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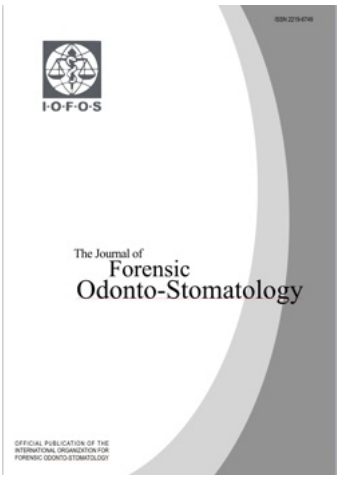

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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Establishing legal threshold of 18-years based on the assessment of mandibular molars using three different methods – An observational study

Sridhar Murali [1], Anjana Bagewadi [2], Lokesh Kumar S [1], Jayapriya Thirupathi [2], Vaishali Keluskar [2]

[1] Department of Oral Medicine, Radiology and Special Care Dentistry, Saveetha Dental College and Hospitals (Saveetha Institute of Medical and Technical Sciences SIMATS), Chennai, Tamil Nadu, India. [2] Department of Oral Medicine and Radiology KAHER's KLE Vishwanath Katti Institute of Dental Sciences, Constituent Unit of KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi, Karnataka, India

Corresponding author: lokemar95@gmail.com

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ABSTRACT

Background: The study evaluates the feasibility of employing the radiographic visibility of the root pulp and periodontal ligament in mandibular molars for age estimation, particularly focusing on the 18 years of age threshold. This study additionally investigates the potential of root canal width reduction in mandibular molars, as a reliable method for forensic age estimation in living individuals.
Materials and methods: A cross-sectional study was conducted to assess the radiographic visibility of the root pulp (RPV) and the root canal width (RCW) of mandibular first, second, and third molars along with the radiographic visibility of the periodontal ligament (PLV) of mandibular third molars, in a sample of 403 individuals aged 16-25 years (220 males and 183 females). Data regarding age for different stages of RPV and PLV and various types of RCW were recorded and observed for sex-based differences. Results obtained were tabulated and descriptive statistics were applied to summarise the findings.
Results: Individuals over 18 years old were classified with higher accuracy using stage 3 of the RPV scoring system in all mandibular molars (first, second, and third) compared to stage 2, which was also effective for the second and third molars. This result held regardless of sex and side examined. Additionally, root canal width (RCW) assessment demonstrated that individuals with RCW types A, B, and C were more likely to be under 18 years old in both sexes. Conversely, individuals with RCW type U on the right side for males and the left side for females exhibited a higher likelihood of being above 18 years old.
Conclusion: The study suggests that the assessment of mandibular molars could potentially serve as an auxiliary tool in age estimation methods, particularly for approximating individuals around the 18 years of age threshold. Further investigation is warranted to explore the potential application of root canal width measurements in forensic age estimation.

INTRODUCTION

Estimating the age of individuals, whether alive or dead, is becoming increasingly significant for forensic purposes because it narrows down the search for the missing person.[1] Age estimation has a wide range of interests and objectives because it necessitates a multi-disciplinary effort to assist in forensic and medico-legal issues. Forensic age estimation is becoming increasingly important in criminal and civil processes, individual identification, immigration, and competitive sports. [2, 3]

Dental age (DA) correlates with chronological age (CA) more closely than any other human biological growth marker (HBGM). [4] Dental maturation refers to the complicated sequences of events that occur during the formation of a crown, root development, eruption of the tooth into the oral cavity, and root apex maturation. Tooth development is a more accurate indicator of dental maturity than tooth eruption. [5] Therefore, examining such occurrences clinically or radiographically can aid in determining CA, which is useful in a variety of fields, including paediatric dentistry, orthodontics, forensic odontology, and human anthropology. [6] Because it is straightforward, non-invasive, and repeatable, radiographic examination is commonly used to determine dental maturity and age (DA). [7, 8]

According to ABFO, orthopantomograms (OPGs) are a component of dental examination and are thus frequently used in the forensic field. OPGs and periapical radiographs show the morphology of posterior tooth roots, the shape and volume of the pulp chamber in teeth, and the relationship between third molars and the surrounding mineralised structures. Probable identification and age determination of a person, ante-mortem, and post-mortem radiographs of teeth can be used to detect caries, bone loss, endodontic treatment, crown and bridge restorations, etc. [9, 10]

Despite their developmental variability, third molars are the most accurate biological indicator of age in adolescents. [11] The agenesis of the third molar is the most common issue when using this tooth to estimate age. [12] When the third molar is congenitally absent or extracted, another approach using teeth other than the third molar [13, 14] would be adequate for discriminating whether a person has reached maturity or not.

Dental age estimation in individuals above 15 years of age and suspected to be older than 18 years is a growing area of concern in forensic science, and age estimation using teeth is accurate up to approximately 15 years of age. [15] In the absence of third molars and other reliable age indicators, it has been proven that mandibular second molars can be used to determine whether the age of an individual is above 18 years or not. [13]

Concerning the legal age of an individual, 18 years is significant because anyone under that age is considered a minor. Since identifying the 18 years of age threshold to distinguish between the status of a minor and an adult is a considerable risk, the proof of achievement of 18 years is of the highest importance in the age estimation of live persons. [16, 17] Two maturity markers, root pulp visibility [18] and periodontal ligament visibility, [18, 19] were commonly used to identify whether an individual had attained 18 years of age or not.

Obliteration or interruption of the root pulp and periodontal ligament develops as age increases because these growth indicators appear to be dependent on mandibular bone development. This led to the discovery of another HBGM in which the relative widths of the distal root canals (RCW) of the lower permanent molars (LL6, LL7, and LL8) were assessed. [20] Based on their evaluation in an orthopantomogram, Roberts et al. [20] divided the relative widths into three stages, namely: RCW-A, RCW-B, and RCW-C.

Owing to the scarcity in the literature, the present study assesses the mandibular molars for age estimation using three methods. It aims to determine whether a threshold of 18 years of age can be obtained based on the radiographic assessment of the visibility of mandibular molars.

MATERIALS AND METHODS

A retrospective analysis was conducted using digital orthopantomograms (OPGs) obtained from the archives of the Department of Oral Medicine and Radiology at a private institution. The OPGs were originally collected for treatment planning and other clinical purposes from 403 individuals aged 16-25 years. Informed consent was obtained at the time of taking the radiograph indicating that it will be used for research purposes while maintaining its confidentiality. This retrospective observational study spanned a period of 12 months from June 2021 to June 2022. It was carried out after obtaining ethical clearance from the Institutional Ethical Committee following the ethical standards set by the Declaration of Helsinki (Finland). [21]

OPGs with the presence of lower right and left mandibular molars (first, second, and third molars) that had root apices closed (Demirjian developmental stage H) and radiographs with no evidence of gross pathology affecting the mandible were included. OPGs that do not depict the area of interest with missing molars on either side due to extraction or congenital

absence and those with any systemic disease or any disorder that affects tooth development are excluded from the study. Also, mandibular molars with existing root canal restorations, single roots, or any evidence of infection e.g. pulpitis, periodontitis, etc. are excluded from the study.
OPGs that met inclusion criteria were exported after masking the demographic details of name, age, and gender in a Tagged Image File Format (tiff) and assigned a random number to ensure blinding for the observers. Two observers (MS and JP) were calibrated for Olze et al., [18, 22] and Roberts et al., [20] methods by staging a sample of 10 OPGs that were not part of the final analysis, and assessment of the sample was provided only after obtaining standardisation between them. Demographic details of the included samples are described in Table 1.

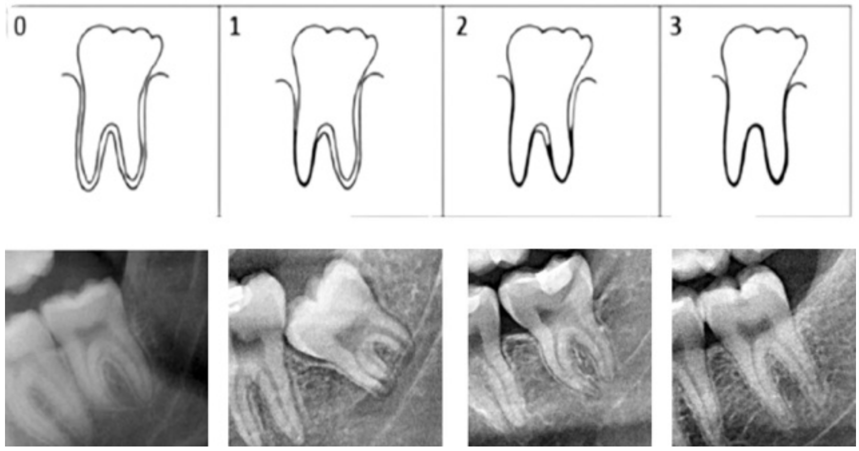
Table 1. Demographic details of the participants described in terms of number and percentage

Category			n	%
Gender		Male	183	45.4
		Female	220	54.6
Age Group (Years)	16-16.9	Male	7	3.83
		Female	14	6.36
	17-17.9	Male	9	4.92
		Female	15	6.82
	18-18.9	Male	24	13.11
		Female	19	8.64
	19-19.9	Male	19	10.38
		Female	30	13.64
	20-20.9	Male	19	10.38
		Female	34	15.45
	21-21.9	Male	25	13.66
		Female	29	13.18
	22-22.9	Male	28	15.30
		Female	27	12.27
	23-23.9	Male	12	6.56
		Female	19	8.64
	24-24.9	Male	40	21.86
		Female	33	15.00
Gender			**Mean**	**SD**
		Male	21.54	2.72
		Female	21.20	2.71
		Total	21.35	2.72

Olze Method of radiographic visibility of root pulp and periodontal ligament
Olze et al., [18] were first to devise a classification system based on radiographic visibility of root pulp in mandibular third molars. He devised a four-stage system based on the visibility of the lumen in the root canals on an orthopantomogram as depicted in Figure 1.

1. Stage 0: Visibility of the lumen is noted to the apex in all the canals.
2. Stage 1: Visibility of the lumen is not till the apex in one of the canals.
3. Stage 2: Visibility of the lumen is not till the apex in both the canals or one of the canals is virtually invisible at full length.
4. Stage 3: Visibility of the lumen is completely absent in both canals.

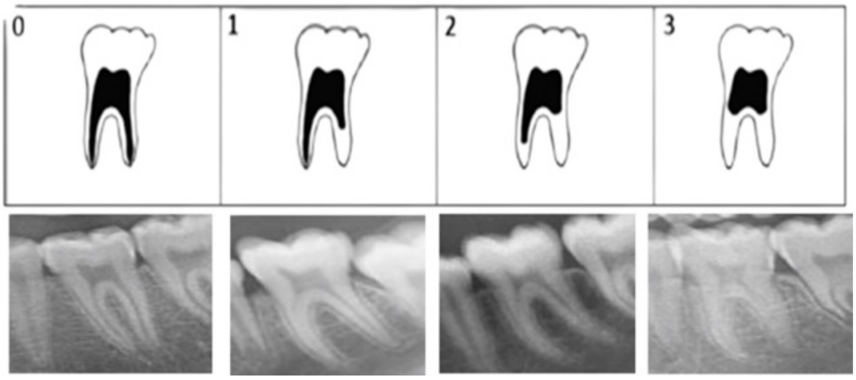
Figure 1. Radiographic visibility of the root pulp according to stages devised by Olze et al., [18] depicted in the mandibular left first molar.



Later, Olze et al., [22] classified the radiographic visibility of periodontal ligament in completely mineralised mandibular third molars. A four-stage system similar to the radiographic visibility of root pulp was utilised as depicted in Figure 2.

1. Stage 0: Visibility of the periodontal ligament is noted along the complete length of the root.
2. Stage 1: Visibility of the periodontal ligament is partially obliterated in one root and from apex till more than half of the other root.
3. Stage 2: Visibility of the periodontal ligament is obliterated along the entire length of one root or the part of the root in both roots.
4. Stage 3: Visibility of the periodontal ligament is completely obliterated in both canals.

Figure 2. Radiographic visibility of the periodontal ligament according to stages devised by Olze et al., [22] depicted in the mandibular left third molar



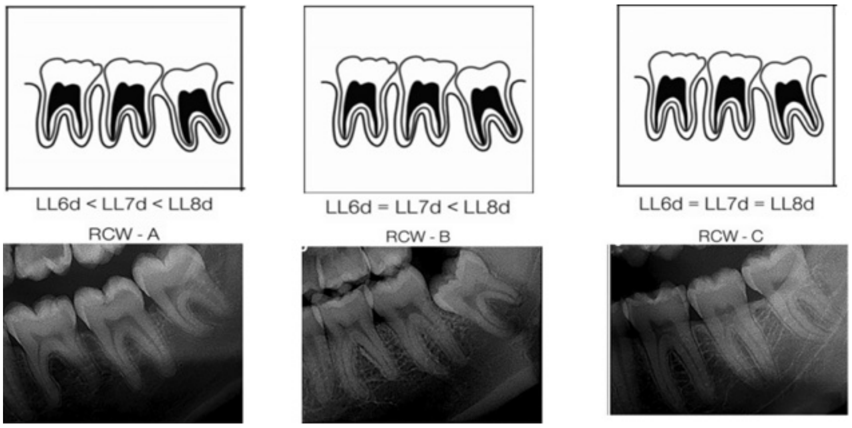
Roberts method:

Roberts et al., [20] proposed an identification marker based on the mesio-distal widths (m-d) of the root canals of left mandibular molars. The distal root canals of the mandibular first, second, and third molars were considered a pattern in predetermined 3 stages as depicted in Figure 3.

1. RCW-A (Root canal width - A): The m-d width of the mandibular first molar (LL6) is narrower than the m-d width of the mandibular second molar (LL7) which in turn is narrower than the m-d width of mandibular third molar (LL8), read as LL6 < LL7 < LL8.
2. RCW-B (Root canal width - B): The m-d width of the mandibular first molar (LL6) is equal to the m-d width of the mandibular second molar (LL7) which in turn is narrower than the m-d width of the mandibular third molar (LL8), read as LL6 = LL7 < LL8.
3. RCW-C (Root canal width - C): The m-d width of the mandibular first molar (LL6) is equal to m-d with of mandibular second molar (LL7) which in turn is equal to the m-d width of the mandibular third molar (LL8), read as LL6 = LL7 = LL8.

4. RCW-U (Root canal width – U): Root canal patterns that met the inclusion criteria and did not fall under any of the stages mentioned above are classified as unknown.

Figure 3. Radiographic and schematic representation of the three categories described by Roberts et al., [20] Abbreviations RCW Root canal width LL6 Mandibular left first molar LL7 Mandibular left second molar LL8 Mandibular left third molar RL6 Mandibular right first molar RL7 Mandibular right second molar RL8 Mandibular right third molar



To assess repeatability and inter-observer agreement, two observers (MS and JP) evaluated all the OPGs. To assess intra-observer agreement, one observer (MS) observed 100 OPGs in a random sample twice after 6 months. The three methods mentioned above were applied to obtain dental ages, which were then statistically assessed after being imported into Microsoft Office Excel 2016.

Statistical analysis:
The Statistical Package for the Social Sciences (SPSS) 21.0 for Windows was used for statistical analysis (SPSS Inc., Chicago, IL, USA). The significance level for analysis was set at 5% ($p < 0.05$). Individual age for each sample was calculated before anonymisation by an observer (LK) who was not part of the assessment. The date of exposure was subtracted from the date of birth and it was recorded as chronological age. Mean, standard deviation, minimum, median, maximum, lower quartile, and upper quartile were determined for root canal visibility, periodontal ligament visibility, and root canal width wherever applicable. Also, the applicability of these methods in classifying the individuals who have attained major status (≥ 18 years) was determined. To evaluate intra-observer and inter-observer reliability, Cohen's kappa statistics were applied.

RESULTS

Age-related trend changes are noted similarly in the mandibular first, second, and third molars on the left side of males and females. Inter-examiner reliability for radiographic visibility of the root canal of the first, second, and third mandibular molars on both sides was 0.84, 0.86, and 0.82, respectively. Intra-examiner reliability revealed that radiographic visibility of the root canal was 0.82, 0.84, and 0.83, respectively for mandibular first, second, and third molars. Cohen's Kappa values for radiographic visibility of the periodontal ligament of third molars were 0.84, and 0.82 for inter- and intra-observer reliability, respectively. Based on the results obtained, substantial agreement was noted for assessed parameters, thereby confirming its reliability.
The study found a complete absence of Stage 0 (no radiographic visibility of root pulp) in both mandibular first molars and second molars of males, as well as mandibular first molars of females. Notably, the minimum age for mandibular first, second, and third molars in males did not exceed 16 years. However, intriguingly, stages 0 and 3 were not observed until after 19 years of age in mandibular second molars. Additionally, a consistent trend was observed across both sexes, with a gradual increase in participant age as the stage of root canal visibility progressed (higher stages indicating more closure). Detailed information on mean, minimum, and maximum values for root canal visibility stages in mandibular left first, second, and third molars for both males and females are presented in Table 2.

Table 2. Radiographic visibility of root canal in mandibular first, second, and third molars for both sexes on the left side Abbreviations: N – Number of participants SD – Standard Deviation

Sex	Tooth	Stage	N	Mean	SD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Males	36	1	54	20.72	2.61	16.56	18.66	20.31	22.46	27.37
		2	87	21.56	2.63	16.11	19.44	21.14	23.07	27.31
		3	42	22.57	2.78	16.46	20.36	22.73	24.16	28.26
	37	1	122	21.16	2.70	16.11	18.76	21.23	22.71	27.37
		2	42	21.91	2.47	16.46	20.16	21.71	23.54	28.26
		3	19	23.22	2.82	16.61	21.80	24.07	25.43	26.48
	38	0	6	18.02	1.52	16.56	17.06	17.93	18.05	20.84
		1	10	17.83	1.19	16.11	16.77	18.09	18.45	19.58
		2	32	19.70	1.78	16.99	18.43	19.17	20.89	24.86
		3	135	22.41	2.46	16.88	20.66	22.47	24.43	28.26
Females	36	1	72	20.46	2.57	15.76	18.87	20.16	21.83	27.97
		2	105	21.28	2.81	15.82	20.06	20.91	22.59	28.75
		3	43	22.22	2.39	17.55	20.59	22.34	23.68	27.86
	37	0	3	20.40	1.32	19.27	19.68	20.08	20.97	21.85
		1	140	20.78	2.79	15.76	18.91	20.32	22.42	28.75
		2	58	21.66	2.53	16.59	19.56	21.87	23.22	27.86
		3	19	22.94	1.95	19.14	21.76	22.58	24.49	26.46
	38	0	26	18.46	1.46	15.76	17.47	18.52	19.07	21.11
		1	25	18.54	1.65	15.82	17.15	18.88	19.46	21.85
		2	50	21.05	1.72	17.04	20.18	21.19	22.05	24.21
		3	119	22.41	2.61	16.89	20.31	22.37	24.41	28.75

On the contra-lateral side, the study similarly documented an absence of stage 0 radiographic visibility of the root canal in both first molars, regardless of sex, and in male mandibular second molars. The minimum age for all evaluated male subjects was consistently below 18 years (<17 years) across all teeth and stages examined.

Conversely, female subjects exhibited a minimum age below 18 years for all stages in mandibular first and third molars, except stages 1 and 3 in mandibular second molars, where the minimum age exceeded 18 years. Mean values of root canal visibility along with minimum and maximum values for females are presented in Table 3.

