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# EDITORIAL

This has been a year of change for the Journal, and I should like to thank Jules Kieser (and Glynny Kieser) for their editorial work over the last 2 years. This year has been a steep learning curve for me and my assistant editor Charles Maybury, but we are beginning to see the light at the end of the tunnel!

I should like to welcome, on behalf of the JFOS, two new members to our Editorial Board: Irena Dawidson (Sweden) and Helen Liversidge (United Kingdom). I am sure that they will both become energetic and proactive members of the Board. Several members have retired from the Board this year, and Cyril Thomas, Lis Andersen Torpet and Hakan Mornstad are thanked for their contributions to the JFOS over the years.

Earlier this year the list of reviewers was updated and I am delighted that there was such a positive response from them: their hard work and considerable time commitment is much appreciated – long may it continue!

I hope that this edition contains something of interest to all the readers: not only those with experience in the forensic dental field, but also inexperienced colleagues and those from different forensic disciplines. The range of papers in this issue offer basic, straightforward information to encourage those starting on their forensic careers, to more complex and statistical issues. If you have been thinking about “putting pen to paper” please put this into action: even if your manuscript is not accepted, there is much to learn from the reviewer comments to help you in the future. It is extremely important that potential authors continue to submit papers so that international research, developments, ideas and case reports can be shared, increasing our knowledge base.

Errors and controversial cases over the years have brought the forensic sciences under close scrutiny and criticism; never before has it been so important to promote quality research, international co-operation and standardisation. We have much to learn.

Kind regards for the festive season.

Judy Hinchliffe (BDS, Dip F Od, Hon FFFLM)
Editor

# SURVIVAL OF BATCH NUMBERS WITHIN DENTAL IMPLANTS FOLLOWING INCINERATION AS AN AID TO IDENTIFICATION

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## ABSTRACT

Dental implants have become a popular choice of treatment in replacing individual lost teeth or entire dentitions. The physical properties of high corrosion resistance, high structural strength and high melting point, suggest the retention of intact implants following most physical assaults. As the implants are machine made, they lack the individualisation required for their use as identifiers of the deceased, however the Straumann™ Company (Waldenburg, Switzerland) has recently released information that within the chamber of their implants they have laser etched batch numbers. The number of implants with the same batch number varies from 24 to 2400. The purpose of this study was to ascertain if the batch number was still identifiable following intense heat exposure in a furnace. A Straumann™ Standard Plus 3.3 x 8 mm implant, with no healing cap nor abutment attached was incinerated to 1125 degrees Celsius. Another Straumann™ Standard Plus 3.3 x 8 mm implant was also incinerated in the same way as the first implant but with an abutment attached. The results indicated that the first implant had totally oxidised within the internal chamber whilst the second implant following the removal of the abutment revealed an intact identifiable batch number. If the companies constructing implants were to place individual serial numbers rather than batch numbers on these implants then the potential exists for a new approach to be established for the identification of the deceased.

**(J Forensic Odontostomatol 2010;28:1:1-4)**

**Keywords**
Human identification, dental implants, incineration, batch numbers, serial numbers

**Running Title**
Batch numbers within implants

## INTRODUCTION

A dental implant is a prosthetic device that is inserted into the upper or lower jawbone, onto which an artificial tooth, crown or bridge can be anchored. Dental implants are typically constructed from titanium.[1] More recently some manufacturers are constructing their implants from zirconia[2,3], or a combination of titanium and zirconia[4] The placement of titanium implants has become widespread throughout the world with over 460 different implant types available to dentists. In some countries the growth of implants placed within patients is greater than 1% per year.[5] The likelihood of implants present in the deceased in the future would also increase at this rate within those countries.

Implants lack the individuality of hand crafted restorations as they are mass produced. However, since 2010 Straumann™ has been laser etching batch numbers within the chamber of their implants. The number of implants with the same batch number varies between 24 to 2400. (*Per com.* Schuler M, Head Clinical and Scientific Affairs, Straumann™ company). Although this number is still quite high it reduces the frequency from many thousands in some cases.

The Victorian bushfires of 2009 highlighted the fragility of and lack of dental postmortem remains. Postmortem radiographic images of intact dental implants surrounded by lost dental remains were noted. Dental implants made from titanium have a melting point greater than 1650ºC[6] and those made from zirconia have a melting point greater than 1850ºC[7]. This physical property of extremely high melting point[8,9] could potentially assist in the identification of victims where there is lack of other scientific evidence such as DNA or fingerprints[10] and loss of the fragile dental remains.

In the cases of extreme incineration of victims who have been treated with implants it is important to ascertain if the implant batch or serial numbers within the implant chambers survive the incineration process sufficiently that their numbers can be identified. The authors decided to test the

Straumann™ dental implants which contain batch numbers following incineration in a temperature controlled kiln. The hypothesis was that the batch numbers of pre- and post-incineration implants could be reliably compared.

## MATERIALS AND METHODS

The Straumann™ Company kindly donated implants for this study. The implants selected from the donations were Straumann™ Standard Plus 3.3 x 8 mm. The composition of the implants was commercially pure titanium. The same type of implant was used in a previous study and its oxide layer determined by elemental analysis to contain titanium, oxygen with only trace amounts of other elements.[11] One implant had neither abutment nor healing screw attached, whilst another implant had an abutment finger tight screwed onto it. Both implant batch numbers were imaged using a WILD Heerbrugg™ (Leica Microsystems, Wetzler, Germany) microscope attached with a digital camera (Nikon Coolpix 5900, Tokyo, Japan).

The implants were placed in an INFI-TROL™ (K.H. Huppert, Chicago, USA) kiln designed to heat porcelain restorations. The temperature within the INFI-TROL™ kiln was monitored with a digital thermometer Model N19 - Q1437 (Dick Smith, Chullora, Australia), with a temperature range of -200 to 1,370°C (±0.5%) using K-Type thermocouples.

The implants were heated to 1125 ºC and left at this temperature for five minutes. Photographs of the implants within the kiln were taken at 100 ºC intervals commencing at 600 ºC. At the conclusion of the experiment, the kiln was switched off and the door opened to allow the implants to cool off slowly. At room temperature the implants were again photographed before removal. They were then examined using light microscopy and the inner chambers of the implant bodies digitally imaged.

## RESULTS

The batch number is clearly visible within the implants before firing as shown in Fig. 1. Following firing of the first implant without the abutment, it can be seen in Fig. 2. that the number is totally obscured by the oxidation layer that formed. In Fig. 3, the implant which had the abutment screwed on and subsequently removed shows the number still visible, although not as clearly as in Fig. 1. There was a slight change to a straw colour in Fig. 3. as well as friction markings near the first thread.

## DISCUSSION

The results indicated that there was clearly a marked difference to whether the batch number could be observed between the uncovered implant and the abutment attached. As both implants were heated under the same conditions, it is assumed that the tightening of the abutment precluded sufficient oxygen from entering the chamber to form a thick oxide layer. The straw colour (Fig. 3.) indicated that there was a small amount of oxidation and probably this phenomenon was due to the amount of oxygen retained within the chamber following sealing with the abutment. The friction markings indicated that the contact area is away from the area where the batch number is etched. This allays the concern that the number is not damaged by the screwing and unscrewing of the abutment. This is a small initial study which needs to be repeated with many more implants to substantiate the current findings.

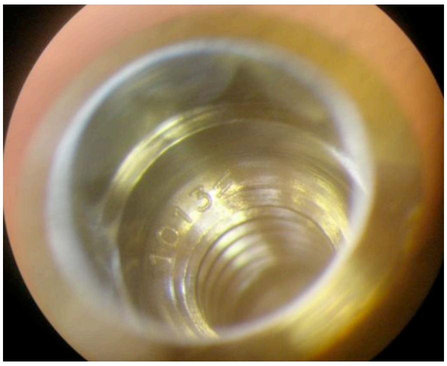

***Fig. 1**. Batch number clearly visible within implant before incineration.*

***Fig. 2**. Implant without abutment following incineration. Number not visible.*

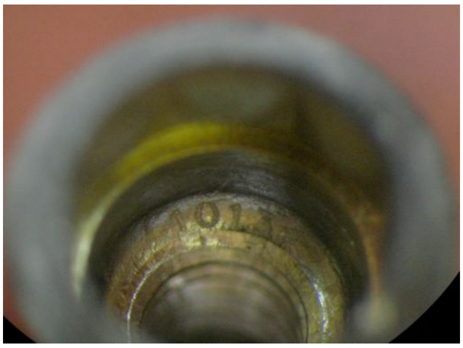

***Fig. 3**. Implant with abutment after incineration and following removal of abutment. Number visible.*

The oxidation layer formed following firing of the second implant created a sufficient bond between the abutment and the implant which necessitated a wrench to separate the two even though it was initially finger tight. At this temperature metal fusion had not occurred, however at higher temperatures fusion might develop especially if the implant was exposed directly to a flame. Difficult mechanical sectioning of the implant might be required to view the batch number in such a case.

It was suggested that perhaps the oxidation crust could be easily removed from the first implant to reveal the number beneath the first implant, however attempts to do so proved fruitless. Perhaps a gentle oxidation removal liquid might be of benefit. Where there is an oxidation layer the survival of the identifying batch number will depend on the depth of the etched number and the thickness of the oxidation layer. It would be unlikely that batch number would be preserved within an oxidised layer but this would need to be confirmed by more sophisticated image analysis techniques.

During treatment with dental implants the inner chambers of the implants would be sealed either with a healing cap initially or some form of abutment restoration. Depending upon the skill of the surgeon, it is assumed that the healing caps should be tightly screwed down so that the healing cap would act similarly to an abutment and hence produce the same results although further testing with healing caps is required.

Currently there are several internet search websites to assist in the recognition of dental implant types.[11-13] Where there is lack of circumstantial evidence indicating who the victim is, the identification of the implant type could assist the identification team.[14] This is especially relevant where the implant type is rare as there could be only one or two surgeons placing these types of implants in that jurisdiction. The number of implants, the widths and lengths of them together with the information from the company agent suppliers of those specific implants could narrow the search to find the dental surgeon which inserted them. Linking the batch numbers to the notes of the surgeons would increase the weight of evidence linking the identification of that victim. The ideal would be that the companies producing the implants etch an individual serial number within each implant.

## CONCLUSIONS

Extreme heat will destroy teeth and conventional dental restorative materials, as well as other scientific identifiers in victims. Due to their physical properties, implants will resist thermal insult although the lack of uniqueness of mass produced objects limits the use of implants in identification. The addition of batch numbers within implants and the ability of these implants to retain their numbers following high temperature assault would increase the weight of evidence.

This small study indicated that batch numbers within Straumann™ implants survived heating to 1125 ºC where an abutment was attached. If the companies could be convinced to insert serial numbers on each implant this could help establish a new approach to identify deceased persons.

## *ACKNOWLEDGEMENTS*

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# MATCHING SIMULATED ANTEMORTEM AND POSTMORTEM DENTAL RADIOGRAPHS FROM HUMAN SKULLS BY DENTAL STUDENTS AND EXPERTS: TESTING SKILLS FOR PATTERN RECOGNITION

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## ABSTRACT

The aim of this study was to evaluate the ability of undergraduate dental students to match simulated ante- and post-mortem radiographs in human skulls with “experts” as controls for the 1)number of post-mortem images needed for a match, 2)accuracy of the matches, and 3)time spent for a match. A film bitewing was recorded in each side of 51 dentate dry human skulls (a.m.-images) and digital images of the teeth were recorded using a sensor (p.m.-images). 102 correctly matching and 102 non-matching image pairs were constructed. Ten students and three experts scored the image pairs as: “certain match”, ”certain non-match”, or ”uncertain”. None of the experts but half of the students made false positive scores. Half of the students performed just as accurately as the experts. All students (except one who made 8 false positive results) asked for more p.m.-images than did the experts before deciding on a match, however, all students, but one, also spent less time per image pair than did the experts before deciding on a match (P<0.001).

This simulated test sample may identify dental students and dentists with abilities for pattern recognition and thus help in the decision on who might be included as part of a forensic dental team when extra help is needed.

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**Keywords:** forensic sciences; dental identification; radiography, digital

**Running title:** Matching simulated antemortem and postmortem radiographs

## INTRODUCTION

Dental records, and in particular radiographs, are one of the most reliable methods of victim identification after disasters. After the Asian tsunami (2004), the identification of missing persons with dental records was significantly higher than among those without.[1-3] Teeth and dental restorations are to some extent resistant to fire,[4] and identity was established through dental evidence in 92% of burn victims (292 cases)[5] and in 39%[6] and 88% respectively, of flight crash victims.[7] Radiographs were available in 71% of fire victims in Scandinavia sampled over a 10-year period;[5] these were mostly bitewings, which are the most frequent radiographs taken in general dental practice.[8]

Radiographic identification is based on the recognition of characteristic patterns when comparing the antemortem (AM) and postmortem (PM) images, and dental work facilitates the matching.[9] Improvements in dental health status leading to more individuals without dental restorations may interfere with the discrimination potential[5] when merely anatomic features such as the shape of crowns, pulp chambers and roots, the pattern of alveolar bone trabeculae and crest of the alveolar bone can be used.

