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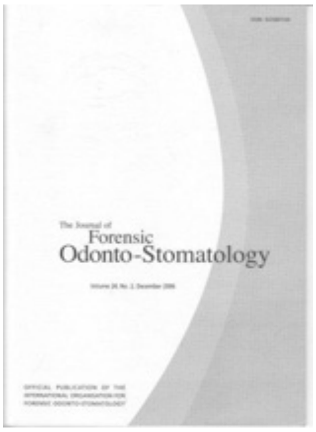

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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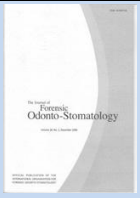

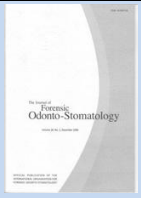

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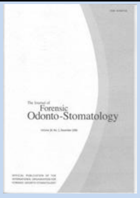

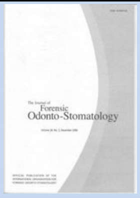

JOURNAL of FORENSIC ODONTO-STOMATOLOGY

VOLUME 34 Number 1 July 2016

SECTION IDENTIFICATION

The Use of Intraoral Radiographs for Identification of Edentulous Patients Rehabilitated With Implants

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The author declares to have no conflict of interest.

ABSTRACT

The aims of this study were; i) to determine the accuracy by which two intra-oral radiographic examinations performed on patients with edentulous mandibles treated with dental implants can be matched. ii) to determine whether prosthodontic supra-construction is important for matching. iii) to investigate whether there is a difference between oral and maxilla-facial radiologists (OMR) and dental practitioners, not specialized in oral and maxillofacial radiology (NOMR), regarding their ability to match.

The specific features of the radiographs used by the operators to acquire a match were also investigated.

Intra-oral radiographic examinations from 59 patients were utilized. Radiographic examinations from 47 patients carried out at placement of the supra-construction and at subsequent follow-up examinations were used as "ante-mortem" and "post-mortem" records respectively. Examinations from 12 patients were added to the "post-mortem" records without "ante-mortem" records being available.

The study was divided into two parts. In Part One all "ante"- and "post-mortem" records had the supra-construction masked and in Part Two it was visible. Seven dentists (4 OMR, 3 NOMR) were instructed to specify on what basis each matching was made on the confidence of a three-graded scale

OMR had 93.2 % and 98.5 % accuracy in Parts One and Two respectively. NOMR had 63.8 % and 87.9 %. Bone anatomy was the most commonly used feature by OMR to obtain a match. For NOMR it was the appearance of the fixtures. OMR reported higher confidence in their ability to match the examinations. This study indicates that OMR could be a valuable resource in cases of identification where dental implants are a feature of the post-mortem dental records.

KEYWORDS*: *dental identification, forensic odontology, dental implants, intra-oral radiographs, edentulous patients

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INTRODUCTION

The mother of Emperor Nero, Agrippina (15-59 A.D.), was able to identify a murdered rival, Lollina Paulina, due to the victim´s characteristic dental arch. To this day, this is the first documented case of dental identification.[1]

Almost two thousand years later, in 1965, Per-Ingvar Brånemark (1929-2014) incorporated the first dental implant in a human patient.[2]

Today, dental implants are widely used in rehabilitation for edentulousness and there are more than 460 different implant system available worldwide,[3] with the *ad modum* Brånemark procedure being the most common implant technique in Sweden.[2]

Due to the increasing number of people rehabilitated with oral implants, it is inevitable that any future human disaster will contain human remains with oral implants to be a feature of the recovered jaws. Despite the increasing popularity of oral implant treatments few studies have investigated the accuracy of establishing person-identity based on intra-oral radiographs of edentulous jaws treated with implants.

In a study by Borrman *et al*, the question was raised whether the accuracy in establishing the identity of persons by intra-oral radiographs was dependent on the observer's field and degree of specialisation in dentistry.[4] In later studies it was concluded that dentist's specialized in oral and maxillofacial radiology had a higher accuracy rate than general practitioners or dentists specialized in other fields.[5-7]

The aims of this study were; *i*) to determine the accuracy by which two intra-oral radiographic examinations taken on separate occasions on patients with edentulous mandibles treated with dental implants could be matched. *ii*) To determine to what extent prosthodontic supra-construction used in implant treatment was of importance in the identification process. *iii*) To determine whether there was a difference between oral and maxillofacial radiologists and dental practitioners not specialized in oral and maxillofacial radiology regarding their ability to match the radiographic examinations.

Because of the lack of dental status in these edentulous cases where only morphological differences and the implant features were used to base the interpretation, it is of interest to know which features the operators used to make their matches. Against this background the study also investigated those features of the intra-oral radiographs that operators most commonly used in making a match.

MATERIALS AND METHODS

The study material was collected from the implant archives at the department of Oral and Maxillofacial Surgery at the University hospital of Umeå (Norrlands universitetssjukhus, NUS), Sweden. Inclusion criteria comprised two intra-oral radiographic examinations taken on separate occasions of patients with edentulous mandibles treated with four implants of Nobel Biocare Brånemark system 3.75 mm. Study material from 59 patients (29 males and 30 females) fulfilled the inclusion criteria.

The radiographic examinations were carried out by different examiners using different X-ray machines. Thus the study material simulated a potential real-life scenario where radiographs taken ante-mortem for placement of implants were compared to radiographs taken post-mortem from a location where decedants were discovered of unknown identity. The mean age of the patients was of 73 years (minimal age 32 years and maximum age 96 years). All of the intra-oral radiographs were anonymized. The study was performed in accordance with the principles of Helsinki Declaration.

Two intra-oral radiographic examinations performed on different occasions from each of 47 patients (23 males and 24 females) served to simulate “ante-mortem” and “post-mortem” material respectively. The radiographic examinations available at placement of the supra-construction were used as “ante-mortem” records and subsequent follow up radiographic examinations taken some time following placement of the supra-construction served as “post-mortem” records. Each radiographic examination comprised up to eight intraoral radiographs.

All four implants were visible in the “ante-mortem” records. Some implants had failed following placement and, as a result, the number of implants demonstrated in the “post-mortem” records varied between two and four. Intraoral radiographic examinations of the remaining 12 patients were added to create a difference in number between the number of “post-mortem victims” and the number of “ante-mortem missing persons” in an attempt to add further rigour to the study. Each “ante-mortem” record was assigned a two-digit number and gender. The “post-mortem” records were each assigned gender and a randomized three-digit number.

The examinations were compiled in two Microsoft PowerPoint presentations (Microsoft PowerPoint 2010, Microsoft Corporation, Redmond, WA, USA), one presenting ”ante-mortem” examinations and the other presenting ”post-mortem” examinations. The two presentations were installed on a computer in a dedicated observation room at the Department of Oral and maxillofacial radiology of NUS. The two presentations could be studied simultaneously and the participants could freely scroll through the records and zoom in and out each radiograph according to their preference. A printed version of all records was also available in the observation room. Three participants were unable avail themselves of the facility of the dedicated observation room and were given the PowerPoint presentations on USB drives for interpretation on their own computers.

Four oral and maxillofacial radiologists (OMR) and four dentists not specialized in oral and maxillofacial radiology (NOMR) were invited to participate in the study. Each participant received written instructions about the study and an answer form in which they were asked to individually link each ”ante-mortem” record to a matching ”post-mortem” record. They were also informed that there were twelve "post-mortem" records with no corresponding "ante-mortem" records.

The participants were instructed to specify on what basis each matching was made; anatomy, fixture, supra-construction (Part Two), or other observation and to grade the confidence of every match on a three-graded scale: "certain", "probable" and "possible".

The study was divided into two parts. In Part One all "ante "and "post-mortem" records had the supra-construction masked The masking was performed in Microsoft PowerPoint using built in drawing tools and ready-made shapes; the criterion was that no part of the supra-construction should be visible, thus the bottom of the masking border was placed in the abutment region of each implant (Fig. 1).In part two, the supra-constructions were visible. This was done to investigate whether the supra-construction is of significance in the identification process. Part Two was carried out following completion of Part one. The same set of records was used both times.

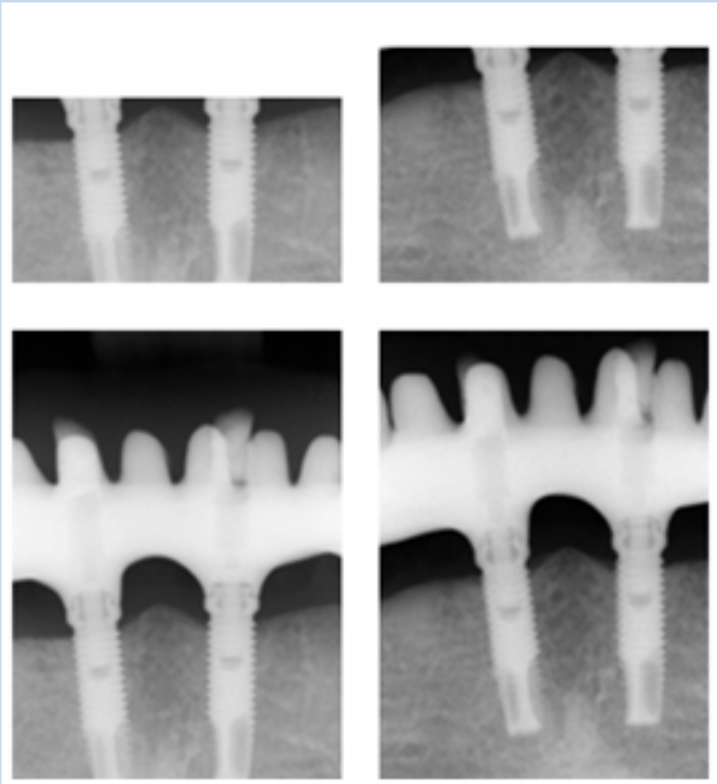


Fig.1: Example of radiographs of the same patient and region from different dates. Upper row; when the supra-construction was masked (Part One). Lower row; when the supra-construction was visible (Part Two). Upper left: masked "ante-mortem". Upper right: masked "post-mortem". Lower left: unmasked "ante-mortem". Lower right: unmasked "post-mortem"

The numbers of correct matches were registered and the results compared using non-parametric tests (Fisher's exact test, McNemar's test). A positive match was considered correct irrespective of graded confidence level. A blank answer was counted as an erroneous match. The level of significance was set at 5 %.

RESULTS

One participant from the NOMR group did not submit any answer form. In total 3 participants from the NOMR group and 4 participants from the OMR group completed the study.

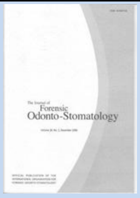
Out of 47 possible matches the average number of correct matches in Part One was 43.8 for OMR and 30.0 for NOMR. The difference between the groups was statistically significant (p = 0,001) (Table 1). One participant from the OMR group did not submit matches for all 47 cases but left 12 cases blank. All other participants submitted complete answer forms.

In Part Two, the average number of correct matches was 46.3 for OMR and 41.3 forth NOMR group. The difference was not statistically significant (p = 0,111). Both groups had a higher accuracy in part two, and the increase in accuracy was statistically significant for both groups.
The largest increase in accuracy was seen in the NOMR group (Table 1).

