the variables sasba_iris, listo_iris and lsli_iris and moderate correlations were observed for the variables slbsba_iris and lssto_iris.

Fig. 2 expresses a graphic representation of the human facial growth, especially the progressive growth of nose, mouth and ear illustrating the morphometric alterations detected with aging in males and females.
ICC test reached high value for the intra- and inter-rater reliability (>0.75).

DISCUSSION

In the present study, the variables were calculated in relation to the diameter of the Iris, which is considered a highly stable anatomic structure.[3,15] Based on the low variation of the diameter of the iris with aging, it may be used as a reliable reference to investigate proportional facial growth. In specific, the diameter of the iris was measured linearly from the landmarks iris lateral to iris medial, which are considered landmarks with low variability in facial metric analysis.[3] The final quantification of the diameter of the iris was achieved taking the mean linear measurement between the two years.
Once the diameter of the iris was obtained, this study investigated the proportional growth of facial structures described in the literature with high variation with age: the ear, nose and mouth.[6,9] The analysis of these structures is relevant to support further studies on age estimation through facial aging and is justified on the scarce scientific literature testing and validating techniques in the field. In fact, most of the studies involving age estimation and facial aging are limited considering the sample size and quality.[6,9] In the present study, an expressive (n=700) and highly standardized sample was collected from a database of the Brazilian Federal Police.
Currently, no standard is established to set up studies on the morphometric variations within facial aging through imaging.[8,14] Efforts are made to simulate with drawings and computer graphics the facial variations with aging. However, the analysis of these simulations relies on a considerably subjective procedure. Yet subjective procedures may influence on the reliability of these analyses as tools in the forensic routine – especially in cases of missing persons, in which contemporary photographs of the face may not be available in databases.[6]

Table 2 – Proportional variations of the means of the 13 variables in relation to the diameter of the iris, distributed according to sex and age range

	Age range		Age range		Age range		Age range		Age range		Age range	
	20-30		**20-40**		**20-50**		**20-60**		**20-70**		**20-80**	
Sex	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
Ratio												
v01_nsn_iris	-2.1%	3.1%	0.0%	-0.5%	-2.9%	4.1%	0.1%	3.2%	3.0%	2.6%	1.1%	7.5%
v02_alal_iris	1.4%	2.3%	4.8%	3.0%	4.4%	-1.1%	7.3%	6.8%	14.4%	10.5%	17.3%	11.7%
v03_nprn_iris	-2.7%	1.9%	-3.0%	-3.3%	-5.1%	2.2%	-2.4%	-0.6%	1.1%	0.4%	-1.6%	5.9%
v04_prnsto_iris	6.3%	6.0%	7.9%	1.3%	9.6%	6.5%	10.7%	13.2%	12.5%	8.5%	11.0%	10.6%
v05_snsto_iris	9.0%	5.3%	5.1%	-2.7%	10.5%	7.5%	9.9%	11.2%	12.5%	7.3%	9.4%	9.3%
v06_lssto_iris	1.1%	-5.7%	-0.8%	-16.5%	-10.4%	-32.2%	-20.3%	-32.6%	-19.9%	-34.4%	-18.7%	-40.3%
v07_listo_iris	-8.5%	-13.1%	-12.6%	-27.2%	-28.7%	-43.4%	-35.7%	-48.2%	-47.0%	-51.8%	-39.8%	-64.5%
v08_chch_iris	4.7%	7.2%	3.4%	5.0%	8.6%	8.8%	2.8%	11.2%	5.7%	6.7%	4.4%	4.4%
v09_lsli_iris	-4.9%	-10.4%	-8.6%	-23.2%	-22.4%	-40.0%	-30.5%	-43.3%	-38.1%	-45.8%	-32.9%	-56.6%
v10_sasba_iris	3.0%	3.1%	2.7%	3.2%	4.5%	13.2%	12.2%	14.5%	15.7%	20.6%	17.8%	27.0%
v11_slbsba_iris	-3.3%	-1.3%	0.9%	11.4%	0.6%	18.6%	25.8%	18.4%	14.3%	24.1%	25.3%	38.7%
v12_ngn_iris	0.1%	1.9%	0.0%	-4.2%	-2.4%	-1.4%	-1.5%	-0.5%	-0.6%	-2.4%	-1.6%	-0.5%
v13_sngn_iris	3.3%	0.0%	-0.6%	-9.9%	-2.2%	-7.6%	-4.9%	-6.2%	-6.9%	-10.0%	-6.6%	-12.3%

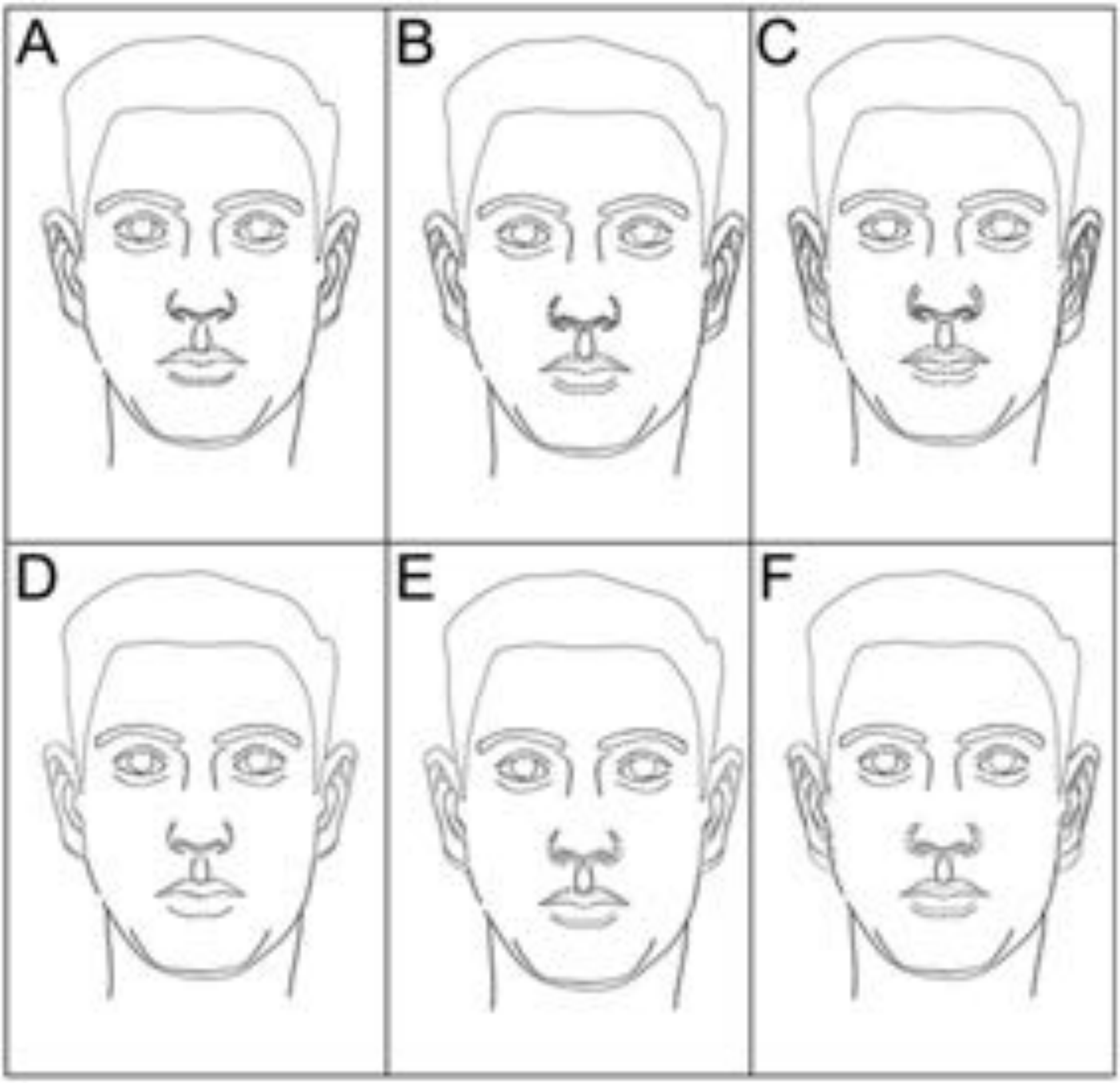
F: female; M: male; age range expressed in years; 13 variables described in Table 1.