Matching of the AM and PM radiographs is undertaken by forensic odontologists following a disaster, and a fundamental requirement is that they make few matching errors. A false positive match may be crucial whilst a false negative match may be less critical since alternative methods may subsequently contribute to the correct match. Specialist training may secure a low number of matching errors. Previous studies have shown that a forensic specialist made fewer false positive matches than a general dentist and a dental student.[10,11] One report states that forensic odontology cannot be carried out by dentists without the proper training, but in disasters of a large scale where there are thousands of fatalities, non-specialists also participated in the identification process.[12] The ability for pattern recognition in volunteers participating in victim identification may be tested in a simulated situation before they take part in true forensic work.

## AIM

The aim of this study was to evaluate the ability of dental students to match simulated antemortem and postmortem dental radiographs from human skulls with “experts” as controls, to assess whether inexperienced volunteers possess the same ability for pattern recognition as experts.
Factors for consideration:
1) Number of post-mortem images needed for a match
2) Accuracy of the matches
3) Time spent for a match

## MATERIALS AND METHODS

Fifty-one consecutively numbered, dentate dry human skulls were selected for the study. Twenty-five percent of the skulls were from young individuals judged from the minimal tooth wear and developing third molars, and 75% were from older individuals with moderate to extensive tooth wear. None of the teeth had restorations.

### Recording of conventional film (antemortem images)

Four bitewings (BW) (an anterior and a posterior exposure in both the left and the right side) were exposed using Kodak Insight (Eastman Kodak Company, Rochester, N.Y., USA) dental film size 2 (31x41 mm) with one film in each paper pack and placed in a film holder (Kwik Bite, Hawe Neos Dental, Bioggio, Schweiz). After insertion of the film holder, the mandible was fixed in occlusion on the holder (Fig. 1). Exposure settings were standardized (Gendex 1000 dental unit, 15 mA, 65 kV, 32 cm f-f distance, rectangular tube collimation, 12 mm acrylic soft tissue simulation). Exposure time varied between 0.26 and 0.34 seconds to obtain a subjectively judged adequate density and contrast in the image. Film processing was semi-automated in a Periomat Plus (Zenith Dental, Agerskov, DK) processing machine. If approximal surfaces overlapped into the dentine, the image was retaken.

***Fig. 1.*** *Antemortem film exposure in a skull.*

### Recording of digital images (postmortem images)

Four of the 51 skulls had loose teeth (premolars or molars), and these teeth were removed (simulated tooth extraction) to give variation to the sample. Four BWs were recorded as for the antemortem images using a Schick CMOS sensor size 2 (Schick Technologies Inc., N.Y., USA) and a sensor holder (CDR Universal holder, Schick Technologies Inc., N.Y., USA). Further, periapical images were recorded in the premolar and molar regions in both jaws. During these recordings the sensor was supported by wax when positioned behind the teeth and jaw bone. Exposure settings were standardized as for film, and exposure time was 0.18 to 0.22 seconds. The digital images were saved in their original software (Schick CDR, DICOM for Windows, version 3.5.0.145) and thereafter exported to .tiff (tagged image file format).

### Scoring antemortem and postmortem image pairs

For the AM image, the BW film that displayed the highest number of teeth (either the anterior or the posterior image from each side of the jaw) was chosen. This film was mounted in a frame and numbered with the skull number. The corresponding post-mortem digital BW from the same side of the same skull was selected. These two images were defined as a match. In this way 102 (51 skulls x 2 sides) correctly matching image pairs were produced. Thereafter, 102 non-matching image pairs were produced by pairing a film and a digital image from the same side of a jaw haphazardly, though not mixing a “young” and an “older” skull. In total, the study sample thus consisted of 204 image pairs, 50% matching and 50% non-matching.

Thirteen participants took part in the study: ten fourth-year pregraduate dental students (Stud01 - Stud 10) who after an invitation to the whole group volunteered to participate, and three “experts” served as controls: one radiologist (Exp02) and two forensic specialists (Exp01 and Exp03) who had worked for more than a decade with forensic sciences (among other tasks, both participated in the victim identification after the tsunami in Thailand 2004-2005).[13] A dedicated program (UniScore, Erik Gotfredsen, School of Dentistry, Aarhus University, Aarhus, Denmark) was developed to display the digital images and score the image pairs. The digital images were blinded with regard to skull number and displayed on a laptop computer (Fujitsu Siemens, 17” monitor, resolution 1024x768 pixels, 32 bit color depth) in 1:1. When displaying a digital image the program stated which film BW was to be paired with the digital image. The film was viewed with a viewer (magnification 1.5) on a light box. The observer scored the image pair as being a: "certain match," "certain non-match," or "uncertain" (i.e. cannot decide whether or not this is a match).

It was decided by each individual participant when and for how long they would work at any given time. Each participant logged in using an individual code, and the program kept track of the image pairs that had been scored by that participant. The remaining images were mixed and shown in a random sequence for every log in. When all participants had decided on the 204 image pairs (1st session), those image pairs that had been scored as "uncertain" were re-evaluated in a 2nd session. In this 2nd session, the same digital post-mortem BW plus the other digital BW that had been taken from the same side of the jaw (if the BW shown in the first session was the anterior, then also the posterior was shown and *vice versa*) were displayed and used for the comparison with the film BW. Again, "uncertain" scores were re-evaluated in a 3rd session, in which all post-mortem images, including periapicals and BWs, were displayed and used in the scoring.

Time taken for scoring an image pair was recorded without the participants' knowledge.

### Data treatment

The number of image pairs scored as a "certain match," "certain non-match" and "uncertain" were counted for each participant in the three scoring sessions, and the following statistics were calculated: True positive (TP) = score "certain match", which was correct; False positive (FP) = score "certain match," which was not correct (it was not a match); True negative (TN) = score "certain non-match," which was correct; False negative (FN) = score "certain non-match," which was not correct (it was a match).

Differences between the experts and students in the number of images scored as "uncertain" *vs.* "certain" ("certain match" + "certain non-match") were analyzed by chi-squared tests, along with the differences between the participants in the number of correct (TP+TN) *vs.* incorrect (FP+FN) scores.

Time consumption for matching an image pair ranged from 11 seconds to five minutes; 99% of the scores had taken between 11 and 195 seconds. Since the participants did not know that time was recorded, they may have left the program or have been disturbed without logging out, and the histogram distribution of seconds showed interval breaks after 99%. Therefore, the time for the last 1%, equaling 48 scores in total, was set to 195 seconds, which was thus defined as the longest time used for scoring an image pair.

## RESULTS

### Number of postmortem images needed to decide on a match

No participant was able to decide if all image pairs were a "certain match" or a "certain non-match" in the 1st session, and a large number of image pairs were scored as "uncertain" (Table 1). Two of the experts scored the lowest number of "uncertain" matches (11%). One student (Stud07) scored fewer "uncertain" cases than did the third expert, but this student made 16 false scores. The remaining nine students scored significantly more "uncertain" cases than the experts ($P<0.05$). In the 2nd session all students still scored a number of cases "uncertain" (range 2-15 cases) while only one of the experts scored "uncertain" (in four cases). In the 3rd session, where periapical images were also available, all observers had decided on either a "certain match" or a "certain non-match."

### Accuracy of matches

In Table 1 the number of correct matches, TP and TN, and the number of incorrect matches, FP and FN, can be seen for the three sessions. None of the experts scored FP while one student made one FP, three students made two FP and one student made 13 FP scores. All observers (except Stud09) made FN scores. Since the total number of incorrect scores was rather small (Table 1), only the difference between the experts and Stud07 was statistically significant ($P<0.02$).

### Time consumption

In Table 2 the mean time use per image pair and the total time consumption for each participant can be seen. All students except Stud02 spent less time per image pair than did the three experts ($P<0.001$) (Fig. 2). Stud02, Stud03 and Stud10 spent significantly more time than the remaining students ($P<0.001$), and Stud02 more time than the two others. The remaining students did not differ significantly.

## DISCUSSION

Victim identification is frequently based on radiography of the teeth when ante-mortem radiographs are available. The post-mortem exposures may be obtained either by film or by digital receptors. The conventional film demands special facilities, such as developing machines with chemicals that are dependent on clean water, constant temperature, electricity etc. Waiting for the film to be developed may also be an inconvenience, particularly if many retakes are needed. The problems with film development have been described after the air flight crash in 1980, where film development was performed in hotel rooms.[6] Digital dental radiography demands electricity, a digital receptor and a pc, and since image capture takes but a few seconds, the number of images and retakes are not important delaying factors. In the identification of victims after the tsunami in Thailand in 2004, Thai forensic odontologists used a digital sensor to record the post-mortem images (Alan Richards, personal communication). Some ante-mortem radiographs of the missing persons were transmitted in digital form to Thailand using secure wireless transmission protocols,[14] but most ante-mortem images were film-based. The present experiment imitated the situation where ante-mortem images are film-based and post-mortem images are recorded digitally. The Schick CMOS sensor is one of the most commonly sold dental sensors, and was therefore used in the present study for the post-mortem images. In the future most intraoral radiographs will be digital as dentists worldwide are changing to digital receptors.[15,16] It may be that the larger digital image as displayed on a monitor even facilitates the pattern recognition in ante- and post-mortem radiographs compared to a 3x4 cm dental film. This was not part of the present study, but may be interesting for a future investigation.

In our study there were no anatomical differences between the AM and PM images except in cases, where a tooth had been “extracted”. It may therefore be anticipated that it would be an easy task to match the pairs of radiographs correctly. However, there were no dental restorations in the skulls, and tooth wear was quite alike in many of them. While we appreciate that this simulated situation does not resemble true forensic victim identification, this test sample seemed to be useful to distinguish between participants with strong and weaker abilities for pattern recognition.

The number of images needed before an observer could decide on a match or non-match was larger for the students (except for one) than for the experts in our study. Using film, this would result in a slower identification process and additional costs while using a digital receptor, capturing additional images is extremely fast[15,17] and with little extra costs. The difficulties in positioning the sensor for a bitewing examination in a patient[18] may be less severe in the deceased (or when fragmentation occurs) where the jaws can be fixed in occlusion by various methods.

The majority of the students spent less time for scoring an image pair since they apparently sooner scored “uncertain” when in doubt. This may strategically be an efficient approach, particularly when working with a digital receptor where many exposures can be performed in a short period of time, and radiation dose need not be considered.

When visually matching dental radiographs of teeth with no restorations or other dental work, a correct match depends on the observer’s ability for pattern recognition, that is, to analyze and compare the anatomical pattern of crowns, roots, pulp chambers, interproximal marginal bone, etc. Knowledge of distortion in the image due to differences in projection geometry between the ante- and post-mortem images also plays a role. It has been stated that forensic odontology should not be carried out by general dentists without specialist training.[19] Inexperience of the operator is suggested to have lead to errors in the comparative dental analyses that followed the Asian tsunami of 2004.[20] The pregraduate dental students in our study had all passed the same exams including a course in radiology. However, there were large differences between them with respect to the number of false scores made during matching. A false positive match may be unfortunate since in the real situation this means that the deceased is identified and returned to the bereaved family, while a false negative match means that the body is still under investigation, and other methods may aid this. The experts made no FP scores. Half of the students made FP scores, and in particular one student made a large number. The other half of the students made no FP, and made no more FN than the experts. These students seemingly were stronger in pattern recognition than the other students. It may seem odd that the periapical images helped the observers to decide on a match when they were not able to do so when the two postmortem bitewings were compared with the antemortem bitewing. It may be that the projection geometry in the periapicals in some instances was more equal to that of the ante-mortem BW, and the radiographic patterns were therefore more alike. The fraction of incorrect matches was in accordance with previous studies on simulated antemortem and postmortem matching of bitewing radiographs. In another study a forensic specialist, a dentist, and a student matched film bitewings taken several years apart in patients (adults and children) with and without fillings. Also in that study the forensic specialist performed more accurately (2 FP matches) than the other observers (8 FP matches).[11] The number of FN recordings in that study depended on the time interval between the BW to be matched, the longer time between them, the more FN scores.[10] In children it should be more difficult to recognize the radiographic pattern, which changes with growth, the longer the time period between the radiographs, and therefore more FN would be expected.

## CONCLUSIONS

In this study of matching simulated antemortem and postmortem radiographs, dental students needed more post-mortem images before deciding on a match than did experts. However, the students spent less time in scoring an image pair than did the experts. Half of the students were less accurate than the experts; in particular they scored false positives, which the experts did not. Half of the students performed just as accurately as the experts. This limited simulated test sample may identify dental students and dentists with abilities for pattern recognition and thus help in the decision as for who might be included as part of a forensic dental team when extra help is needed.

## *ACKNOWLEDGEMENT*

Pregraduate dental students Eva Swenson and Anne Voigt Lauridsen are thanked for recording the radiographs and acting as participants.