Table 1 - Number of correctly matched cases (mean, min and max) in the group of Oral and Maxillofacial Radiologists (OMR) and the group of dental practitioners (NOMR) when the supra- structure was masked (Part One) and visible (Part Two) respectively

Category	**Number of correctly matched cases out of 47 (mean, max, min**	**%**	**P-value**
Part One			
OMR NOMR	43.8, (35, 47) 30.0, (21, 38)	93.2 % 63.8 %	0.001[1]
Part Two			
OMR NOMR	46.3, (45, 47) 41.3, (38, 45)	98.5 % 87.9 %	0.111[1]
Difference Part One vs. Part Two OMR NOMR		+ 5.7 % + 37.8 %	<0.001[2] 0.002[2]
[1] Fisher's exact test [2] McNemar's test			

The feature most commonly used for identification in Part One differed between the OMR and NOMR groups. On average, the OMR group most often utilized the bone anatomy as an identification marker (72 % of the cases) whereas the NOMR group utilized the same feature in only 30 % of the cases. Instead of utilizing the



bone anatomy most of the NOMR group used the appearance of the fixture as an identification marker (59 %).

In Part Two, the features most commonly used for identification in the OMR group were, firstly, information relating to bone anatomy (65 %) and, secondly, the design of the supra-construction (59 %). The features most commonly used for identification in the NOMR group were, firstly, information about the fixtures (67 %) and, secondly, the design of the supra-construction (60 %) (Fig. 2).

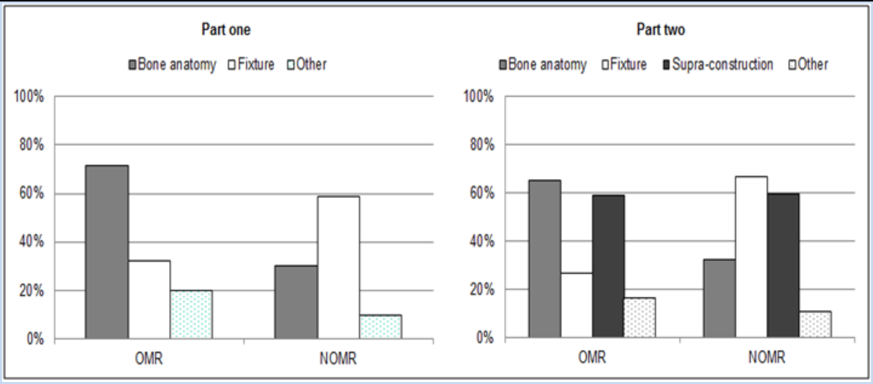


Fig. 2: The proportion of cases in which different features (*i.e.* bone anatomy, fixture, and supra-construction) were used by Oral and Maxillofacial Radiologists (OMR) and dental practitioners (NOMR) when matching cases. Part One; when the supra-construction was masked. Part Two; when the supra-construction was visible

For the feature category named "other", participants reported characteristics such as foreign bodies, gut-feeling and decisions made by elimination method (Fig. 2).

In both Parts One and Parts Two, on average, OMR more frequently graded their matches with certainty than did NOMR. Both groups reported an increased certainty in Part Two. The largest average increase in certainty from Part One to Part Two was seen in the NOMR-group (Fig. 3). All cases reported as "identified with certainty" were correct, except for one assessment in Part One and two assessments in Part Two all submitted by the NOMR group.

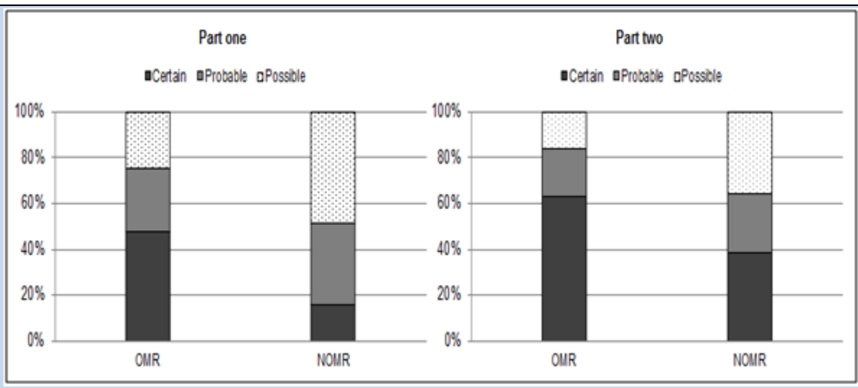


Fig. 3: Proportion of matched cases reported as certain, probable and possible by Oral and Maxillofacial Radiologists (OMR) and dental practitioners (NOMR). Part One; when the supra-construction was masked. Part Two; when the supra-construction was visible

DISCUSSION

The findings of this study suggest that it is possible to match intra-oral radiographs taken on different occasions of edentulous mandibles treated with the same implant system. Access to information about the supra-construction increases accuracy and confidence but is not, according to our findings, essential in order to obtain correct matches.

Dentists specialized in oral and maxilla-facial radiology were superior to dentists not specialized in oral and maxillofacial radiology. In both parts of the study the OMR group showed a higher mean total number of correctly matched cases compared to the NOMR group. In Part One the difference was statistically significant.

An interesting finding was that the OMR group more often utilized the anatomy of bone as an identification marker than did the NOMR group. This was a consistent finding throughout both parts of the study and could indicate that radiologists are more familiar with using and interpreting a wide spectrum of landmarks in intra-oral radiographs. This has previously been discussed by Borrman *et al.*[4, 7]

In Part Two, in the majority of cases, both groups utilized information about the supra-constructions in the identification process, but as described above, the two groups differed in how they collated the information with other features.

The largest increase in accuracy from Part One to Part Two was seen in the NOMR group. This could imply that information about the supra-construction is of greater importance for those with less radiographic experience than it is for dentists specialized in oral and maxillofacial radiology. The participants in the OMR group used bone anatomy, unique for each individual, in the majority of cases as a basis for their matching. This means that that, for the OMR group, important information in the material was present in both parts of the study and could explain the similar results achieved for each of the two parts. Another explanation could be that the OMR group already had a high accuracy in part one, making a large increase in improbable.

Apart from the fact that the participants in the NOMR group achieved a lower success rate in matching the examinations than the OMR group, they were also less confident when matching the cases. It may be assumed that they the NOMR group were uncomfortable and inexperienced in these circumstances. An earlier study has discussed the difficulty of studying oral radiographs in jaws treated with oral implants.[8]

Both groups reported increased confidence in Part Two, suggesting that the visible supra-constructions was an aid in the decision making process. NOMR showed the largest increase in confidence from Part One to Part Two. This might strengthen the idea that information about the supra-construction is of greater importance for dentist with less experience in radiology.

In this study the participants had no information about the time of date for each radiograph. Such information could be of use in the identification process, for instance, to evaluate bone loss and other alterations over time. Therefore it would be desirable to present such information in future studies.

Many of the intra-oral radiographs used in this study were of different brightness and contrast and this fact may have complicated interpretation. Future studies should consider the use of radiographic viewing programmes designed with an option for participants to alter brightness and contrast settings according to preference.

In reality, and in a variety of circumstances when victim identification becomes paramount, it is often possible to take supplementary ”post-mortem” radiographs to better depict the area of interest and to “mimic” more closely the settings and projections of the ante-mortem images.[2] The ethical considerations of this study based on living patients precluded this possibility but, nonetheless, it is plausible to believe that the participants would have obtained an even higher accuracy should this option have been available.

CONCLUSION

This study show that it is possible to correctly match radiographic intra-oral examinations performed on different occasions of edentulous mandibles treated with oral-implants and that dentists specialized in oral and maxillofacial radiology do so with higher accuracy than dentists not specialized in oral and maxillofacial radiology.

This suggests that oral and maxillofacial radiologists could potentially be a valuable resource in future identification occasions where dental implants were a feature of the post-mortem dental records. Due to the increasing number of people rehabilitated with oral implants, it is inevitable that any future human disaster identification process will contain human remains where oral implants are a feature of the recovered jaws.

ACKNOWLEDGEMENT

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SECTION IDENTIFICATION

Behavior In Vitro Of The Dentin-Enamel Junction In Human Premolars Submitted To High Temperatures: Prediction Of The Maximum Temperature Based On Logistic Regression Analysis

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The authors declare that they have no conflict of interest.

ABSTRACT

Objective: The aim of the study was to provide scientific evidence that would permit DEJ separation to be used as a parameter to estimate the temperature to which burnt, carbonized or incinerated cadavers or human remains had been subjected. Materials and methods: A descriptive pseudo-experimental study was carried out in vitro using cone beam tomography to determine the physical behavior of the dentine-enamel junction in 60 human premolars submitted to high temperatures (200°C, 400°C, 600°C, 800°C and 1000°C). Results: Spearman's concordance and correlation index was used to determine the relationship between longitudinal separation of the dentine-enamel junction (mm) and temperature (°C) and a simple linear regression model developed to show that once micro- and macrostructural changes are initiated in the enamel and dentine. Conclusions: The dentine-enamel junction begins to separate from the cervical towards the occlusal as temperature increases.

KEYWORDS: forensic science, forensic odontology, dental tissues, dentine-enamel junction, high temperatures, medico-legal documentation

INTRODUCTION

In the past five years increasing numbers of studies have described changes in dental tissues and dental materials to show repetitive patterns and allow correlation between in vitro and in vivo observations. The objective of these studies has been to generate useful markers for forensic odontological identification and documentation of medico-legal autopsies of individuals who have died as a result of the high temperatures generated in, for example, catastrophic fires, car and airplane crashes, terrorist attacks, bombings, post-mortem cremation to impede identification of the individual and ante-mortem cremation as a method of torture.

Teeth are able to resist temperatures of up to 1200°C without important loss to the dental macrostructure[1]; complete cremation of a cadaver between 700 – 800°C for 1 hour or more is reported in the literature without loss to the dental macrostructure of the teeth[2]. However there have been few investigations into the changes that occur in the dental tissues when teeth are submitted to high temperatures. The earliest studies focused principally on the macroscopic description of changes in color, fissures and cracks, fragmentation and bursting of the mineralized dental tissues[3-6]. They did not identify microscopic markers with sufficient scientific reliability to allow these results to be applied to the documentation of medico-legal autopsies during the identification of cadavers or burnt, carbonized and incinerated human remains.

However, in some of these studies it was reported that as temperatures increased enamel separated from dentine at the level of the dentine-enamel junction (DEJ)[5,7]. This finding has been associated with combustion of the organic matrix (a non-fibrillar component consisting of glycoproteins, glucosaminoglycans and proteoglycans, together with a fibrillar-collagen component) and physical-chemical changes in the inorganic component (crystals of calcium hydroxyapatite) of the enamel and dentine, producing reduction in the tissue volume and loss of continuity solution in the DEJ.