Table 3. Radiographic visibility of root canal in mandibular first, second, and third molars for both sexes on the right side Abbreviations: N – Number of participants SD – Standard Deviation

Sex	Tooth	Stage	N	Mean	SD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Males	36	1	56	20.61	2.57	16.56	18.45	20.15	22.41	26.10
		2	95	21.72	2.60	16.11	19.79	21.71	23.49	27.37
		43	32	22.65	2.91	16.61	20.25	22.77	25.22	28.26
	47	1	136	21.29	2.67	16.11	19.03	21.36	22.82	27.37
		2	36	21.86	2.67	16.61	19.83	21.94	23.54	28.26
		3	11	23.64	2.83	18.61	22.62	25.03	25.47	26.48
	48	0	8	17.74	1.45	16.11	16.74	17.78	18.02	20.84
		1	7	17.72	0.75	16.46	17.27	17.92	18.35	18.46
		2	38	20.08	2.10	16.61	18.65	19.68	21.31	25.61
		3	130	22.41	2.45	17.19	20.68	22.44	24.49	28.26
Females	46	1	63	20.41	2.69	15.76	18.58	20.16	21.84	26.78
		2	116	21.18	2.64	15.82	19.46	20.90	22.59	28.75
		3	41	22.44	2.57	17.55	20.33	22.60	24.04	28.08
	47	0	2	19.68	0.57	19.27	19.47	19.68	19.88	20.08
		1	144	20.66	2.71	15.76	18.83	20.32	22.34	28.75
		2	58	21.99	2.45	17.20	20.20	21.90	23.28	27.86
		3	16	23.31	2.18	19.14	22.19	23.52	25.47	26.46
	48	0	28	18.48	1.63	15.76	17.19	18.52	19.39	21.75
		1	23	18.57	1.60	16.02	17.39	18.34	19.46	21.85
		2	55	20.61	1.78	17.04	19.22	20.79	21.83	24.21
		3	114	22.67	2.46	16.89	20.80	22.47	24.61	28.75

In the current investigation, the radiographic visibility of the periodontal ligament in mandibular third molars was evaluated, as these teeth are the only ones still developing within the included age cohorts. In males, a progressive increase in minimum age was observed with advancing PDL visibility stages. Stage 1 visibility in the right mandibular third molar was associated with an age exceeding 18 years. Conversely, females exhibited stage 3 visibility in the right mandibular third molar as the initial indicator of being above 18 years. A similar trend of increasing age with advancing PDL visibility stages (0-3) was observed in females. Detailed descriptive statistics (mean, standard deviation, lower and upper quartiles) are presented in Table 4.

Table 4. Radiographic visibility of periodontal ligament in mandibular third molars on both sides for males and females Abbreviations N – Number of participants, SD – Standard Deviation

Sex	Tooth	Stage	N	Mean	SD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Males	38	0	19	18.55	2.11	16.11	17.91	18.25	18.50	26.05
		1	26	21.09	2.20	17.71	18.75	21.18	22.21	25.61
		2	61	21.07	2.68	16.46	19.24	20.65	22.79	27.37
		3	77	22.81	2.32	16.61	21.28	22.68	25.03	28.26
	48	0	19	18.52	2.12	16.11	17.78	18.25	18.55	26.05
		1	28	21.42	2.20	18.41	18.99	21.93	22.61	25.61
		2	54	21.11	2.79	16.46	19.24	20.48	23.04	27.37
		3	82	22.57	2.38	16.61	20.95	22.61	24.42	28.26
Females	38	0	51	18.86	1.99	15.76	17.45	18.80	19.78	26.70
		1	23	19.92	2.18	16.60	18.40	19.61	21.49	24.21
		2	74	21.74	2.53	16.91	20.04	21.19	23.16	28.08
		3	72	22.70	2.21	18.71	20.90	22.44	24.22	28.75
	48	0	50	18.77	1.95	15.76	17.45	18.73	19.54	26.70
		1	30	20.30	2.17	16.60	18.90	20.01	21.88	24.21
		2	75	21.67	2.4	16.91	20.21	21.19	22.98	28.08
		3	65	22.92	2.22	18.95	21.77	22.60	24.80	28.75

Utilizing Roberts method, the study discovered that the minimum age for categories RCW-A, RCW-B, and RCW-C was under 18 years of age for both sexes and assessed sides. This study included an additional category, RCW-U, which does not correspond to the original Roberts classification. Notably, the minimum age for RCW-U exceeded the 18 years of age threshold for males on the right side and females on the left side. Further details on mean age, minimum and maximum ages, and upper and lower quartiles for all Roberts method categories are provided in Table 5.

In the assessment of majority status, the root canal visibility of mandibular first, second, and third molars on both sides was evaluated for males and females. The analysis of the left side mandibular molars indicated an increasing probability of individuals being above 18 years of age with advancing stages. Stage 3 of visibility in all molars demonstrated a high likelihood of individuals having reached the age of majority in both sexes. Similarly, Stage 2 of the mandibular left second and third molars showed comparable precision in determining majority status. Detailed frequencies of individuals classified as having attained majority status based on left side mandibular molar visibility are presented in Table 6.

Table 5. Patterns of Root canal width visibility of mandibular first, second, and third molars on both sides and both sexes. N – Number of participants, SD – Standard Deviation, RCW – Root Canal Width, A, B, C, U stands for different types of RCW.

Sex	Side	Type	N	Mean	SD	Minimum	Lower quartile	Median	Upper quartile	Maximum
Males	Right	RCW-A	22	19.39	2.38	16.56	17.98	18.34	20.45	26.05
		RCW-B	40	19.47	1.65	16.11	18.57	19.49	20.55	23.05
		RCW-C	84	22.74	2.53	16.61	21.31	22.68	24.76	28.26
		RCW-U	37	22.35	2.24	18.06	20.81	22.03	24.36	26.13
	Left	RCW-A	20	19.06	1.91	16.56	18.00	18.26	19.75	22.79
		RCW-B	38	19.89	2.28	16.11	18.64	19.41	21.11	26.05
		RCW-C	81	22.76	2.51	16.61	21.40	22.68	24.76	28.26
		RCW-U	44	21.85	2.34	17.92	20.25	21.32	23.68	26.13
Females	Right	RCW-A	41	19.67	2.51	15.76	17.43	19.59	21.30	25.61
		RCW-B	56	19.71	1.95	16.02	18.45	19.42	20.86	23.86
		RCW-C	76	22.68	2.33	17.20	20.99	22.59	23.97	27.97
		RCW-U	47	21.90	2.72	16.57	20.12	21.20	23.19	28.75
	Left	RCW-A	51	19.30	2.18	15.76	17.43	19.14	20.85	24.74
		RCW-B	36	20.18	2.59	16.02	18.45	19.95	21.15	26.24
		RCW-C	78	22.37	2.50	17.20	20.64	22.56	23.86	27.97
		RCW-U	55	21.95	2.37	18.51	20.24	21.25	23.14	28.75

Table 6. Stage distribution according to majority status (< 18 years or ≥ 18 years) based on root canal visibility of mandibular first (36), second (37), and third molars (38) for both males and females on the left side Abbreviations n = number of participants, % indicates frequency of distribution

Root Canal Visibility	Status	36		37		38	
		Males n (%)	Females n (%)	Males n (%)	Females n (%)	Males n (%)	Females n (%)
Stage 0	< 18 years	-	-	-	0 (0)	5 (35.72)	11 (42.31)
	≥ 18 years	-	-	-	3 (100)	14 (73.68)	15 (57.69)
	Total	-	-	-	3 (100)	19 (100)	26 (100)
Stage 1	< 18 years	9 (16.67)	14 (19.44)	13 (10.66)	25 (17.86)	2 (7.69)	11 (44.00)
	≥ 18 years	45 (83.33)	58 (80.56)	109 (89.34)	115 (82.14)	24 (92.31)	14 (56.00)
	Total	54 (100)	72 (100)	122 (100)	140 (100)	26 (100)	25 (100)
Stage 2	< 18 years	4 (14.50)	13 (12.38)	2 (2.63)	1 (1.72)	8 (2.63)	3 (6.00)
	≥ 18 years	83 (85.50)	92 (87.62)	40 (97.37)	57 (97.37)	53 (97.37)	47 (94.00)
	Total	87 (100)	105 (100)	38 (100)	58 (100)	61 (100)	50 (100)
Stage 3	< 18 years	3 (7.14)	2 (4.65)	0 (0)	0 (0)	1 (1.30)	4 (3.36)
	≥ 18 years	39 (92.86)	41 (95.35)	11 (100)	19 (100)	76 (98.70)	115 (96.64)
	Total	42 (100)	43 (100)	11 (100)	19 (100)	77 (100)	119 (100)

In the corresponding analysis on the right side, the data revealed that for both sexes, the likelihood of an individual reaching the 18 years of age threshold was greater in stage 3 for all mandibular molars in females than in males. Notably, stage 2 of the mandibular first molar exhibited a higher frequency of correctly classifying individuals according to majority status. Interestingly, stage 1 of mandibular second and third molars demonstrated a superior frequency rate compared to stage 2 in terms of root canal visibility. The frequency of individuals achieving majority status with mandibular left first, second, and third molars is outlined in Table 7.

Table 7. Stage distribution according to majority status (< 18 years or ≥ 18 years) based on root canal visibility of mandibular first (46), second (47), and third molars (48) for both males and females on the right side Abbreviations n = number of participants, % indicates the frequency of distribution

Root Canal Visibility	Status	46		47		48	
		Males n (%)	Females n (%)	Males n (%)	Females n (%)	Males n (%)	Females n (%)
Stage 0	< 18 years	-	-	-	0 (0)	6 (31.58)	12 (42.86)
	≥ 18 years	-	-	-	2 (100)	13 (68.42)	16 (57.14)
	Total	-	-	-	2 (100)	19 (100)	28 (100)
Stage 1	< 18 years	7 (12.50)	15 (23.81)	13 (9.56)	28 (19.44)	1 (3.57)	11 (47.83)
	≥ 18 years	49 (87.50)	48 (76.19)	123 (90.44)	116 (80.56)	27 (96.43)	12 (52.17)
	Total	56 (100)	63 (100)	136 (100)	144 (100)	28 (100)	23 (100)
Stage 2	< 18 years	7 (6.00)	13 (11.21)	3 (11.17)	4 (6.90)	8 (11.17)	4 (7.27)
	≥ 18 years	88 (94.00)	103 (88.79)	33 (88.83)	54 (93.10)	46 (88.83)	51 (92.73)
	Total	100 (100)	116 (100)	403 (100)	58 (100)	54 (100)	55 (100)
Stage 3	< 18 years	2 (6.25)	1 (2.44)	1 (5.26)	0 (0)	1 (1.22)	2 (1.75)
	≥ 18 years	30 (93.75)	40 (97.56)	18 (94.74)	16 (100)	81 (98.78)	112 (98.25)
	Total	32 (100)	41 (100)	19 (100)	16 (100)	82 (100)	114 (100)

DISCUSSION

In medico-legal practice, accurate age estimation by forensic odontologists is crucial for the appropriate management of individuals in various legal contexts, particularly regarding juvenile justice proceedings. [23] This is due to the distinct legal ramifications associated with age thresholds such as 14, 16, 18, and 21 years. [23] Beyond the legal sphere, age determination also plays a pivotal role in establishing eligibility for educational opportunities, employment, marriage, and social benefits (e.g. pension), highlighting its broader societal significance. [16]

In forensic evaluations requiring age verification, particularly for legal proceedings involving minors, accurate assessment of the 18 years of age threshold is crucial. While third molar development has been proposed as a method for age estimation, its reliability has been challenged due to documented cases of early mineralisation in individuals below 18 years old. [24-26] Olze et al., [27] addressed this challenge by proposing a novel method for assessing pulp visibility (secondary dentine formation) in orthopantomograms, aiming to enhance forensic certainty in dental age estimation.

The progressive deposition of secondary dentine throughout life diminishes the pulp chambers and root canals, readily observable on dental radiographs. [28-29] Inspired by this age-related phenomenon, Olze et al. [18, 22] devised two novel dental techniques to evaluate the radiographic visibility of root pulp and periodontal ligament in lower third molars for forensic age estimation. Due to inherent variations in third molar development, employing a combination of these methods yielded superior age-predictive accuracy compared to single-method approaches. [30] Considering the documented frequency of missing mandibular third molars (up to 28% [31], 46-60% [3]), which could skew results, this study adopted a differentiated assessment approach. Root pulp visibility was assessed bilaterally in all mandibular molars, while PDL visibility was assessed bilaterally only in mandibular third molars.

Mandibular first molars were pioneered for age estimation by Balla et al., [14] to evaluate their radiographic visibility as maturity markers. They observed that the minimum age was approximately 14 years in males and females for stages 0 and 1, irrespective of the side assessed. Interestingly, the minimum age was over 18 years for stage 3 in males compared to females (16 years approximately). Mantapuri et al., [32] further supported this age range, with stages 0 and 1 reported in the age of 12 years in both males and females. This study, however, found a complete absence of stage 0 in both sexes. Notably, the minimum age for stages 1, 2, and 3 was higher in both sexes compared to previous reports, with minimum age reaching 20, 21, and 22 years respectively.

Balla et al. [13] investigated the root pulp visibility in mandibular second molars as an age indicator. Their findings revealed that at 14 years of age, stage 0 was noted, regardless of sex. Stage 1 was observed at a minimum age of 14.74 years, whereas stage 2 was observed above 18 years in males and below 18 years in females. In this study, a near-identical minimum age of approximately 21 years was noted for both stages 1 and 2 in males and females. While stage 0 was present in a limited number of females, it was absent in males. Notably, stage 3, absent in the previous report, presented with a minimum age of approximately 23 years in both sexes, in the current study.

Previous investigations by many researchers [2-3, 18-19, 33-35] have explored the relationship between root pulp visibility (RPV) in mandibular third molars and chronological age in males. These studies reported minimum ages for RPV stages 0-3 ranging from 16.3 to 18.2 years for stage 0, 16.5 to 21.0 years for stage 1, 18.1 to 25.3 years for stage 2, and 19.1 to 26.5 years for stage 3. Similarly, in these studies, the minimum ages for RPV stages 0-3 ranged from 16.3 to 18.8 years for stage 0, 16.1 to 21.6 years for stage 1, 18.1 to 23.4 years for stage 2, and 21.2 to 27.7 years for stage 3. Consistent with these findings, this study identified minimum ages of approximately 16 years for RPV stages 0, 2, and 3 in male participants, and minimum ages of 15 years for stage 0, and 16 years for stage 1 and 2 in female participants, respectively. Also, stage 3 of PLV in females was found above the 18 years of age threshold.

The mesio-distal width of mandibular molars on the left side, as assessed by Roberts et al., [20] serves as a potential human biological growth marker for identifying individuals exceeding the 18 years of age threshold. Their study identified three distinct patterns (RCW-A, RCW-B, and RCW-C) with a minimum age over 18 years for categories RCW-C, irrespective of sex. These findings were corroborated by Davidson et al., [36] who further introduced a new category (RCW-U) for teeth not readily classified in the initial types. The results of this study were in concordance with previous studies, with the minimum age for all patterns (RCW A, B, and C) was 16 years in males for both sides, whereas 15 years was noted for RCW-A, 16 years for RCW-B, and 17 years for RCW-C in females for both sides.

This study evaluated the predictive accuracy of mandibular molar pulp visibility stages for age classification (<18 years vs. ≥18 years). Stage 2 was identified as the potential threshold in mandibular first molars, with previous reports [14] indicating 20.4% and 16.9% false positive rates for males and females, respectively. Similar rates of false positives were noted in this study (10.25% for males and 11.8% for females), suggesting that stage 2 may be a reliable indicator of age within acceptable error margins.

In a study by Balla et al., [13] the mandibular second molar demonstrated high accuracy in sex classification, with 100% for males and 79.2% for females. Notably in this study, left side molars displayed superior predictive power compared to right side counterparts, with 97% accuracy for both sexes on the left, compared to 88% and 93% for males and females on the right, respectively.

This study was limited by an imbalance in group sizes between males and females, and the exclusion of individuals with incomplete root formation, which resulted in a narrower age range (16-25 years) compared to the potential population (up to 40 years). Also, the participant cohort was above 16 years questioning the credibility of the assessment in the age group below it for assessment of mandibular first and second molars. Predictive classification using ROC curve analysis could not be carried out due to improper distribution of samples in various age groups and the stages of pulp visibility seen. The prospective design was necessitated by radiographic regulations, but future retrospective studies with a wider age range and consideration of factors like ethnicity and dietary habits could provide more generalised findings on the radiographic visibility of root pulp and periodontal ligament in mandibular molars.

CONCLUSION

The present study demonstrates a significant positive correlation between increasing stages of pulp and periodontal ligament visibility in mandibular molars and the attainment of legal adulthood (18 years of age). This correlation holds irrespective of the specific tooth assessed.

Key findings include:

- Mandibular first molars exhibiting stage 2 or 3 pulp visibility exhibit a high probability (> 90%) of belonging to individuals above 18 years of age.
- Stage 2 pulp visibility in mandibular second molars compared to stage 1 shows a significantly higher likelihood (> 94%) of exceeding the legal age threshold.
- Both root pulp and periodontal ligament visibility in mandibular third molars can be used for age estimation with increasing accuracy from stage 2 to 3.
- Application of Roberts method to assess mandibular molar root canal width reveals that individuals categorised as RCW-C have a substantially higher probability of being above 18 years old.

These findings suggest the potential of mandibular molar visibility and root canal morphology as reliable indicators of legal age in forensic and anthropological contexts.

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Correlation of spheno-occipital synchondrosis and mandibular condylar cortication with chronological age using computed tomography in Indian population– A cross-sectional study

Sridhar Murali[1], Anjana Bagewadi[2], Santosh D Patil[3], Jayraj Malik[2], Anabelle Fernandes[2], Lokesh Kumar S[1], Jayapriya Thirupathi[2], Vaishali Keluskar[2]

[1] Department of Oral Medicine, Radiology and Special Care Dentistry, Saveetha Dental College and Hospitals (Saveetha Institute of Medical and Technical Sciences SIMATS),Saveetha University, Chennai, Tamil Nadu, India. [2] Department of Oral Medicine and Radiology KAHER's KLE Vishwanath Katti Institute of Dental Sciences, Constituent Unit of KLE Academy of Higher Education and Research, Belagavi, Karnataka, India. [3]Department of Radiodiagnosis Jawaharlal Nehru Medical College, Constituent Unit of KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi, Karnataka, India.