***Table 1:*** *Outcome for the 13 participants matching 204 image pairs during the three sessions*

| **1st session** | **True positive** | **False positive** | **True negative** | **False negative** | **"Uncertain"** | **Total** |
|---|---|---|---|---|---|---|
| Exp01 | 64 | 0 | 92 | 4 | 44 | 204 |
| Exp02 | 86 | 0 | 93 | 2 | 23 | 204 |
| Exp03 | 89 | 0 | 89 | 3 | 23 | 204 |
| Stud01 | 80 | 0 | 74 | 3 | 47 | 204 |
| Stud02 | 49 | 0 | 89 | 1 | 65 | 204 |
| Stud03 | 47 | 0 | 86 | 0 | 71 | 204 |
| Stud04 | 58 | 0 | 74 | 2 | 70 | 204 |
| Stud05 | 71 | *1* | 66 | 2 | 64 | 204 |
| Stud06 | 67 | 0 | 76 | 5 | 56 | 204 |
| Stud07 | 87 | *8* | 72 | 3 | 34 | 204 |
| Stud08 | 54 | 0 | 62 | 2 | 86 | 204 |
| Stud09 | 69 | 0 | 71 | 0 | 64 | 204 |
| Stud10 | 52 | *1* | 69 | 1 | 81 | 204 |
| **2nd session** | | | | | | |
| Exp01 | 29 | 0 | 10 | 1 | 4 | 44 |
| Exp02 | 13 | 0 | 10 | 0 | 0 | 23 |
| Exp03 | 8 | 0 | 14 | 1 | 0 | 23 |
| Stud01 | 13 | 0 | 24 | 0 | 10 | 47 |
| Stud02 | 38 | 0 | 11 | 2 | 14 | 65 |
| Stud03 | 49 | 0 | 16 | 3 | 3 | 71 |
| Stud04 | 36 | 0 | 29 | 0 | 5 | 70 |
| Stud05 | 19 | *1* | 31 | 2 | 11 | 64 |
| Stud06 | 26 | 0 | 24 | 0 | 6 | 56 |
| Stud07 | 11 | *5* | 16 | 0 | 2 | 34 |
| Stud08 | 42 | *2* | 38 | 1 | 3 | 86 |
| Stud09 | 30 | 0 | 26 | 0 | 8 | 64 |
| Stud10 | 42 | 0 | 24 | 0 | 15 | 81 |
| **3rd session** | | | | | | |
| Exp01 | 3 | 0 | 1 | 0 | 0 | 4 |
| Stud01 | 5 | 0 | 5 | 0 | 0 | 10 |
| Stud02 | 10 | 0 | 3 | 1 | 0 | 14 |
| Stud03 | 2 | 0 | 1 | 0 | 0 | 3 |
| Stud04 | 5 | 0 | 0 | 0 | 0 | 5 |
| Stud05 | 6 | 0 | 4 | 1 | 0 | 11 |
| Stud06 | 3 | 0 | 3 | 0 | 0 | 6 |
| Stud07 | 0 | 0 | 2 | 0 | 0 | 2 |
| Stud08 | 2 | 0 | 1 | 0 | 0 | 3 |
| Stud09 | 2 | *2* | 4 | 0 | 0 | 8 |
| Stud10 | 6 | 0 | 9 | 0 | 0 | 15 |

***Table 2:*** *Mean, minimum and maximum time (seconds) to assess one image pair by each participant and total time spent for matching all 204 image pairs (minutes)*

| | **Mean sec.** | **Min. sec.** | **Max. sec.** | **Total min.** |
|---|---|---|---|---|
| Exp01 | 79 | 24 | 195 | 331 |
| Exp02 | 62 | 18 | 195 | 235 |
| Exp03 | 66 | 17 | 195 | 248 |
| Stud01 | 41 | 14 | 195 | 176 |
| Stud02 | 67 | 16 | 195 | 315 |
| Stud03 | 52 | 15 | 195 | 240 |
| Stud04 | 40 | 12 | 195 | 185 |
| Stud05 | 35 | 11 | 195 | 163 |
| Stud06 | 38 | 11 | 195 | 170 |
| Stud07 | 31 | 11 | 176 | 125 |
| Stud08 | 38 | 13 | 133 | 185 |
| Stud09 | 41 | 16 | 195 | 188 |
| Stud10 | 47 | 14 | 195 | 233 |

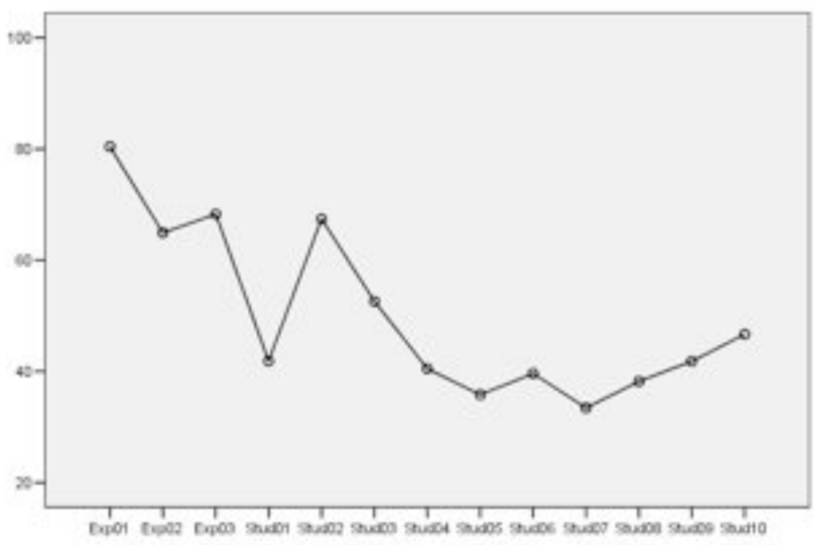

***Fig. 2.*** *Average time spent (seconds) by each participant in scoring an image pair.*

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# AGE ESTIMATION FROM DENTAL CEMENTUM INCREMENTAL LINES AND PERIODONTAL DISEASE

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## ABSTRACT

Age estimation by counting incremental lines in cementum added to the average age of tooth eruption is considered an accurate method by some authors, while others reject it stating weak correlation between estimated and actual age. The aim of this study was to evaluate this technique and check the influence of periodontal disease on age estimates by analyzing both the number of cementum lines and the correlation between cementum thickness and actual age on freshly extracted teeth. Thirty one undecalcified ground cross sections of approximately 30 µm, from 25 teeth were prepared, observed, photographed and measured. Images were enhanced by software and counts were made by one observer, and the results compared with two control-observers. There was moderate correlation ((r)=0.58) for the entire sample, with mean error of 9.7 years. For teeth with periodontal pathologies, correlation was 0.03 with a mean error of 22.6 years. For teeth without periodontal pathologies, correlation was 0.74 with mean error of 1.6 years. There was correlation of 0.69 between cementum thickness and known age for the entire sample, 0.25 for teeth with periodontal problems and 0.75 for teeth without periodontal pathologies. The technique was reliable for periodontally sound teeth, but not for periodontally diseased teeth.

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**Keywords:** Age estimation. Incremental lines. Forensic Dentistry. Dental cementum. Human identification.

**Running Title:** Age estimation, dental cementum incremental lines and periodontal disease.

## INTRODUCTION

Age estimation through dental parameters can be of valuable assistance in human identification. It may also help in other situations such as: determining the legal liability of teenagers and adults of unknown age, assist adoption processes, release retirement funds for adults of unknown age as well as support research in Archeology and Paleodemography.

In individuals aged between zero and 12 years, the formation and development of the human dentition has well-defined stages which show strong correlation with chronological age. Therefore, this correlation can be used with a good degree of confidence in estimating the age of these young individuals.[1] However, this technique becomes more challenging when it is necessary to estimate the age of adult individuals with completely developed dentitions.

Dental cementum is a mineralized tissue of continuous apposition and the measurement of its thickness can help estimate the age of an individual.[2] Histological analysis of the quantity and quality of incremental lines of cementum (LC) deposited around the roots of human teeth can also help estimate the age of an individual. The LC used for estimating age are best observed in the acellular cementum, present in the cervical and middle thirds of the roots. Counting the number of alternating dark and light LC, plus the average age at which the analyzed tooth erupts provides an estimate of the chronological age of an individual. This technique was first described by Scheffer[3] and Laws,[4] who observed alternating light and dark patterns in the teeth of sea mammals: the patterns more evident in the dentine than in cementum. These patterns were found to be correlated with annual seasonal changes in feeding patterns and times of mating, allowing researchers to estimate the age of those animals with accuracy. In 1982, Stott et al.[5] applied the technique to humans, finding a positive correlation between estimated and known age.

Some authors reported failure applying the technique to humans[6-8] whilst others reported moderate correlation between age and number of LC.[9-11] Although the ultrastructural nature of LC (and the biological processes that form and change them) are not yet fully understood,[12-13] several authors report a significantly low margin of error in age estimations for this technique.[14-19]

Technical improvements[20-21] and new technologies to help differentiate the lines have been proposed,[22-23] but a decreased accuracy of the technique in more advanced ages[7, 24-25] and the influence of periodontal diseases[15-16] are still factors that require better understanding. The purposes of this study was to evaluate the correlation between the number of LC, cementum thickness and age, analyzing the influence of periodontal health on the age estimates as well as the influence of image quality and observational variation.

## MATERIALS AND METHODS

Fifty-five freshly extracted teeth were obtained from 42 individuals of known age. Teeth were collected from the Dentistry College Clinic of the University of São Paulo, and from two private dental offices. The subjects answered an anamnetic questionnaire about their general health and previous known systemic conditions. All teeth were extracted for elective dental treatment. The reasons for the extractions were divided into: coronal destruction caused by caries (a), irreversible loss of periodontal attachment (b), destruction caused by caries associated with periapical processes (c), impaction (d), and orthodontic indications (e). Undecalcified cross sections of approximately 30 µm were prepared following the techniques proposed by Maat et al.[20-21] The middle third of the root was the region chosen for sectioning. Areas with evident dental calculus were avoided for sectioning. The resulting slides were mounted and photographed under an optical microscope and the resulting digital images were enhanced using *Image J* software, version 1.43s,* aiming to enhance the LC present in the image (by contrast enhancement), without alteration.[22] For measurements of cementum thickness, the scale was defined on the software, according to information provided by the manufacturer of the microscope and digital camera used for the photographs.

To analyze the possible influence of image quality on the counts, the images were classified into:[16]

1. Low quality, low contrast, many artifacts and irregular aspect of LC.
2. Moderate quality, with adequate contrast, some artifacts in the image, with regular lines.
3. Desired quality, with almost no artifacts, good contrast, regular and well defined LC.

The counting of LC was made according to Kagerer and Grupe,[16] where an observer had his scores confirmed by two other independent members (control observers) of our research group. The observer performed three counts in regions with the best visualization of the LC, and the result was considered the average between the three counts. The ages of tooth eruption[26] considered in this study were specific to the geographic region of the research (São Paulo, Brazil). The cementum thickness was measured by software in the three regions where the counts were made and in two additional different regions, and the result for cementum thickness was the average between the five measurements.

The age of the individuals and the reason for tooth extraction were not known to the observers. This data was disclosed after the age estimations and measurements were performed by the observer. The intra and interobserver variability was calculated by intraclass correlation coefficient. The correlations between known and estimated ages, and between cementum thickness and known age were calculated by Pearson's correlation coefficient. [1]

## RESULTS

Of all prepared teeth, 24 teeth from 17 individuals showed no LC suitable for counting, and were discarded. The resulting sample included 31 teeth from 25 individuals with known ages between 17 and 77 years, with a mean age of 44.2 years.
The sample was divided into:-

1) Teeth extracted for any reason - but not periodontal disease, and
2) Teeth extracted because of periodontal disease.

Known and estimated ages, analyzed teeth, image grades and reasons for extraction are shown in Tables 1 and 2, respectively. Pearson´s correlation coefficient was (r) = 0.74, p<0.01, for the first group and (r) = 0.06 for the second group. For the entire sample, the correlation coefficient was (r) = 0.59.
Average thicknesses (in micrometers) for teeth extracted for periodontal reasons and non-periodontal reasons is shown in scatter plots (Graphics 1 and 2), with simple linear regression calculations for the expected values according to age.

### Observational variation

For the assessment of intraobserver variation, all three observers counted five images twice with an interval of five days between counts. The intraclass correlation coefficient (c) measured the repeatability between counts. The repeatability between the counts was considered excellent[27] for the three observers. Scores for observer, control

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[1] ** Developed by Wayne Rasband, NIH, USA*

observer 1 and control observer 2 were 0.89, 0.98 and 0.83, respectively.

The correlation between known and estimated ages for the sample divided into images of grades 1 and 2 was 0.79 (n=17, $p<0.01$) and 0.59 (n=12, $p<0.05$), respectively. Grade 3 images were not considered because of their small number (n=2). Correlations for the sample divided into ages above or below 50 years old were 0.03 (n=13) and 0.51 (n=12, $p<0.01$), respectively.

The interobserver variability was also assessed (by intraclass correlation coefficient) between counts made by the observer and control observers, as seen in Table 3. The repeatability between observers' scores was considered good,[27] with a coefficient of 0.70.

## DISCUSSION

During the application of the technique, observers faced many difficulties that may bias the counts, for example: variation in thickness of the LC, blurry LC on the images, overlay of the same line at different levels (that could be interpreted as two lines), lack of definition of the cementum-dentine junction and cementum resorptions (that may decrease the thickness of the cementum). In some cases, discrete changes in alternating shades of gray, highlighted by the software´s image enhancement, were the criteria used by observers to count a particular line. Despite the positive correlation found between estimated and real ages, these difficulties may have accounted for a more moderate correlation coefficient value. Even for well calibrated observers, the subjective component will always be present in the counts, even in the images with superior quality.

As repeatability for intraobserver variation was excellent, results suggest that differences between counts made from the same image, by the same observer on different occasions did not have a major influence on the errors found, as reported by other authors.[9, 17]

Despite a significant influence of subjectivity on the scores, intraclass correlation coefficient for interobserver variation was considered average/good to excellent[27] demonstrating adequate agreements. Results suggest that the counts made by observer and control observers may vary significantly in relation to actual age, but that this variation is not predominantly due to inconsistencies among observers.