The purpose of the present study was to use cone beam tomography to determine the in vitro behaviour of DEJ in human premolars submitted to high temperatures so that a predictive marker of the maximum temperature to which the tooth had been subjected could be established. This marker could be used for forensic odontological identification in medico-legal necropsies

MATERIALS AND METHODS

This was a cross-sectional, pseudo-experimental and descriptive study in vitro that used cone beam tomography to describe the behavior of the DEJ in human premolars, to determine whether a causal relationship exists between the phenomenon of enamel-dentine separation and high temperatures. The null hypothesis was that human premolars, on being subjected in vitro to high temperatures, did not present significant changes in the DEJ.

SAMPLE

Once authorization was obtained from the Human Ethics Committee of the Universidad del Valle according to the Declaration of Helsinki, 60 premolar teeth were collected. The teeth were obtained from patients attending the oral and maxillofacial surgery clinics of the Universidad del Valle for orthodontic extractions. All of the teeth were caries free, fracture free and had not been subject to previous dental treatment

The teeth were washed with non-sterile water to eliminate the remains of blood and tissues, and then immersed in 5% chloramine T (100 g sodium tosylchloramide diluted in 2L distilled water) for fixing. The teeth remained in chloramine T for 1 week and were subsequently immersed in saline solution at room temperature to maintain conditions of 100% humidity according to the norms of ISO/DIS 11405:2003[8].

Finally the teeth were classified and distributed randomly into different groups that would be subjected to a range of high temperatures. As a control group 10 teeth were not submitted to high temperatures to establish criteria of normality (Table 1).

Table 1. Classification and distribution of the sample

Groups	Control	Intervention				
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Temperature	0°C	200°C	400°C	600°C	800°C	1000°C
Number of teeth	10	10	10	10	10	10

APPLICATION OF HIGH TEMPERATURES

Teeth of the intervention group were placed individually in trays coated with Cera-fina® Whipmix® and introduced in groups of 10 into an earthen oven (Thomas® Small Benchtop 1256®) previouslv calibrated to five different ranges of temperature (200ºC, 400ºC, 600ºC, 800ºC, 1000ºC) with a rate of increase of 10ºC per min from an initial temperature of 28ºC until each of the test temperatures was reached. For example, 10 teeth corresponding to the 200ºC group were exposed to a temperature range of 28 - 200ºC, the oven was allowed to cool to room temperature and the teeth removed. Ten teeth of the 400ºC group were then subjected to a temperature range of 28 - 400ºC, the oven allowed to cool as before and the teeth removed. The same procedure was used in succession for the 600ºC, 800ºC and 1000ºC groups. This protocol was standardized by the Oral and Maxillofacial Surgery Study Group of the School of Dentistry at the Universidad del Valle[7].

The in vitro model considered was carried out in an oven rather than in a direct flame against the background that in the various literature reports the maximum temperature reached was 1000°C over a period of 25-30 min and then subsequently falling to 500°C. At this stage all the oxygen had been consumed by combustion and the organic materials reduced to carbon (carbonization) or until the compounds of calcium, phosphates, silica or other oligoelements were sinterized (incineration)[9]. This in situ "muffler effect" is comparable to the changes undergone by the perioral tissues and facial musculature, as well as the bone, dental and periodontal tissues[3].

MEASUREMENTS

After being submitted to high temperatures the teeth were embedded in acrylic autopolymerized resin (New Stetic®) to facilitate their manipulation in the tomograph i-CAT® 3-D Cone Beam Dental Imaging System. The tomographs were taken longitudinally (in the buccal-lingual plane), with a voxel size of 0.2, exposure time of 14.7 s, tomographic section thickness of 1 mm, distance between sections of 1 mm, real-size images of 1:1 and 0% transverse distortion.

A preliminary study was carried out with five premolars which were each submitted to a specific temperature (200°C, 400°C, 600°C, 800°C and 1000°C respectively) with one premolar as a control. This was done in order to calibrate the observation equipment and standardize it for the observer according to the behavior of the dental tissues (enamel and dentine) and

DEJ at each specific temperature. The expert and observer used i-CAT® 3-D Dental Imaging System software to measure the length of the DEJ separation, represented by a radiolucent space between the enamel and dentine, from the most apical enamel of the cervical third of the tooth up to the longitudinal projection in the occlusal direction. In the same way the level of occlusal separation was correlated with the coronal thirds (cervical, medial and occlusal) of each sample (Figure 1). These measurements were used to estimate the degree of concordance by means of the intra-class correlation coefficient using the Epidat® 4.0 software and assuming a confidence level of 95.0%. The results show a correlation of 0.9455 for the inter-observer criterion (observer vs. expert) and 0.9658 for the intra-observer criterion (observer vs. observer). The values 0 = no correlation, 1 = direct correlation and -1 = inverse correlation were used as references.

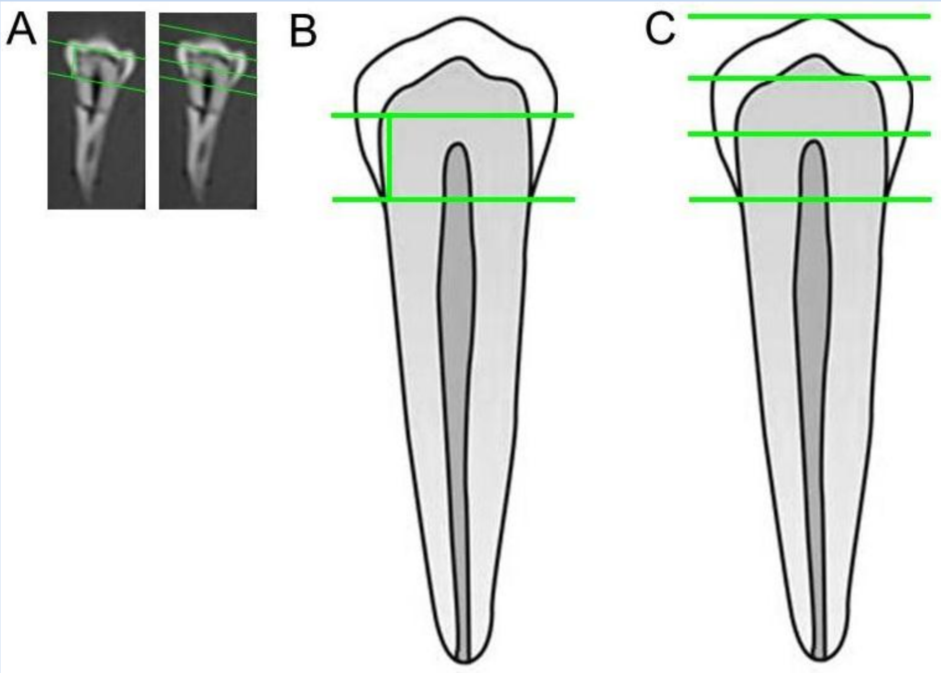

Fig. 1: Measurement of the extension of the radio lucid band that is compatible with the length of the separation of the DEJ from the cervical region to the occlusal region, and its correlation with coronal dental thirds. A. Cone Beam Tomography of a tooth subjected to high temperatures; B. A premolar tooth diagram in which the measurement taken of DEJ from the cervical region to the occlusal region is observed; C. A premolar tooth diagram in which the coronal dental thirds are observed.

STATISTICAL ANALYSIS

The measurements for DEJ separation in the cervico-occlusal direction were processed with the software IBM SPSS Statistics® 21 by means of descriptive (measurements of central tendency) and non-parametric statistics. The a priori plan of analysis involved systematization of the variables (Table 2) and analysis of satisfaction of the assumed statistics to determine whether the variables were dependent or independent. The non-parametric Kolmogorov-Smirnov test was used to evaluate normality of the sample distribution, with $P > 0.05$ being taken as normal. Levene's test was also used to contrast the homogeneity of variances, the

null hypothesis being P >0.05 when variances are equal.
Taking the temperature as an independent variable and the extent of the right and left sides of the DEJ separation and the coronal thirds as dependent variables, the Kolmogorov-Smirnov test confirmed the null hypothesis that there is normality in the extent of the right side of the DEJ separation (P = 0.886) and that of the left side of the DEJ separation (P = 0.442). Levene's test indicated that the alternative hypothesis could be accepted because there was inequality of variances in the extent of the right (P = 0.015) and left (P = 0.002) sides of the DEJ separation.

Spearman's correlation coefficient determines the level of association or dependence between an ordinal and numerical variable (in this case temperature and extent of the right and left sides of the DEJ separation), or between two ordinal variables (temperature vs. right and left coronal thirds). Interpretation of Spearman's correlation coefficient indicated that the values here varied between -1 and +1 (the more the coefficients approached a value of a +1, the greater the degree of correlation between the associated variables; 0 signified that no correlation existed). The null hypothesis corresponded to P > 0.05 when there was no correlation.

Finally a simple linear regression model was developed in which the extents of the right and left sides of the DEJ separation (numerical dependent variables) were analyzed as a function of the temperature (independent ordinal variable) to which the teeth were subjected. The null hypothesis corresponded to P > 0.05 when the model was not applicable.

RESULTS

When a tooth is exposed to high temperatures it may undergo structural changes that depend the maximum temperature reached, so that it may remain intact at 200°C, burnt at 400°C (change of color and formation of fissures and cracks), carbonized at 600°C (reduced to carbon by incomplete combustion), incinerated between 800°C and 1000°C (reduced to ashes), and finally explodes at 1200°C (radicular and coronal burst)[4-7].

With respect to the junction between the enamel and the dentine, at 200°C no macro-structural changes were observed that affected the DEJ when teeth were compared before and after being submitted to high temperatures, apart from a loss of shine (opacity of the enamel) associated with dehydration (Figure 2A). At 400°C the enamel turned brown and opaque (revealing the coronal dentine that presented signs of combustion of the extracellular organic matrix); while in the cervical region loss of continuity of the enamel was observed due to longitudinal and transverse fractures, as well as its separation from the dentine, indicating loss of DEJ continuity (Figure 2B). At 600°C the enamel became chalk-white due to combustion of the inorganic matrix; the coronal dentine that was visible through the fragmented enamel appeared grayish due to the change of phase from carbonized to incinerated while in the cervical region fragmentation of the enamel could be observed due to deepening of surface cracks and fissures, suggesting loss of DEJ continuity (Figure 2C). At 800°C the incinerated enamel assumed a grayish color since the coronal dentine became transparent in certain regions even when carbonized, while in the cervical region greater separation of the enamel from the dentine was observed due to loss of DEJ continuity (Figure 2D). Finally, at 1000°C the crowns of the teeth turned white due to incineration of the enamel and dentine. DEJ separation was much more obvious in the cervical region due to bursting and separation of the enamel, as well as the reduced volume of the root compared to that of the crown (Figure 2E).