Corresponding author:
anjanabagewadi455@gmail.com

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KEYWORDS

Occipital Bone;
Sphenoid Bone;
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ABSTRACT

Background: Forensic age estimation is a procedure which utilises many methods to estimate the age of both living and deceased individuals, including those who have died in natural disasters or man-made catastrophes. The pattern and closure of spheno-occipital synchondrosis (SOS) fusion, along with subchondral ossification of the mandibular condyle, can be used to estimate age.
Aim and objectives: This study aims to estimate age using computed tomographic (CT) images of spheno-occipital synchondrosis fusion (SOS) and mandibular condylar cortication (MCC), and to correlate these findings with chronological age.
Materials and methods: The present study included 435 CT images of individuals aged 10-25 years. SOS fusion was assessed using a four-stage system, and MCC was assessed bilaterally using a three-stage system on the sagittal plane. Data on fusion stages and cortication types were entered along with chronological age, and then statistically analysed.
Results: SOS fusion stage 2 occurred at similar age in males (19.82 ± 2.67 years) and females (19.23 ± 2.93 years). Earlier fusion of other stages was observed in females by a mean age of 2 years. MCC was completed 1 year earlier in females, with statistically significant differences ($p \leq 0.001$). When comparing cortication types and different fusion stages, only type II cortication showed statistically significant differences compared to different fusion stages ($p \leq 0.001$).
Conclusion: Mandibular condylar cortication (MCC) and spheno-occipital synchondrosis (SOS) fusion were positively correlated with chronological age, suggesting that these parameters can be used as an adjunct method for age estimation.

INTRODUCTION

Forensic personal identification (FPI) is a core area of forensic sciences and technologies. It deals with the development and implementation of appropriate techniques to identify live and recently deceased subjects, and decomposed human remains at a crime scene for criminal investigation.[1] Despite significant advances in diagnostic methods, FPI remains a challenging task, especially when dealing with skeletal remnants or decomposing body parts. The identification of human remains becomes progressively challenging with increasing postmortem interval (PMI) due to degradation of physical characteristics, further complicated by the influence of both biotic and abiotic factors on the decomposition process.[2, 3] Primary FPI methods include fingerprint analysis, DNA profiling, and dental comparisons.[4] However, these methods have limitations when analysing decomposed skeletal remains or when antemortem and post-mortem records are unavailable.[4,5] In these scenarios, anthropological techniques can be employed for the estimation of population characteristics including age, sex, stature, and ancestry, along with individual identifiers such as moles and cicatrices scars.[6] Accurate estimation of any of these biological profiles is crucial for personal identification, as it narrows down the search for a missing person.[7]

Bone age estimation is a reliable forensic tool, as demonstrated by both quantitative and qualitative studies.[8] Previous studies have used cranial suture closure, dentition, epiphysis and ossification centres, and the articulating surfaces of the ox coxae (pubic symphyses and auricular surface).[9] Methods for evaluating these indicators have evolved, with computed tomography (CT) and magnetic resonance imaging (MRI) now being used in addition to other macroscopic and conventional radiographic examinations.[10]

The spheno-occipital synchondrosis (SOS), also known as the basilar suture, is a growth center between the occipital and sphenoid bones that plays a role in the development of the craniofacial region. Due to its late ossification phase, it is a valuable source of both therapeutic and forensic data.[11] Forensic age estimation by considering the pattern and timing of SOS fusion can provide an upper and lower age limit in adolescents and aids in adult age estimation.[12]

Age estimation using a single bone is found unreliable in previous studies.[13-14] Therefore, this study investigated the use of cortication around the condyle, another reliable factor, to estimate age. The development of the mandibular condyle is closely related to the development and growth of the mandible, with morphological changes in size and remodeling occurring at specific ages.[15] A recent study demonstrated that the displacement of condylar cartilage by bony tissue is not observed until the attainment of adult skeletal maturity.[16] Assessment of the aforementioned parameters can be performed using macroscopic examinations involving a cadaver or radiographic examinations such as CT, MRI, CBCT, or a combination of any of these.[17-20] However, CT scans provide the most precise and accurate images of these factors. Bayrak et al.,[21] evaluated the relationship between mandibular condylar cortication (MCC), spheno-occipital synchondrosis (SOS), and chronological age. As there can be variations among different populations and ethnic groups, this study proposed to assess the correlation between chronological age, SOS, MCC using computed tomography in the Indian population.

MATERIALS AND METHODS

This was a retrospective cross-sectional study of 435 individuals aged 10 to 25 years. CT scans were collected from the archives of a private medical institution between January 2023 till July 2023. CT scans were acquired using a 256-slice GE Revolution Evo® CT machine with the following protocol: 120kVp; 280 mAs; window level of 4000 AU; scan time of 12 s.

Continuous sagittal sections of thickness 0.1 mm with a field of view of 20-25 cm, showing the skull base and mandibular condyle, were included for assessment. The scans were exported in a tagged image file format (*tiff), blinding the patients' demographic details, such as name, age, and sex, revealing the area of interest for assessment by two observers. This study was conducted in accordance with the ethical standards of Declaration of Helsinki, including all amendments and revisions.[22]

This study included CT scans of patients with no evidence of congenital or developmental deformities involving the skull base and mandible; no history of previous trauma or treatment; and no technical errors, motion blur, or artifacts. The CT scans of patients with systemic diseases or temporomandibular joint disorders were excluded. Demographic characteristics of the included subjects are described in the Table 1.

Measurement of parameters

Sagittal sections were selected as they reveal the complete visibility of the mandibular condyle and provide insight into its cortication status. The degree of SOS fusion was assessed in the midsagittal plane because it was clearly visible in that section. Two dentomaxillofacial radiologists

Table 1. Demographic details of the participants described in terms of number and percentage.

Category			n	%
Gender		Male	284	65.3
		Female	151	34.7
		Total	435	100
Age Group (years)	Male	10-14	28	9.9
		15-20	71	25.0
		21-25	185	65.1
		Total	284	100
	Female	10-14	16	10.6
		15-20	42	27.8
		21-25	93	61.6
		Total	151	100
Mandibular Condylar Cortication	Right	Type I	46	10.6
		Type II	143	32.9
		Type III	246	56.6
		Total	435	100
	Left	Type I	48	11.0
		Type II	140	32.2
		Type III	247	56.8
		Total	435	100
Spheno-occipital synchondrosis		Stage 0	41	9.4
		Stage 1	14	3.2
		Stage 2	68	15.6
		Stage 3	312	71.7
		Total	435	100

evaluated the SOS fusion and MCC based on the staging systems described by Franklin and Flavel et al., [23] and Bayrak et al., [24], respectively. To assess intra-observer reliability, one observer randomly assessed a sample of 100 CT images after a period of one month. MCC was assessed based on three stage grading system as depicted in Figure 2

Type I: Absence of cortication observed on mandibular condyle.

Type II: Bone on the mandibular condylar surface appears at a lower density than structure around condyle (cortical areas).

Type III: The surface of mandibular condyle appears at higher or similar density than surrounding cortical areas.
Four stages of Spheno-occipital synchondrosis fusion assessment as depicted in Figure 1.

Statistical analysis:
The stages of SOS fusion and MCC with respect to sex and chronological age were entered into Microsoft Excel® software (Microsoft, Redmond, WA, USA). The chronological age was calculated by subtracting the date of birth from the date of exposure. Statistical Package for the Social Sciences (SPSS) software (version 22.0, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Quantitative variables were represented using mean and standard deviation whereas qualitative variables were expressed as numbers and percentages.
The Kolmogorov-Smirnov test was used to assess the distribution of the data and its normality. One-way ANOVA with post hoc Tukey's test was performed to determine the difference between the stage of SOS fusion and MCC and sex in accordance with chronological age. Linear regression analysis and Pearson correlation tests were used to determine the relationship between chronological age, sex, SOS fusion, and MCC. Inter-examiner and intra-examiner reliability was assessed using Cohen's kappa statistics. The level of significance was set at 5% ($p \leq 0.05$).

RESULTS

Cohen's kappa coefficient demonstrated substantial inter- and intra-examiner agreement in assessing mandibular condylar cortication (MCC) and spheno-occipital synchondrosis (SOS) (k = 0.78 and 0.81, respectively).
No sex- or side-specific differences were observed in the minimum age for type I MCC, which was 10 years. Type II MCC began at 14 years in both sexes, with the exemption of the right side in males, where it began as early as12 years.
Females exhibited Type III MCC onset 3 years earlier (16 years) compared to males (19 years). Statistically significant differences were noticed for MCC across all types and sides, as detailed in the Table 2.
The minimum age for stage 0 and 2 fusion was identical for both males and females. However, stage 1 fusion occurred significantly earlier in females compared to males, with a mean difference of 5 years ($p \leq 0.001$). Notably, stage 3 fusion occurred significantly earlier in males by 3 years than females ($p \leq 0.001$), as shown in Table 3. These findings suggest the existence of distinct and sex-specific timing of SOS fusion across various stages as depicted in Table 3.
This study examined the relationship between the different stages of SOS and MCC in both the male and female subjects on both the sides. On right side of MCC, statistically significant differences were observed in both males and females for Type II MCC and across all stages of SOS. ($p \leq 0.001$ and $p = 0.029$, respectively). Notably, females lacked Stage 0 SOS with Type II and III MCC, while those with Type I MCC lacked Stage 2 SOS. Both males and females with Type III MCC on right side did not present with Stage 1 SOS. Table 4 describes the mean values, along with minimum and maximum age limits, for different stages of MCC and SOS. Similarly, on the left side, statistically significant differences were observed in both sexes for Type II MCC and across all stages of SOS. ($p \leq 0.001$ and $p = 0.018$, respectively). Additionally, Type I MCC and Stages of SOS (Stage 0, 1, and 3) revealed a statistically significant difference ($p = 0.002$) in females. Notably, females showed an absence of Stage 0 SOS in Type I and II MCC, while those with Type I MCC lacked Stage 2 SOS. Stage 1 SOS was absent in both males and females with Type III MCC. The mean values, along with minimum and maximum age limits, for different stages of MCC and SOS are described in Table 5. A very strong positive correlation was observed between MCC on the right and left sides for both genders ($p \leq 0.05$). Other assessed parameters also exhibited a strong positive correlation between them for both genders (see Table 6).
This study evaluated the variation in the type of MCC on both sides in 246 images. Type I MCC was observed on both sides in all but one image, where type II MCC was noted on the left side. In 9 of the 246 images with type III MCC, type II MCC was noted on the left side. Other possible variations in the types of MCC between the right and left sides are described in Table 7.

Figure 1. Stage 0: Completely open. Stage 1: Closed superior border. Stage 2: Complete fusion with visible fusion scar. Stage 3: Complete fusion with no visible scar.
Stage assessment system of Spheno-occipital synchondrosis devised by Franklin and Flavel.et al.[23]

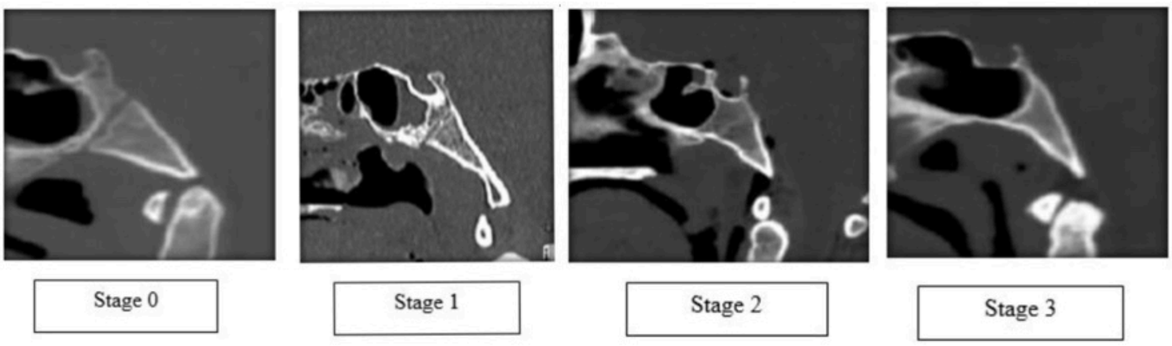

Figure 2. Mandibular condylar cortication assessment based on 3-stage system devised by Bayrak et al. [24]

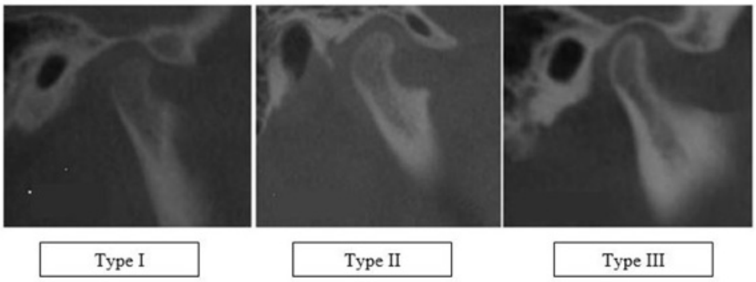

Table 2. Types of MCC with respect to the sides and gender. Superscript a, b, and c represent type I, II, and III MCC, respectively Superscript ab, ac, and bc denotes association between type I, II, and III MCC based on post hoc analysis n - number of patients SD - Standard deviation Min - minimum, Max – maximum. ** denotes highly statistically significant difference ($p \leq 0.001$) Statistical test used: One-way ANOVA with post hoc Tukey test.

Sex	Mandibular Condylar Cortication	Right					Left					p
		n	Mean	SD	Min	Max	n	Mean	SD	Min	Max	
Males	Type I $^{ab, ac}$	32	12.84	3.18	10	24	33	12.76	3.09	10	24	≤0.001**
	Type II ^{bc}	91	19.63	2.67	12	25	87	19.71	2.55	14	25	
	Type III	161	23.14	1.53	19	25	164	23.09	1.57	19	25	
	Total	284	20.85	3.93	10	25	284	20.85	3.92	10	25	
Females	Type I $^{ab, ac}$	14	10.93	1.07	10	13	15	11.47	2.37	10	19	≤0.001**
	Type II ^{bc}	52	18.54	2.39	14	24	53	18.72	2.41	14	24	
	Type III	85	22.69	1.72	16	25	83	22.67	1.86	16	25	
	Total	151	20.17	4.03	10	25	151	20.17	4.03	10	25	
Total	Type I $^{ab, ac}$	46	12.26	2.85	10	24	48	12.35	2.91	10	24	≤0.001**
	Type II ^{bc}	143	19.23	2.62	12	25	140	19.34	2.54	14	25	
	Type III	246	22.98	1.61	16	25	247	22.95	1.68	16	25	
	Total	435	20.62	3.97	10	25	435	20.62	3.97	10	25	

Table 3. Stages of SOS with respect to sex for included studies. n - number of patients. SD - Standard deviation. Min - minimum, Max – maximum. ** denotes highly statistically significant difference ($p \leq 0.001$) Statistical test used: One-way ANOVA

Spheno-occipital synchondrosis		n	Mean	SD	Min	Max	p
Male	Stage 0	29	12.62	3.34	10	24	≤0.001**
	Stage 1	12	16.67	1.44	15	19	
	Stage 2	33	19.82	2.68	14	25	
	Stage 3	210	22.39	2.28	10	25	
	Total	284	20.85	3.93	10	25	
Female	Stage 0	12	10.83	0.94	10	13	≤0.001**
	Stage 1	2	14.50	6.36	10	19	
	Stage 2	35	19.23	2.93	14	25	
	Stage 3	102	21.71	2.64	13	25	
	Total	151	20.17	4.03	10	25	
Overall	Stage 0	41	12.10	2.96	10	24	≤0.001**
	Stage 1	14	16.36	2.34	10	19	
	Stage 2	68	19.51	2.81	14	25	
	Stage 3	312	22.17	2.42	10	25	
	Total	435	20.62	3.97	10	25	

Table 4. SOS stages and its corresponding types of MCC on the right side. n - number of patients. SD - Standard deviation. Min - minimum, Max – maximum. * stands for statistically significant values at p ≤ 0.05. ** denotes highly statistically significant difference (p ≤ 0.001) Statistical test used: One-way ANOVA.

Mandibular Condylar Cortication	Spheno-occipital synchondrosis	Males					P
		N	Mean	SD	Min	Max	
Type I	Stage 0	25	12.16	3.00	10	24	0,137
	Stage 1	4	15.50	1.00	15	17	
	Stage 2	1	16.00	-	16	16	
	Stage 3	2	14.50	6.36	10	19	
Type II	Stage 0	3	13.33	1.16	12	14	≤0.001**
	Stage 1	8	17.25	1.28	16	19	
	Stage 2	23	19.00	2.34	14	23	
	Stage 3	57	20.54	2.27	15	25	
Type III	Stage 0	1	22.00	-	22	22	0,200
	Stage 2	9	22.33	1.66	19	25	
	Stage 3	151	23.19	1.52	20	25	
Mandibular Condylar Cortication	**Spheno-occipital synchondrosis**	**Female**					**p**
		n	**Mean**	**SD**	**Min**	**Max**	
Type I	Stage 0	12	10.83	0.94	10	13	0,092
	Stage 1	1	10.00	-	10	10	
	Stage 2	-					
	Stage 3	1	13	-	13	13	
Type II	Stage 0	-					0.029*
	Stage 1	1	19.00		19	19	
	Stage 2	23	17.57	1.85	14	21	
	Stage 3	28	19.32	2.55	14	24	
Type III	Stage 0	-					0,549
	Stage 2	12	22.42	1.68	19	25	
	Stage 3	73	22.74	1.73	16	25	

Table 5. SOS stages and its corresponding types of MCC on the left side. n - number of patients. SD - Standard deviation. Min - minimum, Max – maximum. * stands for statistically significant values at p ≤ 0.05. ** denotes highly statistically significant difference (p ≤ 0.001) Statistical test used: One-way ANOVA.

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Mandibular Condylar Cortication	Spheno-occipital synchondrosis	Males					p
		N	Mean	SD	Min	Max	
Type I	Stage 0	27	12.22	2.90	10	24	98
	Stage 1	4	15.50	1.00	15	17	
	Stage 2	1	16.00	-	16	16	
	Stage 3	2	14.50	6.36	10	19	
Type II	Stage 0	1	14.00	-	14	14	≤0.001**
	Stage 1	8	17.25	1.28	16	19	
	Stage 2	25	19.04	2.46	14	23	
	Stage 3	53	20.51	2.31	15	25	
Type III	Stage 0	1	22.00	-	22	22	237
	Stage 2	8	22.25	1.75	19	25	
	Stage 3	155	23.14	1.56	20	25	
Mandibular Condylar Cortication	**Spheno-occipital synchondrosis**	**Female**					**p**
		n	**Mean**	**SD**	**Min**	**Max**	
Type I	Stage 0	12	10.83	0.94	10	13	0.002*
	Stage 1	1	10.00	-	10	10	
	Stage 2	-					
	Stage 3	2	16.00	4.24	13	19	
Type II	Stage 0	-					0.018*
	Stage 1	1	19.00	-	19	19	
	Stage 2	24	17.71	1.94	14	21	
	Stage 3	28	19.57	2.52	14	24	
Type III	Stage 0	-					806
	Stage 2	11	22.55	1.70	19	25	
	Stage 3	72	22.69	1.89	16	25	

Table 6. Correlation between the MCC on both sides, SOS, chronological age and gender. Statistical test used: Pearson Correlation. rp is Pearson correlation coefficient * denotes statistically significant values at $p \leq 0.05$

	MCC Right (r_p)		MCC Left (r_p)		SOS (r_p)		Chronological age (r_p)	
	Male	Female	Male	Female	Male	Female	Male	Female
MCC Right (r_p)	1	1	.934*	.963*	.723*	.709*	.815*	.861*
MCC Left (r_p)			1	1	.754*	.694*	.821*	.836*
SOS (r_p)					1	1	.785*	.746*
Chronological age (r_p)							1	1

Table 7. Variations in the types of MCC on right and left sides.