Table 3 – Means of the 13 variables used in the present study distributed by sex and age range

Age range		**20.0**		**30.0**		**40.0**		**50.0**		**60.0**		**70.0**		**80.0**	
Variable	**Sex**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
v01_nsn_iris	F	4.28	0.34	4.19	0.35	4.28	0.35	4.16	0.33	4.29	0.36	4.41	0.34	4.33	0.41
	M	4.31	0.40	4.44	0.45	4.29	0.40	4.48	0.37	4.45	0.42	4.42	0.41	4.63	0.45
v02_alal_iris	F	2.91	0.28	2.95	0.24	3.05	0.24	3.03	0.25	3.12	0.22	3.33	0.28	3.41	0.32
	M	3.26	0.22	3.34	0.25	3.36	0.21	3.23	0.94	3.48	0.29	3.60	0.35	3.64	0.31
v03_nprn_iris	F	3.38	0.34	3.29	0.35	3.28	0.37	3.21	0.33	3.30	0.35	3.42	0.38	3.33	0.39
	M	3.38	0.42	3.45	0.44	3.27	0.39	3.46	0.38	3.36	0.41	3.40	0.46	3.58	0.44
v04_prnsto_iris	F	2.70	0.26	2.87	0.26	2.91	0.34	2.96	0.33	2.99	0.27	3.04	0.31	3.00	0.37
	M	2.96	0.28	3.14	0.28	3.00	0.26	3.15	0.31	3.35	0.35	3.21	0.41	3.28	0.38
v05_snsto_iris	F	1.82	0.27	1.98	0.23	1.91	0.25	2.01	0.25	2.00	0.21	2.05	0.26	1.99	0.32
	M	2.04	0.22	2.15	0.23	1.98	0.22	2.19	0.39	2.27	0.29	2.19	0.32	2.23	0.31
v06_lssto_iris	F	0.48	0.10	0.49	0.10	0.48	0.12	0.43	0.12	0.38	0.12	0.38	0.10	0.39	0.14
	M	0.50	0.13	0.47	0.12	0.42	0.13	0.34	0.10	0.34	0.12	0.33	0.12	0.30	0.12
v07_listo_iris	F	0.89	0.15	0.81	0.16	0.77	0.15	0.63	0.18	0.57	0.16	0.47	0.20	0.53	0.16
	M	0.85	0.20	0.74	0.19	0.62	0.23	0.48	0.17	0.44	0.14	0.41	0.17	0.30	0.16
v08_chch_iris	F	3.93	0.28	4.11	0.31	4.06	0.30	4.26	0.46	4.04	0.32	4.15	0.39	4.10	0.37
	M	4.01	0.35	4.30	0.37	4.22	0.36	4.37	0.32	4.46	0.52	4.28	0.47	4.19	0.47
v09_lsli_iris	F	1.36	0.20	1.29	0.21	1.24	0.22	1.05	0.27	0.94	0.23	0.84	0.24	0.91	0.23
	M	1.34	0.29	1.20	0.27	1.03	0.31	0.80	0.25	0.76	0.23	0.72	0.26	0.58	0.24
v10_sasba_iris	F	4.67	0.54	4.81	0.34	4.79	0.38	4.87	0.38	5.24	0.39	5.40	0.43	5.50	0.42
	M	4.70	0.40	4.85	0.41	4.85	0.38	5.33	0.40	5.39	0.51	5.67	0.49	5.97	0.44
v11_slbsba_iris	F	1.57	0.23	1.52	0.21	1.58	0.21	1.58	0.22	1.97	0.68	1.79	0.25	1.97	0.28
	M	1.45	0.23	1.43	0.25	1.62	0.19	1.72	0.25	1.72	0.26	1.80	0.30	2.01	0.33
v12_ngn_iris	F	6.96	0.42	6.96	0.43	6.96	0.50	6.79	0.50	6.85	0.40	6.92	0.51	6.85	0.50
	M	7.18	0.44	7.32	0.56	6.88	0.54	7.08	0.51	7.14	0.56	7.01	0.49	7.15	0.54
v13_sngn_iris	F	2.70	0.31	2.79	0.26	2.68	0.32	2.64	0.34	2.57	0.26	2.51	0.34	2.52	0.38
	M	2.88	0.32	2.88	0.33	2.60	0.34	2.66	0.45	2.70	0.36	2.60	0.39	2.53	0.37

SD: Standard deviation; F: female; M: male; age range expressed in years; 13 variables described in Table 1.

Figure 2 - Graphic illustration of the progressive growth of ear, nose and mouth with ageing, based exclusively in the metric analyses (variables) considered in the present study
Caption: Growth of ear, nose and mouth in the ages of 30, 50 and 80 year old are represented for males (from A to C, respectively) and females (from D to F, respectively). Faces outlined in black indicate the age of 20 years old (initial), while the color outlines indicate the variations with ageing.



The scientific literature reports several signs of aging in the human face, such as degeneration of cartilage, loss of elasticity and facial flaccidity. More detailed alterations are reported for specific facial structures, such as nose growth downwards – resulting from the decrease in fat tissue, gravity and retraction of the columella; ear growth – especially the ear lobes influenced by gravity; and progressive loss of lip thickness influenced by the alveolar bone resorption and reduction of perioral muscle tonus.[9-13,16] On the other hand, there is no quantitative information of these facial alterations in the literature. The present research provides innovative outcomes that contribute to the study of facial growth with aging.

In relation to the nose, the variables "nsn" (from nasion to subnasal), "alal" (from right alar to left alar), "npm" (from nasion to pronasal) and "prnsto" (from pronasal to stomion) (Table 1) represented the height, width, vertical upper length, and vertical lower length of the nose, respectively. The most evident variations were observed in variables "alal" and "prnsto" – especially in subjects aged >50 years old. While in one hand the literature lacks information on the progressive increase in nose width ("alal") with age, on the other hand it indicates that the vertical lower length of the nose (distance from the nose to the mouth) decreases with age.[9] Despite that, only the nose width in females reached differences significant statistically in relation to age (p<0.05). When analyzing sex separately, all the variables except "nprrn" reached differences significant statistically (p<0.05). More evident alterations were observed in males.

Considering the age range of 20 years old, the only variables with means statistically significant higher in males than females were "alal" and "prnsto", which indicate that at this period men present the nose larger and with a distance from the mouth higher than women. In the age range of 30 years old, all the variables reached means significantly higher in men than women, while within the range of 40 years old nose width ("alal") becomes larger (p<0.05) in males again. In the age range of 50 years old, the statistical significance of the variables invert and "alal" becomes the only nose-related variable with no difference between males and females. In the following age ranges (60 and 70 years old), "alal" and "prnsto" reveal higher mean values in males than in females (p<0.05), as well "nsn" exclusively for the range of 60 years old. Finally, in the age range of 80 years old, all the variables related to the nose have means higher in men than women (p<0.05).

The present study also detected alterations related to the lip. The variables used to assess these alterations were "snsto" (from subnasal to stomion), "lssto" (from upper lip to stomion), "listo" (from lower lip to stomion), "chch" (from right cheilion to left cheilion), and "lsli" (from upper lip to lower lip) (Table 1). The morphological structures represented by these variables consisted of the total height of upper lip (distance from the nose), height of the upper lip (mucous portion), height of the mucous portion of the lower lip (mucous portion), width of the lips, and height of both lips (mucous portion), respectively. The most evident alteration observed was the decrease in the mean value of the variable "lsli". This finding corroborates the literature[6,8] and is translated as the reduction in the height of both lips (loss of lip thickness) with age. In men, the lips reduced vertically up to 56.6%, while in females it reached 32.9%. All the variables related to the lips revealed differences significant statistically between males and females. Once more, the alterations were more evident in men.

For patients aging 20 years old, no different significant statistically (>0.05) were observed between males and females considering the variables "lssto", "listo", "chch" and "lsli". The opposite was observed for the variable "snsto" (p<0.05), which presented higher mean values in men. It indicates that the upper lip trends to be thicker in males at this age range. Next, in the age range of 30 years old, males presented mean values above females not only for "snsto" but also considering the width of the mouth ("chch") (p<0.05). On the other hand, females presented the mucous portion of the lower lip (listo) larger than males (p<0.05). In the age of range of 40 years old, "snsto" was not different between males and females (p>0.05). At this stage, females presented lip proportion larger than males (p<0.05) – including "lssto", "listo", and "lsli". The only variable remaining higher in males was the width of the mouth ("chch"). In the following age range (50 years old), the width of the mouth was not different between males and females, while the mean of "snsto" became higher in males again. The other variables remained with mean values higher in females (p<0.05). Within 60 years old, the variables observed higher in men were the same observed higher in the age range of 30 years old (p<0.05). The variables "listo" and "lsli" were higher in females, while "lssto" was not different between sexes. For subjects aged 70 years old, two variables ("listo" and "chch") did not reach differences significant statistically between males and females, while "snsto" was higher in males and "lssto" and "lsli" in females (p<0.05). In the last age range (80 years old), "chch" did not result in differences between sexes. The only variable with higher mean value in men was "snsto", while the others were higher in females (p<0.05).