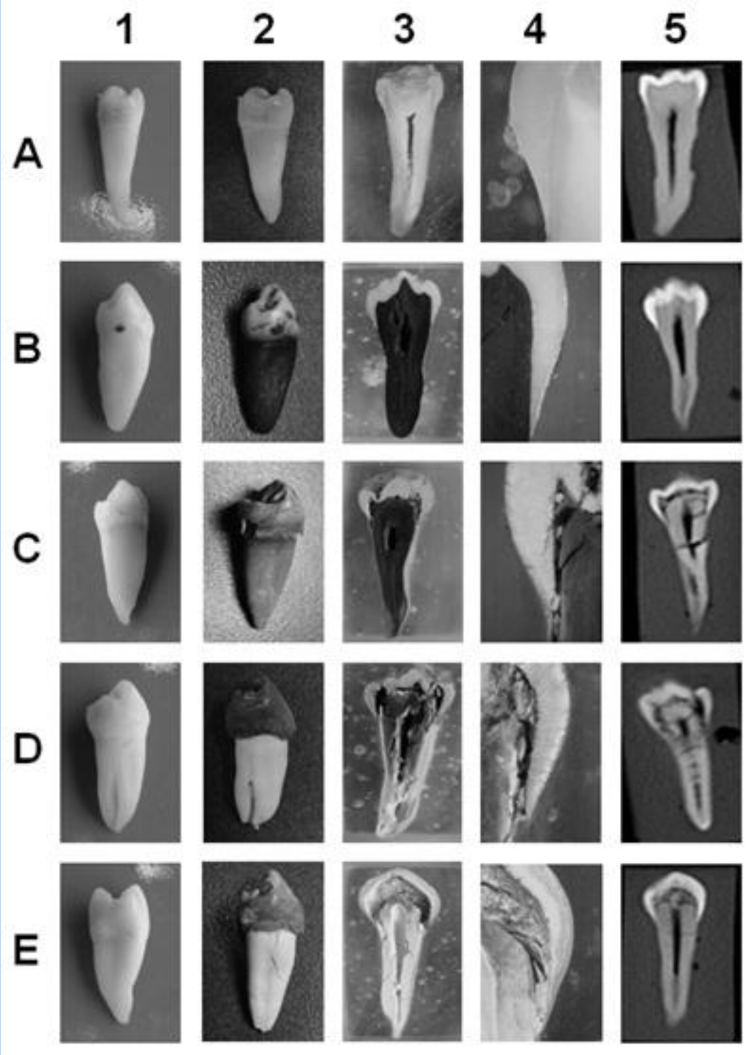

Fig. 2: Premolars subjected to high temperatures. A. 200°C; B. 400°C; C. 600°C; D. 800°C; and E. 1000°C. 1. Photograph of a tooth before being subjected to high temperatures; 2. Photograph of a tooth after being subjected to high temperatures; 3. Sagittal section of a tooth placed on acrylic resin and after being subjected to high temperatures; 4. Details of the separation of the DEJ in a tooth submitted to high temperatures and placed on acrylic resin; and 5. Cone Beam Tomography of a tooth subjected to high temperatures; a radio lucid band is seen to illustrate the separation between the enamel and dentine at the DEJ.

Based on these results Spearman's coefficient of correlation and concordance supported the alternative hypothesis, since there were dependent correlations between temperature and the extent of the right and left sides of the DEJ separation (P = 0.000). A dependent correlation was observed between the extent of right side of the DEJ separation with the coronal right (P = 0.000) and left (P = 0.002) thirds and the extent of the left side of the DEJ separation with the coronal right (P = 0.001) and left (P =0.002) thirds. Dependent correlations were also observed between temperature and the right (P = 0.001) and left (P = 0.002) coronal thirds (Table 3).

The simple linear regression model (adjusted using the R2 determination coefficient) showed that separation of the DEJ on the right is explained by a 40% change in temperature and that of the left side by a 64% change, allowing us to accept the alternative hypothesis (P = 0.000). In the same way it was determined that for each 200°C rise in temperature, DEJ separation widened from 0.72 - 0.76 mm (Table 4). Thus the simple linear regression model developed in this study is valid and useful, the null hypothesis being rejected in all cases.

Table 2. Systematization of variables

Variables	Definition	Type	Measurement Scale	Indicator
Temperature.	Independent variable	Ordinal	Qualitative	Temperature in °C at which separation of the DEJ is observed.
Separation of the right side DEJ.	Dependent variable	Numeric	Quantitative	Separation of the DEJ in mm from the most apical side of the enamel.
Separation of the left side DEJ.	Dependent variable	Numeric	Quantitative	Separation of the DEJ in mm from the most apical side of the enamel.
Right coronal third.	Dependent variable	Ordinal	Quantitative	Longitudinal region of the crown that involves separation of the DEJ.
Left coronal third.	Dependent variable	Ordinal	Qualitative	Longitudinal region of the crown that involves separation of the DEJ.

Table 3. Spearman´s correlation coefficient

Variables	Temperature	Separation of the right side DEJ	Separation of the left side DEJ	Right coronal third	Left coronal third
Temperature	1.000				
Separation of the right side DEJ	0.069 *P* = 0.000	1.000			
Separation of the left side DEJ	0,832 *P* = 0.000	0.610 *P* = 0/000	1.000		
Right coronal third	0.642 *P* = 0.000	0.874 *P* = 0.000	0.584 *P* = 0.000	1.000	
Left coronal third	0.687 *P* = 0.000	0.564 *P* = 0.000	0.803 *P* = 0.000	0.556 *P* = 0.000	1.000

DISCUSSION

Moreno et al[10] argued that separation of the DEJ began at 200°C, becoming more evident at 400°C with bursting of the cervical enamel. At 600°C and 800°C they described the complete separation between enamel and dentine at the level of the cervical and median thirds. Finally between 1000°C and 1200°C the enamel fragmented and separated totally from the dentine like a cap. The authors concluded that this fracturing phenomenon at the DEJ level could be associated with alteration of the extracellular matrix of both mineralized tissues. Enamel has a high inorganic content (96 - 99%) composed of a large quantity of calcium phosphate in the form of hydroxyapatite crystals, so that on being subjected to high temperatures it rapidly loses its scarce inorganic content, producing a strong contraction that induces changes in the organization of these crystals. Dentine has a high inorganic content and contains 12% water. It is protected by enamel in the crowns of the teeth, permitting a certain margin of thermal contraction compared to enamel, causing the latter to fracture at the DEJ level and separate at temperatures of over 400°C.

Although other authors have described this phenomenon none have explained it micro-structurally (4). Ferreira et al (11) pointed out that significant differences exist in the separation of the enamel and dentine at the level of the DEJ. Aramburo et al[12] and Vasquez et al[13] observed that this pattern of fracture between the enamel and dentine at the DEJ level is gradual as temperature increases.

In the present study, the spontaneous separation of the enamel and dentine by the action of temperature behaved as reported in the literature. At 200°C, although no tomographic changes were observed, there was combustion of the organic matrix and cohesive failures of the dentine in proximity to the DEJ. The process of carbonization of the mineralized tissues was initiated between 400°C and 600°C; the enamel rapidly lost its scarce inorganic content and hydroxyapatite crystals gradually increased in size and fused with each other (synterization). The dentine lost volume through dehydration and combustion of the inorganic component. This difference in the dimensional contraction of the dentine is the reason why the enamel separates at the level of the DEJ, initially in the cervical third of the tooth and then gradually up to the medial third of the tooth. This can be observed in tomographs as a radiolucent band. Finally between 800°C and 1000°C, the enamel is totally compacted and the dentine gradually begins the process of incineration of its inorganic component and synterization of the scarce inorganic component. Contraction of the dentine is much more evident and the enamel separates completely allowing a much wider radiolucent band to be observed in the tomographs.

Using cone beam tomography it was possible to determine that a gradual separation of the DEJ moving in a cervical/ occlusal direction was initiated between 200°C and 400°C and became more obvious as the temperature increased until total separation was reached at 1000°C. This constant repetitive phenomenon allowed correlation of the separation of the DEJ in the different coronal thirds as a function of temperature, thus making it possible to predict the behavior of DEJ separation as temperature increased. Thus, Spearman's correlation coefficient showed a relationship between temperature, extent of the separation of the DEJ on the right and left sides and the coronal right and left thirds. In this way the simple linear regression model predicts that on increasing the temperature DEJ separation advances from the cervical third progressively up to the occlusal third at approximately a rate of 0.72 - 0.76 mm per 200°C.

CONCLUSIONS

This in vitro study demonstrates that significant changes occur in the DEJ of human premolars when they are submitted to high temperatures in vitro, and that longitudinal separation of the DEJ constitutes a useful parameter to estimate the temperature to which a tooth has been subjected.

This research may eventually contribute to the documentation of medico-legal autopsies in cases of identification where forensic odontology is used and where decedents have been subject to burning, carbonization or incineration. The collection and analysis of a tomography, could demonstrate the behavior of the DEJ and measure the separation of the enamel and dentine represented by the radiolucent space. The features of this radiolucent space could contribute to the estimation of the temperature to which the tooth had been subjected; below 200°C the DEJ shows no changes, between 400°C and 600°C it is separated in the cervical third of the tooth, and between 800°C and 1000°C it is separated in the occlusal third of the tooth together with other changes such as the complete separation of the enamel in the form of a cap.

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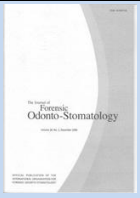

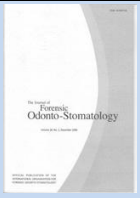

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SECTION IDENTIFICATION

Discrimination Potential of Root Canal Treated Tooth in Forensic Dentistry

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The authors declare that they have no conflict of interest.

ABSTRACT

Forensic Odontology is a vital component of forensic science and one branch involves the application of dental science to the identification of unknown human remains. The aim of this study is to investigate the discriminatory potential for identification of the radiographic morphology of obturated single root canals. Thirty periapical radiographs of patients having endodontic treatment of single rooted canals were selected randomly from the data bank of the digital X- ray system present in the restorative department, University of Science and Technology, Sudan. The post-operative radiographs were considered as an ant-mortem data "Set 1". Ten radiographs from the thirty were reprinted, labelled from (A-J) and considered as a post-mortem data "Set 2". This post-mortem group of 10 radiographs "Set 2" would be compared with the ante-mortem group of 30 radiographs comprising "Set 1". These two sets of radiographs would be examined by 40 dentally trained personnel. The thirty radiographs comprising "Set 1" and the 10 radiographs comprising "Set 2" were provided to each of the examiners who were asked to match the individual post-mortem radiographs ("Set 2") with the ante-mortem radiographs ("Set1"). The result demonstrated that 34 examiners achieved a success rate of 100%, 4 examiners achieved a success rate of 97.5% (1 mismatch) and 2 examiners achieved a success rate of 95% (2 mismatches). The radiographic images of obturated single-rooted teeth in this study were shown to have highly- specific morphological features. It is proposed that, in cases where the ante and post-mortem radiographs of a single-rooted obturated canal show similar morphology, this commonality of morphology can be used as a tool in the identification process.

KEYWORDS: root canal treatment, identification, forensic dentistry.