		MCC Left Side			
	Cortication type	**Type I (%)**	**Type II (%)**	**Type III (%)**	**Total**
MCC Right Side	Type I	45 (10.3)	1 (0.2)	0	46 (10.5)
	Type II	3 (0.7)	130 (29.9)	10 (2.3)	143 (32.9)
	Type III	0	9 (2.1)	237 (54.5)	246 (56.6)
	Total	48 (11)	140 (32.2)	247 (56.8)	435 (100)

DISCUSSION

Establishing an individual's identity is essential in cases of catastrophic events, such as manmade or natural disasters. [25] Age estimation in living individuals is of extreme importance in living or dead individuals for various purposes, such as identification, obtaining civil rights and benefits from society, and medico-legal purposes. [26] Hence, forensic age estimation (FAE) has become an integral part of the forensic medicine field that focuses on utilizing an accurate method of estimating the chronological age of the person. [27] This helps in the identification of both living and deceased individuals and can be used to create a biological profile that can be compared to missing persons for the latter. [28]

With the recent surge in the need for identification and age estimation in living individuals for legal purposes, forensic anthropology has been extended to include this area of study. [29] Age estimation procedures are implemented to accurately categorize individuals as adults or children with protected legal status, ensuring transparency through reproducible methodologies and verifiable results for expert review and evaluation of clinical interpretations. [30]

A variety of methods can be used to estimate chronological age, including height and weight measurements, pubertal status, dentition, and dental findings. [31] While these methods offer valuable insights, no single approach can definitively ascertain chronological age with complete accuracy. [27] This highlights the need for novel approaches and improved accuracy in age estimation techniques.

The expanding role of computed tomography (CT) in forensic medicine extends beyond post-mortem examinations, finding application in anthropological studies and age estimation of living individuals. [32] Notably, integrating skeletal changes revealed by CT scans with other age-related indicators, such as dental development, enhances the accuracy and confidence in age estimation compared to relying solely on traditional methods. [33]

Kadesjö et al., [34] compared the effective dose of cone-beam computed tomography (CBCT) and multislice CT (MSCT) for temporomandibular joint (TMJ) examinations using thermoluminescent dosimetry (TLD) measurements. Before dose optimization, the bilateral effective dose was 184 µSv for CBCT and 113 µSv for MSCT. Following optimization, the CBCT dose was reduced by 50% compared to MSCT, resulting in effective doses of 92 µSv and 124 µSv, respectively. This suggests that optimization strategies demonstrate considerable potential for significantly reducing radiation doses associated with both dentomaxillofacial CT and CBCT examinations.

Considerable variation in the closure of SOS among males, females, and different ethnicities has provoked interest in the research field to further assess the fusion degree as an accurate age estimation method. [10] Also, the mandibular condyle, an integral component of the mandible varies according to sex with respect to its growth, development, shape, or morphological appearance. [35] Developmental changes, such as cortication can be detected on radiographs, and the assessment of these changes can be used to correlate with the age of the individual.

Assessment of SOS fusion was performed using a four-stage system devised by Franklin and Flavel et al., [23] utilizing CT in 312 Australian individuals. In males, the SOS was open (Stage 0) at a mean age of 10.28 ± 3.30 years and complete fusion (Stage 3) occurred at mean age of 19.83 ± 2.94 years. In females, the SOS was open at a mean age of 8.62 ± 2.40 years, and complete fusion (Stage 3) occurred at a mean age of 18.62 ± 3.55 years. Other fusion stages (stages 1 and 2) also occurred earlier in females than in males.

Hisham et al., [36] assessed fusion of SOS using a four-stage system in a CT scans of 500 Malaysian individuals. Stage 0 fusion occurred earlier in females (9.33 ± 2.69 years) than in males (10.26 ± 2.45 years). Stages 1 and 2 also occurred earlier in females than in males, by approximately 2 years and 1 year, respectively. Stage 3 fusion occurred at similar ages in both sexes, with mean age of 20.84 ± 2.84 years in males and 19.78 ± 3.35 years in females.

Two Turkish studies assessed the SOS using a four-stage system on CT scans. [18, 37] Sinanoglu et al., [18] found that stage 0 and stage 2 fused at similar age in males and females, whereas stage 1 and stage 3 fused 1-2 years earlier in females. Kocasrac et al [37] found out that all fused earlier in females, except for stage 0, which fused earlier in males.

In this study, the SOS fusion stages were generally earlier in females than in males. Stage 0 fusion occurred approximately 2 years earlier in females (10.83 ± 0.94 years) than males (12.62 ± 3.34) years. [23] Stage 1 and 3 fusion also occurred earlier in females, by 1-2 years. Stage 2 fusion occurred at similar ages in males and females (19.82 ± 2.68 years and 19.23 ± 2.93 years, respectively). Complete fusion (Stage 3) occurred at the age of 22.39 ± 2.28 years in males and 21.71 ± 2.64 years in females. The results in this study correspond to previously published studies [18, 23, 36, 37] that showed earlier fusion of SOS in females, suggesting that females attain skeletal maturity earlier than males do.

Lei et al., [38] were the first to classify the formation of cortical bone around mandibular condyle using CBCT based on presence of a bony layer in the periphery of the mandibular condyle. They found that the initial signs of subchondral bone formation were seen at 12-13 years for girls and 13-14 years for boys, with complete cortical bone formation by 21 years for females and 20 years for males.

Bayrak et al., [24] developed a novel method of mandibular condylar cortication (MCC) classification, which identified three stages based on the density difference of cortical bone surrounding the mandibular condyle and areas adjacent to condyle. Type I cortication was seen at a mean age of 14.14 years for males and 13.01 years for females, suggesting that there is no evidence of cortical bone formation below this age category. They concluded that the type of MCC may vary for the same individual when the right and left sides are considered, and that the cortication process occurs later in males than in females.

Ma et al., [39] investigated the morphology and cortication of the mandibular condyle using cone-beam computed tomography (CBCT) from 1010 temporomandibular joints (TMJ). They found that the majority of mandibular condyles exhibited a planar morphology anteriorly, while a convex morphology was more prevalent posteriorly. The mean age for males and females with no evidence of cortication was 15.11 ± 2.71 and 14.25 ± 2.60 years, respectively. Complete cortication of the mandibular condyle was observed at a mean age of 23.63 ± 3.36 years for males and 23.86 ± 3.73 years for females.

MCC was assessed on both sides in this study. Irrespective of the side, Type I cortication was seen at a mean age of 12-13 years in males and 10-11 years in females. MCC was complete at a mean age of 23 years in males and 22 years in females. These results are consistent with those reported by Lei et al., [38], Bayrak et al., [24], and Ma et al. [39]

Seo et al., [40] assessed the cortication of the mandibular condyle using Cone Beam Computed Tomographic (CBCT) images of 829 Korean individuals. The prevalence of no cortication was higher in males (30.84%) compared to females (19.74%). Conversely, females exhibited a higher prevalence of complete cortication (35.59%) compared to males (26.48%). These findings suggest sex-based differences in mandibular condyle cortication patterns, potentially influencing susceptibility to temporomandibular joint disorders.

Bayrak et al., [21] were the first to assess the relationship between SOS fusion stages and different types of MCC using CBCT. They found a positive correlation between these parameters, with statistically significant differences for both males and females. Similarly, in this study, a very strong positive correlation was seen for both sexes between right- and left-side MCC and SOS, as well as between chronological age, sex, and the measured parameters. This suggests that MCC, along with SOS fusion, can be used as a reliable method for estimating age.

The findings from this study converge with the previous study [41] on the promising use of these parameters in forensic settings, with a key strength being their consistent results across observers. The authors acknowledge that the health, nutrition, growth, and developmental status of bones, as well as ethnicity, can influence the closure of the SOS. This can impart variability with respect to age; hence, this method should be used as an adjunct to other more accurate age estimation methods. Additionally, the limited sample size and uneven distribution of age groups among ethnicities may limit the generalizability of the findings.

Future studies should include an equal number of individuals in both groups to accurately compare the differences in age for closure of spheno-occipital synchondrosis as well as mandibular condylar cortication. Additionally, an improved sample size with more data from studies that utilized computed tomography for the assessment of both parameters in the Indian population is needed to create a population-specific dataset for future forensic purposes.

CONCLUSION

This study evaluated the reliability of the SOS and MCC for age estimation which was demonstrated by the positive correlation between chronological age, sex, and assessed parameters, thereby revealing it to be used reliably for age estimation. Calculating an age range of the included individuals for various stages of MCC and SOS based on maximum and minimum ages can be considered. To assess the generalizability of these findings and explore potential population-specific variations, further research is warranted comparing these observations across diverse demographic subsets. Additionally, future studies should investigate the potential of cone-beam computed tomography (CBCT) as a comprehensive imaging modality for evaluating the mandibular condyle and middle cranial fossa.

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Compliance with Ethical Standards: This retrospective study was conducted on patients who underwent routine radiological investigation. Consent was obtained from all patients prior to radiographical exposure.

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Forensic odontology-related awareness, knowledge, and attitude among dentists and dental students in two Egyptian universities: a survey-based study

Fatma Nada Khalifa[1,2], Amira Fathy El-Gazzar[3,4], Walaa Samir[5], Fatma Mohamed Hassan[1]

[1]Forensic Medicine and Clinical Toxicology department, Faculty of Medicine, Cairo University, Cairo, Egypt. [2]Forensic Medicine and Clinical Toxicology department, Faculty of Medicine, Badr university in Cairo, Badr city, Cairo, Egypt. [3]Public Health and Community Medicine department, Faculty of Medicine, Assiut University, Assiut, Egypt. [4]Public Health and Community Medicine department, Faculty of Medicine, Badr university in Cairo, Badr city, Cairo, Egypt. [5]Oral Radiology department, Faculty of Dentistry, Beni-Suef University, Beni-Suef, Egypt.

Corresponding author: fatmahasan.2010@cu.edu.eg

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KEYWORDS

Awareness; Dentists; Dental students; Egypt; Forensic odontology; Knowledge

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ABSTRACT

Aim and objectives: This study evaluated the awareness, knowledge, and attitude regarding forensic odontology (FO) among dental students and graduates at Beni-Suef University and Badr University in Cairo, Egypt.
Methods: This cross-sectional study included 316 dentists and dental students in their final year of undergraduate study. An online self-administered questionnaire in English was used to obtain participants' responses.
Results: Less than half of the participants (47.5%) were aware of the branch of dentistry named FO. The highest percentages of correct answers in the knowledge assessment were reported in dental age estimation questions, while the lowest percentages were in items related to the use of FO in confirming sexual abuse and using palatal rugae as a marker in forensic identification. The most common source of knowledge was the internet (52.0%). Only 11.1% of the participants received FO courses in their colleges. Studying FO as a postgraduate course was interesting to 67.4% of the participants. The knowledge score was significantly associated with the participants' gender, graduation status, and educational level.
Conclusion: This study revealed a lack of awareness of FO as a branch of dentistry among dental students and dentists in Egypt. Increasing awareness and knowledge of FO could be achieved by integrating FO into the dental curriculum at both undergraduate and postgraduate levels.

INTRODUCTION

According to the World Dental Federation (FDI), forensic odontology (FO) refers to the area of dentistry concerned with properly handling and examining dental evidence and appropriate evaluation and demonstration of dental findings in favor of justice.[1] FO processes, reviews, analyzes, and presents dental evidence to provide scientific proof and unbiased data to judicial proceedings.[2]
The identification of human remains using dental records, age and gender identification of the living and deceased, analyzing bite marks, providing evidence of abuse, and expert witness testimony in court using forensic dental evidence are just a few of the immense applications of FO.[3] Several techniques are utilized in FO, including rugoscopy, cheiloscopy, tooth impressions, bite mark analysis, radiography, photography, and

molecular procedures.[4]
Dentists are actively engaged in applying FO, so they should possess adequate expertise and knowledge of FO while dealing with dental records, studying bite marks, interpreting radiographs, conducting DNA analysis of tooth pulp, and assessing tooth morphology.[2,5] Courses, demonstrations, and lectures presented by the American Academy of Forensic Science (AAFS) represent fundamental training sources for forensic dentists. Professional certification in FO is offered by the American Board of Forensic Odontology (ABFO), a division of the AAFS.[6,7]
Despite the importance of FO to criminal justice, it is still underdeveloped in many countries.[8] Several studies conducted in Saudi Arabia revealed a lack of sufficient knowledge of FO among dental practitioners and students.[5,9,10] In the United Arab Emirates, a study conducted at Ajman University indicated inadequate knowledge of FO among dental students.[8]
In Egypt, there is a significant literature gap regarding FO, and no study has evaluated the knowledge or attitude of dentists or dental students toward FO. This study aimed to bridge this gap by assessing the awareness, knowledge, and attitude toward FO among dental students and graduates at Beni-Suef University and Badr University in Cairo, Egypt.

PARTICIPANTS AND METHODS

This cross-sectional study was conducted among dentists and dental students in their final year of undergraduate study at two Egyptian universities, Beni-Suef University and Badr University in Cairo, from March 2023 to June 2023.

Study tool:
A semi-structured questionnaire in the English language was developed after reviewing previous literature and included the following parts:
Part 1: Demographic and professional characteristics of the participants, including gender, age, years of clinical experience, level of education, and work sector (private or governmental).
Part 2: Awareness of FO and whether the participants had attended FO courses were assessed by two closed-ended questions with yes or no answers. The actual knowledge was evaluated by 15 close-ended statements sourced from published literature,5,9,10 with three answers: yes, no, or do not know.
Part 3: Attitude toward FO was assessed using six questions with answers rated on a 5-point Likert scale (strongly disagree, disagree, neutral, agree, and strongly agree).
The questionnaire underwent piloting and validation to identify any problems in the questions that could lead to biased answers. Cronbach's alpha of reliability for the knowledge and attitude sections was 0.74 and 0.76, respectively.

Study participants and sample size:
All dentists and dental students who were willing to participate in their final year at Beni-Suef University and Badr University in Cairo were included in the study. The sample size for students was calculated using Epi-Info. Based on the expected level of knowledge (30%) and a confidence interval of 95%, the sample size required was determined to be 175 students. For dentists, they were totally included.

Data collection technique:
An online self-administered questionnaire on Google Forms was utilized. The questionnaire link was sent to the official email addresses of the dentists and dental students, accompanied by a cover letter explaining the study's aim and assuring the anonymity of participants' responses.

Ethical statement and approval:
The study received approval from the research ethics committee of the Faculty of Dentistry, Beni-Suef University (approval number #REC-FDBSU/06042013-04/KF). All data-gathering processes were conducted confidentially following the Helsinki Declarations of biomedical ethics. Participants were informed about the anonymous nature of the survey, and their participation was voluntary. Only those who agreed were given access to the questionnaire.

Statistical analysis:
Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) statistical software version 25 (International Business Machines IBM, SPSS Inc, NY). Descriptive statistics (frequencies and percentages) were used to demonstrate categorical sample characteristics and responses, while the arithmetic mean, and standard deviation (SD) were used to present the outcomes.

The Shapiro-Wilk test was employed to test normality, with a significance value greater than 0.05 (0.2) indicating normal data distribution.
The summated score of the 15 actual knowledge statements was obtained for each study participant (correct responses scored 1, wrong and do not know responses scored 0). The scores were then converted into percentages classified into four categories: ≤50 (poor), 50-70 (moderate), 70-90 (good), and ≥ 90 (very good).11 The attitude score was calculated on a scale from 1 (strongly disagree) to 5 (strongly agree), with the maximum score being 30, indicating a positive attitude.
Bivariate analysis was conducted using Pearson's chi-square ($\chi 2$) to identify the association between demographic factors and awareness of FO and knowledge levels. Statistical significance was set at $P < 0.05$.

RESULTS

This study included 316 dentists and dental students, with a mean age of 24.38±7.15 years. The majority of the participants (258, 81.6%) were between 18 and 28 years old, more than half of them (172, 54.43%) were females, and (144, 45.57%) were males. More than half of the participants (175, 55.4%) were undergraduates, while (72, 22.8%) graduated less than 5 years ago and (69, 21.8%) graduated 5 years or more. Regarding educational level, (175, 55.4%) were dental students, (60, 19%) had bachelor's degrees, (40, 12.6%) had master's degrees, and (41, 13%) had doctorate degrees. Membership in the governmental sector was represented by (200, 63.3%) of participants, while (116, 36.7%) were from the private sector (Table 1).

Table 1. Demographic and professional characteristics of the study participants

Variable	Frequency N=316	%
Age (years)		
18-28	258	81.6
>28-38	34	10.8
>38-48	20	6.3
>48	4	1.3
Mean ± SD	24.38±7.15	
Minimum-maximum/range	18-54/36	
Gender		
Male	144	45.57
Female	172	54.43
Graduation status		
Undergraduate	175	55.4
Graduated (˂5 years)	72	22.8
Graduated (≥ 5 years)	69	21.8
Level of education		
Dental student	175	55.4
Bachelor degree	60	19.0
Master degree	40	12.6
Doctorate degree	41	13.0
Work or faculty sector		
Private	116	36.7
Government	200	63.3

SD: Standard deviation

Regarding participants' awareness of FO, (150, 47.5%) reported that they were aware of the branch of dentistry named FO, while (166, 52.5%) did not know about it (Figure 1). Figure 2 shows that (35, 11.1%) of the participants took FO courses in their colleges.

Regarding responses to knowledge items among participants who were aware of FO, the percentages of correct replies for each question varied. The highest percentages were reported in questions related to age estimation; an individual's dental age can be estimated by the eruption status (135, 90%), FO can be used to estimate age (133, 88%), and an individual's dental age can be estimated by radiography (127, 84.7%). The lowest percentages of correct answers were reported in questions regarding whether FO can confirm sexual abuse (77, 51.3%) and whether palatal rugae can be used as a marker in forensic identification (80, 53.3%).

The reported sources of knowledge in FO were the internet (78, 52.0%), followed by undergraduate and postgraduate lectures (51, 34%), books (31, 20.7%), scientific articles and journals (30, 20.0%), and seminars and workshops (19, 12.7%). The mean knowledge score was 10.7±3.1. The knowledge percentage score was categorized as "poor" for (23, 15.3%), "moderate" for (25,16.7%), "good" for (55, 36.7%), and "very good" for (47, 31.3%) of the respondents (Table 2).

Participants' attitudes toward FO, as presented in Table 3, revealed that (113, 75.3%) of those who knew FO as a branch of dentistry agreed about the importance of FO in recognizing culprits and deceased individuals, and (118, 78.7%) agreed on the importance of keeping dental records. Only (13, 8.6%) reported that their knowledge of FO is adequate, while (96, 64%) reported inadequate knowledge of FO. Studying FO as a postgraduate course was interesting for (101, 67.4%) participants, but only (50, 33.4%) wanted to specialize in FO. The mean attitude score was 22.5±3.1.

The association between participants' demographic and professional characteristics and awareness of FO as a dentistry branch revealed significant differences between various subgroups. Older participants, females, those with five years or more of experience, those with doctorate degrees, and those working in the public sector were more aware of FO than their comparable groups (p-value = 0.000) (Table 4).

Figure 1. A pie chart representing the distribution of participants (n=316) regarding awareness of forensic odontology as a branch of dentistry.