When the sample is divided into images of grades 1 and 2, contrary to expectations, results show that the less positive correlation between known and estimated ages is associated with the higher quality (grade 1) images ((r) = 0.59), and not to the lower quality (grade 2) images ((r) = 0.79). Thus, the quality of the images cannot be considered the main criterion to explain inaccurate age estimates (Figs 1 and 2). A similar finding was also reported in another study.[15]

The sample split into individuals younger and older than 50 years also shows a remarkable decrease of the correlation (0.51 and 0.03, respectively) between known age and estimated age, suggesting that the technique is less effective at older ages, as reported by other authors.[7-8, 24-25] Apparently, periodontal problems are the major source of error of estimated ages. The correlation for the entire sample ((r) = 0.59, $p<0.01$) increases if teeth with periodontal problems are excluded ((r) = 0.74, $p<0.01$) and decreases significantly when only teeth with periodontal problems are considered ((r) = 0.06). The same applies to the mean errors in years (Tables 1 and 2). These findings also agree with other studies,[14, 16] but when the actual ages of individuals with periodontal problems (n=12) are analyzed, it is noticed that all of them are 50 years old or more. This fact, coupled with the decrease of accuracy of the technique with increasing age,[7-8, 24-25] may have contributed to the extremely high mean errors found. Moreover, any previous periodontal treatment (such as dental scaling) may have caused the removal of cementum, impairing age estimates. Pathological alterations of cementum in periodontitis may compromise the results for this group: the spatial arrangement of collagen fibers can be changed,[28] or their destruction may result in thinner cementum layers[29] and, consequently, less LC.

Subjects 2, 3 and 8 had cementum thickness measurements that were lower than might be expected. However, these three suffered from diabetis (condition recorded when the tooth was donated, but not disclosed to the observer until after the age estimation was undertaken). These smaller average thicknesses for diabetic patients agree with the findings of other authors.[30]

Results for cementum thickness suggest that a correlation exists between cementum thickness and actual age, as it also does between LC and actual age, however, this correlation may decrease if individuals have periodontal problems, diabetes and older ages.

The teeth of individuals 9, 10 and 19 had highly overestimated ages (mean errors of 11.7, 10.6 and 10.4 years, respectively). These teeth were periodontally sound third molars and were extracted because of caries or impaction. Because third molars may have significant variation in time of eruption, root/crown morphology and maybe quality and quantity of LC, an analysis excluding these teeth was made. Without these three outliers, the correlation for periodontally sound teeth rises to (r) = 0.80 (p <0.01). This finding further strengthens the hypothesis that periodontally sound teeth can have their ages estimated from LC.

In this study, sample size was small due to difficulties in finding available independent collectors of teeth (to avoid counting bias due to the observer and control observers having contact with the donors). Short period of time granted to access the dental clinics (two days a week, from October to December, 2009) also hampered collection of a large number of teeth. Although pilot studies for slide preparation were conducted in our research group, this technique is not widely applied in Brazilian forensic human dental examination and research. Therefore, the authors faced many histotechnical difficulties as this is the first validation of the LC technique in the country. In addition, counts were made on one microscopic slide, by one observer at one occasion. This analysis design can be up to 799% less efficient than counting eight slides by one observer at one occasion.[17] However, the preliminary results suggest a significant correlation between estimated and known age for periodontally sound teeth and a weak correlation for age estimates of periodontally diseased teeth, agreeing with previous studies carried out on larger samples in other countries.

## CONCLUSIONS

- Estimating age by counting the LC added to the tooth´s mean eruption age can be a reliable method for teeth without periodontal diseases, with a mean error of 1.6 years for the sample. When examining teeth with periodontal diseases, the results provide an underestimation of age, with an average error of 22.6 years for the sample. The correlation for the entire sample ((r) = 0.58, p <0.001) was moderate, but weaker for teeth with periodontal problems ((r) = 0.06) and stronger ((r) = 0.74, p<0.01) for periodontally sound teeth.
- A similar moderate correlation between cementum thickness and known age was found ((r)= 0.69, p<0,01), but it was lower in teeth with periodontal diseases ((r)= 0.25), and higher in teeth without periodontal problems ((r)= 0.76).
- The accuracy of the technique decreased with increasing age of the individuals analyzed.
- The image quality, and the intra-and inter did not provide the major sources of error.

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***Table 1:*** *Age estimates for teeth extracted for any reason other than periodontal disease. Pearson´s correlation coefficient (r) = 0.74, p <0.01*

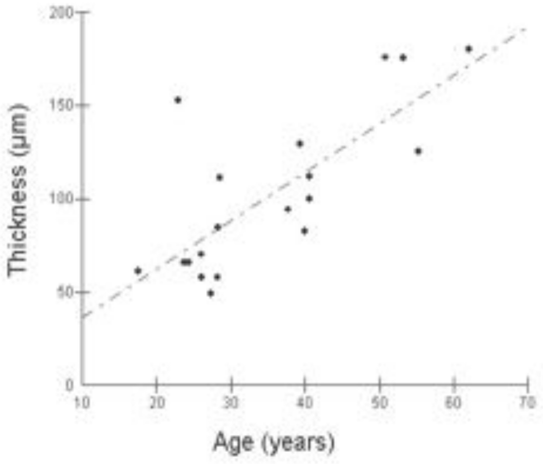
| Individual | Tooth | Known age: years | Estimated age: years | Reason for extraction | Image grade |
|---|---|---|---|---|---|
| *1* | 25 | 40.6 | 39.1 | a | 2 |
| | 14 | 40.6 | 35.8 | a | 2 |
| *5* | 47 | 26.1 | 24.8 | c | 1 |
| | 46 | 26.1 | 27.1 | c | 2 |
| *6* | 23 | 23.7 | 21.3 | d | 1 |
| *7* | 35 | 55.2 | 42 | a | 1 |
| *9* | 48 | 17.6 | 29.3 | e | 1 |
| *10* | 18 | 27.4 | 38 | a | 1 |
| *12* | 17 | 37.8 | 43.1 | a | 1 |
| *13* | 24 | 39.3 | 35.1 | a | 2 |
| *14* | 24 | 28.2 | 28.1 | a | 1 |
| *15* | 13 | 53.2 | 44 | a | 1 |
| *16* | 43 | 28.5 | 33.8 | c | 1 |
| *17* | 38 | 24.3 | 27.6 | d | 1 |
| *18* | 26 | 62.1 | 40.5 | a | 2 |
| *19* | 18 | 22.9 | 33.3 | a | 1 |
| *21* | 16 | 40 | 25.8 | a | 1 |
| *22* | 23 | 50.8 | 46.3 | a | 1 |
| *23* | 25 | 28.3 | 29.5 | a | 1 |
| *average* | - | *35.4* | *33.8* | | |

***Table 2:*** *Age estimates for all teeth extracted because of irreversible loss of periodontal attachment (reason for extraction b). Pearson´s correlation coefficient (r) = 0.06.*

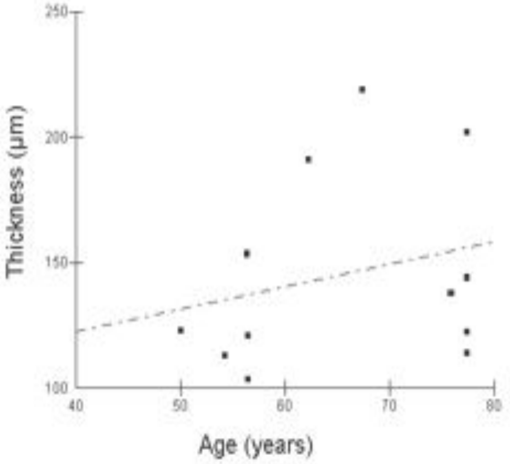
| Individual | Tooth | Known age: years | Estimated age: years | Image grade |
|---|---|---|---|---|
| *2* | 33 | 77.3 | 45.8 | 2 |
| | 32 | 77.3 | 39.1 | 3 |
| | 31 | 77.3 | 41.8 | 2 |
| | 43 | 77.3 | 55.5 | 1 |
| *3* | 22 | 56.4 | 33.5 | 2 |
| | 23 | 56.4 | 24 | 2 |
| *4* | 34 | 67.3 | 50.5 | 1 |
| *8* | 11 | 75.8 | 26.8 | 1 |
| *11* | 38 | 62.2 | 63.3 | 2 |
| *20* | 14 | 54.2 | 47 | 2 |
| *24* | 16 | 56.3 | 45.5 | 3 |
| *25* | 27 | 50 | 43.1 | 2 |
| *average* | - | *65.7* | *43.1* | |

***Table 3:*** *Age estimates for observer (O) and control observers A and B (COA and COB, respectively)*

| Individual | Known age: years | O | COA | COB | Individual | Known age: years | O | COA | COB |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 40.6 | 39.1 | 37.1 | 43.5 | *11* | 62.2 | 63.3 | 42.3 | 70.3 |
| | 40.6 | 35.8 | 35.8 | 37.1 | *12* | 37.8 | 43.1 | 45.8 | 44.1 |
| *2* | 77.3 | 45.8 | 41.8 | 48.1 | *13* | 39.3 | 35.1 | 35.1 | 35.1 |
| | 77.3 | 39.1 | 26.8 | 40.1 | *14* | 28.2 | 28.1 | 27.5 | 26.8 |
| | 77.3 | 41.8 | 41.8 | 36.1 | *15* | 53.2 | 44 | 34 | 57 |
| | 77.3 | 55.5 | 52.5 | 63.5 | *16* | 28.5 | 33.8 | 44.1 | 44.8 |
| *3* | 56.4 | 33.5 | 36.1 | 38.5 | *17* | 24.3 | 27.6 | 31 | 29 |
| | 56.4 | 24 | 30.6 | 26 | *18* | 62.1 | 40.5 | 30.5 | 41.5 |
| *4* | 67.3 | 50.5 | 48.8 | 57.5 | *19* | 22.9 | 33.3 | 33 | 40.3 |
| *5* | 26.1 | 24.8 | 27.1 | 32.1 | *20* | 54.2 | 47 | 50 | 53.6 |
| | 26.1 | 27.1 | 27.5 | 31.1 | *21* | 40 | 25.8 | 28.8 | 29.8 |
| *6* | 23.7 | 21.3 | 36 | 47 | *22* | 50.8 | 46.3 | 42.6 | 57 |
| *7* | 55.2 | 42 | 47.6 | 47.3 | *23* | 28.3 | 29.5 | 35.5 | 37.5 |
| *8* | 75.8 | 26.8 | 27.5 | 30.1 | *24* | 56.3 | 45.5 | 42.5 | 51.1 |
| *9* | 17.6 | 29.3 | 32.6 | 36.3 | 25 | 50 | 43.1 | 43.1 | 45.1 |
| *10* | 27.4 | 38 | 39.6 | 41.6 | *average* | *47.1* | *37.4* | *37.3* | *42.5* |



***Graphic 1:*** *(upper) - Cementum thickness (µm) and actual age, teeth extracted for non-periodontal reasons (n = 19). Pearson´s correlation coefficient (r) = 0.75, p <0.01*



***Graphic 2:*** *(below) - Cementum thickness (µm) and actual age, for teeth extracted for periodontal reasons (n = 12). Pearson´s correlation coefficient (r) = 0.25*