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INTRODUCTION

Dental Comparison is the main method used for the identification of victims in cases of mass disaster where there are large numbers of victims. Forensic dentistry plays a basic role in the identification of individuals who cannot be identified by routine methods.[1] Forensic dental identification depends mainly on the recognition of common concordant features by comparison of ante-mortem and post-mortem dental records with no irreconcilable differences demonstrated between the two sets of records. [2]

The vital role of Forensic Odontology in identification is based on the unique characteristics and arrangements of the teeth of different individuals. [3] Although establishment of individual identity by the use of forensic odontology had been extremely useful and reliable, it is totally dependent on the presence of ante-mortem records. [4]

Keiser-Nielsen assigned 12 concordant features between ante-mortem and post-mortem dental records to establish positive identification; [2] although this is not an accepted universal standard. Keiser-Nielsen considered an extraordinary feature occurring in more than 10% of circumstances, could consider as a unique feature to make a positive identification. [2] Obtaining ante-mortem dental information from written dental records is commonly used but it is unreliable as a “stand alone” method because of the possibility of the inclusion of recording errors or misinterpretation. Capturing and recording ante-mortem dental data, including the morphological features of teeth, surrounding structures and physical details before and after any dental treatment is well recognised as being an optimal way to preserve dental information. Dental radiographs are ideal as one of the tools for this purpose. The radiographic images captured from a patient can be duplicated precisely in the future should this prove necessary and the process of duplication is non-operator specific. Against this background radiographic imaging became an extremely significant role in the comparison process in personal forensic identification. [5]

Dental radiology has played a key role in the identification of victims in many cases. In 1973, the identification of 73% of the 35 burned victims in Hotel Hafnia, Denmark was achieved by eight dental surgeons forming part of the identification team. [6] The identification of American victims of Operation Desert Storm was largely dependent on forensic dental radiology. 244 out of the 251 victims were identified based on the availability of dental records including panoramic radiographic images. [7]

Several studies using dental radiographs are recorded regarding the successful use of dental restorations for the purpose of identification. In relation to the pattern of the amalgam restoration, the measure of uniqueness of patterns of amalgam restoration in the upper and lower dentition was investigated by Philips who found that patterns of amalgam restoration in the first molar were relatively common and therefore had a low measure of uniqueness. However if the pattern of the amalgam restoration in the first molar was combined with the patterns in one or more other teeth, then uniqueness increased markedly and improved the likelihood of identification of that person. [8]

In a study by Borman and Grondahi (1990), the radiographic appearance of teeth and restorations of two sets of bitewing radiographs were compared by seven dentally-trained observers. The question asked was whether the radiographic image of a single compound amalgam restoration

in a posterior teeth was unique. All seven observers were asked to identify all of the cases where simple restorations were present. The results showed that mistakes were made by a total of five of the seven observers. [9]

The relatively recent trend for aesthetic dentistry has resulted in the introduction of tooth-coloured composite materials to replace amalgam. This has opened up a new area of research regarding the radiographic assessment of composite materials for purposes of identification. Nonetheless if it can be demonstrated that both the ante and post-mortem radiographs of a single composite restoration in the same tooth show the same morphology, this uniqueness can be used for purposes of identification. [10, 11]

Post-operative endodontic radiographs provide a rich source of information that may be a unique identifying feature for a particular tooth and individual. This is because of the rare frequency of variation between the radiographic appearance of an endodontic filling compared to that of an intra- coronal restoration. [5]

Bonavilla et al (2008) conducted a study to evaluate which endodontic obturation materials were capable of withstanding high temperatures such as those to which an incinerated victim would have been exposed. This information was used to compile a database regarding the use of root canal filling materials as an aid in forensic identification. [12]

Savio C. et al carried out a study to evaluate the radiographic appearance of unrestored teeth, restored teeth and endodontically treated teeth after exposure to different ranges of high temperatures. The outcome showed significant retention of radiographic appearance and features of endodontic treatment were recognizable up until 1100 C. [13]

The aim of this present study is to investigate the discriminatory potential of the radiographic appearance of obturated single root canals for purposes of identifications.

MATERIALS AND METHODS

This was a cross-sectional hospital-based study conducted in the dental hospital of the Faculty of Dentistry, University of Science and Technology in Sudan. Thirty periapical radiographs of patients having endodontic treatment of single-rooted canals were selected randomly from the data bank of the digital x- ray system present in the restorative department. The post-operative radiographs were printed, the crown area was cut from the radiographs, labelled from (1 – 30) and considered as an ant-mortem data “Set 1” (Figure 1, 2).

Ten radiographs from the thirty were reprinted, labelled from (A-J) and considered as a post-mortem data “Set 2” (Figure 3). This post-mortem group of radiographs “Set 2” would be compared with the thirty radiographs comprising “Set 1”.

Fig.1: A sample radiograph represents set (1)

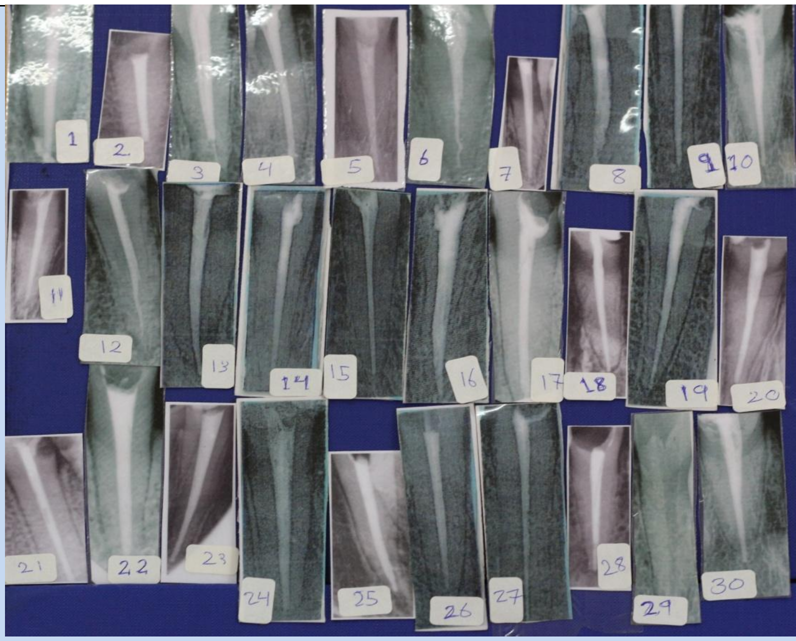

Fig.2: Radiographs represent ante-mortem labelled from (1-30)

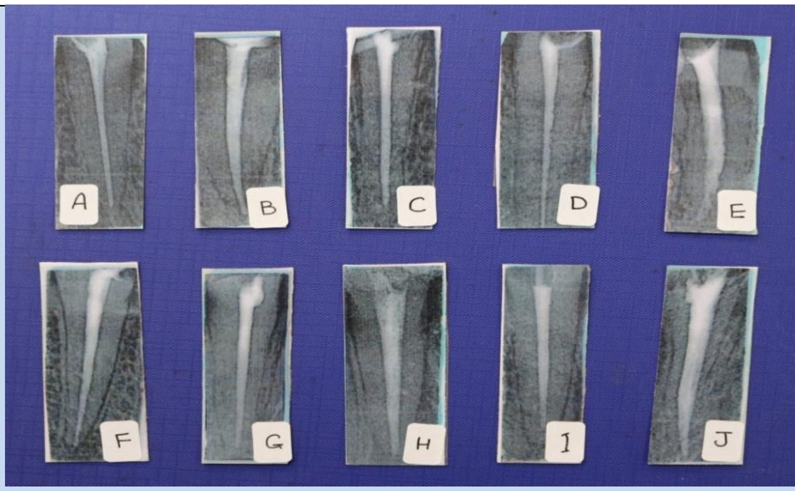

Fig.3: Radiographs represent post-mortem labelled from (A-J)

Both sets of radiographs were examined by 40 dentally trained personnel. The thirty radiographs comprising "Set 1" and the 10 radiographs comprising "Set 2" were supplied to each of the examiners who were required to match the individual post-mortem radiographs ("Set 2") with the ante-mortem radiographs ("Set1"). The examiners included three Maxilla-Facial Surgeons, one Periodontist, two Orthodontists, two Endodontists, three Paedodontists, three Prosthdontists, thirteen General Practitioners, nine Dental Assistants and four Dental Laboratory Technicians. Their success rates of matching the radiographic images were recorded.

Descriptive statistical analysis was performed using SPSS version 21 to obtain the means and standard deviations of the scores of the examiners. The One way ANOVA Test was used to compare the mean scores among the different categories of participants involved in the study. This research was approved by the Research and Ethics Committee of the Faculty of Dentistry, University of Science and Technology, Sudan.

RESULTS

34 examiners achieved a success rate of 100%, 4 examiners achieved a success rate of 97.5% (1 mismatch) and 2 examiners achieved a success rate of 95% (2 mismatches). Of the four examiners who achieved a success rate of 97.5% (1 mismatch) two were Specialists (one Maxillo-Facial Surgeon and one Endodontist) and two were Auxiliary Staff (one Dental Assistant and one Laboratory Technician). Of the two examiners who achieved a success rate of 95% (2 mismatches) both were Specialists (Maxillo-Facial Surgeon and Endodontist). (Table 1)

The mean score of the participants was 9.78 $\pm$ 0.53. The minimum gained score as 8 while the maximum was 10. The most frequent score was 10. (Table 2)

Based on the category of the observers, the percentage of full identification among General Dentists was the highest (100 %), followed by Auxiliary staff (84.6%). Specialists had the lowest score (71.4%). (Table 3)

Statistical analysis showed a significant difference in the mean score of Specialists when compared with that of General Dentists and other Auxiliary Staff (P = 0.038). The mean score of the Specialists was significantly lower than that the General Dentists and Auxiliary Staff (Dental Assistants and Laboratory Technicians). The General Dentists achieved the highest mean score of 10. (Table 4)

Table 1: The Results of the Examiners

Examiner	Specialty	Score out of 10
1	Maxillofacial surgeon	8
2	Maxillofacial surgeon	9
3	Maxillofacial surgeon	10
4	Orthodontist	10
5	Orthodontist	10
6	Paedodontist	10
7	Paedodontist	10
8	Paedodontist	10
9	Endodontist	9
10	Endodontist	8
11	Prosthdontist	10

12	Prosthdontist	10
13	Prosthdontist	10
14	Periodontist	10
15	General dentist	10
16	General dentist	10
17	General dentist	10
18	General dentist	10
19	General dentist	10
20	General dentist	10
21	General dentist	10
22	General dentist	10
23	General dentist	10
24	General dentist	10
25	General dentist	10
26	General dentist	10
27	General dentist	10
28	Dental assistant	10
29	Dental assistant	10
30	Dental assistant	9
31	Dental assistant	10
32	Dental assistant	10
33	Dental assistant	10
34	Dental assistant	10
35	Dental assistant	10
36	Dental assistant	10
37	Laboratory technician	10
38	Laboratory technician	10
39	Laboratory technician	9
40	Laboratory technician	10

Table 2: General Score Statistics

Mean	9.78
Median	10
Mode	10
Std. Deviation	0.53
Minimum	8
Maximum	10

Table 3: Identification successful rate among participants

Category	Percentage (%)
Specialist	71.4
General Dentist	100
Auxiliary staff	84.6

Table 4: Comparison of scores between professions

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P value
					Lower	Upper			
Specialist	14	9.6	0.76	0.203	9.06	9.94	8	10	0.038*
General Dentist	13	10	0	0	10	10	10	10	
Auxiliary staff	13	9.85	0.376	0.104	9.62	10.07	9	10	
Total	40	9.78	0.53	0.084	9.61	9.94	8	10	

*P-*value is significant*

DISCUSSION

This study demonstrated that 34 out of the 40 dentally trained examiners were able to match the 10 radiographic images of obturated single-rooted canals from "Set 1" and "Set 2" of the same tooth with a success rate of 100%. This group of 34 represented 85 % of the total of the sample size of examiners who participated in the study. Statistically the chance of being able to correctly match the 10 radiographs from "Set1" (30 radiographs) to their counterparts in "Set 2" (10 radiographs) represents a chance in 1 in 30,045,015 [8] and it is unlikely that this outcome could be ascribed to chance. The indication was that the discriminatory characteristics of the radiographic images of obturated single root canals is so significant that it could be unique and used as a tool for purposes of identification.