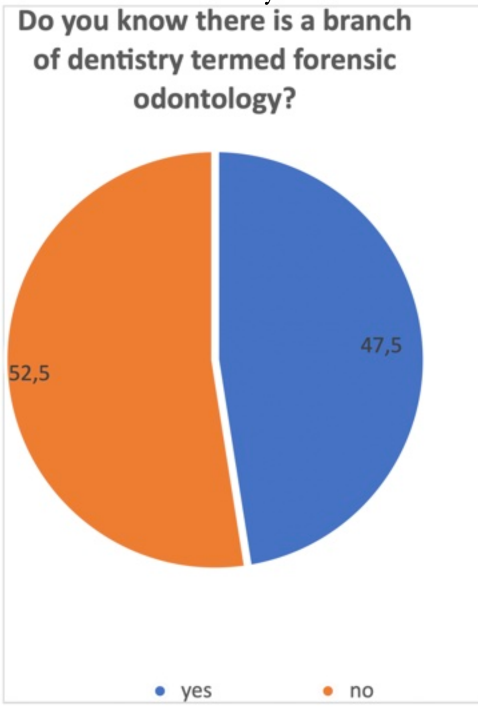

Figure 2. A pie chart representing the distribution of those who took a forensic odontology course in their colleges.

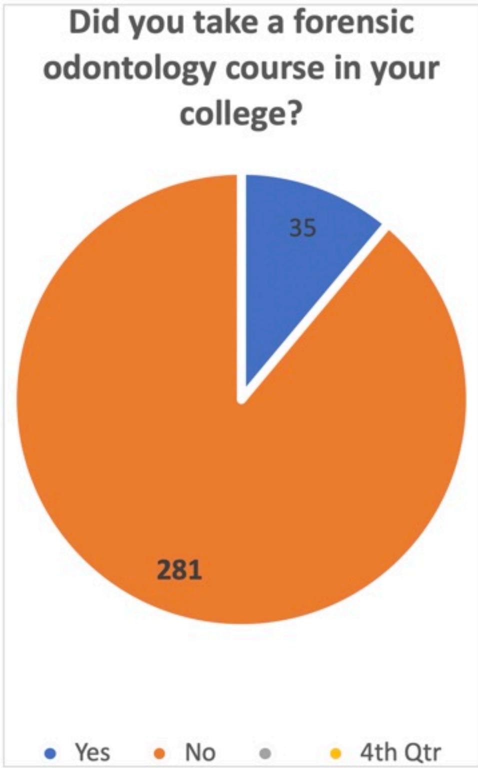

Table 2. Participants' responses to forensic odontology knowledge items

Variable	Frequency N=150	%
Forensic odontology aids in physical violence identification		
yes	115	76.7
No	8	5.3
I do not know	27	18
Forensic odontology can be used to estimate age		
yes	133	88.7
No	7	4.7
I do not know	10	6.7
Forensic odontology can be used for gender identification		
yes	111	74
no	11	7.3
I do not know	28	18.7
Forensic odontology can confirm child neglect		
yes	111	74
no	11	7.3
I do not know	28	18.7
Forensic odontology can confirm sexual abuse		
yes	77	51.3
No	21	14.0
I do not know	52	34.7
Analysis of bite-mark patterns aids in identifying criminals		
yes	118	78.7
No	5	3.3
I do not know	27	18
Dental patterns are unique identifiers		
yes	115	76.7
no	6	4.0
I do not know	29	19.3
An individual has a unique jaw structure		
yes	89	59.3
no	24	16.0
I do not know	37	24.7
An individual has a unique lip print		
yes	84	56.0
No	9	6.0
I do not know	54	36.0
DNA can be extracted from the teeth of a deceased person		

yes	118	78.7
no	7	4.7
I do not know	25	16.7
Palatal rugae can be used as a marker in forensic identification		
yes	80	53.3
no	3	2.0
I do not know	67	44.7
Practicing forensic odontology needs a permit or certification		
yes	117	78.0
no	2	1.3
I do not know	31	20.7
An individual dental age can be estimated by radiography		
Yes	127	84.7
no	6	4.0
I do not know	17	11.3
An individual dental age can be estimated by the eruption status		
yes	135	90.0
No	5	3.3
I do not know	10	6.7
The most accurate method to identify individuals		
DNA comparison	88	58.7
Fingerprints	35	23.3
Physical anthropological exam of bone and teeth	18	12.0
Visual identification	9	6.0
What was your source of information in forensic odontology? [A]		
Undergraduate or postgraduate lectures	51	34.0
Internet	78	52.0
Scientific articles and journals	30	20.0
Books	31	20.7
Seminars and workshops	19	12.7
Others	9	6.0
Total knowledge score		
Poor knowledge	23	15.3
Moderate knowledge	25	16.7
Good knowledge	55	36.7
Very good knowledge	47	31.3
Knowledge score (mean ± SD)	10.7±3.1	

A: Participants reported more than one answer.
SD: Standard deviation

Table 3. Participants' attitudes towards forensic odontology

Variable	Frequency N=150	%
Forensic odontology is helpful in recognizing culprits and dead persons		
Strongly agree	35	23.3
agree	78	52.0
Neutral	23	15.3
Disagree	6	4.0
Strongly disagree	8	5.3
It is important to keep patients' dental records		
Strongly agree	58	38.7
agree	60	40.0
Neutral	20	13.3
Disagree	5	3.3
Strongly disagree	7	4.7
My knowledge of forensic odontology is adequate		
Strongly agree	2	1.3
agree	11	7.3
Neutral	41	27.3
Disagree	68	45.3
Strongly disagree	28	18.7
I am interested in studying forensic odontology, if there is postgraduate study		
Strongly agree	37	24.7
agree	64	42.7
Neutral	34	22.7
Disagree	10	6.7
Strongly disagree	5	3.3
I want to be a specialist in forensic odontology		
Strongly agree	19	12.7
agree	31	20.7
Neutral	68	45.3
Disagree	19	12.7
Strongly disagree	13	8.7
I want to participate in workshops and seminars in forensic odontology		
Strongly agree	32	21.3
agree	54	36.0
Neutral	36	24.0
Disagree	12	8.0
Strongly disagree	16	10.7
Attitude score (mean ± SD)	22.5±3.1	

SD: standard deviation.

Table 4. Association between demographic and professional characteristics of the participants and awareness that forensic odontology is a branch of dentistry

	Knows (N=150)	Does not know (N=166)	P-value
Age (years)			
18-28 (N=258)	106 (41.1)	152 (58.9)	0.000*
>28-38 (N=34)	26 (76.5)	8 (23.5)	
>38-48 (N=20)	14 (70.0)	6(30.0)	
>48 (N=4)	4 (100)	0 (0)	
Gender			
Male (N=144)	65(45.1)	79(54.8)	0.000*
Female (N=172)	84(48.8)	88 (51.16)	
Graduation status			
Undergraduate (N=175)	67 (38.3)	108 (61.7)	0.000*
Graduated (<5 years) (N=72)	33(45.8)	39 (54.2)	
Graduated (≥ 5 years) (N=69)	49(71.0)	20(29.0)	
Level of education			
Dental student (N=175)	60(34.3)	115 (65.7)	0.000*
Bachelor degree (N= 60)	32 (53.3)	28 (46.7)	
Master degree (N=40)	29(72.5)	11 (27.5)	
Doctorate degree (N=41)	32(78.0)	9(22.0)	
Work or faculty sector			
Private (N=116)	52(44.8)	64 (55.2)	0.000*
Public (N=200)	98 (49.0)	102 (51.0)	

*P-value is statistically significant.

The association between demographic and professional characteristics of the respondents and knowledge scores revealed significant differences regarding gender, graduation status, and educational level (P-value = 0.04, 0.002, and 0.002, respectively) (Table 5).

Table 5. Association between demographic and professional characteristics of the participants and knowledge scores

	Poor	Moderate	Good	Very good	P-value
Age (years)					
18-28 (N=100)	20(20.0)	15(15.0)	32(32.0)	33(33.0)	0.09
>28-38 (N=30)	2(6.7)	6(20.0)	10(33.3)	12(40.0)	
>38-48 (N=16)	2(12.5	2(12.5)	10(62.5)	2(12.5)	
>48 (N=4)	0 (0.0)	0(0.0)	3(75.0)	1(25.0)	
Gender					
Male (N=69)	9(13.0)	6(8.7)	25(36.23%)	29(42.0%)	0.04*
Female (N=81)	13(16.0)	18(22.2)	30(37.0%)	20(24.7%)	
Graduation status					

Undergraduate (N=70)	19(27.1)	14(20.0%)	22(31.4%)	15(21.5)	0.002*
Graduated (<5 years) (N=30)	3(10)	5(16.7)	12(40.0)	10(33.3)	
Graduated (≥ 5 years) (N=50)	3(6.0)	7(14.0)	21(42.0)	19(38.0)	
Level of education					
Dental student (N=65)	18(27.7)	13(20.0)	21(32.3)	13(20.0)	0.002*
Bachelor degree (N=30)	2(6.7)	3(10.0)	12(40.0)	13(43.3)	
Master degree (N=27)	1(3.7)	5(18.5)	7(25.9)	14(51.8)	
Doctorate degree (N=28)	1(3.6)	3(10.7)	16(57.1)	8(28.6)	
Work or faculty sector					
Private (54)	3(5.5)	9(16.7)	21(38.9)	21(38.9)	0.06
Public (96)	19(19.8)	15(15.6)	34(35.4)	28(29.2)	

*P-value is statistically significant.

DISCUSSION

FO is a significant branch of dentistry that plays a crucial role in identifying the living and deceased and investigating abuse and criminal acts. Awareness and knowledge of FO are essential among dental practitioners.[5,12] Practicing FO has gained importance in many developed countries; however, it is struggling in developing countries like Egypt.

This study is the first to evaluate awareness, knowledge, and attitude toward FO among dentists and dental students in Egypt.

An important finding in this study is that 52.5% of the participants were unaware that there is a branch of dentistry termed FO, which can be explained as FO is not included in the undergraduate or postgraduate academic curricula in Egypt. Among the participants, only 11.1% reported they had FO courses in college. Similarly, several studies conducted in various countries, such as Saudi Arabia,[3,5] UAE,[8] and Pakistan,[13,14] reported inadequate knowledge and awareness of FO among dental practitioners or students. Dietrichkeit Pereira et al. [15] stated that one of the primary causes of the lack of knowledge of FO is that it is not a part of the mandatory undergraduate curriculum in dentistry. On the other hand, several studies in India, such as Sahni et al. [16] in Delhi, Rahman et al. 2017 [11] in Bhubaneswar, and Ram et al. [17] in Chennai, reported sufficient knowledge of FO among dental teaching staff, surgeons, and students, respectively. The Dental Council of India curriculum includes 30 hours of theoretical and practical FO sessions for the third and final years of the Bachelor of Dental Surgery program, which explains the adequate knowledge of the dental community in India.[18]

Regarding responses to knowledge of FO items among participants who knew that FO is a branch of dentistry, the majority correctly answered questions related to dental age estimation. This finding is consistent with Almutairi et al. [9] and Hashim et al. [8] However, Preethi et al. [19] reported that around half of their study participants did not know how age can be estimated using teeth.

The importance of FO can be related to the resistance of dental tissue to high temperatures and decomposition, making it a suitable source for DNA.[20] Fortunately, more than three-quarters of dentists and dental students who knew FO was a branch of dentistry acknowledged this fact, which is consistent with the results of Gupta et al.,[21] Hashim et al.,[8] and Tahir et al.[13]

The majority of dentists and dental students in this study agreed about the importance of FO in identifying physical abuse and child neglect; however, only half of them approved its role in sexual abuse cases. Intimate partner violence might include bite marks that require expert dental consultation.[22] Therefore, dentists must have sufficient knowledge, a trained eye, and professional skills for such consultations.

Palatal rugae are unique, and their shape remains constant throughout life, making them an alternate identification source when other approaches are challenging.[23,24] Nearly half of the participants in this study did not know that palatal rugae could be used in forensic identification, a similar result to that reported by

Almutairi et al.[9] Additionally, almost half were unaware that each individual has a unique lip print and jaw structure, possibly due to a lack of exposure to FO in Egypt.

In this study, although more than half of the dental students and dentists were unaware of FO's existence as a branch, the majority who knew this fact had a sufficient knowledge score; 31.3% were classified as very good and 36.7% as good.

Regarding participants' attitudes toward FO, most of those who knew FO was a branch of dentistry agreed about the importance of FO in recognizing culprits and deceased persons and the importance of keeping dental records. This finding is consistent with Ali et al.,[14] who reported that 87% of their study participants believed dental records are valuable in identifying the deceased and crime suspects. Additionally, Preethi et al. [19] found that most dental practitioners know the value of maintaining dental records.

A dental record is maintained for forensic purposes and may be used as a future reference for practitioners, as consumer court evidence, and for dental insurance. The increased public awareness regarding legal issues in healthcare necessitates dental practitioners being more knowledgeable about dental records.[25]

In this study, the minority (8.6%) believed that their information about FO is adequate. Similarly, Abdul et al. [3] reported that 93.2% of undergraduate dental students and 83.4% of graduates acknowledged their lack of knowledge about FO. Hashim et al. [8] found that 93.1% of participants agreed they are missing adequate knowledge in FO.

Despite studying FO as a postgraduate course being interesting for more than two-thirds of participants who knew FO as a dentistry branch, only about one-third wanted to be specialists in FO. This finding could be related to the absence of job opportunities in FO in Egypt, which discourages dentists from choosing FO as a profession. Similar results were reported by Abdul et al.,[3] Hashim et al.,[8] and Al-Abdaly et al.[5] On the contrary, Hannah et al. found that 89.6% of participants believe that FO has good scope as a profession.[26]

Regarding the association between the demographic and professional characteristics of the participants and awareness and knowledge of FO, older participants, females, those with five years or more of experience, those with doctorate degrees, and those working in the public sector were more aware that FO is a branch of dentistry than their comparable groups. Additionally, the knowledge score was significantly associated with gender, graduation status, and educational level.

This is in accordance with Almutairi et al.,[9] who found that experience above 5 years, Ph.D. education, and working in the government sector were significantly associated with higher actual knowledge. Additionally, Tahir et al. [13] reported higher awareness among consultants and specialists than undergraduates and postgraduate residents and the older age group than the younger ones. They attributed this difference to consultants' better clinical experience and access to more opportunities for ongoing medical education.

CONCLUSION AND RECCOMANDATIONS

This study revealed a lack of awareness of FO as a branch of dentistry among dental students and dentists in Egypt. However, those aware that FO is a branch of dentistry showed adequate knowledge of various FO items. The knowledge score was significantly associated with the gender, graduation status, and educational level of the participants. This study highlights the importance of integrating FO into the dental curriculum at the undergraduate and postgraduate levels. Dental institutions should offer FO training through workshops, seminars, regular education programs, and in-field training in forensic centers to enhance the knowledge and skills of graduates. Moreover, offering more job opportunities in FO will increase interest in attending and participating in various education and training programs.

Strengths and limitations

This study was the first to evaluate FO awareness, knowledge, and attitude among dentists and dental students in Egypt. Egypt has 68 dentistry faculties, including state-funded, national, and private institutions. However, this study was conducted in only two educational institutions, so the sample does not represent all dentists and dental students in Egypt. The study can serve as a benchmark for future studies advocating the importance of FO.

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Analysis and comparison of tooth wear in late antiquity and early middle age in populations that lived in continental and coastal Croatia using digitized VistaMetrix method

Ana Družijanić [1,5], Ivan Galić [1,2], Marin Vodanović [3], Mario Šlaus [4], Jelena Dumančić [3], Marija Roguljić [1,5], Ana Glavina [6], Andrea Gelemanović [7], Hrvoje Brkić [3]

[1] University of Split School of Medicine, Split, Croatia. [2] Department of Oral Surgery, Clinical Hospital Split, Split, Croatia. [3] Department of Dental Anthropology, School of Dental Medicine, University of Zagreb, Croatia. [4] Anthropological Center, Croatian Academy of Sciences and Arts, Zagreb, Croatia. [5] University of Split School of Medicine, Department of Periodontology, Split, Croatia. [6] Dental Outpatient Clinic Split, Split, Croatia. [7] Mediterranean Institute for Life Sciences (MedILS), Split, Croatia

Corresponding author: ana.druzijanic8.1.ad@gmail.com

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KEYWORDS

Tooth wear;
Dental age;
Tooth attrition;
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ABSTRACT

Background: Tooth wear is a non-pathological loss of hard tissues on the incisal and occlusal tooth surface. In archaeology, the loss of dental tissue through attrition is associated with living opportunities and habits, availability, characteristics and methods of food preparation. In forensics, tooth wear is used to estimate the dental age on cadavers. Material and methods: For this study, we used an archaeological sample from two sample collections. In this study, tooth wear was compared in archaeological samples of well-preserved maxilla and mandible specimens (n=392) from Croatian coastal and continental populations from Late Antiquity (LA) and the Early Middle Ages (EMA). The computer system VistaMetrix 1.38 was used to analyse the abrasion and attrition of hard dental tissues. The Shapiro-Wilk and chi-square tests were performed for categorical data to test the difference between two historical periods and two geographical locations, while the Kruskal-Wallis test was performed for continuous data. Results: There was a statistically significant difference in the proportion of tooth wear in total teeth area ($P < 0.001$) when comparing continental and coastal Croatia in LA and coastal Croatia between LA and EMA ($P = 0.006$ and $P < 0.001$, respectively). Samples from coastal Croatia from the LA period had the lowest percentage of tooth wear with a median of 8.35%, while samples from coastal Croatia from the EMA had the highest percentage of tooth wear with a median of 18.26%. Our results generally show greater tooth wear in the EMA period in male subjects.Conclusion: The results of the tooth wear research obtained with the Vista Metrix software can contribute to the study of life circumstances and changes that the analysed population has experienced in its historical development.

INTRODUCTION

One of the methods used to assess dental age is to analyse tooth wear. It can be used after the age of 20, when the teeth begin to wear on the occlusal surfaces and incisal edges due to their function. Tooth wear analysis is regularly used in forensic and archaeological analyses. [1,2] During lifetime, tooth enamel is subject to wear due to physical and mechanical influences, which is known as tooth abrasion. This phenomenon is known as tooth wear. It occurs during the chewing of food and can occur in varying degrees and patterns. The horizontal form of enamel abrasion is more common and affects the incisal edges and occlusal surfaces of premolars and molars, while vertical enamel abrasion occurs on the vertical surface of the teeth, particularly the incisors. [3-6] In contrast to abrasion of the enamel, attrition means the gradual and regular loss or physiological deterioration of tooth structures without the action of abrasive agents. These changes can be a reliable indicator of the lifestyle and dietary habits of a certain population. Frequent consumption of solid foods increases abrasion and tooth wear is more pronounced. The abrasion angle can also provide important information about the lifestyle of a particular population. For example, straight abrasion is characteristic of hunting and harvesting populations, while the dentition of those engaged in agriculture is characterised by oblique abrasion. [7] In previous studies, tooth wear has been used as one of the parameters for age estimation or to distinguish samples from different historical periods. [8,9] However, it is mainly assessed using visual scales that determine tooth wear according to the complex stages of tooth tissue involvement.