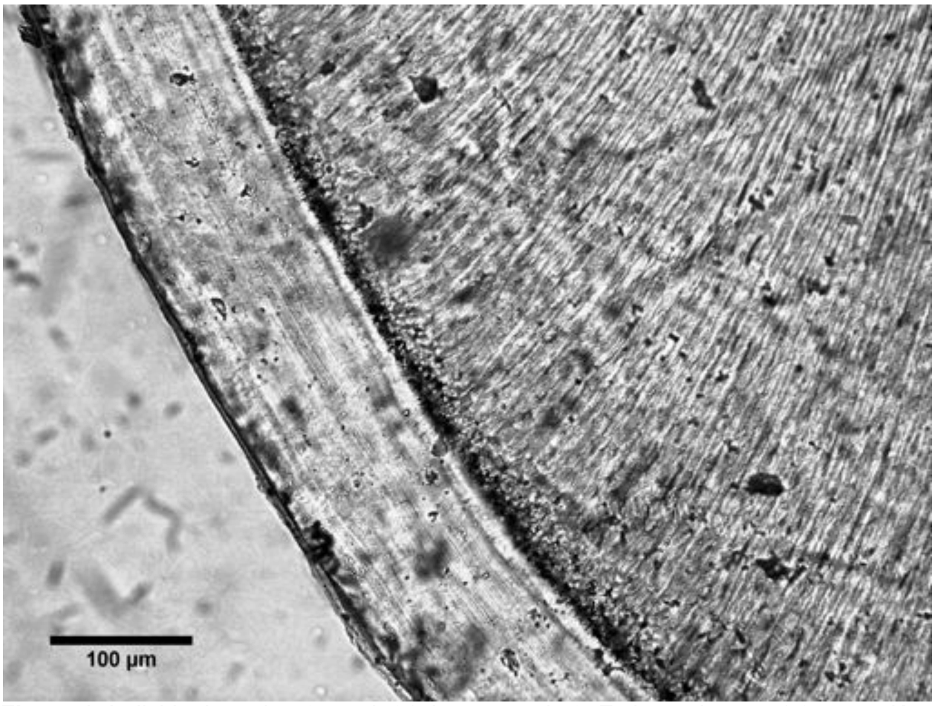


***Figure 1:*** *Individual number 2, tooth 31, image grade 2. Estimated age: 41.8 years. Known age: 77.3 years*

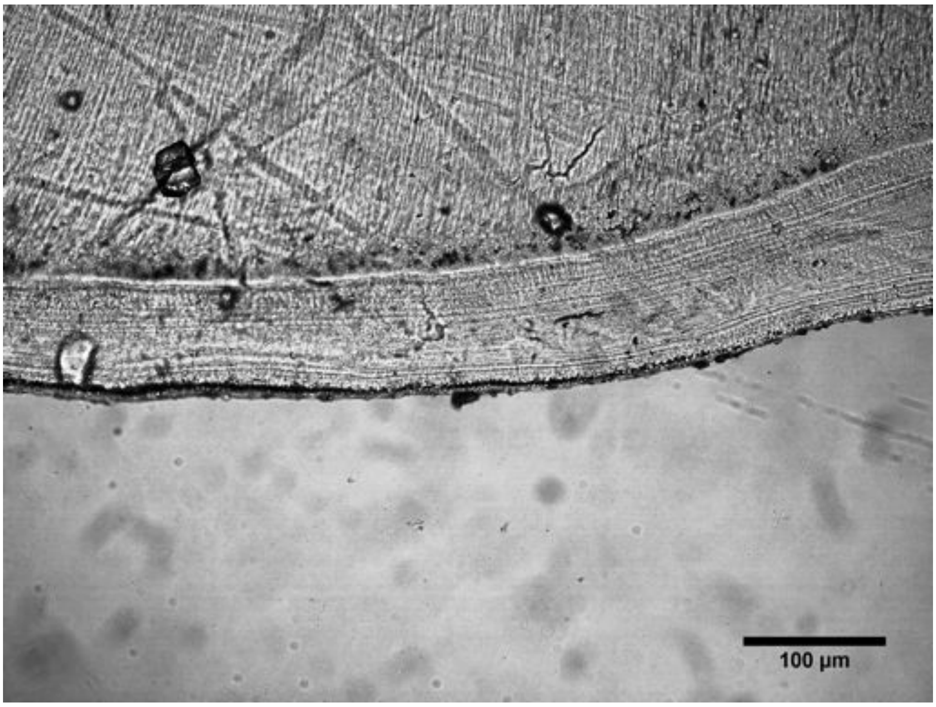


***Figure 2:*** *Individual number 22, tooth 13, image grade 1. Estimated age: 46.3 years. Known age: 50.8 years*

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# SEX DISCRIMINATION POTENTIAL OF PERMANENT MAXILLARY MOLAR CUSP DIAMETERS

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## ABSTRACT

The purpose of the present investigation was to assess the potential usefulness of permanent maxillary molar cusp diameters for sex discrimination of poorly preserved skeletal remains. Cusp diameters were measured from standardized occlusal view photographs in a sample of black South Africans consisting of 130 males and 105 females. Results demonstrated that all cusp dimensions for both first and second maxillary molars exhibited significant sexual dimorphism ($p < 0.001$). Univariate and multivariate discriminant function equations permitted low to moderate classification accuracy in discriminating sex (58.3%-73.6%). The allocation accuracies for cusp diameter measurements were as high as, and even surpassed, those observed for conventional crown length and breadth dimensions of the same teeth. The most accurate result (73.6%, with a sex bias of only 0.5%) was obtained when all cusp diameters from both maxillary molars were used concurrently. However, only slightly less accurate results (~70.0%) were achieved when selected dimensions from only one of the molars, or even a single cusp, were utilized. Although not as reliable at predicting sex as other skeletal elements in black South Africans, the derived odontometric standards can be used with highly fragmentary skeletal material, as well as immature remains in which crown formation of the maxillary molars is complete.

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**Keywords:** sex determination; dentition; discriminant function analysis; South Africa

**Running title:** Sex discrimination from maxillary molar cusp diameters

## INTRODUCTION

The determination of sex is central to the process of establishing a personal identification from human skeletal remains. Not only does accurate sex diagnosis effectively cut the number of possible matches in half, but also methods for estimating age-at-death and stature are often sex dependent. The most reliable results are obtained from morphological and metric analyses of the bony pelvis and skull.[1] Measurements of the long bones, particularly those of the femur and humerus, may also provide highly accurate sex assessments.[2]

It is often the case in forensic practice, however, that the only available criterion for discriminating sex is the measurement of the permanent dentition, given that teeth are more resistant to taphonomic degradation than osseous material.[3] As a result, odontometric standards to facilitate sex diagnosis have been developed for diverse population groups. The most commonly used dental measurements in forensic sex assessment studies have been mesiodistal and buccolingual crown diameters. For example, Acharya and Mainali[4] reported accuracy rates ranging from 62% to 83% for a Nepalese sample using combinations of crown length and breadth dimensions, while Ates et al.[5] obtained classification accuracies between 68% and 81% for similar measurements in a Turkish sample. More recently, Prabhu and Acharya[6] examined the dentition of an Indian sample and observed allocation accuracy results of 63% and 75% for the maxillary and mandibular teeth, respectively, using multivariate discriminant function analysis. Comparable sex classification percentages have also been achieved for a contemporary Turkish sample utilizing diagonal crown measurements, such as mesiobuccal-distolingual and distobuccal-mesiolingual diameters.[7] However, correct measurement of these dental dimensions may not be possible with highly fragmentary remains in which broken or incomplete tooth crowns are the only materials available for study.

Alternative approaches for measuring tooth size have also been devised, in which intra-coronal components, such as intercusp distances, cusp areas, and cusp diameters, are studied instead of the whole crown. In several recent investigations, cusp measurements of the permanent maxillary molars displayed levels of sexual dimorphism comparable to, and even greater than, those reported for conventional crown length and breadth dimensions.[8-10] These results suggest that metric analysis of intra-coronal dimensions may provide an alternative, yet effective, method of dental sex assessment. Therefore, the objective of the current study was to test the hypothesis that significant sexual dimorphism is present in permanent maxillary molar cusp diameters, for which reasonably accurate odontometric standards for discriminating the sex of poorly preserved skeletal remains can be produced.

## MATERIALS AND METHODS

The dental material used in this investigation was drawn from the Raymond A. Dart Collection of Human Skeletons, housed in the Department of Anatomical Sciences, University of the Witwatersrand, and the Pretoria Bone Collection, curated in the Department of Anatomy, School of Medicine, Faculty of Health Science, University of Pretoria. These documented skeletal series were derived primarily from donated and unclaimed cadavers of 20th century black (indigenous) and white (European) South Africans used for medical teaching and research.[11,12]

The permanent maxillary molars of 235 black South Africans of known sex (130 males, 105 females) were examined in the present investigation. The selected sample consisted of specimens simply labeled as ‘black’ or ‘South African Negro’, as well as identified individuals from the Zulu, Sotho, Tswana, Xhosa, and Swazi population groups, given that they constitute the majority of the indigenous remains in the two skeletal collections. For each group, roughly an equal number of males and females was selected. Although some degree of morphological variation has been observed between these ethnic groups, the subdivisions within the South African black population are disappearing.[13] Furthermore, in many forensic situations the local population is not known, and thus a model incorporating a variety of ethnic groups will be of more practical value. The age-at-death for the sample ranged between 12 and 78 years, with a mean age of 42.8 ± 13.9 years for males and 35.6 ± 13.4 years for females. The recorded dates of death for the sample ranged from 1927 to 2000.

This study examines the first and second maxillary molars. The third molar was not considered given that in many cases this tooth was absent due to agenesis, incomplete maturation, or antemortem/postmortem loss. In addition, the occlusal surface of the distal molar was often complicated by numerous accessory wrinkles and grooves, and thus cusp boundaries were not clearly discernible for a number of specimens in which the third molar was present. Tooth crowns in which the main fissures separating cusps were obscure, due either to dental restorations or marked occlusal wear, were excluded from the analysis. Only molars possessing all four principal cusps (protocone, paracone, metacone, hypocone) were utilized. This included teeth exhibiting hypocone expression consistent with grades 3.5-5 (moderate to very large cusp) according to the Arizona State University Dental Anthropology System.[14] Molars on the left side of the maxilla were examined unless one of these teeth was missing or damaged, in which case the antimere from the right side was utilized.

Cusp diameters were measured from standardized occlusal view photographs obtained for individual teeth using the macro setting of a digital camera (Nikon Coolpix S610). The camera was mounted on a tripod and a leveling device was used to ensure a consistent camera angle. Each tooth was oriented such that the buccal and, where possible, mesial and distal cervical enamel lines were perpendicular to the optical axis of the camera. The molar crown was positioned in the center of the image seen through the camera’s LCD monitor to minimize parallax error. A millimeter scale, placed parallel to the tooth and in approximately the same horizontal plane as the occlusal surface, was included in each photograph for calibration. The computer-assisted image analysis software program ImageJ[·] was subsequently used to take measurements from the uploaded digital photographs.

On the occlusal view photographs, the diameter of each individual cusp was determined by measuring the diagonal distance from the central pit to the most distant point located along the outer margin of the crown corresponding to the relevant cusp (**Fig. 1.**). Following Yamada and colleagues,[10,15,16] the central pit or fovea located at the bottom of the central fossa was defined as the point of intersection of the primary occlusal fissures. Although the location of the central pit will be affected by the relative position of the cusps, it is a key feature of all maxillary molars that is readily identified on occlusal view photographs[15] and provides an appropriate

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[·] Distributed freely by the National Institutes of Health, USA (http://rsbweb.nih.gov/ij/)

landmark for assessing the size of individual cusps.[10,16] It should be noted that cusp diameters do not necessarily cross over the cusp tips. Maximum mesiodistal and buccolingual crown diameters were also measured from the digital images. All measurements were rounded to the nearest tenth of a millimeter (0.1 mm).

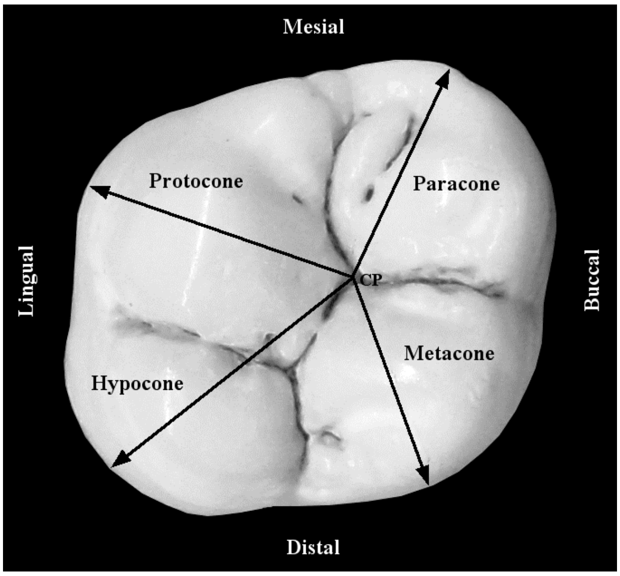

***Fig. 1:.*** *Representative example of an occlusal view photograph of a permanent maxillary molar. Arrows illustrate cusp diameters, defined as the distance from the central pit (CP) to the most distant point on the crown margin (tip of arrow) of the corresponding cusp.*

An important consideration in this research is the repeatability of these rather small dental dimensions. Previous investigations have demonstrated that methods for measuring cuspal dimensions from digitized images have relatively low levels of measurement error both within and between observers.[9,15,17] In the present study, measurement precision was evaluated from double determinations in which the entire photogrammetric process (crown orientation, image capture, central pit identification and measurement) was repeated for 30 teeth selected at random from the original sample. There was no indication of systematic methodological errors between the two sets of values, based on paired $t$-tests ($p > 0.05$ for all dental dimensions). In addition, the relative technical error of measurement (rTEM), which quantifies the extent of random error,[18] ranged from 1.4% to 1.7% for the four cusp diameters. These results suggest that methodological errors were small and unlikely to bias the results.

Subsequent to data collection, the D'Agostino-Pearson omnibus test was conducted for all variables in order to determine whether or not the data were normally distributed.[19] Following this, general descriptive statistics and percentages of sexual dimorphism were calculated for all dental dimensions. Between-sex comparisons for each measurement were performed using the independent-sample $t$-test. The crown and cusp measurement data were then subjected to direct discriminant function analyses to develop a set of equations for determining sex. To address the differential preservation of forensic remains, various multivariate functions were generated for both maxillary molars taken together (M1 and M2) for more complete remains, as well as for each tooth separately (M1 or M2) for more fragmentary dentitions. Discriminant analysis was also performed on individual measurements (e.g., M1 paracone diameter) to obtain formulae for diagnosing sex from partial tooth crowns. The reliability of each of the derived discriminant functions was assessed using the leave-one-out classification procedure. This cross-validation technique classifies each individual of a sample by the functions derived for all specimens other than that specimen itself, and thus provides less biased classification estimates for the study sample. The advantage of employing the leave-one-out method over a split-sample (or holdout) method, particularly when relatively small datasets are concerned, is that the former procedure effectively uses all of the data for both developing a discriminant function and evaluating its performance, which increases the chance of generating an accurate classification model.[20]

## RESULTS

The results of the D'Agostino-Pearson omnibus tests demonstrated that all measurements had a normal distribution ($K^2 < 1.05$, $p > 0.59$). The descriptive statistics of crown and cusp diameters for both maxillary molars are shown in **Table 1**. Mean values were significantly different between the sexes ($p < 0.001$), with male values exceeding those of females for all observed dimensions. The percentages of sexual dimorphism revealed that among cusp diameters the protocone and hypocone displayed the greatest dimorphism in both maxillary molars. In fact, protocone diameter was more dimorphic than mesiodistal and buccolingual dimensions in both the M1 and M2, as was hypocone diameter in the second molar.