This finding confirmed that endodontically-treated teeth are an important tool for purposes of identification. [5] Many other studies have also highlighted the importance of radiography in human identification by, for example, comparison of trabecular bone patterns, comparison of the morphology of the frontal sinus and comparison of the bones of the maxilla. [14, 15] It is proposed that an endodontically-treated tooth should be considered as a comparative ante and post-mortem landmark for purposes of identification.

Based on the category of the observers, the percentage of full identification among General Dentists was the highest (100 %), followed by Auxiliary staff (84.6%). Specialists had the lowest score (71.4%). (Table 3) Moreover, the specialists had significantly the lowest mean score among all participants (p value). This suggests that comparing the general pattern of the obturated canal and the surrounded bone in ante and post-mortem radiographs might depend more on general outlines rather than the presence of detailed features upon which Specialists can tend to focus.

It is common practice that, during forensic comparison between radiographic images, the similarities of the features that are common in both images are compared. [5] Failure to match the radiographs by some of the examiners could be as a result of different positioning of the tube of the X-ray machine when the ante and post-mortem X-rays were captured and the consequent difference between the orientation of the images. [5] Further research is needed.

The results of this study demonstrate that the morphology of an obturated single root canal is easily identifiable by comparison of ante and post-mortem radiographs. Obturation of single-rooted teeth using gutta-percha creates a unique pattern that can be easily recognised using radiographs. This study also suggests that it is highly unlikely for two obturated single-root canals to have exactly the same radiographic appearance.

CONCLUSION

The radiographic images of the obturated canals of single-rooted teeth in this study were shown to have highly specific morphological features that could act as a potential aid for purposes of identification. The discriminatory potential of the unique morphology of the obturated canal of a single-rooted tooth could be used for evidence-based decision making in Forensic Dentistry.

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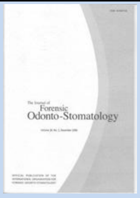

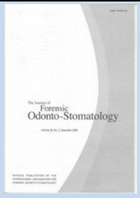

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SECTION BITE MARKS

Three-dimensional Validation of the Impact of the Quantity of Teeth or Tooth Parts on the Morphological Difference Between Twin Dentitions

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ABSTRACT

BACKGROUND: The number of teeth involved in cases of bite-mark analysis is generally fewer in comparison to the number of teeth available for cases of dental identification. This decreases the amount of information available and can hamper the distinction between bite suspects. The opposite is true in cases of dental identification and the assumption is that more teeth contribute to a higher degree of specificity and the possibility of identification in these cases. Despite being broadly accepted in forensic dentistry, this hypothesis has never been scientifically tested.

OBJECTIVE: The present study aims to assess the impact of the quantity of teeth or tooth parts on morphological differences in twin dentitions.

MATERIAL AND METHODS: A sample of 344 dental casts collected from 86 pairs of twins was used. The dental casts were digitized using an automated motion device (XCAD 3D® (XCADCAM Technology®, São Paulo, SP, Brazil) and were imported as three-dimensional dental model images (3D-DMI) in Geomagic Studio® (3D Systems®, Rock Hill, SC, USA) software package. Sub samples were established based on the quantity of teeth and tooth parts studied. Pair wise morphological comparisons between the corresponding twin siblings were established and quantified.

RESULTS: Increasing the quantity of teeth and tooth parts resulted in an increase of morphological difference between twin dentitions. More evident differences were observed comparing anterior vs. entire dentitions ($p<0.05$) and complete vs. partial anterior dentitions ($p<0.05$).

CONCLUSION: Dental identifications and bite-mark analysis must include all the possibly related dental information to reach optimal comparison outcomes.

KEYWORDS: forensic dentistry, bitemark, dental identification, morphology, 3D morphometric comparison

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INTRODUCTION

Bitemarks are patterned impressions of human[1] or animal[2] teeth on skin[1], objects[3] or foodstuffs[4]. Bitemark analysis involves a comparative procedure to match dentitions of potential suspects with the associated patterned mark or injury[1,3,5,6]. In cases of dental identification, ante-mortem (AM) dental records of a known person are compared with post-mortem (PM) dental records of an unknown person in an attempt to identify similarities between both sets of records[3]. Both bitemark analysis and dental identification rely on the quality and quantity of the available dental evidence. In bitemark analysis, the quality and quantity of the evidence are dependent upon the nature of the injury itself. Information can also be extrapolated from the teeth once the injuries are shown to be dental patterned marks[1].

The quality of the dental evidence is not only related to standards for the registration of images of the patterned mark but also to classification and analysis. Specifically, a higher quality of analysis is achieved using three-dimensional (3D) registration of dental evidence as opposed to the use of two dimensional (2D) imaging technology[7]. Moreover, evidence based on tooth morphology will be more prevalent than that based on dental treatment and pathology in the near future[7], becoming more important for the identification of victims (dental identification) and suspects (bitemark analysis). Dental evidence is more useful when combining information from different teeth[3,8]. In this context, it has always been assumed that the quality of the evidence is directly related to the amount of teeth and tooth parts available for analysis.

Bitemark analysis is generally performed using 2D image registration[1,3]. However, it is also feasible in 3D, with surface scanning[9] and photogrammetry[10].

The evidence registered is essentially based on tooth morphology, including information on dental shape, size angulation and position of the teeth[10]. The analysis of these evidences varied according to the contemporary technology available including the separate investigation of dental shape using transparent foils[11], the separate analysis of dental shape (hollow contours), size (metric measurements) and angulation (polygons) using 2D digital overlays[12], and the combination of all evidences using 3D superimpositions[13,14].

In most cases of bitemark analysis the quantity of available evidence is usually limited, often consisting on the indentations of the incisal edges of the maxillary and mandibular six anterior teeth[15–20]. In most cases of bitemark analysis fewer teeth are available compared to dental identification cases[6,8]. This is one of the reasons why dental identification cases are considered to offer less legal challenge than cases of bitemark analysis in Court. However, the impact of the quantity of teeth and tooth parts affecting the differences between human dentitions has never been scientifically tested. This study, involving twin siblings, where any differences between the dentitions would be expected to be minimal[21,22], is based on the pair-wise comparisons of the dental morphology following controlled and systematic modifications in the quantity of teeth and tooth parts available for comparison.

The present research aims to quantify the morphological differences between the dentitions of twin siblings using different quantities of teeth and tooth parts.

MATERIALS AND METHODS

The present research was approved by the local Committee of Ethics in

Research under the protocol number: 19575613.2.0000.0020.

The studied sample consisted of 86 pairs of twins (n=172), of which 39 pairs (n=78) were monozygotic (M) and 47 pairs (n=94) were dizygotic (D). From each of the included subject (n=344) dental impressions of the maxillary (n=177) and the mandibular arch (n=177) were taken using alginate (Jeltrate Dustless®, Dentsply®, York, PA, USA) and cast in plaster (Durone®, Dentsply®, York, PA, USA). The study models obtained were digitalized as .STL files using an automated motion device with angular laser scanning (XCAD 3D® (XCADCAM Technology®, São Paulo, SP, Brazil). The .STL files were imported in a personal computer (HP Pavilion®, Hewlett-Packard®, Palo Alto, CA, USA) for duplication, using the copying and pasting command tools of the operating system (Windows 10®, Microsoft Windows, Redmond, USA). The final sample consisted of 688 .STL files. These files were imported in Geomagic Studio® (3D Systems®, Rock Hill, SC, USA) software package (GS) and stored as digital cast files (DCF).

The study was divided in 3 parts (Figure 1). In Part 1, the DCF from the original 86 pairs of twins (n=172) were copied. Using GS, the original images were cropped and reduced to include the clinically visible crowns of the six anterior teeth (Group Ant.). The copied images were cropped a second time and reduced to include the clinically visible dental crowns of all of the anterior and posterior teeth (Group All). In Part 2, monozygotic twin pairs with completely erupted permanent teeth were selected (14 mandibular and 19 maxillary pairs of dentitions). The DCF of these subjects were cropped to include the crowns of 10 teeth, namely the six anterior teeth and the first and second premolars (Group 10). This group was duplicated twice. The DCF of the first duplicate were cropped to include the crowns of 8 teeth

namely the six anterior teeth and the first premolars (Group 8), while in the second duplicate the DCF were cropped to include crowns of the 6 anterior teeth (Group 6). Part 3 used the same sample as Part 2. The DCF were cropped to include the entire morphology of the crowns of the 6 anterior teeth (Group Compl.). This group (Group Compl) was duplicated and the duplicated DCF cropped with a section parallel to the horizontal plane at the level of the highest interdental papilla (Group Crop.). All the crown cropping procedures were performed in GS, placing pre-cropping points along the cemento-enamel junction of all of the teeth including the areas of interest.

Within each group all the possible pair-wise morphological comparisons between DCF were accomplished using GS automated superimposition. The pair-wise differences were calculated in GS and expressed in millimeters as four quantification values: the maximum positive deviation (max.+); the maximum negative deviation (max.-); the average deviation (average); and the standard deviation of the average (SD). To combine the four quantification values their Euclidean distance from origin (zero) was calculated with the following formula: $\text{Distance}=\sqrt{\text{Max+}^2+\text{Max−}^2+\text{Average}^2+\text{Standard deviation}^2}$. In this context, the least morphological difference between pair-wise compared DCF occurs when the distance value is equal to zero. The log-transformed distances were compared between groups using a linear mixed model with Sidak[23] correction for multiple hypotheses, separately for mandibular and maxillary DCF. The statistical tests were performed with significance rate of 5% using S+® 8.0 (Tibco®, Palo Alto, California, USA) software package.