Morphometric alterations in the ear were also investigated in this study. The variables related to these alterations were "sasba" and "slbsba", which represent the distances between the landmarks supra-auricular and sub-auricular (height of the

Figure 3 - Outcomes of Pearson's correlation coefficient for the association of variables in fuction of age in females (Variables described in Table 1).

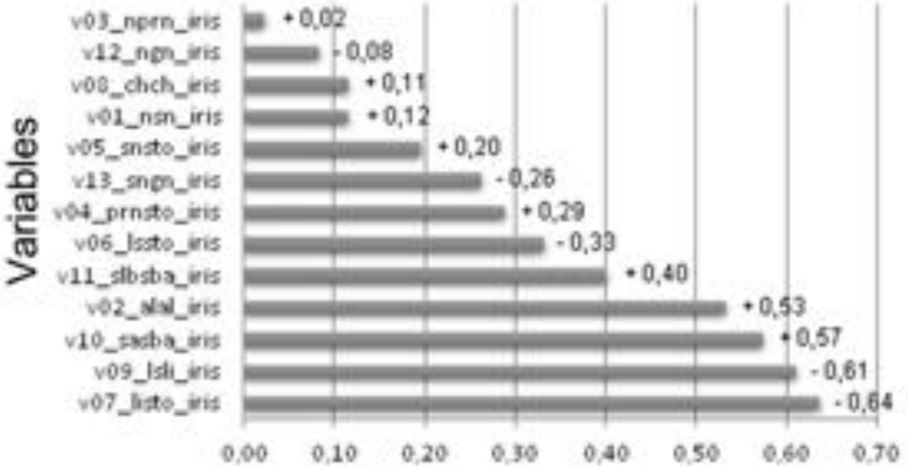
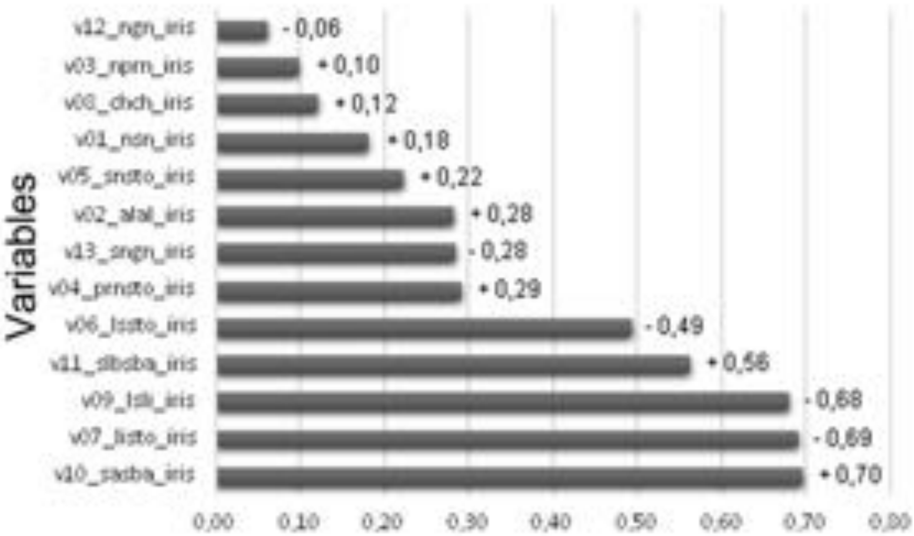


Figure 4 - Outcomes of Pearson's correlation coefficient for the association of variables in fuction of age in males. (Variables described in Table 1).



ear) and between the sub-auricular and the upper ear lobe (height of the ear lobe), respectively. Confirming the scientific literature, both variables increased with age and were manifested clinically with the enlargement of ear and ear lobe.[11] Moderate correlations with age ranges were observed for these variables. All the morphometric variations were different statistically between males and females ($p<0.05$). Specifically, in males the lower portion of the ear increased in size in 38.7%, while in females this portion increased in 25.3%.

The morphometric variations of the ear observed in relation to age indicated that in the range of 20 years old the ear lobe ("slbsba") of females was larger than males ($p<0.05$). No difference significant statistically was observed for "sasba" in relation to sex. In the age ranges of 30 and 40 years old, no difference was observed between sexes considering both variables. In subjects aged 50 years old, both variables reached mean values considerably higher in males ($p<0.05$). In the range of 60 years old, no difference significant statistically was observed for the variable "sasba", while "slbsba" was higher in females than males. In the last age ranges (70 and 80 years old), no difference ($p>0.05$) was observed between males and females considering the variable "slbsba", while "sasba" was higher in males. It reveals an inversion on the size of the ear lobe, which is larger in females in the age range of 20 years old and becomes larger in males in the age ranges of 70 and 80 years old.

Besides the nose, mouth and ear, the present study investigated two variables related to the height of the human face: "ngn" and "sngn", which indicate the distance between nasion and gnation (2/3 of the face), and between subnasal and gnation (1/3 of the face), respectively. A trend on the decrease of the lower portion (1/3) of the human face was observed with age. In males aged 80 years old, the decrease in mean values reached 12.3%, while it reached 6.6%. According to the literature, the reduction may be justified on the loss of muscle tonus and teeth as well alveolar bone resorption.[6,12,13] In specific, major variations were observed among males. In the age ranges of 20, 30, 50, 60 and 80 years old "ngn" was had mean values considerably higher in males ($p<0.05$). In the ranges of 20 and 60 years old "sngn" was also higher in males ($p<0.05$). Variable "sngn" was not different between sexes in subjects aged in the ranges of 30, 50 and 80 years old ($p>0.05$). Lack of differences significant statistically between males and females considering both variables were observed in the age ranges of 40 and 70 years old.

It is important to note that, the metric analysis performed in this study was restricted to the facial structures known for having more evident alterations in relation to age.[6,9] Moreover, these structures were analyzed bidimensionally in photographs. In future studies, three-dimensional analyses are encouraged to improve the investigation of facial alterations with aging, enabling simulations and morphometric assessment with more contemporary imaging, such as facial scanning and photogrammetry. On the other hand, bidimensional photoanthropometry – used in this study, represented a reliable tool for facial morphometrics in a forensic context.

CONCLUSION

The validation of alternatives for the assessment of age through facial analysis is essential to improve the routine of police services and medico-legal institutes. The present study supports these alternatives with the application of a non-expensive and practical approach.

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Geometric morphometric analysis of sexual dimorphism in the mandible from panoramic X-ray images

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KEYWORDS

Geometric morphometrics;
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ABSTRACT

The human mandible is routinely utilised as part of the assessment of biological identity in forensic anthropological and odontological practice. The research introduces a novel geometric morphometric technique to investigate and quantify shape variation in the morphology of the mandibular corpus and ascending ramus and consequently highlights the potential for forensic purposes. Human mandibles from digital clinical orthopantomogram X-ray images, based on a sample of 50 male and 50 female adults from a modern Italian population, were examined. Three fixed landmarks were applied to the symphysis and condyle and 50 semi-landmarks re-sampled along the inferior corpus and the posterior ramus. Symmetrical reflection was applied yielding 200 configurations of 53 landmarks. Shape analyses were undertaken via: Procrustes superimposition; principal components analysis to investigate patterns of variation; classification using linear discriminant analysis with leave-one-out cross-validation; partial least squares (PLS) to test for structural modularity; and finally, retitle page sampling and re-analysis following PLS to optimize shape classification criteria. Stepwise re-sampling of landmarks reached an optimum cross-validated classification of 94.0% based on 25 landmarks; the results are strongly significant and suggest that the shape relationship between the mandibular corpus and ramus offers significant potential for forensic identification purposes using this method.

INTRODUCTION

The human mandible is routinely utilised as part of the assessment of biological identity in forensic anthropological and odontological practice.[1] Various authors have pointed to the utility of odontological methods in morphological and metric features of teeth,[2] but also traditionally recovered morphological, metric, and non-metric traits in the mandible, including discrete areas such as symphyseal morphology and shape,[3-6] gonial angle, gonial inversion and eversion,[7-10] ramus flexure,[11,12] overall shape from elliptical Fourier transforms[13-15] and discriminant functions based on linear dimensions[16,17] amongst others in the assessment of biological sex and ancestry.