Results of the direct discriminant function analyses for dimensions of the maxillary first molar are provided in **Table 2**. The highest cross-validated classification accuracy rate was observed for buccolingual breadth at 73.6% (F7). The addition of mesiodistal length to the equation (F1) did not increase the overall accuracy; however, it did provide a lower sex bias (or difference between male and female allocation rates). The combination of the two mesial cusp diameters (F2) yielded an overall sex prediction rate of 71.9%, as did the combination of lingual cusp diameters (F4). The most effective individual cusp diameter was the protocone (F8, 71.1%), followed by the hypocone (F11, 69.4%).

The cross-validated classification accuracies for functions derived from second molar dimensions are generally lower than those observed for the first molar (**Table 3**). The combination of the two lingual cusps (F15) provided the best overall result for the M2 with a sex prediction success rate of 71.9%, a value identical to that observed for M1 dimensions. Classification accuracies were reduced slightly for distal (F13, 69.8%) and mesial (F14, 69.4%) cusp combinations. Separate functions for protocone diameter (F19) and hypocone diameter (F22) yielded similar results, as both provided 70.2% accuracy in allocating sex.

The functions incorporating dimensions from both maxillary teeth did not generally provide increased sex classification accuracy rates over those achieved utilizing dimensions for only the first molar (**Table 4**). However, the function incorporating all cusp diameters from the M1 and M2 (F24) yielded the best overall accuracy rate observed in this study at 73.6%, with a minimal sex bias of only 0.5%.

## DISCUSSION

In this study, digital photogrammetric methods were used to collect cusp diameter data for a black South African sample. All cusp measurements, for both the first and second maxillary molars, were highly sexually dimorphic in this group. These results are generally consistent with a previous investigation concerning sex dimorphism of maxillary molar cusp diameters in modern Japanese.[10] As with the Japanese, dimensions of the second molar displayed more dimorphism than the first molar in black South Africans. Likewise, in both studies the greatest percentage of sexual dimorphism was observed in hypocone diameter of the second molar. Additionally, hypocone diameter was the second most dimorphic cusp dimension of the M1 crown in Japanese and black South Africans. Although the abovementioned results are in general agreement, there are some notable differences between the Japanese study and the results of the current investigation. For example, in the present study protocone diameter displayed the highest percentage of sexual dimorphism among all first molar dimensions. In contrast, the diameter of the M1 protocone was the least dimorphic measurement in the Japanese sample.[10] Also, metacone diameter was the least dimorphic cusp for both the M1 and M2 in black South Africans, which was not the case in Japanese dentitions. The apparent difference in the pattern of sexual dimorphism between these two geographically disparate populations is likely due to a combination of environmental and genetic factors, given that dental sexual dimorphism is strongly influenced by sex-linked genes.[21, 22]

The results of the current research confirm the general trend in which molar teeth that develop later in ontogeny display greater sexual dimorphism compared to members of the same tooth class that develop earlier.[8,10,15,23] The results also support, for the most part, the ontogenetic hypothesis that sex differences tend to be more pronounced in later-formed, distal crown components of the maxillary and mandibular molars than in earlier-formed, mesial crown components.[8,10,15,24] However, sexual dimorphism was not always greater in the later-developed distal cusps of the first or second maxillary molars in black South Africans, a result consistent with previous findings based on molar cusp diameters in modern Japanese.[10] These latter observations, therefore, support the recent work of Guatelli-Steinberg et al.[22] which demonstrated that the timing of dental crown formation, and the related changes in sex hormone concentrations, has only a minor role in generating crown size sexual dimorphism.

Cusp diameter measurements of the maxillary first and second molars provide low to moderate sex discrimination, with overall classification accuracies for the derived discriminant functions ranging between 58.3% and 73.6%. These classification results are comparable to those reported in a prior study concerning sex allocation in black South Africans based on odontometric data. Specifically, Kieser and Groeneveld[25] achieved sex identification rates of 70.2% for males and 66.7% for females utilizing crown length and breadth diameters of the maxillary tooth row. In the current study, the sex classification percentages obtained for cusp diameters of the maxillary M1 and M2 were as high as, and even surpassed, those observed for conventional crown length and breadth dimensions of the same teeth. The most accurate allocation results for cusp diameter measurements were obtained when both maxillary molars were used concurrently. However, only slightly less accurate results were achieved when only one of the molars, or even a single cusp, was utilized.

It should also be mentioned that the current research formed part of a larger study that also evaluated the degree of sexual dimorphism present in basal cusp areas of the maxillary molars and the utility of these measurements in diagnosing sex. Univariate and multivariate discriminant function analyses incorporating cusp area measurements for the same South African sample yielded similar allocation accuracies, ranging from 59.6% to 74.0% (Macaluso, In press [ref 31]). As in the current study, mesial and lingual cusp combinations for the M1 and M2 provided some of the highest sex prediction accuracy rates. These results are unsurprising given that both investigations utilized datasets which represent measures of cusp size for the same set of teeth. However, cusp diameters demonstrated higher levels of accuracy for functions derived from maxillary second molar dimensions in comparison to those obtained utilizing cusp area data. An additional benefit of using cusp diameters, over cusp areas, is that these dimensions do not require access to specialized equipment or computer programs. Although the cusp diameter measurements used in this study were derived from digital photographs, equivalent odontometric data can be obtained using conventional measuring techniques, such as sliding calipers.[10,16] Therefore, digital photogrammetric methods are not required to make use of the sex discriminatory capacity of maxillary molars cusp diameters, which is not the case for basal cusp areas.

The classification accuracies obtained for cusp diameter measurements of the permanent maxillary first and second molars are lower than those observed in previous, non-dental, sex allocation studies of black South Africans.[13,26-29] Therefore, it is recommended that the derived discriminant functions be used as an adjunct to more reliable sex discriminating methods when additional skeletal elements are available for examination. Nonetheless, the odontometric formulae derived in the current investigation are of interest to forensic scientists given that tooth crowns are often preserved, even in situations where the recovered skeletal remains are incomplete or badly damaged, such as dismemberments, cremations, and mass disasters.

In addition, a number of previously established odontometric approaches require a complete maxillary and/or mandibular dentition or at least some portion of the anterior tooth row, such as the canines. However, in many instances the dentition is too fragmented by perimortem (e.g., trauma, burning) and/or postmortem (e.g., weathering, soil acidity) factors to allow for the measurement of each tooth in either dental arcade. Likewise, the anterior teeth may not be available for examination. The odontometric standards developed in this study that employ dimensions of the multi-rooted maxillary molars, therefore, may be of particular value given that these teeth are less frequently lost postmortem than the anterior teeth, which possess only a single root.[30] Furthermore, various antemortem and taphonomic processes can differentially affect the dentition thus rendering conventional mesiodistal and buccolingual crown dimensions useless, yet allowing individual cusp diameters to be accurately measured. For example, a particular postmortem insult may fracture the enamel along the buccal aspect of a molar crown, given its greater exposure to the external environment, while leaving the lingual portion of the tooth unaffected. Therefore, the utility of the odontometric formulae developed in this study is enhanced by the fact that they may be used with even partial tooth crowns.

Additionally, the sex discriminating standards developed in this study can be used with both adult and immature specimens since the crowns of the permanent dentition develop at an early age and, once formed, remain unchanged during the growth process. Therefore, cusp diameter measurements may provide a useful indication as to the sex of a subadult individual in which the secondary sex features of the skeleton are not yet defined. This is important given that the inability to diagnose the sex of juvenile skeletal material with satisfactory accuracy continues to be an important limitation to forensic identification. However, in view of the metric variation that exists between human populations, caution is warranted when attempting to apply the odontometric standards derived in this study to an individual from a different population.

## CONCLUSION

The present study demonstrated that the level of sexual dimorphism in the cusp diameters of the permanent maxillary molars in black South Africans was sufficiently large to determine sex with an accuracy of 58.3%–73.6%, utilizing univariate and multivariate discriminant function analyses. These allocation accuracy rates are lower than those obtained for other skeletal elements in black South Africans, and thus the derived formulae should only be used as an adjunct to more reliable sex discriminating methods if additional remains are preserved. However, the odontometric standards developed in this study have particular value in situations where the recovered skeletal material is highly fragmentary due to perimortem and/or postmortem insults. In these cases, cusp diameter measurements of the maxillary first and second molars can be used to diagnose the sex of complete or partial tooth crowns, even when conventional mesiodistal and buccolingual crown dimensions cannot be accurately recorded. Furthermore, the derived classification models may provide a useful indication as to the sex of immature remains in which the secondary sexual features of the skeleton are not fully expressed.

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***Table 1:** Descriptive statistics, t-values, and percentages of sexual dimorphism for maxillary molar crown dimensions*

| Variables † | Males ($n$ = 130) | | | Females ( $n$ = 105) | | | Sex Dimorphism | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range | *t*-values* | %‡ |
| M1 | | | | | | | | |
| MD diameter | 11.09 | 0.57 | 9.5-12.6 | 10.63 | 0.53 | 9.4-12.4 | 6.380 | 4.33 |
| BL diameter | 11.50 | 0.59 | 10.1-13.4 | 10.89 | 0.52 | 9.6-12.3 | 8.370 | 5.60 |
| Pr diameter | 6.56 | 0.38 | 5.6-7.6 | 6.18 | 0.40 | 5.1-7.5 | 7.510 | 6.15 |
| Pa diameter | 5.59 | 0.34 | 4.8-6.8 | 5.34 | 0.27 | 4.6-6.0 | 6.290 | 4.68 |
| Me diameter | 5.52 | 0.32 | 4.6-6.4 | 5.36 | 0.32 | 4.7-6.3 | 3.900 | 2.99 |
| Hy diameter | 7.23 | 0.44 | 6.0-8.5 | 6.89 | 0.43 | 5.8-8.0 | 5.950 | 4.93 |
| | | | | | | | | |
| M2 | | | | | | | | |
| MD diameter | 10.94 | 0.76 | 9.3-12.6 | 10.38 | 0.57 | 9.1-12.0 | 6.530 | 5.39 |
| BL diameter | 11.70 | 0.71 | 10.0-13.5 | 11.18 | 0.61 | 9.6-12.6 | 6.970 | 4.65 |
| Pr diameter | 6.73 | 0.47 | 5.6-8.3 | 6.31 | 0.43 | 5.3-7.4 | 7.110 | 6.66 |
| Pa diameter | 5.85 | 0.44 | 5.0-7.1 | 5.58 | 0.40 | 4.7-6.7 | 4.650 | 4.84 |
| Me diameter | 5.25 | 0.38 | 4.2-6.1 | 5.09 | 0.43 | 4.3-5.9 | 3.460 | 3.14 |
| Hy diameter | 7.10 | 0.56 | 5.3-8.3 | 6.56 | 0.54 | 5.0-8.0 | 7.450 | 8.23 |

† MD: mesiodistal; BL: buccolingual; Pr: protocone; Pa: paracone; Me: metacone: Hy: hypocone.
‡ Sexual dimorphism % = [male mean/female mean-1] x 100.
*All significant at $p < 0.001$.

***Table 2:** Discriminant functions for determining sex from crown dimensions of the maxillary first molar*

| Function number | Discriminant function † | Sectioning point | Male accuracy | Female accuracy | Overall accuracy |
|---|---|---|---|---|---|
| F1: crown diameters | y = (0.570)(MD) + (1.427)(BL) - 22.223 | -0.061 | 72.3% | 75.2% | 73.6% |
| F2: mesial cusps | y = (1.847)(Pr) + (1.565)(Pa) - 20.378 | -0.059 | 68.5% | 76.2% | 71.9% |
| F3: distal cusps | y = (0.407)(Me) + (2.108)(Hy) - 17.132 | -0.042 | 68.5% | 69.5% | 68.9% |
| F4: lingual cusps | y = (02.154)(Pr) + (0.527)(Hy) - 17.484 | -0.054 | 70.8% | 73.3% | 71.9% |
| F5: buccal cusps | y = (2.815)(Pa) + (0.753)(Me) - 19.529 | -0.045 | 64.6% | 71.4% | 67.7% |
| F6: mesiodistal | y = (1.802)(MD) - 19.619 | -0.045 | 60.0% | 67.6% | 63.4% |
| F7: buccolingual | y = (1.790)(BL) - 20.099 | -0.058 | 76.2% | 70.5% | 73.6% |
| F8: protocone | y = (2.585)(Pr) - 16.515 | -0.053 | 72.3% | 69.5% | 71.1% |
| F9: paracone | y = (3.217)(Pa) - 17.629 | -0.044 | 68.5% | 66.7% | 67.7% |
| F10: metacone | y = (3.113)(Me) - 16.962 | -0.027 | 58.3% | 61.0% | 58.3% |
| F11: hypocone | y = (2.291)(Hy) - 16.208 | -0.042 | 68.5% | 70.5% | 69.4% |

†Abbreviations are the same as in Table 1.