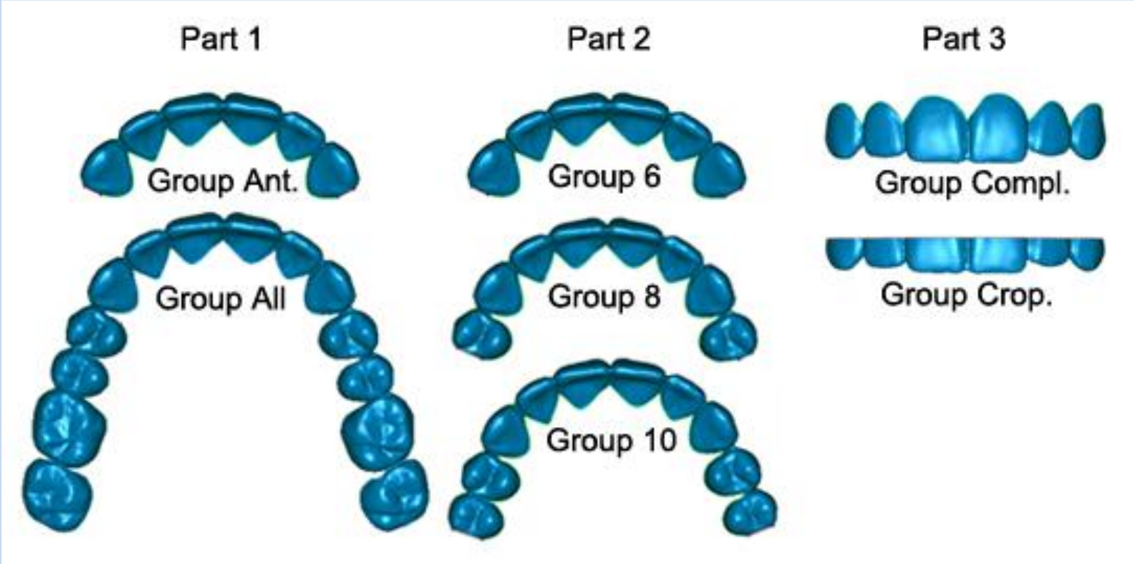


Fig.1: Studied DCF areas of interest in each study part
DCF: digital cast files; Part 1 – Group Ant.: anterior dentition; Group All: entire dentition; Part 2 – Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Part 3 – Group Compl.: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns. DCF in Part 1 and 2 represented in 2D occlusal view and in Part 3 in 2D buccal view. Occlusal and buccal views are merely illustrative. Entire dental crowns were used and compared in a 3D environment in all study parts.

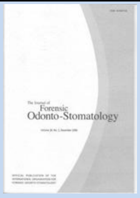
RESULTS

In study Part 1, the mean Euclidian distance observed comparing DCF in Group All was statistically significantly higher than the mean Euclidian distance observed comparing DCF in Group Ant., both for the maxilla and the mandible (p=0.0001) (Table 1; Figure 2).

Table 1 – Comparison of mean Euclidean distances, arch specific for each studied group

Dental arch	Part	Groups	Mean	*p*
Maxillary	1	Ant. vs. All	4.98 vs. 6.43	0.0001
	2	6 vs. 8	3.38 vs. 3.54	0.9088
		6 vs. 10	3.38 vs. 3.64	0.7843
		8 vs. 10	3.54 vs. 3.64	0.9931
	3	Compl. vs. Crop.	3.38 vs. 2.57	0.0027
Mandibular	1	Ant. vs. All	4.29 vs. 7.89	0.0001
	2	6 vs. 8	2.95 vs. 3.17	0.8858
		6 vs. 10	2.95 vs. 3.51	0.5145
		8 vs. 10	3.17 vs. 3.51	0.9135
	3	Compl. vs. Crop.	2.95 vs. 2.21	0.0122

Part 1 – Group Ant.: anterior dentition; Group All: entire dentition; Part 2 – Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Part 3 – Group Compl.: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns. *p*-values obtained with a linear mixed model using Sidak[23] correction for multiple hypotheses. Significance rate set at 5%.



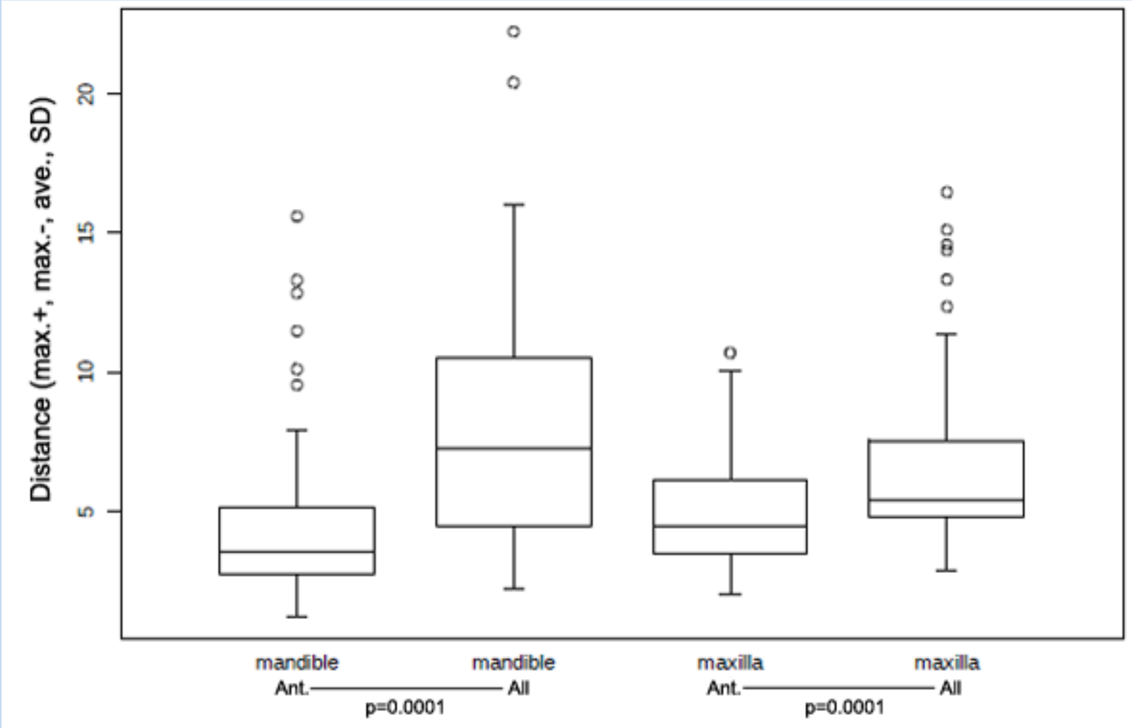


Fig. 2: Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups Ant. and All
DCF: Digital cast files; Group Ant.: anterior dentition; Group All: entire dentition; Mean Euclidean distance for maxillary DCF: 4.98 (Group Ant.) and 4.43 (Group All); Mean Euclidean distance for mandibular DCF: 4.29 (Group Ant.) and 7.89 (Group All); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; *p*-values obtained with a linear mixed model using Sidak[23] correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups Ant. and All for maxillary and mandibular DCF: 0.0001 (*p*).

In study Part 2, the mean Euclidean distance observed comparing DCF in Groups 6, 8 and 10 gradually increased in the maxilla as well as in the mandible. No statistically significant differences were observed between Groups (p>0.05) (Table 1; Figure 3).

In Part 3, the mean Euclidean distance observed comparing DCF in Group Compl. was statistically significant higher than Group Crop., both for the maxilla (p=0.002) and the mandible (p=0.012) (Table 1; Figure 4).

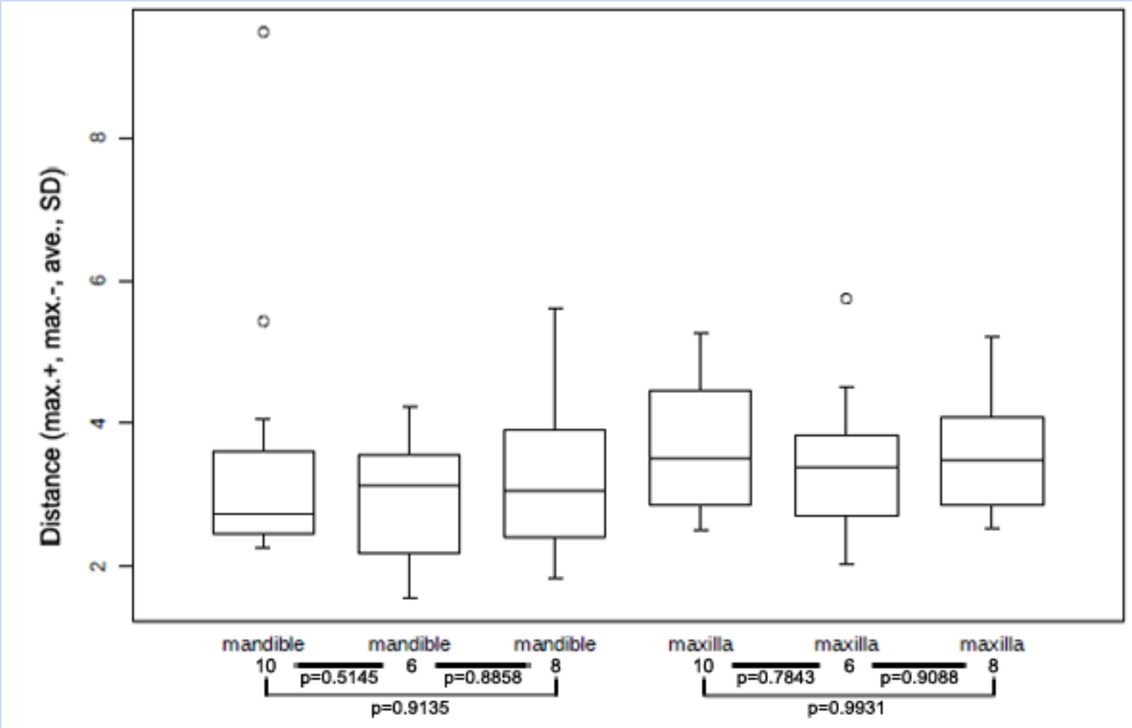


Fig. 3: Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups 6, 8 and 10
DCF: Digital cast files; Group 6: anterior dentition; Group 8: anterior dentition and first premolars; Group 10: anterior dentition and first and second premolars; Mean Euclidean distance for maxillary DCF: 3.38 (Group 6), 3.54 (Group 8), and 3.64 (Group 10); Mean Euclidean distance for mandibular DCF: 2.95 (6), 3.17 (8), and 3.51 (10); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; *p*-values obtained with a linear mixed model using Sidak[23] correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups 6, 8 and 10 for maxillary and mandibular DCF: >0.05 (*p*).