Insights into historical periods, lifestyles, political and religious aspects, art, philosophy, economics and other aspects of society can be obtained by applying modern multidisciplinary techniques. [10] The material heritage provides a rich source of information. In addition to buildings, weapons, tools, jewellery and art, skeletal remains such as teeth are also a source of information as they record the life experience of an individual. [11] They can provide information about living conditions, eating habits, work activities, dental and general health. Changes in the orofacial system, such as dental caries, loss of hard dental tissues due to abrasion and wear, as well as changes in the alveolar bone and orthodontic anomalies, are very useful for analysing and reconstructing the lives of people from different historical periods. [12, 13] Human teeth from individuals of different ages at the time of death can be preserved for thousands of years. Therefore, the dentition and teeth are often the subject of research in bioarchaeology and palaeostomatology and are used in forensic sciences. [14, 15] Analysing bone remains of the jaw and preserved teeth, including a detailed diagnosis and interpretation of diseases of the dentition itself and the dental system, can help in reconstructing the way of life of ancient people. They can also provide information about the type and origin of the food that ancient people consumed. [16] In addition, a careful and detailed examination of the oral cavity and teeth can reveal many details about a person's health status, work activities and lifestyle, which is extremely important for forensic odontology. [17]

The difference in diet between the two historical periods, Late Antiquity (LA) and the Early Middle Ages (EMA), is probably reflected in the loss of hard tissue. The consumption of various vegetables and fruits characterises the LA period. The diet included many raw fruits and nuts, which have an abrasive potential. People in LA also consumed different types of meat, which required vigorous chewing. In contrast, the diet in EMA was generally much milder and consisted of grains and other carbohydrate-rich foods, while meat and protein-rich foods were rare. Consumption of such food did not require vigorous chewing, which is reflected in the lower amount of tooth wear in samples from EMA compared to those from LA. [18, 19]

Some professional activities also leave traces in the oral cavity and on the teeth. Mechanical, chemical and thermal injuries to the mucosa and hard dental tissues are often the result of performing certain activities. [10] For example, professional brass players and craftsmen such as glass blowers, fish net makers, carpenters and tailors may have characteristic mechanical damage on their teeth due to gripping objects between their teeth during their daily work. [19]

This research is based on well-preserved osteological collections of known sex and skeletal age owned by the Croatian Academy of Sciences and Arts.

The aim of this study was to analyse and compare tooth wear in two different historical periods, LA and EMA, in populations living in two different geographical locations, the continental and the Croatian coastal area, using the new digitised VistaMetrix method.

Based on the obtained results, the possibility of their use for dental age estimation in forensic and archaeological research will be determined.

MATERIALS AND METHODS

Samples

Skeletal remains curated in the Osteological Collection of the Croatian Academy of Sciences and Arts (HAZU) were used in this study. This research was approved by the Ethics Committee of the School of Dental Medicine, the University

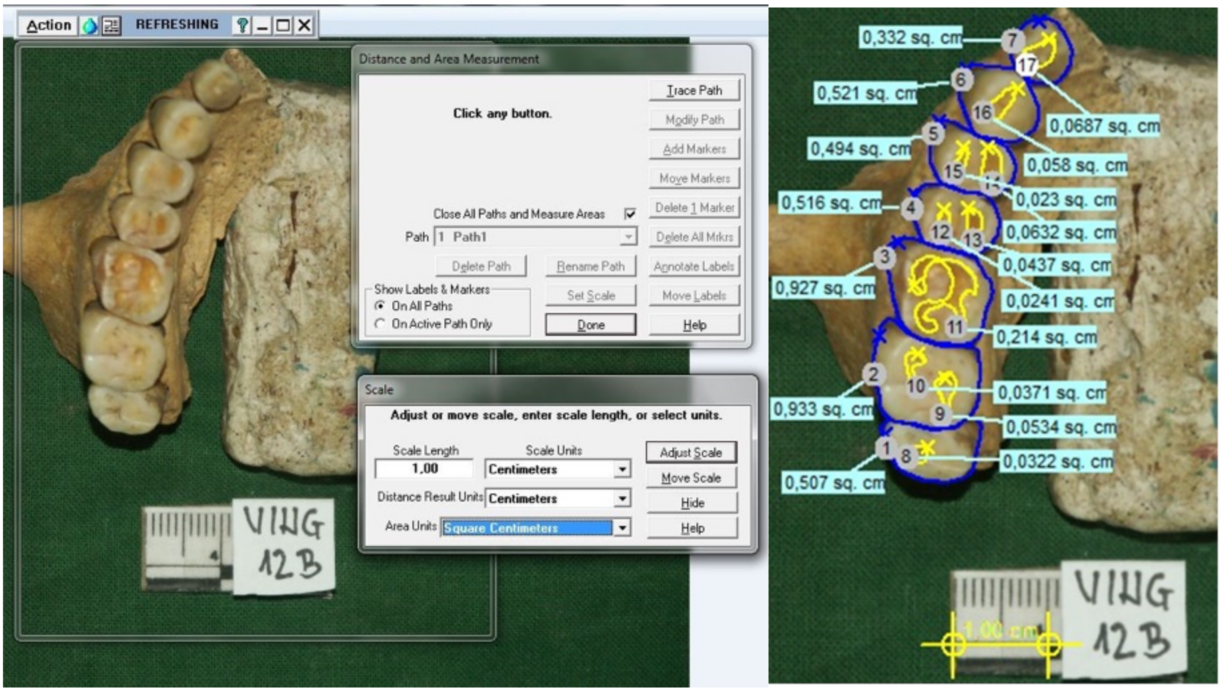
of Zagreb, at the 18th regular session held on June 4th 2020, decision number 05-PA-30-XVIII-6/2020. The samples originate from seven archaeological sites in Croatia, four from inland (continental) and three from coastal regions of Croatia and belong to two historical periods: Late Antiquity (LA) and Early Middle ages (EMA).
The remains from the LA period originate from four inland (Zmajevac, Štrbinci, Osijek, and Vinkovci) and one coastal site (Zadar). The EMA samples were from one coastal site – (Velim Velištak) and one continental site (Radašinovci). From each of the sites, a number of skeletons were picked as a random sample to represent all of the sites equally.
The sex and osseal age were determined for each skeleton on the basis of the skeletal bones. This data comes from the archives of the Croatian Academy of Sciences and Arts. On the basis of this data, the examined sample according was staratified to sex (male and female) and three age groups: young age (18-30 years), middle age (30-50 years) and old age (50 and older).
The sample consisted of 130 remains of male and 75 remains of female skeletons from all the sites and both historical periods. Regarding the exact anatomical structure, 182 maxillary remains and 210 mandibular remains were included in the sample.
Unfortunately, due to the relatively poor preservation skulls of the individuals under 15 years old, and the impossibility of performing measurements, only teeth and skulls from adults were used in this analysis.
Digital photographs of the maxillary and mandibular remains were made with a LUMIX DMC-TZ50 photo camera (Panasonic, Osaka, Japan) at a 90-degree angle. A 1cm calibrated scale was included in each photography. All jaw samples were occlusally photographed with the help of a fixed camera holder at the same distance. With the help of a lens (85 mm : 3,5 G ED), and a circular flash (Sigma EM-140 DG), the recording was performed by one researcher (M.V.). Resolution and file format of the images acquired was 300dpi, 24 million pixels, Figure 1.
The measurements were taken from 392 photographs of teeth and jaw specimens using the VistaMetrix 1.38 computer system (SkillCrest LLC, Tucson AZ, USA).
Regarding the teeth, only the teeth present in the maxillary and mandibular remains were included in the study. Excluding factors were the teeth lost ante and post-mortem and teeth lost

Figure 1. Schematic representation of the recording of occlusal surfaces of skeletal remains in this research

during the archaeological excavation process. Since this study aimed to analyze tooth wear, teeth affected with severe carious lesions were also excluded, which brings the total number of excluded teeth to 1563 of all teeth from all maxillar and mandibular remains. Regarding the historical periods, the LA period had 739 lost and 26 caries-affected teeth, while the EMA period had 748 lost and 40 caries-affected teeth.
The study included 4408 teeth, of which 1545 molars, 1224 premolars, 647 canines, and 992 incisors.
The computer system VistaMetrix 1.38 consists of a transparent pattern overlaid with a photography or material to be edited and contains a measurement toolbar (Figure 2). VistaMetrix 1.38 allows quantitative measuring on digital images to obtain metric data and differs from the standard methods of qualitative evaluation of tooth wear.
In our study, tooth wear was analysed as the main parameter, and the involvement of hard dental tissues was measured objectively and precisely using computer software. One examiner (A. D.) analyzed all photographs using the VistaMetrix 1.38 software for one month.
Analysis of digital photographs included measurements of areas on each tooth of each sample of maxillary and mandibular remains.

First, using VistaMetrix 1.38 software, the visible biting surfaces of the posterior teeth and the incisal edges of the anterior teeth were marked; they included all visible surfaces of the teeth above the alveolar bone. A blue line bounds each surface. The computer program records the bounded area in cm^2.
Then, on the same teeth, measurements are performed on surfaces that show signs of tooth wear by abrasion or attrition. A yellow line bounds such places, and their surface was also expressed in cm^2. If there is no visible loss of hard dental tissues on the tooth, it was recorded in the photograph with a dot, and in the data analysis, it was expressed as 0 cm^2. Finally, if there are two different tooth wear surfaces on one tooth, they are recorded separately in the photograph, and in the data analysis, such surfaces were added up for individual teeth (Figure 2).
To determine the inter- and intra-examiner agreements, two experienced and well-trained dentists (A. D., H. B.) evaluated the photographs. Before beginning the study, each examiner evaluated 40 randomly selected photography twice at different times to calibrate the methods and examiners.
In addition, 10% randomly selected one month after the initial evaluation were assessed by both examiners twice, one week apart.

Figure 2. Analyzed photography using VistaMetrix 1.38 computer software (SkillCrest LLC, Tucson AZ, USA)



All the obtained data is summarised in an Excel spreadsheet.
For the statistical analysis, we subdivided the sample according to gender, historical period and geographical location. With regard to the known skeletal age, we divided the sample into younger, middle and older ages.
The total tooth surfaces and the surfaces of tooth wear determined in this way are compared with each other in order to determine the differences in tooth wear between the sexes, historical periods, geographical locations and age groups.

Statistical analysis
All statistics were performed with R v3.2.2. (Team RC. R: A language and environment for statistical computing. Vienna, Austria 2013). The normal distribution of the continuous variables was first tested using the Shapiro-Wilk test. As not all variables followed a normal distribution, the results were presented as medians with the interquartile range (IQR). Subsequently, the $\chi 2$ test was performed for categorical data to test the difference between two historical periods and two geographical locations, while the Kruskal-Wallis test was performed for continuous data.

For significant associations, Dunn's post hoc tests were performed for multiple pairwise comparisons. The level of statistical significance was defined as P < 0.05. The interclass correlation coefficient (ICC) was used to determine agreement between and within investigators

RESULTS

Our results showed that there were no statistically significant differences between the two historical periods and geographical locations in terms of sex and estimated skeletal age (P = 0.115 and P = 0.174, respectively). However, when the sample was divided into three age groups: young age, middle age and old age, there was a statistically significant difference in EMA between continental and coastal samples, with the majority of samples in coastal Croatia estimated to be middle-aged, while in continental Croatia the majority of samples belonged to a younger group (post hoc = 0.005) (Table 1).

Table 1. Distribution of jaw remains by sex and estimated skeletal age depending on historical period and geographical location

	Total	Late Antiquity (LA)		Early Middle Ages (EMA)		P*
		Continental (N = 94)	Coastal (N = 35)	Continental (N = 32)	Coastal (N = 44)	
Sex, N (%)						
Male	130 (63.41)	57 (60.64)	28 (80)	17 (53.12)	28 (63.64)	115
Female	75 (36.59)	37 (39.36)	7 (20)	15 (46.88)	16 (36.36)	
Estimated skeletal age, N (%)						
Young age (15-30)	50 (24.39)	23 (24.47)	9 (25.71)	15 (46.88)	3 (6.82)	0.005^{B}
Middle age (31-45)	120 (58.54)	54 (57.45)	22 (62.86)	11 (34.38)	33 (75.00)	
Old age (46+)	35 (17.07)	17 (18.09)	4 (11.43)	6 (18.75)	8 (18.18)	

* $\chi 2$ test with post hoc test (only significant results after pairwise comparisons labelled). Abbreviations for significant post hoc tests: A – LA Continental & LA Coastal; B – EMA Continental & EMA Coastal; C – LA Continental & EMA Continental; D – LA Coastal & EMA Coastal

Comparing coastal and continental Croatia in two historical periods, there was no statistically significant difference in the total number of teeth (P = 0.123) or the total area of all teeth (P = 0.150). However, there was a statistically significant difference in the proportion of tooth wear in the total teeth area (P < 0.001, Table 2).

Furthermore, the post hoc test revealed a significant difference when comparing continental and coastal Croatia in LA and coastal Croatia between LA and EMA (P = 0.006 and P < 0.001, respectively). Overall, samples from coastal Croatia from the LA period showed a minor proportion of tooth wear with a median of 8.35%, whereas samples from coastal Croatia from the EMA had the most significant proportion of tooth wear with a median of 18.26%.

When the samples were stratified by sex, a statistically significant difference was found only in the male population for the proportion of tooth wear to total teeth area, again between continental and coastal Croatia in LA and coastal Croatia between LA and EMA (P = 0.031 and P = 0.001, respectively).

Regarding estimated skeletal age, the proportion of tooth wear to total teeth area was not significant for the young and old age groups, but differed significantly in the middle-aged population group, where it was higher in coastal Croatia from EMA than in coastal Croatia from LA (P<0.001).

The proportion of tooth wear in total tooth area was also compared between these two historical periods and geographical locations, separately for each tooth group for the entire sample and stratified based on sex and age group. Overall, we found a statistically significant difference in all teeth groups except the right canines when we looked at the proportion of tooth wear to total tooth area by historical period and geographic location (Table 3). When the sample was stratified based on sex and age group, a statistically significant difference in tooth wear was found in all tooth groups in the male population and in incisors, right and left premolars, and left molars in the middle-aged population. The same trend was observed in all teeth groups, with a significant increase in tooth wear observed in continental samples compared to Croatian LA coastal samples and in EMA samples compared to LA coastal samples. No statistically significant difference was observed between LA and EMA samples from continental Croatia or between continental and coastal EMA samples.

Table 2. Distribution of the total number of teeth, total area, and tooth wear of all teeth depending on the historical period and geographical location and grouped by sex and estimated skeletal age

	Late Antiquity (LA)		Early Middle Ages (EMA)		P*
	Continental	Coastal	Continental	Coastal	
TOTAL					
Samples (N)	95	37	32	44	
Total number of all teeth, median (IQR)	23 (12)	23 (10)	20.5 (8.25)	22 (10.5)	123
Total area of all teeth (cm^2), median (IQR)	14.04 (6.18)	13.62 (6.69)	12.03 (6.39)	11.73 (6.39)	150
Tooth wear/total area of all teeth (%), median (IQR)	13.98(12.15)	8.35 (7.41)	13.6 (13.94)	18.26(13.03)	<0.001[A,D]
MALE					
Samples (N)	57	28	17	28	
Total number of all teeth, median (IQR)	23 (11)	22.5 (8.5)	21 (8)	21.5 (10.5)	401
Total area of all teeth (cm^2), median (IQR)	14.87 (5.71)	13.56 (6.78)	12.57 (6.53)	12.06 (7.65)	351
Tooth wear/total area of all teeth (%), median (IQR)	14.26 (13.32)	8.3 (5.98)	15.36 (18.6)	20.64 (15.14)	0.001[A,D]
FEMALE					
Samples (N)	37	7	15	16	
Total number of all teeth, median (IQR)	24 (15)	22 (8)	18 (9)	22 (8.75)	368
Total area of all teeth (cm^2), median (IQR)	13.48 (7.51)	13.96 (5.75)	11.97 (3.94)	11.14 (5.19)	443
Tooth wear/total area of all teeth (%), median (IQR)	13.75(11.34)	8.35 (7.28)	9.47 (10.95)	15.97 (9.93)	237
YOUNG AGE					
Samples (N)	23	9	15	3	
Total number of all teeth, median (IQR)	26 (6)	22 (5)	23 (8)	27 (7.5)	243
Total area of all teeth (cm^2), median (IQR)	15.16 (4.33)	14.93 (3.97)	13.67 (5.08)	14.04 (3.97)	529
Tooth wear/total area of all teeth (%), median (IQR)	8.44 (7.43)	5.7 (2.31)	8.78 (6.32)	5.33 (0.69)	48
MIDDLE AGE					
Samples (N)	54	22	11	33	
Total number of all teeth, median (IQR)	25 (12.25)	23 (9.5)	17 (9)	22 (10)	100
Total area of all teeth (cm^2), median (IQR)	14.92 (6.55)	13.56 (7.07)	12.06 (5.14)	11.91 (6.25)	319
Tooth wear/total area of all teeth (%), median (IQR)	13.95 (7.4)	9.29 (5.96)	15.76(14.25)	17.83(13.74)	0.001[D]
OLD AGE					
Samples (N)	17	4	6	8	
Total number of all teeth, median (IQR)	15 (7)	15.5 (11.5)	14.5 (7.75)	15 (7)	861
Total area of all teeth (cm^2), median (IQR)	10 (5.35)	9.94 (5.6)	8.6 (2.79)	7.56 (3.82)	611
Tooth wear/total area of all teeth (%), median (IQR)	30.66 (15.05)	27.39 (16.96)	28.92 (11.65)	22.37 (5.62)	342

* Kruskal-Wallis test with Dunn's post hoc test for significant associations. Abbreviations for significant post hoc tests: A – LA Continental & LA Coastal; B – EMA Continental & EMA Coastal; C – LA Continental & EMA Continental; D – LA Coastal & EMA Coastal

Table 3. The ratio variables of canine teeth with significant differentiating function based on discriminant analysis