***Table 3:** Discriminant functions for determining sex from crown dimensions of the maxillary second molar*

| Function number | Discriminant function [†] | Sectioning point | | Male accuracy | Female accuracy | Overall accuracy |
|---|---|---|---|---|---|---|
| F12: crown diameters | y = (0.727)(MD) + (0.988)(BL) - 19.147 | -0.054 | | 67.7% | 71.4% | 69.4% |
| F13: mesial cusps | y = (1.836)(Pr) + (0.776)(Pa) - 16.465 | -0.053 | | 68.5% | 70.5% | 69.4% |
| F14: distal cusps | y = (0.111)(Me) + (1.774)(Hy) - 12.733 | -0.052 | | 70.0% | 69.5% | 69.8% |
| F15: lingual cusps | y = (1.036)(Pr) + (1.109)(Hy) - 14.382 | -0.055 | | 71.5% | 72.4% | 71.9% |
| F16: buccal cusps | y = (1.817)(Pa) + (1.170)(Me) - 16.469 | -0.036 | | 58.5% | 61.9% | 60.0% |
| F17: mesiodistal | y = (1.463)(MD) - 15.642 | -0.044 | | 62.3% | 70.5% | 66.0% |
| F18: buccolingual | y = (1.504)(BL) - 17.318 | -0.049 | | 70.0% | 65.7% | 68.1% |
| F19: protocone | y = (2.199)(Pr) - 14.391 | -0.050 | | 67.7% | 73.3% | 70.2% |
| F20: paracone | y = (2.343)(Pa) - 13.421 | -0.033 | | 56.2% | 71.4% | 63.0% |
| F21: metacone | y = (2.766)(Me) - 14.327 | -0.024 | | 63.1% | 55.2% | 59.6% |
| F22: hypocone | y = (1.806)(Hy) - 12.382 | -0.052 | | 70.0% | 70.5% | 70.2% |

[†] Abbreviations are the same as in Table 1.

***Table 4:** Discriminant functions for determining sex from crown dimensions of the maxillary first and second molar*

| Function number | Discriminant function[†] | Sectioning point | | Male accuracy | Female accuracy | Overall accuracy |
|---|---|---|---|---|---|---|
| F23: crowns diameters | y = (0.284)(MD M1) + (1.188)(BL M1) + | -0.062 | | 70.0% | 76.2% | 72.8% |
| | (0.373)(MD M2) + (0.158)(BL M2) - 22.238 | | | | | |
| F24: all cusps | y = (0.937)(Pr M1) + (1.151)(Pa M1) + | -0.068 | | 73.8% | 73.3% | 73.6% |
| | (-0.094)(Me M1) + (-0.009)(Hy M1) + | | | | | |
| | (-0.171)(Pr M2) + (0.554)(Pa M2) + | | | | | |
| | (-0.583)(Me M2) + (1.205)(Hy M2) - 19.015 | | | | | |
| F25: lingual cusps | y = (1.468)(Pr M1) + (-0.113)(Hy M1) + | -0.061 | | 70.0% | 69.5% | 69.8% |
| | (0.253)(Pr M2) + (0.958)(Hy M2) - 16.799 | | | | | |
| F26: buccal cusps | y = (2.361)(Pa M1) + (0.494)(Me M1) + | -0.046 | | 63.8% | 67.6% | 65.5% |
| | (0.451)(Pa M2) + (0.366)(Me M2) - 20.112 | | | | | |

[†] Abbreviations are the same as in Table 1.

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# DENTAL AGE ESTIMATION BASED ON THIRD MOLAR ERUPTION IN FIRST NATIONS PEOPLE OF CANADA.

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**ABSTRACT**
Forensic age estimation of living subjects has become an increasing focus of interest in modern society. One main criterion for dental age estimation in the relevant age group is the evaluation of third molar eruption. The importance of ethnic variation in dental development requires population specific data for dental age evaluation. In the present study, we determined the stages of third molar eruption in 347 female and 258 male First Nations people of Canada aged 11 to 29 years based on radiological evidence from 605 conventional orthopantomograms. The results presented here provide data on the age of alveolar, gingival, and complete eruption of the third molars in the occlusal plane that can be used for forensic estimation of the minimum and most probable ages of investigated individuals.

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**Keywords**: Dental age; Tooth eruption; Third molar; First Nations people of Canada

**Running title**: Third molar eruption in First Nations people of Canada

## INTRODUCTION

Forensic age assessment in living subjects has become increasingly important over the last few years.[1-4] The investigated individuals are foreign nationals without valid identification papers whose chronological age is of relevance in legal proceedings. In most countries, the age thresholds of legal importance lie between 14 and 21 years.

In accordance with the updated recommendations for age estimation in criminal proceedings of the Study Group on Forensic Age Diagnostics, for an age estimation a physical examination, an radiograph of the hand as well as a dental examination should be performed. If the skeletal development of the hand is completed, an additional radiological examination of the clavicles should be carried out.[5]

One main criterion for dental age estimation in the relevant age group is the evaluation of third molar eruption. The importance of ethnic variation in dental development requires further population studies in order to reach an adjustment of each method according to the specific population, with an increase in precision and accuracy.[6]

This paper presents statistical measures on the time course of third molar eruption in First Nations people of Canada.

## MATERIALS AND METHODS

A total of 605 conventional orthopantomograms of 347 female and 258 male First Nations people of Canada of known age (11 to 29 years) were analyzed retrospectively. The First Nations people of Canada who were investigated belonged to the Ojibwa tribe. Today, the Ojibwa live in local reservations north of the Lake Superior and the Lake Huron as well as in parts of Minnesota, North Dakota, Wisconsin, Manitoba and Sasketchewan. The orthopantomograms were made during the years 1987-2007. Patient identification number, sex, date of birth, date of exposure, and eruption stages of the third molars were recorded for each individual subject. The age and sex distribution of the study population is shown in Table 1.

The eruption stages were evaluated using the classification of stages by Olze et al.[7] (Fig. 1):

| | |
|---|---|
| Stage A | Occlusal plane covered with alveolar bone. |
| Stage B | Alveolar eruption; complete resorption of alveolar bone over occlusal plane. |
| Stage C | Gingival emergence; penetration of gingiva by at least one dental cusp. |
| Stage D | Complete emergence in occlusal plane. |

Impacted third molars were excluded from the analysis. Mesially, distally and vestibulo-orally angulated third molars were classified as impacted as recommended by Archer[8] and Wolf and Haunfelder.[9] Wisdom teeth with an unclear direction of eruption also were not included in the analysis.

Statistical analyses were performed using the program SPSS 16.0 for Windows. Each individual age was calculated as date of exposure minus date of birth and recorded as years and 1/10 of years. For each stage a minimum and a maximum were found and a median with lower and upper quartiles as well as a mean with standard deviation were calculated. In case of stage D, 50% probability values have been calculated using logistic regression.

## RESULTS

The results of the statistical analysis for females are shown in Table 2 and for males in Table 3. Table 4 presents the 50% probability values for stage D.

For both sexes the obtained data show that within the entire observed age interval, the minima and means of the chronological age increased with increasing stage. Thus, they demonstrate a good correlation between the stages and the chronological ages of the subjects.

Stage B was first achieved by females between 12.4 and 13.1 years and by males between 13.0 and 13.4 years. The earliest appearance of stage C was at age 13.7 years in females and between 13.6 and 18.0 years in males. The occurrence of stage D was first found between 15.9 and 17.4 years in females and between 14.5 and 18.3 years in males.

For stage A the means varied for both sexes between 12.8 and 15.1 years. The means of stage B were between 15.9 and 18.4 years. The means for stage C showed a range between 17.2 and 21.6 years. For stage D the means varied between 20.5 and 22.8 years. The 50% probability values for stage D were found between 18.7 and 23.2 years.

## DISCUSSION

Tooth eruption is a parameter of developmental morphology which, unlike tooth mineralization, can be determined in two ways: by clinical examination and/or by evaluation of dental radiographs. While ‘eruption’ incorporates the entire journey of the tooth from its formation in the alveolar crypts to full occlusion, ‘emergence’ is restricted to the time when any part of the tooth finally clears the gingival margin and becomes visible in the mouth until the stage when the tooth finally comes into occlusion with its partner tooth from the opposing jaw.[10]

Studies on the chronology of third molar eruption are scarce. Rantanen[11] investigated the clinical emergence of third molars in a total of 2218 Finnish males and females ranging in age from 16 to 24 years. The median age of upper and lower third molar eruption in the male subjects was determined to be 21.7 and 21.8 years, respectively, compared to 23.3 and 23.0 years in females. In this study population, the third molars of the male subjects emerged roughly 1.5 years earlier than those of the females.

Levesque et al.[12] determined the age of alveolar and gingival eruption and mineralization state of the third molars based on evidence from 4640 orthopantomograms from 2278 male and 2362 female Franco-Canadians of ages ranging from 7 to 25 years. Alveolar eruption occurred at a mean age of 17.7 years in the investigated females and 17.2 years in the male subjects. Complete clinical emergence of the wisdom teeth occurred at the age of 19.0 years in the female subjects and 18.5 years in the males. Thus, alveolar eruption of the third molars occurred 0.2 years earlier and gingival emergence occurred 0.5 years earlier in the males than in the females.

Müller [13] analyzed third molar emergence in 823 male and female German subjects of ages ranging from 16 to 40 years. The median ages of third molar emergence were found to be 20.36 and 20.29 years, respectively. No emergence of third molars was observed in the group of 16-year-olds; the presence of third molars was first detected in the group of 17-year-olds. More than 50 % of the complete set of third molars had emerged by the age of 21 years.

In a review of literature on growth and development in Japan, Kimura14 provided Japanese statistics on third molar emergence (mean age: 19.8 years in males and 21.0 years in females).

Olze et al.[7] analyzed and compared the chronological course of third molar eruption in German, Japanese, and South African populations. They found that the investigated German population has an intermediate rate of dental development as determined by comparing the different ages of third molar eruption. The defined eruption stages occurred at earlier ages in the investigated South African population, and at later ages in the Japanese population. Statistically significant population differences were observed in males at stages A and B. The South African males were a mean of 3.0 to 3.2 years younger than the German males at these stages of development, and the Japanese males were a mean of 3.1 to 4.2 years older than their South African counterparts. The females exhibited statistically significant population differences at stages A, B and C. The South African women reached the target stages a mean of 1.6 to 1.8 years earlier than the German women, whereas the Japanese women were a mean of 0.9 to 3.3 years older than their German counterparts.

Compared to the data published by Olze et al.[7] the First Nations people of Canada who were examined in the present study are intermediate between South Africans and Germans in most stages and in both sexes. This means that compared to South Africans the First Nations people of Canada show delayed eruption of the third molars. In comparison to Germans and Japanese, however, the First Nations people of Canada show accelerated eruption of the third molars, with the difference between the First Nations people of Canada and the Japanese being more crucial than between the First Nations people of Canada and the Germans. However, due to the different age structure of the studied sample compared to the populations studied by Olze et al.,[7] those statements should be made very carefully.[15]

## CONCLUSION

The results of our study show the necessity of generating population-specific data for forensic age diagnostics in living individuals. According to the authors' knowledge, this research paper is the first study of the time course of third molar eruption in First Nations people of Canada.

Our results provide data on the age of alveolar, gingival, and complete eruption of the third molars in the occlusal plane that can be used for dental age estimation. As the mean values and medians of stage D of the third molar eruption depend on the upper limit of age of the investigated sample, the 50% probability value was also set for this stage. This value refers to the most probable minimum age of a person whose third molars show stage D.