More recently, a number of studies have utilised geometric morphometric (GM) approaches to address issues of biological identity in this anatomical region.[18-23] Defined simply, GM is the statistical analysis of form based on Cartesian landmark coordinates.[24] GM techniques generally involve the capture of homologous landmarks which can be defined as precise locations on biological specimens that hold some functional, structural, developmental or evolutionary significance and are directly comparable between specimens. Landmarks can be recorded as two or three-dimensional co-ordinates which result in a spatial framework of the relative positions of the chosen points in two or three-dimensional space. Such data allows for the statistical analysis of the embedded shape geometry of a biological form through a variety of transformative or computational methods.[25-29] The research presented here utilises geometric morphometric techniques to investigate and quantify shape variation in the morphology of the mandibular corpus and ascending ramus and consequently highlights the potential for forensic human identification. We present the results of a novel morphometric study using clinical panoramic scanning x-radiography, the aim of which was to develop a methodologically and statistically robust means of investigating biological variation in lower jaw morphology from a commonly acquired clinical data source which may be of use in the human identification process.

MATERIAL AND METHODS:

Digital orthopantomogram images (OPG) in TIFF format were acquired of the upper and lower dentulous jaws of 50 Italian male and 50 female participants, derived from a larger clinical image database. The images were captured by odontologists using a panoramic digital device (Planmeca Proline xc) as part of private clinical dental practice, with permission obtained for the anonymised use of resulting OPG for research studies. The specimens utilised were drawn from a larger sample pool, with the age distribution of both sex cohorts matched using exact randomisation (male profile matched to female), resulting in a common demographic profile for both sexes (Fig. 1); this yielded a mean age of 38 years for males (minimum age 20 years, maximum age 68 years) and 37 years for females (minimum age 21 years, maximum age 62 years). The OPG images were stripped of biographical information, and re-labelled with a sequential numerical code which referenced sex and age only. Three type I and type III 2D landmarks were applied to the symphysis and condylar process using TPS Digit software and 50 re-sampled equidistant semi-landmarks were established along the inferior border of the corpus and the posterior border of the ascending ramus; semi-landmarks were anchored anteriorly and posteriorly to fixed homologous points (Table 1). Landmarks were reflected to the opposite side, providing *n* 200 configurations of *k* 53 landmarks (resulting in *n* 100 left and *n* 100 right side, *k* 53 each).

Fig. 1 Age profile of male and female cohorts used in this study. Mean ages: male 38 years, female 37 years. Range: male 20 to 68 years, female 21 to 62 years.

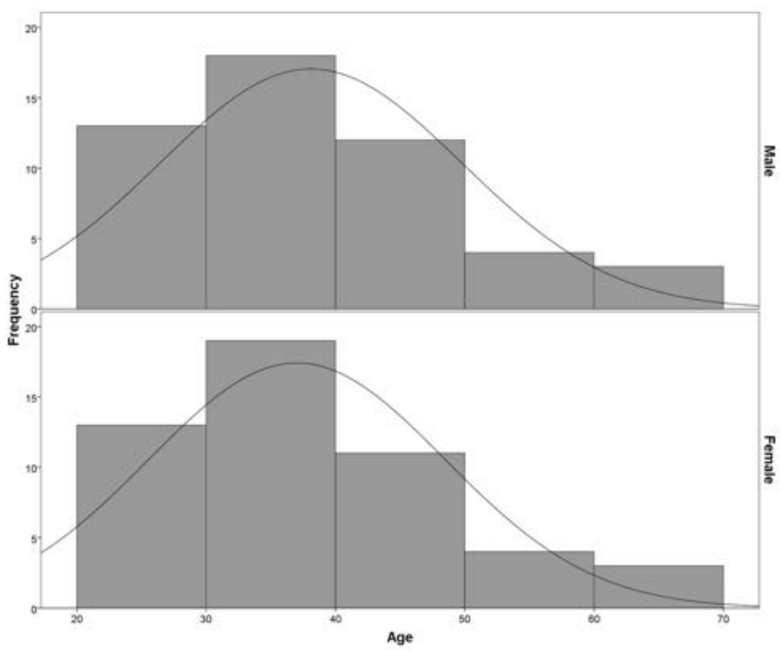


Table 1. Landmark definitions.

Landmark	Description
1	Infradentale
2	Most superior point on mandibular condyle
3	Most anterior point on mandibular condyle
4-33	Equidistant semi-landmark series captured along inferior border of mandibular corpus from gnathion to gonion
34-53	Equidistant semi-landmark series captured along posterior border of ascending ramus from gonion to the most postero-superior point on the mandibular condyle

The statistical shape analyses of the 2D coordinate configurations involved the following stages: (1) partial Procrustes superimposition (GPA) with full tangent space projection; (2) regression of tangent space distance onto Procrustes distance to test for tangent space approximation; (3) assessment of inter and intra-observer measurement error using a 5x5x5 repeat procedure, with Procrustes ANOVA to test for differences in group means; (4) principal components analysis (PCA) to reduce dimensionality and investigate patterns of population variation, with test for significance of shape differences between groups using Procrustes ANOVA; (5) classification using Fisher's linear discriminant analysis (LDA) with leave-one-out cross-validation to assess performance of the classification; (6) partial least squares (PLS) analysis within a single configuration to test for structural modularity; and (7) stepwise re-sampling and re-analysis of dataset following PLS to optimise shape classification criteria. Shape analyses were undertaken using the *shapes* library[30] and complementary *R* statistical routines,[31] partial least squares analysis in *MorphoJ*[32] and additional statistical analyses in SPSS *20.0*.[33]

RESULTS

The resulting two-dimensional coordinate configurations (*n* 200) were subjected to a generalised Procrustes analysis (GPA) with full-tangent space projection and scaling invariance which effectively removes size from the analysis by scaling all configurations against unit centroid size[34] leaving only shape differences between configurations. Potential measurement error of both intra and inter-observer landmark acquisition was assessed by two of the authors (PRQ and JRQ) digitising five individual configurations five times on five separate occasions and then subjecting the resulting configurations to GPA.

Procrustes ANOVA based on Procrustes distance[35] was used to assess the relative magnitude of error from repeat measurements; no significant differences were noted between test runs within and between each observer confirming that measurement error was low.

Further statistical analysis of shape requires that the Procrustes coordinate configurations are projected back into Euclidean tangent space,[34] with statistical analyses carried out within that space using standard multi-variate methods. The appropriateness of tangent space projection was tested by regressing the distance in tangent space onto Procrustes distance; this produced a correlation coefficient of 0.99 indicating that tangent projection and Procrustes approximation were successful. When dealing with such high dimensional data it is natural to reduce the dimension and a commonly used technique is principal components analysis (PCA), carried out by forming the sample co-variance matrix of the residuals and computing the eigenvalues and eigenvectors accordingly. Following GPA, the mandibular configurations were subjected to PCA to explore the relationships within the global sample and specifically the patterns of sexual dimorphism between male and female forms. 102 principal components were

produced ($2k - 4 = 102$ shape variables), allowing for an investigation of overall shape variation in corpus and ramus morphology.
The first 10 principal components account for over 95% of shape variation in the sample (Table 2). Global shape variation is expressed in Fig. 2 by the plot of principal components 1 and 2 and shape variance by vector plots of the first three principal components in Fig. 3. As can be clearly seen, global variation is primarily manifested through the relative size differences in the height of the ascending ramus and placement of the inferior border of the symphysis (PC1), with higher order PCs displaying differential shape patterns in the orientation of the gonial angle compared to the symphysis and ascending ramus (PC2) and flexure of the inferior corpus and projection of the symphysis (PC3).

Table 2. Total population variance explained by principal components analysis (first 10 PC's only).

	Eigenvalue	% var	Cum %
PC 1	0.00099494	36.108	36.108
PC 2	0.00085379	30.985	67.093
PC 3	0.00030537	11.082	78.176
PC 4	0.00017213	6.247	84.423
PC 5	0.00011095	4.027	88.449
PC 6	0.00006511	2.363	90.812
PC 7	0.00006037	2.191	93.003
PC 8	0.00003876	1.407	94.410
PC 9	0.00002743	0.996	95.405
PC 10	0.00002138	0.776	96.181

Fig. 2 Visualization of global population shape variation showing results of principal components analysis based on covariance matrix of *k* 53 landmarks. Graph highlights first two principal axes of variation with markers indicating sex.