	Samples (N)	Late Antiquity (LA)		Early Middle Ages (EMA)		P*
		Continental	Coastal	Continental	Coastal	
INCISORS						
Tooth wear/total area (%), median (IQR)						
Total	1011	15.85 (15.68)	11.74 (9.64)	15 (16.72)	18.84 (12.24)	0.002[A,D]
Male	620	15.94 (12.95)	11.11 (8.19)	19.89 (19.4)	18.42 (17.39)	0.008[A,D]
Female	378	14.16 (16.38)	13.95 (10.53)	12.8 (15.69)	18.92 (7.72)	273
Young age	270	9.85 (7.75)	7.87 (9.45)	9.11 (16.48)	7.1 (0.95)	563
Middle age	603	15.91 (15.46)	13.75 (9.06)	16.02 (12.35)	19.01 (12.2)	0.031[D]
Old age	125	25.97 (13.65)	19.68 (1.39)	28.5 (7.66)	19.66 (7.89)	274
Tooth wear/total area (%), median (IQR) – Right incisors						
Total	513	14.72 (14.22)	10.8 (12.97)	12.86 (15.22)	18.16 (13.68)	0.014[A,D]
Male	317	16.09 (13.93)	10.71 (14.29)	21 (11.64)	17.31 (18.11)	46
Female	188	13.45 (14.36)	13.26 (10.3)	10.38 (8.46)	19.49 (8.69)	133
Young age	139	10.32 (8.61)	7.89 (9.05)	8.9 (9.38)	7.83 (1.05)	494
Middle age	306	14.97 (12.19)	11.18 (16.97)	18 (9.51)	18.64 (14.2)	144
Old age	60	24.89 (17.07)	17.32 (4.06)	24.64 (20.58)	19.25 (8.17)	363
Tooth wear/total area (%), median (IQR) – Left incisors						
Total	498	16.47 (14.13)	10.08 (9.72)	10.51 (18.82)	18.48 (12.39)	0.002[A,D]
Male	303	16.12 (13.22)	8.69 (8.81)	13.03 (18.66)	18.65 (13.62)	0.020[A,D]
Female	190	16.96 (16.49)	11.72 (10.21)	6.5 (9.88)	16.73 (8.91)	143
Young age	131	7.7 (9)	6.32 (11.11)	8.83 (5.36)	5.79 (0.68)	703
Middle age	297	16.52 (13.55)	10.08 (7.97)	9.75 (17.86)	18.65 (11.37)	0.009[A,D]
Old age	65	26.58 (13.4)	21.02 (0.21)	20.69 (10.71)	19.78 (6.7)	230
CANINES						
Tooth wear/total area (%), median (IQR)						
Total	655	10.83 (11.41)	7.47 (7.37)	9.13 (13.77)	12.91 (9.81)	0.022[D]
Male	412	11.21 (10)	7.59 (6.66)	9.81 (11.53)	14.9 (11.61)	0.003[D]
Female	235	8.84 (12.72)	7.93 (8.23)	5.79 (9.97)	8.15 (5.26)	759
Young age	164	8.84 (9.72)	4.21 (4.49)	4.41 (5.12)	4.12 (2.34)	491
Middle age	392	9.45 (7.72)	7.59 (6.91)	10.47 (13.64)	12.81 (10.64)	56
Old age	91	22.27 (16.24)	15.15 (11.8)	15.78 (6.94)	14.92 (4.63)	196
Tooth wear/total area (%), median (IQR) – Right canines						
Total	336	10.69 (11.44)	7.9 (7.9)	9.48 (13.78)	11.55 (12.64)	65
Male	211	11.35 (9.44)	7.2 (7.43)	10.21 (19.3)	14.61 (13.98)	0.004[D]
Female	121	7.76 (15.18)	12.29 (8.15)	8.73 (12.75)	8.04 (4.12)	916
Young age	83	7.22 (7.49)	5.55 (2.56)	6.67 (9.51)	7.13 (2.59)	907
Middle age	202	9.7 (7.88)	8.22 (7.27)	11.2 (18.88)	10.94 (13.88)	50
Old age	47	23.41 (21.36)	14.31 (14.68)	16.94 (7.56)	15.8 (8.79)	403
Tooth wear/total area (%), median (IQR) – Left canines						
Total	319	10 (12)	6.28 (8.21)	8 (9.94)	10.93 (13.18)	0.013[D]
Male	201	11.84 (12.11)	6.53 (8.16)	11.13 (7.7)	16.2 (12.25)	0.008[D]
Female	114	8.68 (13.25)	3.91 (7.55)	2.34 (6.96)	6.69 (3.1)	258
Young age	81	6.78 (10.3)	2.74 (3.3)	2.34 (4.71)	3.65 (4.42)	315
Middle age	190	8.68 (8.6)	6.53 (7.51)	10.22 (8.41)	11.44 (14.12)	143
Old age	44	24.01 (10.23)	14.59 (12.2)	14.02 (7.95)	16.95 (8.57)	210
PREMOLARS						
Tooth wear/total area (%), median (IQR)						
Total	1242	8.3 (10.36)	4.58 (4.75)	7.06 (7.35)	11.12 (12.9)	0.001[A,D]

Male	785	8.3 (12.67)	4.4 (4.95)	12 (9.88)	11.63 (11.34)	0.004^{D}
Female	439	8.49 (9.76)	5.46 (3.31)	6.53 (2.19)	6.69 (10.47)	344
Young age	323	5.82 (5.42)	4.26 (3.64)	6.35 (3.4)	5.42 (2.97)	596
Middle age	749	7.95 (9.08)	4.15 (4.02)	7.54 (10.46)	11.16 (12.43)	0.001^{A,D}
Old age	152	24.62 (22.16)	36.85 (29.42)	15.88 (4.85)	13.84 (10.88)	293
Tooth wear/total area (%), median (IQR) – Right premolars						
Total	613	7.71 (11.12)	4.07 (5.49)	7.48 (10.67)	10.14 (8.91)	<0.001^{A,D}
Male	388	7.44 (10.99)	4.39 (3.84)	8.18 (12.45)	11.31 (10.03)	0.002^{D}
Female	217	8.35 (12.06)	3.9 (4.13)	7.06 (3.15)	6.3 (10.59)	258
Young age	161	5.59 (6.21)	4.72 (2.69)	6.9 (2.96)	3.39 (3.04)	240
Middle age	374	7.38 (11.16)	3.88 (3.54)	5.9 (15.52)	9.54 (8.07)	<0.001^{A,D}
Old age	70	24.69 (24.66)	20.81 (11.96)	16.71 (5.37)	14.46 (7.78)	382
Tooth wear/total area (%), median (IQR) – Left premolars						
Total	629	6.88 (10.91)	4.75 (5.31)	6.25 (5.37)	10.67 (11.6)	0.003^{D}
Male	397	6.88 (11.41)	4.73 (6.51)	7.8 (11.24)	11.02 (10.74)	0.021^{D}
Female	222	6.42 (8.6)	4.75 (2.9)	6.07 (4.85)	8.46 (11.21)	327
Young age	162	4.83 (3.77)	2.86 (2.09)	5.72 (3.74)	4.38 (2.73)	564
Middle age	375	6.71 (7.14)	4.73 (4.19)	9.47 (2.96)	10.62 (12.09)	0.008^{D}
Old age	82	25.11 (22.93)	41.17 (34.25)	11.24 (15.83)	12.87 (9.07)	277
MOLARS						
Tooth wear/total area (%), median (IQR)						
Total	1570	15.5 (15.56)	9.22 (10.51)	15.28 (21.8)	20.75 (23.05)	<0.001^{A,D}
Male	998	15.36 (15.16)	9.59 (8.79)	17.79 (22.18)	24.66 (24.39)	0.003^{D}
Female	547	16.19 (16.76)	8.58 (7.98)	8.41 (17.01)	19.71 (13.84)	395
Young age	445	8.7 (8.22)	5.61 (1.78)	8.21 (5.73)	6.08 (0.41)	81
Middle age	914	15.58 (10.33)	11.91 (7.5)	23.35 (17.53)	19.14 (20.75)	0.011^{D}
Old age	186	34.15 (19.64)	23.1 (13.49)	36.45 (9.29)	32.37 (12.71)	639
Tooth wear/total area (%), median (IQR) – Right molars						
Total	789	14.56 (16.22)	9.8 (9.86)	15.24 (25.07)	18.16 (24.91)	0.018^{D}
Male	494	13.14 (15.54)	9.88 (9.93)	15.59 (26.59)	19.51 (26.35)	0.022^{D}
Female	284	16.24 (16.09)	9.8 (3.21)	12.17 (15.11)	15.69 (17.99)	323
Young age	218	9.95 (9.12)	5.45 (1.79)	11.59 (7.5)	6.3 (0.38)	121
Middle age	466	14.66 (15.37)	10.76 (6.84)	22.15 (20.21)	18.1 (23.08)	91
Old age	94	36.54 (25.57)	17.77 (14.61)	37.63 (9.45)	36.24 (17.85)	732
Tooth wear/total area (%), median (IQR) – Left molars						
Total	781	15.12 (16.36)	8.74 (7.44)	13.28 (20.93)	21.44 (22.2)	<0.001^{A,D}
Male	504	16.55 (15.42)	9.6 (7.34)	19.28 (21.57)	24.07 (24.05)	0.002^{A,D}
Female	263	14.09 (18.29)	7.75 (7.06)	7.68 (18.96)	18.3 (17.06)	120
Young age	227	9.71 (7.92)	6.01 (2.79)	6.98 (6.73)	5.76 (1.12)	168
Middle age	448	14.87 (13.32)	9.95 (7.17)	23.14 (17.66)	21.44 (24.67)	<0.001^{D}
Old age	92	37.25 (21.98)	29.81 (15.23)	34.99 (9.78)	27.76 (8.83)	359

* Kruskal-Wallis test with Dunn's post hoc test for significant associations. Abbreviations for significant post hoc tests: A – LA Continental & LA Coastal; B – EMA Continental & EMA Coastal; C – LA Continental & EMA Continental; D – LA Coastal & EMA Coastal

DISCUSSION

To assess dental age in adults, we use invasive and non-invasive methods that have been scientifically tested. For the rapid assessment of dental age in adults, tooth wear analysis is most commonly used as a non-rapid method. The second method according to Bang and Ramm is the age estimation method, which is based on the translucency of the root dentin. However, this is an invasive method as it requires extraction of the tooth from the alveolus. [19]

Tooth wear is particularly important for studying the relationship between people from different historical periods, their environment and their lifestyles. [20] Ancient people consumed more natural and raw, unprocessed food, which led to a high degree of tooth wear. It has been shown that tooth wear gradually increases with age. Normally, tooth wear dominates in the molars. It starts in the occlusal enamel and gradually extends to the dentin. Examination of teeth from different historical periods, from late antiquity to the Middle Ages, shows that tooth wear is a universal phenomenon. [20,21]

In our study, computer software was used to analyse tooth wear as the most important parameter for the loss of dental structure in continental and coastal geographical areas of Late Antiquity and the Middle Ages. Our results showed a significant difference in tooth wear between the two historical periods and between the two geographical areas. The results showed that the highest tooth wear was recorded in the middle-aged male population on the Croatian coast in the EMA. In terms of tooth groups, the highest tooth wear was found in the incisors, premolars and left molars.

Furthermore, tooth wear increases much more in the coastal later period for the middle adults and remains high in the older age group though it is only the molars that show a marked increase in old age.

The results showed that hard dental tissue loss differed between the two historical periods and between geographical locations, not so much in terms of the number of teeth and total tooth area as much as in tooth wear. We also found the difference between continental and coastal Croatia in LA and coastal Croatia between LA and EMA ($P = 0.006$ and $P < 0.001$, respectively).

Overall, samples from coastal Croatia from the LA period showed a minor proportion of tooth wear with a median of 8.35%, whereas samples from coastal Croatia from the EMA had the most significant proportion of tooth wear with a median of 18.26%. Furthermore, the middle-aged male population had more advanced tooth wear in coastal Croatia in EMA, and the most affected teeth were incisors, premolars, and left molars.

This study used the VistaMetrix 1.38 computer software to estimate tooth wear. This program allows quantitative editing of digital images and provides objective measurements. Unlike other standard methods, like the Scotts method [23], which is visual or qualitative, this computer software provides a more reliable measure of tooth wear expressed in cm^2 on each tooth.

The low price and user-friendliness of this software make it particularly suitable for this type of research. Several studies that have analysed tooth wear on archaeological samples have used less objective and sensitive methods, such as various visual scales. [24, 25] To the best of our knowledge, this is the first study that has analysed tooth wear on human archaeological samples using this software.

Excellent results for intra-rater and interrater repeatability show the applicability and objectivity of this approach to tooth wear analysis. Clements et al. [26], used digital photographs to measure tooth wear and found less than a 2% difference between repeated tests.

Tooth wear and carious lesions are the main potential causes of hard dental tissue loss. From the LA period to the present day, people's dietary and lifestyle habits have changed. It has been shown that tooth wear and loss of dental tissue have decreased with the change in dietary habits.

Our study showed that tooth wear as a proportion of total tooth area was significantly higher in the EMA than in LA, especially in the coastal region. Chazel et al [27] compared the prevalence of apical and dental lesions in an archaeological and modern population (4th to 20th century) and evaluated the influence of environmental factors. They showed that dietary and lifestyle factors in the EMA appeared to be the main risk factors for hard dental tissue loss due to dental care and subsequent caries lesions, which strongly influenced the modern population. [28]

It is well known that ancient populations based their dietary habits on rough, raw and potentially abrasive food. The higher rate of tooth wear in the EMA population may explain why these dietary habits required more vigorous mastication. Moreover, they were familiar with glass and leather manufacturing, the manufacture of fishing gear (e.g. fishing nets) and similar professions that required the use of teeth as tools. [29]

Estimating dental age in adults has proven to be very difficult because it requires knowledge and extensive experience. Compared to qualitative methods of assessment, quantitative methods are much more accurate and show less variation, which is why digital methods are favoured. A study conducted by Gkantidis et al [29] using 3D imaging for tooth wear status showed that the new, powerful 3D imaging methods facilitate the measurement of tooth wear and provide a better understanding of the problem and can be a good tool for assessing dental age.

Today, dental erosion, that impairs tooth wear, is one of the most common oral diseases of the modern man. [30] This makes it difficult to take tooth wear into account when assessing the dental age. Therefore, it should be very careful when diagnosing tooth wear and dental erosion.

Our study had several limitations. Although our sample was representative, this study analyzed archaeological sites only in Croatia. We did not analyze tooth erosion and carious lesions in terms of loss of hard dental tissues, which can also be scope for further research. Although we did not notice the distortion of some images, there is a possibility of it occurring due to the equipment used.The measurer acquired good skills by measuring tooth wear, but it is possible that a minimal error crept in at the blurred boundaries of enamel and dentin.

We predicted a difference in tooth wear between the two observed periods according to the sex and estimated skeletal age and a group of teeth in coastal and continental Croatia populations. Gustafson included Dental wear in his research as one of the parameters in the assessment of dental age in adults. This method is regularly used even today in forensic analyses. Therefore, this research is recommended for further measurements in a recent human for possible forensic analyses. [31]

CONCLUSION

By analysing and measuring the tooth wear with the VistaMetrix computer software, we have obtained results that show the difference between the two historical periods, LA and EMA, in the population of two different geographical locations, continental and coastal Croatia. This finding that could explain changes in dietary and lifestyle habits over two time periods. The obtained results helped to estimate the tooth age of the observed archaeological samples.This software was used for the first time to analyse tooth wear and proved to be a fast, simple and reliable tool for analysing tooth wear. The possibility of using this method is suggested in archaeological and forensic analyzes due to its simplicity

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Virtual dental autopsy: undertaking forensic dental identification remotely using an intra-oral video camera

Shona MN McDonald [1], Gabriel TF Chong [2,3], Andrew H Forgie [4]

[1] Royal Brisbane and Women's Hospital, Queensland, Australia (ORCID 0009-0001-4207-9779). [2] HQ Singapore Armed Forces Medical Corps, Singapore (ORCID: 0000-0003-0953-9531). [3] Forensic Medicine Division, Health Sciences Authority, Singapore. [4] School of Medicine, Dentistry, and Nursing, University of Glasgow, UK.

Corresponding author:
andrew.Forgie@glasgow.ac.uk

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KEYWORDS

Intra-oral video camera (IOVC), Human identification, Oral examination, Telehealth, Disaster victim, Forensic dentistry

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ABSTRACT

Introduction: The aim of this study was to evaluate whether a forensic odontologist working remotely could accurately undertake forensic dental identifications using videos produced by non-dental forensic staff operating an intra-oral video camera (IOVC). The study's aims were to assess the accuracy and time taken to perform remote forensic dental identifications in this manner.

Materials and methods: Eight cadavers from the Centre for Anatomy and Human Identification (CAHID), University of Dundee, UK, were examined by a forensic odontologist via a traditional dental examination. Their dental condition was recorded to serve as ante-mortem records for this study. Videos of each dentition were produced using an IOVC operated by a medical student. Post-mortem records were produced for each dentition from the videos by a remote second forensic odontologist who was not present at the traditional dental examination. The ante-mortem and post-mortem records were then compared, and identification was classified as positively established, possible or excluded.

Results: Established identifications were positively made in all eight cases although there were some non-critical inconsistencies between ante-mortem and post-mortem records. Before the second opinion, 85.6% of the teeth per study subject were charted consistently. After the second opinion, the percentage of consistency increased to 97.2%. Each video on average was about 4.13 minutes in duration and the average time taken to interpret and chart the post-mortem dental examination at the first attempt was 11.63 minutes. The time taken to chart from the videos was greater than is typical of a traditional dental examination.

Conclusion: This pilot study supports the feasibility of undertaking remote dental identification. This novel virtual dental autopsy approach could be a viable alternative to a traditional post-mortem dental examination, in situations where access to forensic dental services is difficult or limited due to geographical, logistical, safety, and/or political reasons.

INTRODUCTION

The term "virtopsy", a combination of the words "virtual" and "autopsy", was first coined by Thali *et al*[1] in 2003. The key concept behind "virtopsy" is to perform post-mortem examinations using non-invasive radiological techniques.[1,2] When this concept is applied to the field of Forensic Odontology, it not only encompasses the use of non-invasive radiological techniques[3-5] but also the use of digital dental technologies and teledentistry,[6-11] and rarely a combination of the two.[12]

Outside the forensic dental setting, there was an uptake of interest in teledentistry during the COVID-19 pandemic. Some healthcare organisations, such as the US Centres for Disease Control and Prevention,[13] advocated the adoption of teledentistry during the pandemic as a means of triaging dental patients to determine whether the patients needed to be physically seen at a dental clinic or not. The objective was to minimise the risk of COVID-19 transmissions between healthcare workers and patients, and reduce the overall burden on the healthcare system during the pandemic.[14] This was necessary during the COVID-19 pandemic as routine dental treatment (in particular aerosol-generating procedures) were deferred in many countries to reduce the risks of transmission.[15] Therefore, during the pandemic, teledentistry offered the ability to provide some form of dental care, even if it was just to provide symptomatic relief, which was better than no care at all;[13] especially for individuals and communities that were underserved before and during the pandemic.[15]

Even before the COVID-19 pandemic, the uptake of telehealth amongst dental professionals had generally lagged behind the medical profession.[16,17] This, in turn, meant that a sudden pivot to teledentistry during the COVID-19 pandemic posed significant challenges in catching-up the technical know-how and closing gaps in equipping when the global supply chains were disrupted.[13] Viewed from this perspective, perhaps then it is not surprising that teledentistry as applied to the field of Forensic Odontology (i.e. as a form of virtual dental autopsy) is also quite nascent. For instance, twenty years have passed since the first paper on "virtopsy" but there have been surprisingly few studies conducted[4,6,7,9-11] and an equally small number of reviews on virtual dental autopsy.[3,5,8,12,18,19] Furthermore, when it comes to the application of teledentistry/ digital dental technology in forensic dentistry, there is no single method that has been universally accepted, in part because of the cost of the equipment.[3]

The recent and arguably still ongoing experience of COVID-19 is a poignant reminder of the risks of infectious disease transmission in both the clinical and mortuary environments. The presupposition is that performing virtual dental autopsies can potentially reduce the number of persons required to physically handle remains with unknown infectious disease status,[12] and so reduce the risks of transmissions amongst forensic professionals. In addition, there may be occasions where access to forensic dental services is difficult or limited due to geographical, logistical, safety, and/or political reasons. In a mass fatality situation, the forensic odontologist(s) would most likely also be subjected to time restrictions, or pressure from the local and/or international community to undertake forensic identifications in an expedient but rigorous manner. There are also jurisdictions where the numbers of forensic odontologists are few and far between to undertake 'peacetime' coronial death investigations, and the manpower shortage is even more acutely felt when it comes to disaster victim identification.[20]

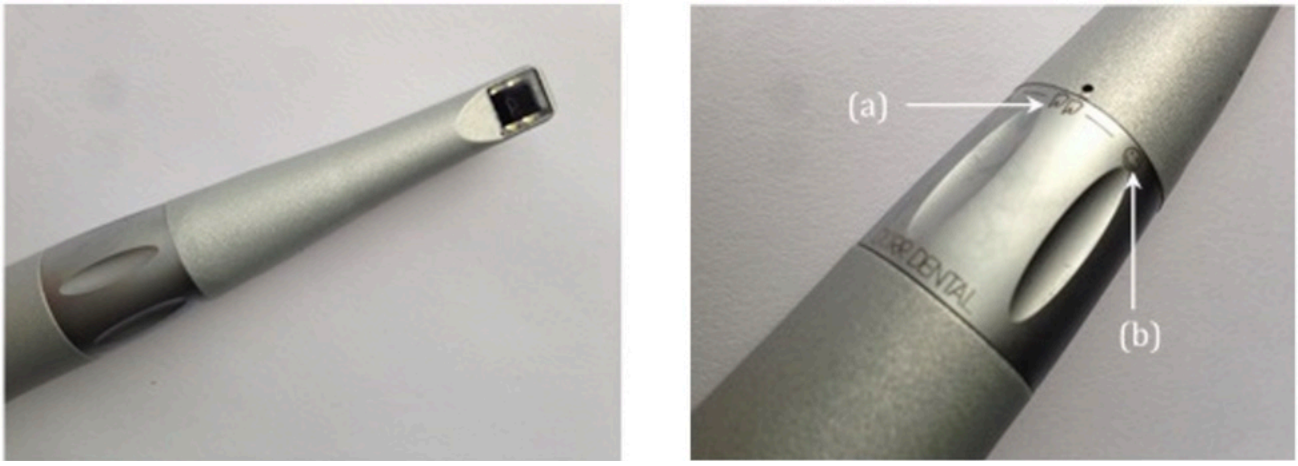
Another group has explored the feasibility of using non-dental forensic staff to capture forensic dental post-mortem images, which were then forwarded to a forensic odontologist who was remotely based.[6] In addition, other researchers have explored using intra-oral video camera (IOVC) systems to undertake virtual dental autopsy.[6,7,9-12] IOVCs were first introduced into clinical dentistry in 1987[8] and have since become increasingly adopted amongst dental professionals in developed countries. A typical IOVC setup consists of an intra-oral camera with a light source that is mounted on a handpiece that can capture, process, and display photographic images and/ or videos. Beyond capturing photos and videos, there are a range of clinical functions for IOVCs which include, but are not limited to, detecting dental caries,[8,21] health education and promotion,[8,22] treatment planning,[23,24] and magnifying the field of vision for example to locate root canal orifices.[8]

This pilot study pushed the boundaries further by evaluating whether a forensic odontologist working remotely could accurately undertake forensic dental identifications using videos produced by a non-dental forensic staff operating an intra-oral video camera (IOVC). The study's aims were to assess the accuracy and time taken to perform remote forensic dental identifications in this manner. A The research question was in a mass fatality incident or remote scenario, are there any possible alternatives to a traditional post-mortem dental examination? In other words, can forensic dentists undertake dental identifications remotely?