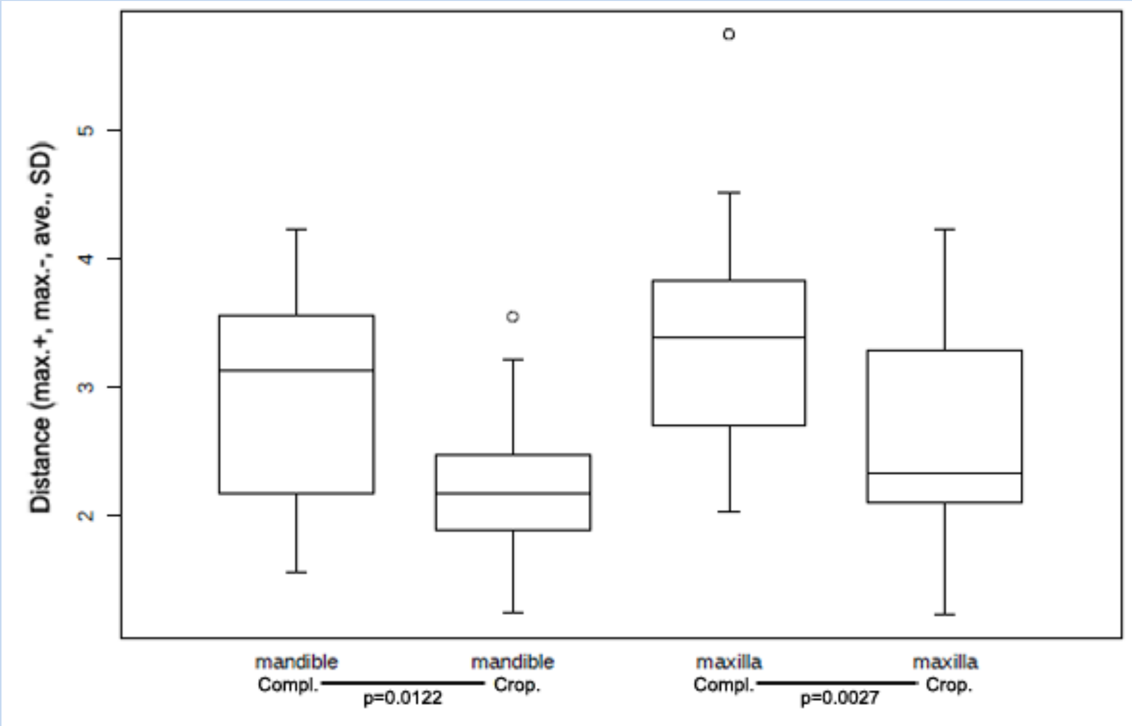


Fig. 4: Boxplots expressing the Euclidean distance of all pair wise DCF comparisons separate for the mandible and maxilla in Groups Compl. And Crop

DCF: Digital cast files; Group Compl..: anterior dentition with complete crowns; Group Crop.: anterior dentition with partial crowns; Mean Euclidean distance for maxillary DCF: 3.38 (Group Compl.) and 2.27 (Group Crop.); Mean Euclidean distance for mandibular DCF: 2.95 (Group Compl.) and 2.21 (Group Crop.); Max.+: maximum positive deviation; Max.-: maximum negative deviation; Ave.: average deviation; SD: standard deviation; *p*-values obtained with a linear mixed model using Sidak[23] correction of multiple hypotheses considering a significance rate set at 5%; Difference between the mean Euclidean distance of Groups Compl. And Crop. for maxillary DCF: 0.0027 (*p*). Difference between the mean Euclidean distance of Groups Compl. And Crop. for mandibular DCF: 0.0122 (*p*).

DISCUSSION

Forensic dentistry is currently using the hypothesis that an increase in the quantity of teeth and tooth parts provides an increase in the amount of dental evidence, increasing the (morphological) differences between subjects. Unlike fingerprint and DNA analysis, dental identification is not governed by the requirement of a minimum number of concordant features[24,25]. Quality assurance guidelines from Forensic organizations, such as the International Organization of Forensic Odonto-Stomatology (IOFOS), recommend that all the combinations of dental evidences available must be explored[8]. In bitemark analysis attempts are made to take into account all of the available evidence[1], but, realistically, in the majority of cases, the analysis is mainly restricted to the incisal morphology of the anterior teeth[10]. In forensic practice the hypothesis that is generally accepted is that increased numbers of teeth correlates to more distinctive identification potential and better comparison outcomes. The inference is that reliability factor in cases of dental identification is better than the reliability factor in of cases of bitemark analysis.

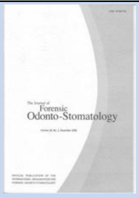
Sibling pairs were sampled in order to have subjects with decreased qualitative differences in dental morphology. It justifies why randomly selected subjects or copied files were not used. Specifically, in the first the highest qualitative morphological differences are expected, while in the second, zero morphological difference will be observed between the corresponding DCF.

Uniqueness is commonly used in the forensic scientific literature to describe human dentitions with converging evidences. However, converging evidences indicate that two dentitions are at most identical but not unique. In fact, uniqueness guarantees that two dentitions in a worldwide population will not be equal. In the context of the present study, lack of uniqueness is translated as the absence of morphological difference (Euclidean distance = zero) between pair-wise compared DCF. In particular, the mean Euclidean distances with highest unique power (6.43 for maxillary and 7.89 mandibular DCF) were observed comparing entire dentitions (Group All, Part 1). By contrast, anterior dentitions with partial crowns (Group Crop., Part 3) revealed the lowest unique power (2.57 for maxillary and 2.21 for mandibular DCF). Generally, this would suggest that an increase in the quantity of teeth and tooth parts increases the Euclidean distances, making dentitions potentially more unique.

In Part 1, the clear statistically significant difference ($p<0.05$) between the DCF of the entire group (Group All) and the anterior group (Group Ant.) (Figure 2) demonstrates that substantial increase in the quantity of teeth relates to increasingly distinctive morphological dental evidence. Specifically, the proportion in the number of teeth between the two groups (Group All/Group Ant.) increased by a factor of 133.33%, meaning that the proportion of mean Euclidean distances increased by 29% for maxillary and 83% for mandibular DCF (Table 1). In Part 2, morphological differences were also observed by firstly phasing in Group 8 (first premolars) and secondly by phasing in Group 10 (second premolars) but no statistically significant results were observed between these group comparisons ($p>0.05$). The proportions in the number of teeth increased by a factor of 33.33% between Groups 8 and 6; by a factor of 66% between Groups 10 and 6; and by a factor of 25% between Groups 10 and 8. This meant that the proportions of mean maxillary Euclidean distances increased by a factor of 4% (Group 8/Group 6); by a factor of 7% (Group 10/Group 6); and by a factor of 2% (Group 10/Group 8). The proportions of mean mandibular Euclidean distances increased by a factor of 7% (Group 8/Group 6); by a factor of 18% (Group 10/Group 6); and by a factor of 10% (Group 10/Group 8). In Part 3, statistically significant differences between groups ($p<0.05$) were observed (Table 1; Figure 4). Part 3, that is the analysis of the proportion in quantity of tooth material included, could be considered less accurate compared to the previous study parts, because the anterior dentition with partial crowns (Group Crop.) were horizontally cropped at the level of the highest interdental papilla, which varied discretely between twin subjects. Assuming that the anterior dentitions were cropped in half – generating a difference in tooth material of 50% between both groups (Group Crop. /Group Compl.), the proportion of mean Euclidean distances increased by a factor of 31% and by a factor of 33% for maxillary and mandibular DCF, respectively.

Analysis of all three parts of the study revealed that higher Euclidean distance values were observed when comparing a larger quantity of tooth material or number of teeth. However, in Parts 1 and 3 statistically significant findings ($p<0.05$) were obtained, differing from Part 2 ($p>0.05$). This difference can be explained by the proportion of tooth material included for analysis. In Parts 1 and 3 the proportion of tooth material increased by at least a factor of 50% between groups, increasing the mean Euclidean distances by up to 83% (mandibular DCF of Part 1). In Part 2 the proportion of increase in tooth material varied between 25-66.66%, increasing the mean Euclidean distances less by only up to 18% (mandibular DCF between Groups G6 and G10). These results suggest that the inclusion of premolars in the anterior dentition provides little additional morphological information of negligible impact upon the mean Euclidean distances with statistical significance. The opposite is observed for the inclusion of all the available teeth in the dental arch (Part 1) and for the analysis of complete (instead of partial) anterior crowns (Part 3).

The use of anterior teeth combined with premolars and molars provides more information that can be used advantageously to differentiate between dentitions. It confirms the hypothesis that more tooth material allows for more combination of evidences[8] contributing to more uniqueness. In the case of bitemark analysis it also confirms the increase in reliability based on the higher quantity of tooth material considered[26]. Even in the absence of statistically significant findings (Part 2), the gradual increase in morphological difference observed adding premolars, represents a clinically significant finding. It suggests that these minor morphological differences can be useful in forensic practice. They allow for positive dental identifications founded on the particular shape of premolar crowns; and for matches between a bitemark and suspect dentitions based on the comparison of the clinically detected premolar morphology. In parallel, the amount of tooth quantity is not exclusively restricted to the number of teeth, but involves also the amount of tooth parts available. In Part 3, the analysis of complete anterior crowns (Group Compl.) increased the morphological difference with 31-33% compared to partial crowns (Group Crop.). In the context of dental identification the quantity of morphological information differs if other tooth parts were considered. More specifically, the gingival half (50%) of the dental crown seems to provide more distinctive morphological information compared to the incisal half (50%). While the incisal half generated up to 33% of morphological difference between DCF, the gingival part is responsible for generating the remaining difference (up to 67%). This can be explained against the background of the inherent genetic influence on the quality of evidence that varies discretely between twin siblings. The quality of evidence may also be modified by non-genetic influence depending on which part of the tooth was included for analysis; for example a nail biting habit that would affect the incisal edge of the tooth or, for example, a periodontal disease that would affect the gingival part of the tooth. In most cases of bitemark analysis the outcomes of study Part 3 are very relevant because the analysis is commonly restricted to the incisal part of the crown[1] (part that impresses the bitten surface). The current



findings suggest that the use of partial anterior crowns hampers the distinction between dentitions compared to the analysis of complete crowns. However, uniqueness of partial anterior crowns (Group Crop.) remains unproved, indicating the need for further investigations.

Regarding comparison of additional tooth material in both the maxillary and mandibular DCF, the mean Euclidean distances increased most on respect of the mandibular DCF. Specifically, in Part 1 the proportion of mean Euclidean distances between mandibular and maxillary DCF increased by a factor of 41%. In Part 2 it increased by a factor of 2% (Group 8/Group 6); 10% (Group 10/Group 6) and 7% (Group 10/Group 8). In Part 3 the increase was 1%. These findings suggest that the trend toward in the morphological differences in mandibular DCF is greater than maxillary DCF regarding of the quantity of tooth material considered. This could infer that that the mandibular DCF is a better determinant in discriminating between dentitions in cases of both human identification and bitemark analysis. Sheets et al.[18] (2011) also justify this finding by reason that dental crowding is more common in the mandibular arch. On the other hand, the lower unique power of maxillary DCF must be considered an important finding for potential sample stratification in studies proving the uniqueness of the human dentition, because apparently uniqueness is more hardly proved within maxillary DCF. Despite this, the morphological difference between dental arches was only prominent in study Part 1 (41% increase). In study Part 2 (2-10% increase) and Part 3 (1% increase) the reduced Euclidean difference between arches suggests that predilection for analysis of specific dental arch in dental identifications and bitemarks must be avoided. It highlights the importance of analyzing and combining all morphological information from both dental arches in dental identification and bitemark analysis.

Further researches in the field should consider firstly using 3D scanning and performing separate comparisons of the dental crowns in each tooth position in order to systematically assess their morphological uniqueness. Secondly, root parts should be tested on their morphological information and uniqueness related to dental identifications. In both cases sibling comparisons on twin samples are recommended, enabling to study the morphological quantity with minimal variation in morphological quality of evidences.

CONCLUSION

The outcome of this research provides evidence that an increase in the quantity of dental information leads to an increase in the number of morphological differences detected between dentitions. The results were based on pair-wise comparison of twin dentitions allowing quality control of the data. The research was based solely on dental morphological data.

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