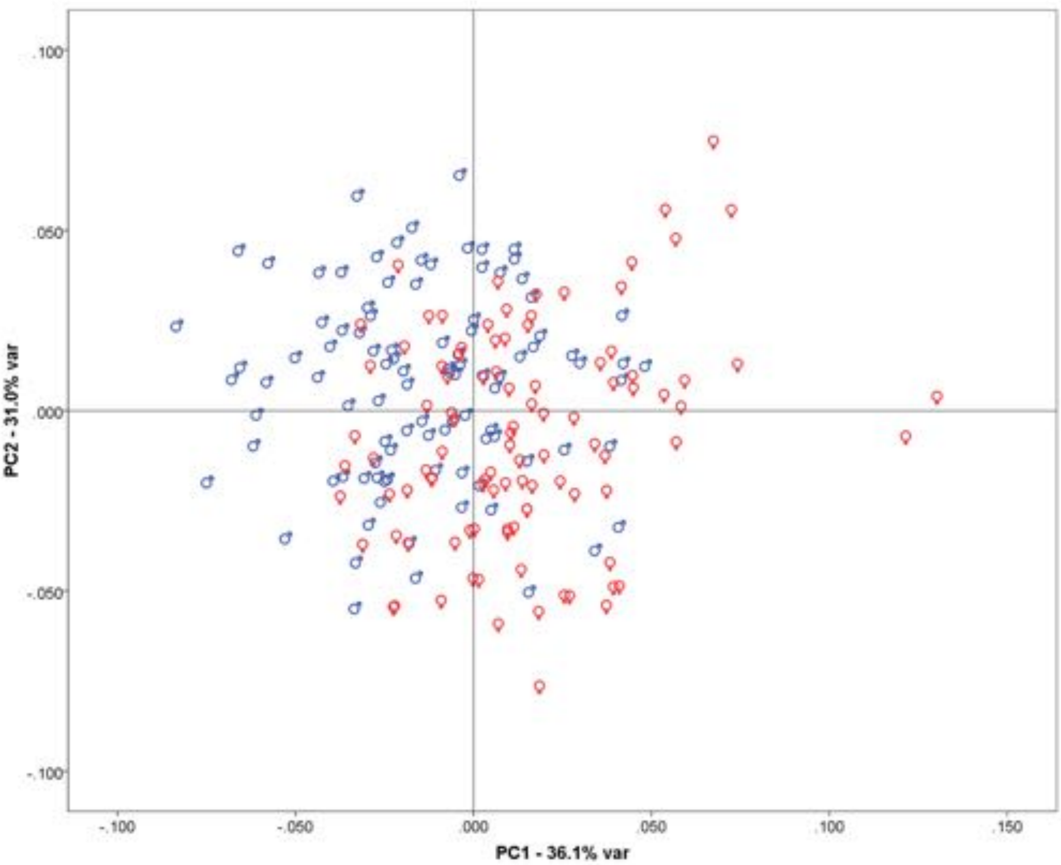
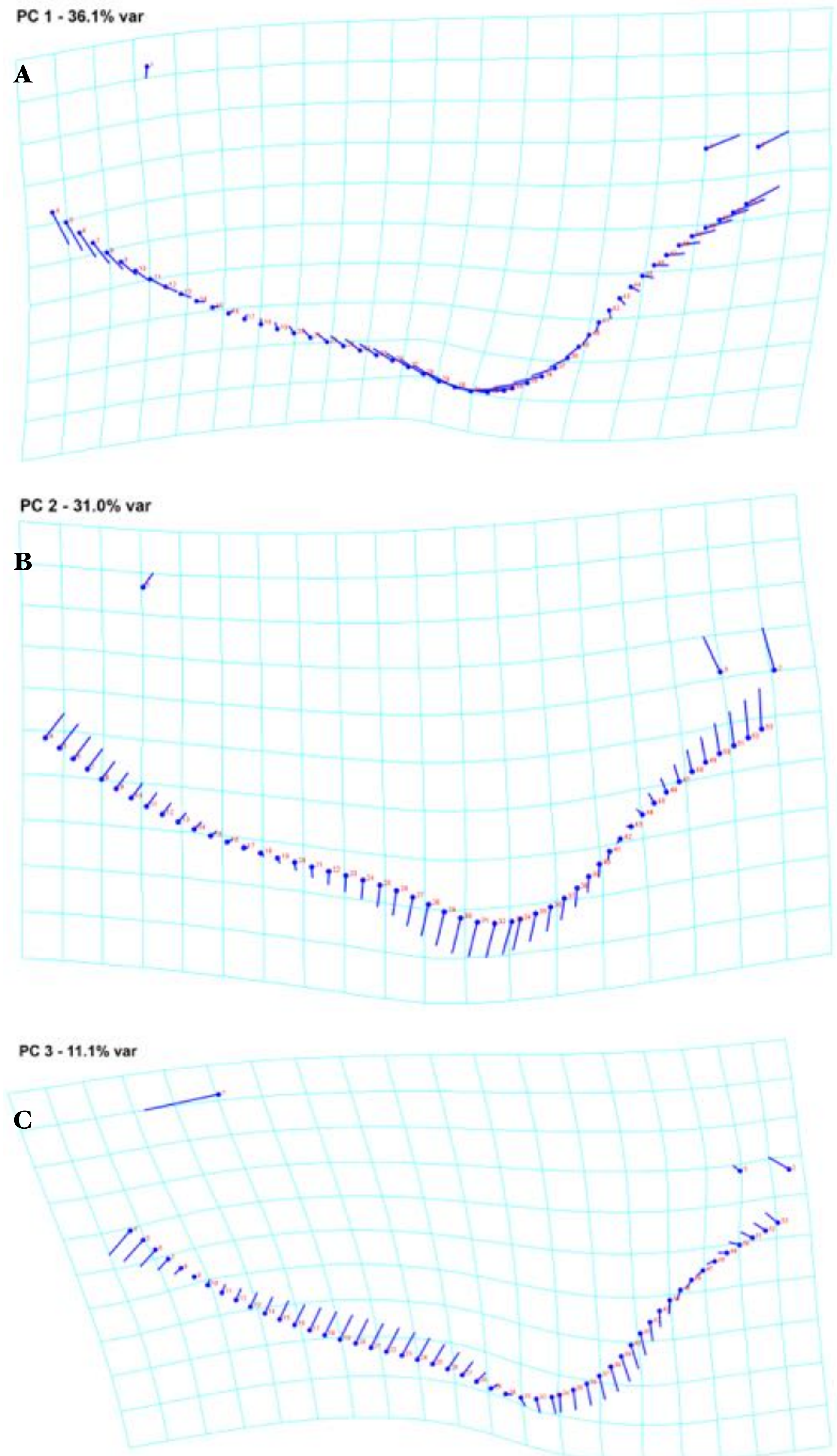


Fig. 3a to 3c Visualization of shape variation along the first, second and third principal component axes showing the extremes of shape variation as vectors compared to the consensus (mean) shape represented by solid circles, with deformation grid applied to assist visualization. Variation represented is PC1 36.11%, PC2 30.99% and PC3 11.10% respectively. Figures generated using *MophoJ*.



The utility of the resulting shape variables as an aid in the assessment of biological sex was undertaken using Fisher's linear discriminant analysis based on the residual shape variables. Classification of specimens based on outlines or semi-landmarks often poses a challenge in GMM analyses in that accurate representation of a curve or surface requires many measurements, but this increase in the number of resulting shape variables dramatically increases the sample sizes necessary to carry out discrimination,[36] otherwise known as the curse of dimensionality.

One way to circumvent this is to convert the shape variables into principal component scores and reduce the dimensionality of the data by analysing a limited number of PC scores from the cases instead of the original data,[37] thus only relatively large group mean differences will be represented by the retained lower order PCs, leaving a proportion of the variance unaccounted for. As a rule of thumb, it is generally valid to have twice the number of specimens in each classification group as there are shape variables and computationally stable results will require many more cases than variables.[36]

In this study we have used PCA to reduce the dimensionality of the shape data and then applied a tuning parameter (p) to limit the number of principal components used in order to optimise the classification, whilst still retaining as large a number of shape variables as possible.

The analysis was undertaken using the *shapes* library[30] on the first p PC scores derived from Procrustes analysis, with reliability of classification based on leave-one-out cross-validation with permutation test for significance. The use of a tuning parameter (rather than the commonly used stepwise entry of independent variables) is to limit the effects of over-fitting of data to the discriminant function which can lead to poor out of sample predictive performance. With high dimensional data it is easily possible to get 100% classification within the training sample on the basis of high-order 'noisy' PCs alone, but very poor out of sample classification; lower order PCs are therefore used as these will not be so affected by the specific noise in the sample. Classification by sex was obtained from the discriminant analysis with p= 10 (thus using PC1 to PC10) with lower classification rates for higher order PCs which were therefore rejected from the analysis.