***Table 1**: Age and sex distribution of the sample*

| Age (Years) | Number Male | Number Female |
|---|---|---|
| 11 | 1 | 9 |
| 12 | 7 | 12 |
| 13 | 8 | 13 |
| 14 | 10 | 18 |
| 15 | 14 | 23 |
| 16 | 19 | 33 |
| 17 | 22 | 33 |
| 18 | 33 | 45 |
| 19 | 26 | 32 |
| 20 | 18 | 28 |
| 21 | 24 | 29 |
| 22 | 17 | 21 |
| 23 | 18 | 22 |
| 24 | 14 | 9 |
| 25 | 10 | 6 |
| 26 | 6 | 3 |
| 27 | 3 | 8 |
| 28 | 6 | 1 |
| 29 | 2 | 2 |

***Table 2***:*Statistical data on the age of emergence of teeth 18, 28, 38 and 48, by stage, in females*

| Tooth | Stage | n | Min | Max | Mean | SD | LQ | Median | UQ |
|---|---|---|---|---|---|---|---|---|---|
| 18 | A | 53 | 11.3 | 25.5 | 15.1 | 2.6 | 13.4 | 15.0 | 16.6 |
| | B | 100 | 13.1 | 27.0 | 18.2 | 2.7 | 16.0 | 18.3 | 19.8 |
| | C | 38 | 13.7 | 27.4 | 20.2 | 2.9 | 18.6 | 19.7 | 22.5 |
| | D | 52 | 17.4 | 29.4 | 22.3 | 2.9 | 20.2 | 22.3 | 23.8 |
| 28 | A | 50 | 11.0 | 23.3 | 14.8 | 2.5 | 12.6 | 14.6 | 16.6 |
| | B | 109 | 13.1 | 27.5 | 18.4 | 2.9 | 16.1 | 18.2 | 20.1 |
| | C | 36 | 13.7 | 27.3 | 20.0 | 3.0 | 18.2 | 19.7 | 21.6 |
| | D | 56 | 17.4 | 29.3 | 22.2 | 3.0 | 19.7 | 21.9 | 24.1 |
| 38 | A | 13 | 11.1 | 17.1 | 13.3 | 1.9 | 11.8 | 13.0 | 14.8 |
| | B | 48 | 12.4 | 26.2 | 17.3 | 2.9 | 15.1 | 16.9 | 18.0 |
| | C | 9 | 13.7 | 23.7 | 17.5 | 3.0 | 15.2 | 16.7 | 19.0 |
| | D | 20 | 15.9 | 27.4 | 20.6 | 3.3 | 18.3 | 19.2 | 22.8 |
| 48 | A | 15 | 11.1 | 17.3 | 13.7 | 2.3 | 11.7 | 12.2 | 15.8 |
| | B | 39 | 12.4 | 26.2 | 16.7 | 2.8 | 14.3 | 16.5 | 18.8 |
| | C | 17 | 13.7 | 23.7 | 18.5 | 2.5 | 16.6 | 18.9 | 20.2 |
| | D | 24 | 15.9 | 29.4 | 21.3 | 3.9 | 18.2 | 19.6 | 25.1 |

n = number of cases
SD = standard deviation
Min = minimum age
LQ = lower quartile
Max = maximum age
UQ = upper quartile

***Table 3:*** *Statistical data on the age of emergence of teeth 18, 28, 38 and 48, by stage, in males*

| Tooth | Stage | n | Min | Max | Mean | SD | LQ | Median | UQ |
|---|---|---|---|---|---|---|---|---|---|
| 18 | A | 29 | 11.7 | 18.9 | 14.9 | 2.0 | 13.0 | 14.8 | 16.8 |
| | B | 61 | 13.4 | 25.4 | 17.8 | 2.2 | 16.5 | 17.8 | 19.1 |
| | C | 18 | 18.0 | 29.8 | 21.6 | 3.0 | 19-4 | 21.5 | 23.5 |
| | D | 83 | 14.5 | 28.8 | 22.2 | 2.6 | 20.7 | 22.1 | 23.7 |
| 28 | A | 26 | 11.7 | 18.9 | 14.6 | 2.1 | 12.8 | 14.6 | 16.6 |
| | B | 81 | 13.4 | 26.7 | 18.1 | 2.5 | 16.5 | 17.9 | 19.3 |
| | C | 30 | 13.6 | 26.3 | 20.6 | 2.7 | 18.7 | 20.8 | 22.5 |
| | D | 73 | 14.5 | 29.8 | 22.8 | 2.8 | 20.9 | 22.5 | 24.8 |
| 38 | A | 5 | 12.2 | 13.5 | 12.8 | 0.5 | 12.4 | 12.9 | 13.2 |
| | B | 20 | 13.0 | 19.3 | 15.9 | 1.7 | 14.7 | 15.4 | 17.2 |
| | C | 3 | 16.7 | 19.5 | 18.1 | 1.4 | 16.7 | 18.3 | 19.5 |
| | D | 20 | 18.0 | 29.3 | 21.6 | 2.8 | 19.6 | 21.2 | 22.6 |
| 48 | A | 5 | 12.0 | 17.1 | 13.6 | 2.1 | 12.1 | 13.0 | 15.4 |
| | B | 20 | 13.0 | 19.5 | 16.2 | 1.8 | 14.9 | 16.3 | 17.5 |
| | C | 3 | 15.0 | 18.5 | 17.2 | 1.9 | 15.0 | 18.0 | 18.5 |
| | D | 17 | 18.3 | 26.3 | 20.5 | 2.1 | 19.0 | 19.6 | 21.3 |

n = number of cases
SD = standard deviation
Min = minimum age
LQ = lower quartile
Max = maximum age
UQ = upper quartile

***Table 4:*** *50% probability values for stage D*

| | Tooth 18 | Tooth 28 | Tooth 38 | Tooth 48 |
|---|---|---|---|---|
| Females | 23.0 | 23.2 | 22.0 | 21.3 |
| Males | 20.5 | 21.6 | 18.8 | 18.7 |

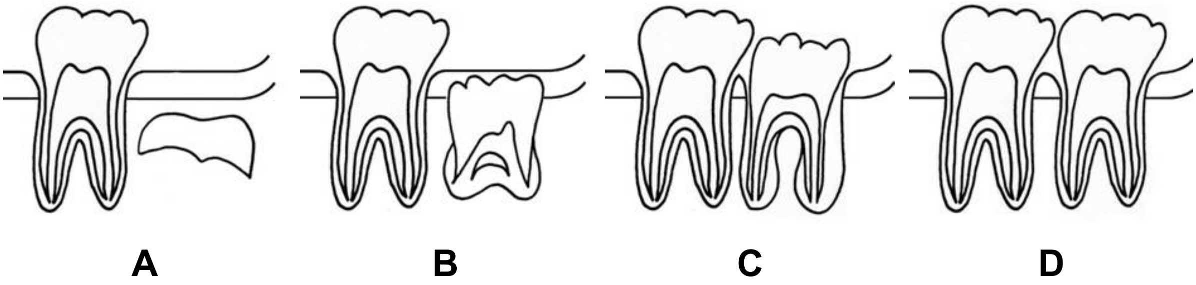

***Fig. 1****: Stages A to D of third molar eruption*

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# DENTAL ANOMALIES AND THEIR VALUE IN HUMAN IDENTIFICATION: A CASE REPORT

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## ABSTRACT

Forensic odontology and anthropology provide valuable support with regard to human identification. In some cases, when soft tissue is destroyed, carbonized or absent for whatever reason, bones and teeth become the only source of information about the identity of the deceased. In human identification, anything different, such as variation from normality, becomes an important tool when trying to establish the identity of the deceased. This paper illustrates a positive identification case achieved by the diagnosis of an anomaly of tooth position, with confirmation using skull-photo superimposition. Even though forensic science presents modern techniques, in this particular case, the anomalous position of the canine played a key role on the identification, showing that the presence of a forensic dentist on the forensic team can be of great value.

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**Running title:** Dental anomalies and human identification

## INTRODUCTION

Anthropologists and odontologists usually have a leading role in the forensic team when dental structures are the only source of information for the identification of human remains. The resistance of teeth and their supporting tissues, even to fire and decomposition, makes them extremely useful for identification purposes.[1-4] In cases of carbonization, advanced decomposition, or partial destruction, all attention turns to the analysis of bones and teeth, and forensic experts need support from the family of suspected victims, on providing clear and complete antemortem medical and/or dental records, to be compared with the remains.[5-9]

For the identification of human remains, anything that distinguishes one person from another, such as a tattoo, or a variation from normality, becomes very important to the forensic team, greatly assisting the identification process. This is the reason why literature shows cases of abnormality, asymmetry and pathology narrowing the search within missing persons files.[3,10] However, few authors discuss the forensic value of dental anomalies that are commonly missed by medical examiners. These variations, analyzed by dental examiners, can potentially lead to a positive identification.[2,3,6,7,11]

In the absence of antemortem information, the forensic team search for alternative sources of reference, such as photographs[12-14] and videotapes[15] for personal features that may be identifiable at the postmortem examination. One of the techniques used in these cases is the skull-photo superimposition. Identification by this method is based on the matching of the outline and positional relationships between anatomical points on the face, and their locations on the skull.[16-20]

This paper reports a recent positive identification case of a Brazilian girl, achieved by the discovery of an anomaly of tooth position and confirmed by skull-photo superimposition, showing the importance of the odontological analysis in this case, along with the anthropological evaluation of personal photographs for human identification.

## CASE REPORT

The remains of a caucasoid female, with an age estimation between 18 and 30 years, was found in an advanced stage of decomposition, on the banks of a river, in São Paulo, SP - BRAZIL. The forensic odontology team noticed that there were five teeth lost postmortem, and no restorations or decay present in any of the remaining dentition, but there was a positional anomaly: the upper left canine (23[*]) was quite buccally displaced (Fig. 1), allowing proximal contact between the lateral incisor (22) and the first premolar (24).

Approximately one month later, a man went to the local Medico-Legal Institute, searching for information about his 23 year-old missing niece. When asked about dental records, the man said she never had dental decay or restorations, but one tooth was "displaced forward." This information drew the attention of the experts, who requested smiling antemortem photographs of the young woman. The images provided were digitalized, stored in a database, and analyzed by the graphic manager Adobe Photoshop,™ allowing the forensic experts to identify the same dental anomaly (23), in the exact position as observed on the skull (Fig. 2), as well as all the other remaining visible teeth.

***Figure 1:*** *Upper dental arch, with buccally placed left canine*

***Figure 2:*** *Submitted antemortem photograph*

After that coincidence, which, according to many authors, is sufficient to establish a positive identification,[3,4,14] the team took pictures of the skull using a digital camera of 6.0 megapixels, in an attempt to reproduce the angle of the face as shown on the photograph (Fig. 3). Following storage in the database, the size of the images ante and

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[*] The dental notation adopted is advocated by the FDI World Dental Federation

postmortem were adjusted, using dental structures, interpupilar distance, and facial contour as size reference, achieving the same scale on both images, followed by the skull-photo superimposition and craniofacial analysis (Fig. 4). Computer-assisted craniofacial superimposition allows the operator to evaluate the fit between the skull and facial images by morphometrical analysis.[19] The correct sizing and positioning of the images is essential - the image of the skull must be in exactly the same scale and angulation as the photograph.

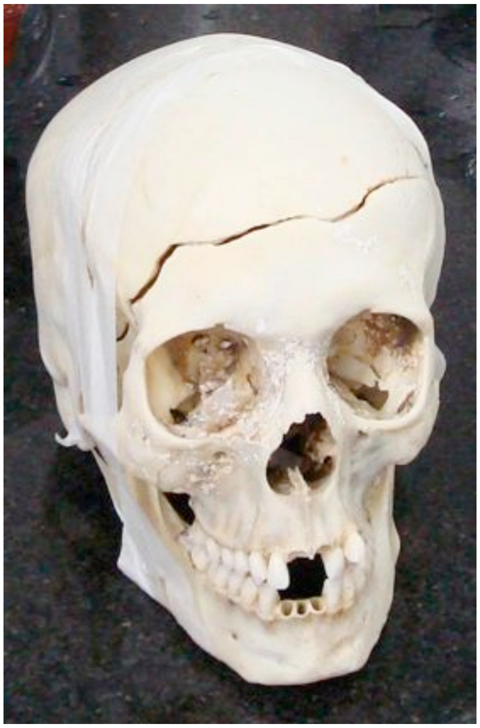

***Figure 3:*** *Skull articulated and placed reproducing the angle of the photo.*

The criteria used to judge the matching between the skull and photo, were the same as those suggested by Austin-Smith and Maples,[21] and presented as follows:

1. The length of the skull considered from bregma to menton fits within the face, and the bregma is covered with hair;
2. The width of the cranium fills the forehead area of the face;
3. The eyebrow follows the supraorbital margin over the medial two-thirds. At the lateral superior one-third or the orbit the eyebrow continues horizontally as the orbital margin begins to curve inferiorly;
4. The orbits completely encase the eye including the medial and lateral folds;
5. The width and length of the pyriform aperture falls inside the borders of the nose;
6. The line of the mandible corresponds to the line of the face
7. The mandible curve is similar to that of the facial jaw; at no point does the bone appear to project from the flesh.
8. The prominence of the glabella and the depth of the nasal bridge are closely approximated by soft tissue covering this area; the nasal bones fall within the structure of the nose, and the imaginary continued line, composed of lateral nasal cartilages in life, conforms to the shape of the nose.
9. The prosthion lies posterior to the anterior edge of the upper lip;
10. The mental protuberance of the mandible lies posterior to the point of the chin.

***Figure 4:*** *Superimposition skull-photo with two degrees of opacity*

## DISCUSSION

Inexperienced observers may not be able to easily notice proportional and feature variation between skulls. However, an expert can demonstrate unlimited variation in shape, size, proportion and detail between skulls, showing that each skull is as individual as each face.[22]

Each dentition is considered to be unique, although to the non-dental eye they all may look the same. Variations in shape, color, position, age changes, wear patterns, caries and periodontitis, and all associated dental restorations and prosthetic work, make the dentition as individual as fingerprints.[2-4,11]

Although forensic odontology and anthropology are extremely valuable when traditional identification methods are unsuitable or have failed (fingerprints, DNA), sometimes they also can be unproductive for various reasons. A very common reason is the absence or inaccuracy of dental records. In these situations, the analysis of any available social and family photographs may help forensic professionals to identify the deceased.[14]

When the anterior dentition is recovered with the skull and a smiling antemortem photograph is available, the shapes of the individual teeth and their relative positions are considered sufficiently distinctive for a positive identification.[14,21] In this particular case, computer-assisted craniofacial superimposition was used to corroborate the positive identification, acting as additional criteria, allowing the team to confirm the identification achieved initially by odontological analysis of a smiling picture. Other elements such as a relationship between the time of the body decomposition and the period of disappearance of the victim, personal characteristics such as sex, age, height, estimated weight were also considered.

## CONCLUSION

This particular positional anomaly of the canine, which played a key role in the identification process, had not been noticed by the medical examiner. This case report, emphasises the value of a forensic dental examiner being present as part of the forensic team during the investigation to seek identification of human remains.

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