Using k 53 landmarks (of which p 10 PCs are retained in the classification) the resulting shape variables successfully classified 85.0% of individuals by biological sex (Table 3). Procrustes ANOVA of shape residuals indicates a statistically significant shape difference between the male and female mean shapes (F 24.33, $p<$ 0.0001).

Table 3. Cross-classification results of sex assessment based on k 53 landmarks and p = 9 PC's (overall cross-validated success rate 85.0%).

	Predicted as male	**Predicted as female**	**Total**
Known male	88.0%	12.0%	100%
Known female	18.0%	82.0%	100%

For the final part of the analysis we investigated the impact of landmark number and location on classification accuracy. The first classification utilised k 53 landmarks from which p = 10 shape variables are retained in the classification. We used a two-block partial least squares analysis (PLS) in order to investigate whether classification accuracy can be improved by utilising either a smaller more constrained anatomical region from the OPG or fewer landmarks overall. PLS examines co-variation between two or more sets of variables and identifies features of shape that most strongly co-vary between blocks; this technique is increasingly being used for studying patterns of integration of parts within single configurations of landmarks thus allowing for an assessment of anatomical or structural modularity.[36-42] In the present study we use an assessment of modularity to investigate whether anatomical regions (specifically the mandibular corpus and ascending ramus) provide better assessment of biological sex if treated as isolated structural units - as seen in previous studies [7,9,11,21,22] – or if they improve sex assessment when combined into a single anatomical module.

To this end we conducted a two-block PLS within the same configuration using *MorphoJ*.[32]

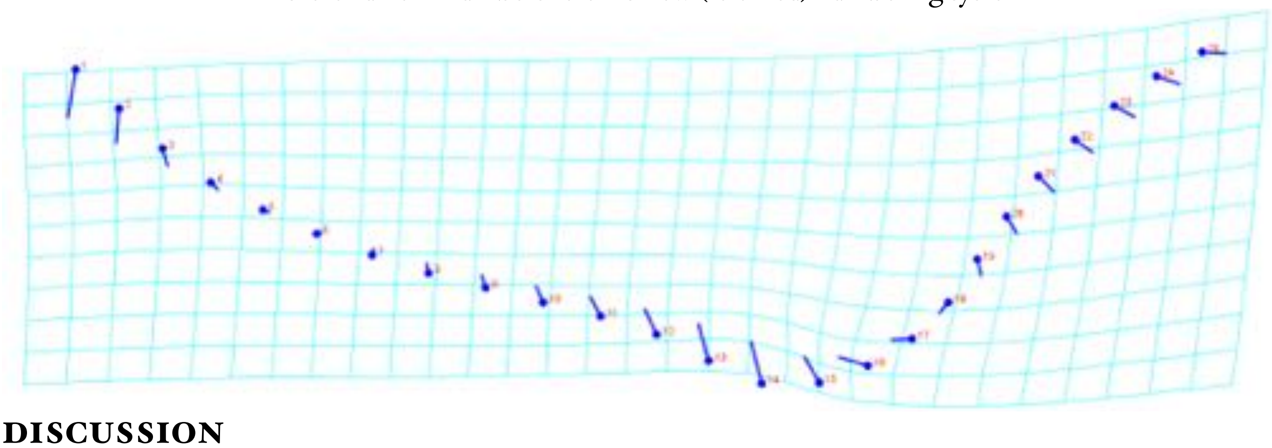
Landmarks were sub-divided into a corpus block (*k* 31 = LM 1 and LM 4 to LM 33) and a ramus block (*k* 22 = LM 2, LM 3 and LM 34 to LM 53). Two-block PLS produced an *RV* coefficient (the measure of co-variance between units) of 0.74, which indicates a strong statistical association between blocks and a high degree of modularity in anatomical structure; an *RV* of 1 implies that one set of variables can be obtained from the other set by rigid rotation and/or reflection.[40] To confirm modularity we performed a series of stepwise exclusion tests, reducing 10% of landmarks at each iteration until no improvement in classification accuracy was noted.

Table 4. Cross-classification results of sex assessment based on *k* 25 landmarks and *p* = 10 PC's (overall cross-validated success rate 92.5%).

	Predicted as male	Predicted as female	Total
Known male	91.0%	9.0%	100%
Known female	6.0%	94.0%	100%

Re-analysed data was based on treatments of dependent (both blocks) and independent units (single blocks); each iteration required a Procrustes refit with PCA in each round of the stepwise procedure. This reached an optimum classification based on *k* 25 landmarks of the dependent blocks (both block sets) with an increased cross-validation of 92.5% of cases by sex (Table 4). Classification rates based on individual treatment blocks were significantly reduced in comparison to the optimal value of *k*. Optimal sex-based shape differences are significant (Procrustes ANOVA, F 24.33, p<0.0001) and visualised in Fig. 4; it should be noted that original fixed landmarks L M 1 to LM 3 were not retained in the optimal configuration, instead the outline of the inferior corpus and posterior ramus is highlighted by *k* 25 retained equidistant landmarks. It can be clearly seen that sex-based differences are localised and most extreme in the inferior displacement of symphysis (LM 1 to LM 3 – pointing of the chin), the degree of incurvature in the corpus just anterior to gonion (LM 8 to LM 16) and posterior displacement in the upper half of the posterior border of the ramus (LM 19 to LM 25) in the female mean shape compared to the male.

Fig. 4 Visualization of shape variation due to maximally-differentiating sexual dimorphism as determined by Fisher's linear discriminant analysis based on optimum *k* 25 landmarks, showing the extremes of shape variation as vectors. Note, variation is expressed from the male consensus (solid circles) to the female mean shape (terminus of vector), with the deformation grid applied to assist visualization. Figure generated using *MophoJ*; note landmark numbers refer to new (retained) numbering system.



DISCUSSION

This investigation was designed to introduce a more standardised method of sex determination in the process of human identification within the field of forensic dental radiology. The efficacy of cross-validated discriminant analyses indicates a very high level of robust and significant classification based on *k* 25 landmarks (92.5% correct overall, 91.0% of males correctly classified, 94.0% of females correctly classified). These results compare extremely well to earlier research (Table 5) using various methods of metric and statistical shape analysis applied to areas of the mandible such as the gonial region,[7,22] ascending ramus,[7,11,21,23] and overall size and shape.[13,14,16,17,19]

Table 5. Comparison of classification success from the present study compared to other published research. *N* indicates dimensions (2D or 3D) and *K* the number of landmarks utilised (if applicable).

Publication	**Classification type**	**Classification success**	***N***	***k***
Balci *et al.,* 2005[11]	Sex assessment based on morphological scoring of traits of ramus flexure using the method of Loth and Henneberg (1996).	Male: 95.6% Female: 70.6% Overall: 90.6%	NA	NA
Franklin *et al.,* 2007[19]	Sex assessment from the subadult mandible based on GMM analysis of overall shape	Male: 55% Female: 65% Overall: 59%	3	21
Franklin *et al.,* 2007[16]	Sex assessment from adult mandible based on GMM analysis of overall shape	Black male: 85.0% Black female: 90.0% Black overall: 87.5% White male: 88.2% White female: 92.3% White overall: 86.7%	3	38
Franklin *et al.,* 2008[17]	Sex assessment from adult mandible based on linear discriminant functions derived inter-landmark distances from 3D shape capture	*All variables:* Male: 83.3% Female: 84.8% Overall: 84.0% *Ramus only:* Male: 69.2% Female: 81.9% Overall: 75.1%	3	NA
Kemkes-Grottenthaler *et al.,* 2002[7]	Sex assessment based on morphological scoring of traits of ramus flexure and gonial eversion using the method of Loth and Henneberg (1996).	*Ramus flexure:* Male: 66% Female: 32% Overall: 59%. *Gonial eversion:* Males: 75.4% Females: 45.2% Overall: 69.3%	NA	NA
Oettlé *et al.,* 2005[21]	Sex assessment from adult mandible based on GMM analysis of ramus flexure	Male: 67.8% Female: 69.9% Overall: 68.9%	2	11
Oettlé *et al.,* 2009[22]	Sex assessment from adult mandible based on GMM analysis of gonial eversion	Male: 73.9% Female: 71.4% Overall: 72.7%	2	7
Pretorius *et al.,* 2006[23]	Sex assessment from adult mandible based on GMM analysis of ramus flexure (component of integrated study)	Male: 67.8% Female: 69.9% Overall: 68.9%	2	11
Schmittbuhl *et al.,* 2001[13]	Sex assessment from adult mandible based on elliptical Fourier analysis (size effects included in the analysis)	Male: 97.1% Female: 91.7% Overall: 94.4%	2	NA
Schmittbuhl *et al.,* 2002[14]	Sex assessment from adult mandible based on elliptical Fourier analysis (size effects normalised in the analysis)	Male: 84.1% Female: 81.2% Overall: 82.7%	2	NA
Present study	Sex assessment of adult mandible based on GMM analysis of outline of inferior corpus and posterior ascending ramus	Male: 91.0% Female: 94.0% Overall: 92.5%	2	25

Orthopantomogram images allow the objective and reproducible collection of 2D images for the analysis of human variation using geometric morphometric techniques. OPG-derived data was chosen for this analysis because it is both abundant (through clinical treatment) and standardised; many orders of magnitude more data of known age, sex and population group is available from clinical OPG sources than is available from dry bone specimens – as such this is a source of biological data which may be of great future utility for research purposes.

This study confirms that (based on OPG imaging) the dentate mandible exhibits significant sexual dimorphism and that skull assessment of unidentified cadavers cannot leave aside the odontological investigation with the benefit of stored radiological images.

Nevertheless, further assessment on a wider sample of OPGs should be carried out in order to increase the predictive accuracy of this novel methodology. This analysis has demonstrated that the use of OPGs provides a robust standardised method of sex assessment and the importance of performing a complete dental radiological assessment during an autopsy with the aim of human identification of unidentified human remains.[43] Stepwise permutation tests and analyses of regional co-variation indicate functional integration in the structure of the mandible, with a high degree of anatomical modularity between the corpus and ramus suggesting that functional ties between the units may co-vary in influencing sex-based morphological expression. Consequently such units should be studied together (an approach only irregularly applied to date, such as in the application of elliptical Fourier transforms [12-14]) and this may allow for the development of identification criteria based on modular unit shape variables which may be applicable for both whole specimens and fragmented remains depending on the forensic situation.

The success of this proof-of-concept study has encouraged us to expand the remit of the research project, and we will be conducting assessment on a larger sample of OPGs in order to increase the predictive accuracy of sexing and to investigate population-level shape differences. We will expand the biological focus of the analysis and investigate within group (sex and population) as well as pooled sample variation. Furthermore, we will investigate methods of translation of shape variables onto dry bone specimens to aid in sex assessment from skeletonised remains.

CONCLUSION

This preliminary morphometric study confirms that mandible exhibits great sex dimorphism and that radiological OPGs images can be used in the forensic human identification process for sex assessment analysing the geometric and allometry relationship between the corpus and the ascending ramus of the mandible. Further assessment on a wider sample of OPGs should be carried out in order to increase the predictive accuracy of this novel methodology.

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Sex estimation in Indians by digital analysis of the gonial angle on lateral cephalographs

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Mandibular/gonial angle,
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ABSTRACT

Objective: Sex estimation of skeletons is important in forensic reconstructive identification. The mandible is a durable component that is suitable to discriminate the sexes while lateral cephalometry is a standardised radiographic technique accepted as a tool in personal identification. Limited data is available for the mandibular/gonial angle as a parameter for sex assessment using lateral cephalometric radiographs. The aim of this study is to determine the gonial angle's accuracy in sexing Indians using a new digital method and statistical approach.
Method: The sample comprised of 304 digital lateral cephalometric radiographs (155 females and 149 males, age between 18-30 years) of Indian subjects. The mandibular/gonial angle was measured on these radiographs using Adobe Photoshop software using tools available therein. The obtained angles for the sexes were subjected to logistic regression analysis (LRA), which forms a composite of weighted independent variables using a multivariate strategy.
Results: The average angle was 122.7° for females and 121.1°for males. LRA produced an accuracy rate of 56.3% in sex assessment, with females being more accurately identified (61.9%) than males (50.3%).
Conclusion: The study demonstrated significant univariate sexual dimorphism among males and females in this population. However, the sex prediction value of this approach was low and thus may not be useful in sex estimation involved in human identification of Indians.

INTRODUCTION

Sex estimation is an important part of post-mortem profiling in human identification. For this purpose, human skeletal material is of prime importance among which the pelvis and skull are two of the more characteristic parts which can aid in the sex identification of an individual. However, in cases where intact skull is not found, mandible alone may play a vital role in sex prediction as it is the most dimorphic bone of the skull. It is durable, resists any type of mutilation and is commonly preserved in forensic, osteological and anthropological contexts.[1,2] Since the mandible is the last skull bone to complete growth, genetic, hormonal or environment factors influence the sexual variations in the mandible. At the age of 14 years mandibular growth becomes stable in females, while it continues for two more years in males.[3] Several sexual dimorphic criteria have been reported in mandibular bone including mandibular ramus height, bi-gonial width, etc., as well as the gonial angle in a few populations.[4] The mandibular/gonial angle is formed by the ramus line which is the tangent to the posterior border of the mandible, and the mandibular line which is the horizontal portion of the inferior border of the mandible through the gnathion.[5,6] The angle of mandible in infants and children is obtuse i.e., 140° or more. In adults it reduces to about 120°-130° because the ramus becomes almost vertical and in old age, the angle again becomes obtuse.[7] Thus, the angle of mandible is frequently used for age estimation of remains recovered from, for example, mass graves. But the significance of mandibular angle as a tool in forensic odontology for sex estimation has seldom been investigated.[8] A few studies on South Africans, Europeans, Americans and Egyptians exist in which the gonial angle has been shown to be sexually dimorphic and demonstrated good sex discrimination accuracy which was in contrast to an Indian study.[4,5,6] Since, skeletal attributes vary among different populations, a need arises for specific standards of assessment.

Radiographic examinations are commonly used for bony structures which are decomposed, cremated or mutilated.[9] Radiologic cephalometric examination of mandible may provide an easy and accurate method in sex estimation by linear and angular measurements. Various studies have claimed skull radiographs as a reliable method for sex estimation with accuracy up to 80% to 100%. Standard cephalometric radiographs allow identification of race and sex of an individual using simple measurements.[10] Limited information is available for the mandibular/gonial angle as a parameter for sex estimation using lateral cephalometric radiographs. A digital method for age estimation on orthopantomographs was conducted and concluded that digital age estimation can be used as a last resort, especially for geriatric age prediction.[11] The aim of this study is to determine how accurate the gonial angle is in sexing Indians; the measurements will be obtained using a digital methodology previously not used in sex assessment as well as a robust statistical approach.

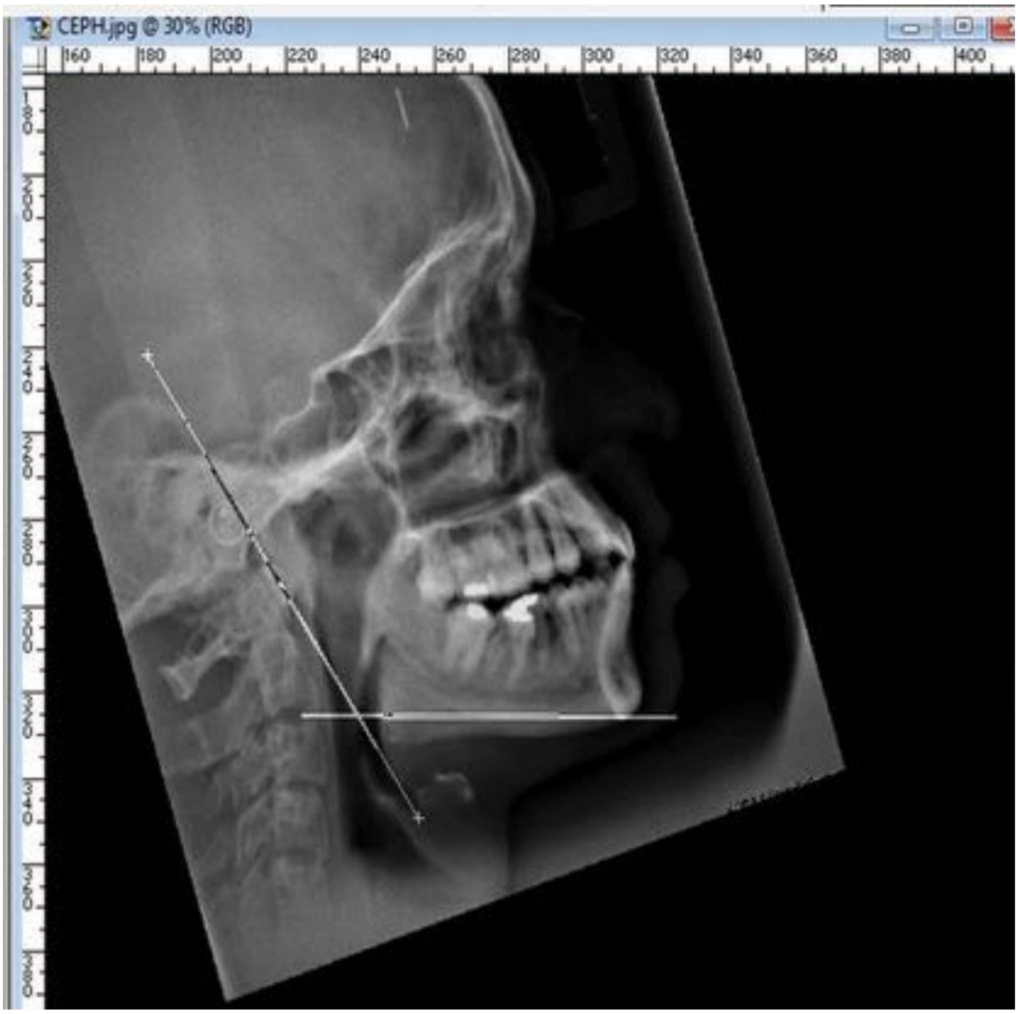
MATERIALS AND METHODS:

After obtaining institutional ethical clearance (Sl. No. 1069 Dated – 25/04/2016), a total of 304 digital lateral cephalometric radiographs (155 females and 149 males) of Indian subjects aged between 18-30 years, which were archived in our department, were used in the study. The radiographs were available in the department as part of a previous study and were taken based on convenience sampling. Individuals with a history of orthodontic treatment or orthognathic surgery, trauma, any systemic disturbance or hereditary facial asymmetry were excluded from the study. The mandibular/gonial angle was measured on these radiographs using Adobe Photoshop CS3 (Adobe Systems Inc., Mountain View, USA) software using a methodology developed by one of the co-authors (ABA), which is briefly described below:

1. Using Ruler Tool (previously called the Measure Tool) in Photoshop a tangential line is marked along the lower border of the body of the mandible.
2. Next go to Image>Rotate Canvas>Arbitrary and click on the latter.
3. The rotation may be done clock-wise (CW) or counter clock-wise (CCW), as appropriate (Photoshop gives its suggestion) which orientates the mandible's lower border horizontally.
4. Activate Photoshop's inbuilt rulers (Ctrl+R in Windows computers; Cmd+R in Macintosh systems); click the cursor within the horizontal ruler at the top of the image and drag to position a line (called 'guide') onto the lower border of the mandible.
5. Next use the Ruler Tool (previously called the Measure Tool) to mark a tangential line along the posterior border of the ramus.
6. The angle 'A' may be noted in the Options bar. Angle between the horizontal line on the lower border of mandible and the tangential line along the posterior border of the ramus gives the Mandibular angle (Figure 1). If the angle is negative, the value must be subtracted from 180 to derive the true gonial angle.

The obtained angle for all subjects was separated for males and females, and descriptive statistics were generated. The sexual dimorphism of the gonial angle among the males and females was assessed using student T-test. The inter- and intra-observer variability were assessed on 50 randomly selected lateral cephalometric radiographs and the readings were subjected to paired t-test. Further, the measurements were subjected to logistic regression analysis (LRA) in SPSS software programme. Bivariate LRA allows predicting group membership (in this case, sex) and forms a composite of weighted independent variables using a multivariate strategy; it also provides a probability of the predicted sex. The level of significance was set at 5% and confidence interval at 95%.

Figure 1: Horizontal line onto the lower border of mandible and tangential line along the posterior border of ramus .



RESULTS

The results demonstrated a mean gonial angle of 122.7° for females and 121.1° for males which were statistically significant i.e. females had a larger gonial angle as compared to the males (Table 1). The results of the paired t-test showed no significant difference between repeat measurements by the same or by different observers (Table 2). The obtained angle for the sexes were subjected to logistic regression analysis (LRA), and an equation was obtained and sex classification accuracy was evaluated (Tables 3 and 4). The results of the logistic regression analysis are depicted in Tables 3 and 4. These include the gonial angle coefficient and the constant for the model in Table 3, which produced an overall accuracy rate of 56.3% in sex assessment, with females being more accurately identified (61.9%) than males (50.3%) (Table 4).

Groups	Mean	Std. Deviation	t value	P value
Male	121.1°	6.4	2.122	0.035
Female	122.7°	6.3		

Table 1 – Descriptive statistics and t-value for the gonial angle in males and females

Test		Mean (Degree)	Difference	t-value	P value
Intraobserver	Evaluation 1	120.7	0.1460	0.280	0.78
	Evaluation 2	120.6			
Interobserver	Observer 1	120.7	-0.1620	-0.312	0.76
	Observer 2	120.9			

Table 2 – Paired sample t-test to assess potential observer variation

	B	S.E.	Wald statistic	df	Sig.	Exp (B)
LRA Coefficient	-0.038	0.018	4.305	1	0.038	0.963
LRA Constant	4.606	2.242	4.222	1	0.040	100.114

Table 3 - LRA equation for the gonial angle

Table 4 – Sex estimation efficacy of gonial angle using logistic regression

Parameter	Males		Females		
Gonial angle	Total No.	Correctly identified	Total No.	Correctly identified	Total Correctly Identified
	149	75 (50.3%)	155	96 (61.9%)	171/304 (56.3 %)

DISCUSSION

The gonial angle is a representation of the form of the mandible. This angle has an important role in predicting growth and it also has specific effects initially on growth, profile changes and the condition of the anterior teeth of the lower jaw.[12] Various studies have been conducted on the gonial angle for age and sex but with contradictory and variable results. There are either environmental or genetic factors controlling the mandibular angle configuration within each population. In previous studies conducted on African, including modern Egyptian populations, the gonial angle of the two sexes was found to be greater in males than females.[6] Usually the mean angle is 3-5 degrees greater in males. The reason for this sex difference may be explained by the fact that men have greater masticatory force than women and generally males have larger mandibles.[13,14] In the present study, the average gonial angle on digital lateral cephalographs was found to be 122.7° for females and 121.1° for males. In this particular population, low sex estimation accuracy (56.3%) was noted which is in contrast to studies done on Egyptians, European- and African-Americans, Anatolians, Australian Aborigines and South Africans but was in agreement with another Indian study and a Lebanese sample.[7] This emphasises the population-specific differences present and the need for further evaluation in the context of genetic and environmental influences.

Sex estimation efficacy of the gonial angle was evaluated using stepwise logistic regression analysis, which demonstrated an accuracy of 56.3%, with females being more accurately identified (61.9%) than males (50.3%). This is in contrast to a study on a European population where discriminant function analysis (another multivariate statistical analysis) defined the gonial angle as one of the best predictors of sex with an accuracy ranging from 83% to 84%.[6] A similar study on European-Americans demonstrated an accuracy rate of 84%-86%. This difference between Indian and European groups may be attributed to geographic/population variation in the gonial angle.

Various studies have measured the gonial angle on dry mandible with use of mandibulometer and on x-ray images with goniometer.[15,16] X-ray imaging is certainly a useful method of carrying out proper comparisons and identifications. One of the most widespread anthropological methods for identification purposes is based on x-ray images of mandible. The determination of the gonial angle has been studied using panoramic radiography and the results have shown that the gonial angle measurement on the panoramic radiographs is an accurate and repeatable measure. A study done by Maryam et al on agreement between panoramic and lateral cephalometric radiographs for measuring the mean gonial angle was found to be insignificant for the sex and age groups.[12] Larheim and Svanaes also stated that both panoramic radiographs and lateral cephalograms were accurate in determining the gonial angle and there was no significant difference between the right and left sides in panoramic radiography.[16] However, most studies believe that lateral cephalograms are a more standardised form of radiograph for skull and mandible measurements. Interestingly, limited data is available for the mandibular/gonial angle as a parameter for sex assessment using lateral cephalometric radiographs.

It is for these reasons that, in the present study, digital lateral cephalometric radiographs were used. The gonial angle was measured in Adobe Photoshop, which provided a simple and an accurate tool for evaluation of the gonial angle on lateral cephalograms. The advantages of this method include the parallelism ensured throughout the technique which simulates as much as possible the measurements that are done on a dry mandible.

Despite several advantages in this method, the accuracy of sex estimation obtained was low, so this approach is not recommended for post-mortem profiling in human identification in Indians. However, it may be useful when no other parameter is available for this purpose.

CONCLUSION

This study demonstrated statistically significant univariate sexual dimorphism in the mandibular angle in a young adult Indian population and a low (males) to moderate (females) sex estimation accuracy using a new digital methodology on lateral cephalograms which is easy to apply and user friendly.

However, since the prediction value of the gonial angle demonstrated a low accuracy rate, it is not recommended for human identification among Indian population.

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