MATERIALS AND METHODS

The intra-oral video capturing software (DBSWIN 4.4 Dental Image Processing and Archiving, Dürr Dental, Bietigheim-Bissingen, Germany) was first installed on a laptop (Samsung NP-R20 Intel® Core™2 CPU, Seoul, South Korea). Pre-testing of the software and intra-oral camera (IOVC; Dürr Dental, Bietigheim-Bissingen, Germany) (Fig. 1) was undertaken on two of the authors' (SM, GC) dentition to verify its image-capturing ability, video standards, and accuracy.

Figure 1. Left: Dürr Dental Intraoral Video Camera. Right: (a) Zoom setting for close up view of surfaces; (b) Overall view setting



Following ethical approval from the University of Dundee, a sample of eight cadavers was identified from the population available at the Centre for Anatomy and Human Identification (CAHID), University of Dundee. As the purpose of the study was to match ante-mortem and post-mortem dental records but not to identify the bodies, the identities of the cadavers remained anonymous and were instead randomly allocated a number for the purposes of this research (numbers 1-8). Eligibility for study inclusion was limited to cadavers with at least one tooth present in each quadrant of the mouth.

Each cadaver was examined in turn, as follows. To facilitate optimal conditions for a traditional post-mortem dental examination, a lamp was positioned to illuminate the mouth. The mandible was opened as far as possible and held in position with a dental prop. The teeth were then cleaned using surgical gauze. Following which, an experienced forensic odontologist (AH) undertook a traditional post-mortem dental examination and dictated the findings to a scribe.

A hard-copy dental chart (odontogram), based on the Fédération Dentaire Internationale (FDI) notation, was used to record the findings. These odontograms charts subsequently served as the ante-mortem records for this study. No radiographs were taken, as protocols in CAHID did not permit this.

After the ante-mortem chart for each cadaver was completed and re-checked by the forensic odontologist (AH), videos of the dentition were recorded a medical student (SM). An IOVC connected via USB to a laptop was used to capture the videos. The medical student handled the IOVC whilst the forensic odontologist handled the laptop. As the IOVC had its own light source, the dental lamp used for the traditional post-mortem dental examination was not required for the intra-oral filming. Seven views were recorded per subject in the following manner:

1. An overall view of the dentition;
2. Upper buccal surfaces (cheek surfaces of teeth);

3. Upper occlusal surfaces (biting surfaces of teeth);
4. Upper palatal surfaces (surfaces of teeth facing the tongue);
5. Lower buccal surfaces;
6. Lower occlusal surfaces; and lastly
7. Lower lingual surfaces (surfaces of teeth facing the tongue)

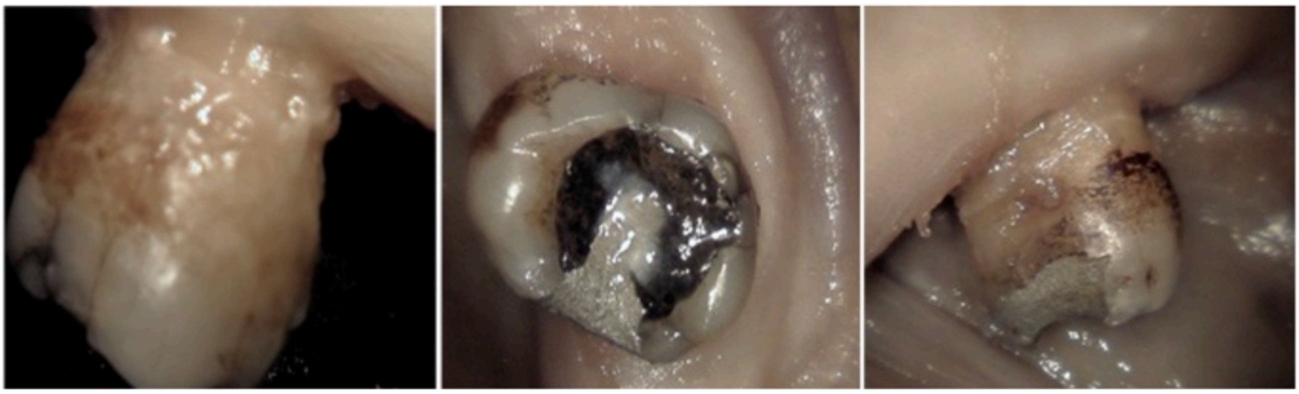
This particular IOVC had three zoom settings; the lowest zoom setting was used for the overall view, whilst the remaining six videos of the buccal, occlusal, and palatal surfaces were recorded at the highest zoom setting (Fig. 1) to optimise the details captured. For the overall view, the lips were reflected with the primary aim of capturing the number and position of teeth present (Fig. 2). Each tooth was thus visible from three viewpoints (i.e. buccal, occlusal and palatal) after a composite viewing of the videos of these three viewpoints (Fig. 3). In select cases, a second take of a surface was recorded where the first take was deemed insufficient (i.e. blurred, jerky, or incomplete); this decision was at the discretion of the forensic odontologist (AH). The videos were then saved to an external hard drive.

The external hard drive was passed to a second forensic odontologist (GC) who was not present at the traditional intra-oral examination and virtual dental autopsy of the subjects. Using only the information provided in the videos, a set of post-mortem records was constructed for each subject (i.e. teeth present, condition of the teeth, type of restorations present and tooth surfaces involved). No limit was set on the time spent nor number of video plays required to produce a post-mortem record.

Once the eight post-mortem records were completed, the authors compared the ante-mortem and post-mortem records. Using the American Board of Forensic Odontology (ABFO) standards, the identifications were subsequently classified as positively established, excluded, or possible.[25] Where discrepancies were found between the ante-mortem and post-mortem records, the videos were re-examined by all the authors and a consensus reached where possible.

Figure 2. Still image from Video 1, Subject 4 to give a general overview of teeth present and treatment present

Figure 3. Three views of a mesio-occlusal restoration on tooth #17 (FDI notation) of Subject 6. From L-R: buccal view; occlusal view; palatal view



RESULTS

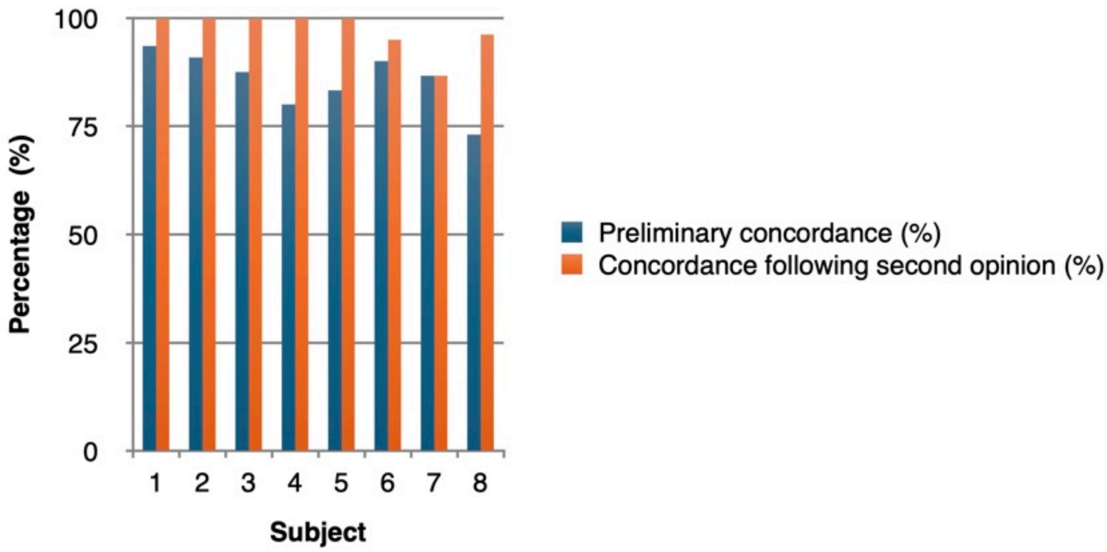
All the post-mortem records were positively matched to the ante-mortem records such that, if required, a definitive dental identification of each cadaver could be established. A perfect match was observed in five out of the eight subjects. In the remaining three, the discrepancies were insufficient to alter the identification decision.

The percentage of teeth charted consistently in the ante-mortem and post-mortem records is shown in Figure 4. Before the second opinion, 85.6% of the teeth per study subject were charted consistently. After the second opinion, the percentage of consistency increased to 97.2%. When comparing the antemortem and post-mortem records, there were initially a total of 23 discrepancies (Table 1), notably the presence or extent of a tooth-coloured restoration. Following re-examination of the videos by the authors, these points of discrepancy were addressed and a consensus reached for 19 out of the 23 discrepancies (82.6%) (Fig. 4).

Table 1. Cumulative frequency of charting inconsistencies, total N= 32

Charting Inconsistencies	Frequency (n)
Presence/absence of amalgam restoration	2
Surface extent of amalgam restoration	2
Presence/absence of composite resin	8
Surface extent of composite resin	7
Presence/absence of veneer	1
Surface involvement/extent of caries	2
Surface involvement of fracture	1

Figure 4. Graph illustrating the accuracy (*i.e.* concordance) of post-mortem charting when compared against ante-mortem records, before and after a second opinion



In total there were four charting differences that could not be resolved with re-examination of the videos, and would require radiograph analysis to arrive at a definitive conclusion (two differences regarding the extent of caries, and two differences regarding the presence or absence of tooth coloured restorations). However, these differences were not significant enough (i.e. irreconcilable) to affect positive identification of the subjects.

Each video on average was about 4.13 minutes in duration (Figure 5) and the average time taken to interpret and chart the post-mortem dental examination at the first attempt was 11.63 minutes (Figure 5). It can be seen from Figure 6 that the combined process was completed in less than 25 minutes per subject (average of 15.76 minutes). When a second opinion was required the length of time taken increased but was not recorded.

Figure 5. Total duration of IOVC videos per subject and the time taken to construct individual post-mortem charts

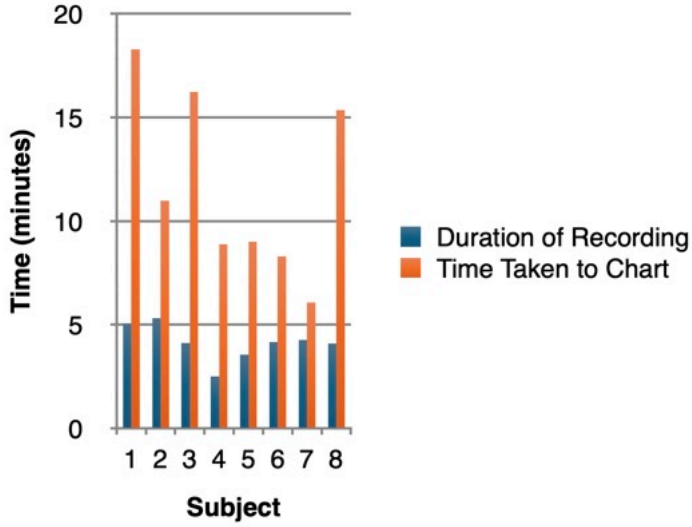
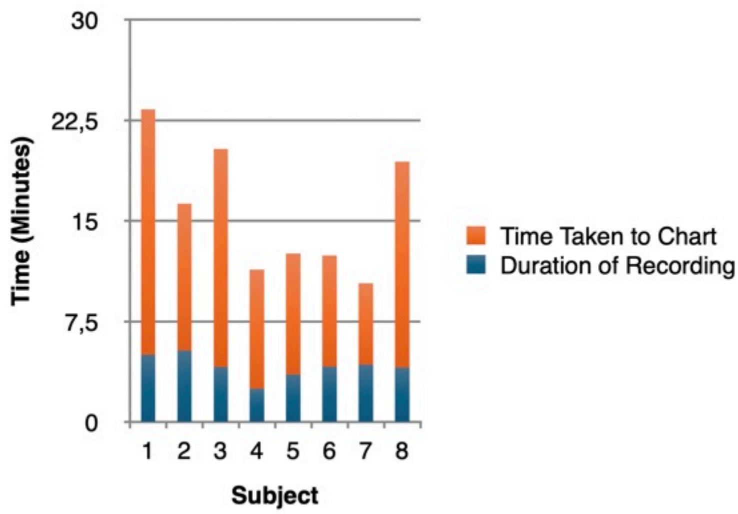


Figure 6. Stacked column representation of Fig 7. to illustrate the total time invested per subject



DISCUSSION

This study shows that (i) a non-dental forensic staff can be trained to competently operate an IOVC to capture intra-oral videos, and furthermore (ii) a forensic odontologist working remotely can rely on these videos to accurately undertake forensic dental identifications with a high degree of certainty. Established identities were positively made in all eight study subjects because of matching ante-mortem and post-mortem records. It is important to highlight that the post-mortem records were charted from the intra-oral videos without any attempt to first calibrate between the non-dental staff (SM) and forensic odontologists (AH and GC),

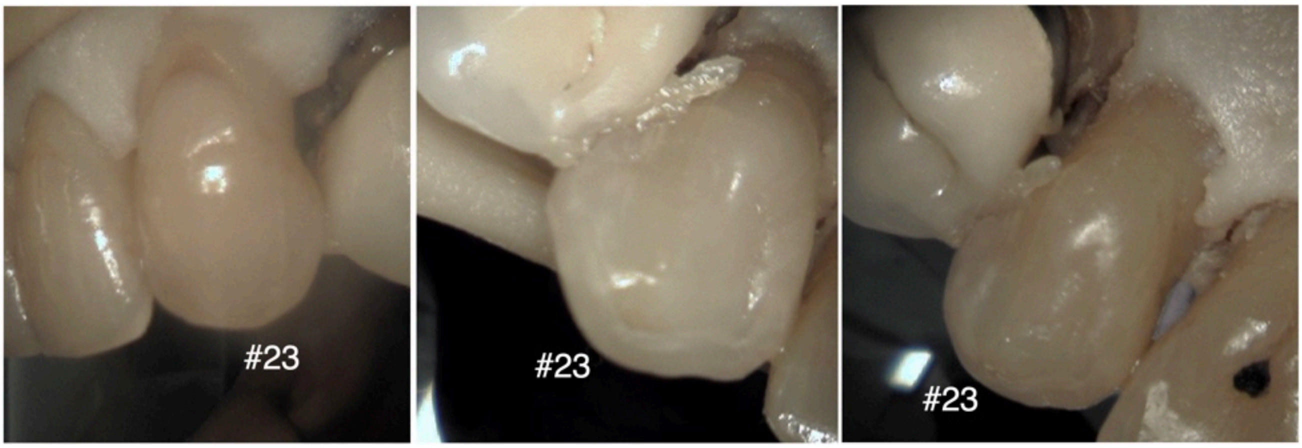
The decision to record seven separate videos per subject was to provide maximum information with minimum disorientation. After some trial and error prior to commencing the study proper, it was found that it was easier to individually record by teeth surfaces rather than by each quadrant (the latter of which proved disorienting and more challenging to interpret). Effective use of the IOVC is a skill that requires development and the early videos were at times jerky, blurry, and of poor quality. This resulted in difficulty interpreting the initial videos. However, as the operator (SM) became more familiar with operating and manipulating the IOVC, the quality of the videos increased. This highlights the importance of appropriate training prior to implementing a new technology.[6,7]

The model of IOVC used for this study was a basic model which lacked specifications that may have enhanced the produced videos, including: an autofocus feature; a dedicated button to capture photographs; an adjustable light source; a microphone; and wireless connectivity. Many of these features are available on newer models of IOVCs; therefore when selecting a particular IOVC brand and model for virtual dental autopsy, consideration should be given as to which specifications will produce the optimum results at the most reasonable cost.

In this study, the videos were asynchronously transferred, in a store-and-forwarded manner, from the non-dental forensic staff to the remotely based forensic odontologist. A case study during the COVID-19 showed the feasibility of using clinical photos taken by physicians, using smartphones which were synchronously transmitted via a general-purpose messaging application, for the purposes of a remote dentist undertaking teledental consultations.[13] When extrapolated to virtual dental autopsies, the assumption is that the real-time synchronous transfer of videos (and images) should also achieve the outcome of undertaking forensic dental identifications remotely. Theoretically, remote dental identification via teledentistry should be possible from any location that is equipped with a computer connected to the internet.

The difficulties associated with charting from the video produced by the IOVC included the difficulty in identifying the presence and extent of tooth-coloured restorations (Fig 7); however, this is also a common experience during traditional visual only post-mortem examinations (i.e. sans radiographs). An additional consideration for this finding is that dentists may chart the same multi-surface restoration differently depending on their perception of the extent of surface involvement (e.g. involving the mesial-occlusal surfaces as opposed to mesial-occlusal-distal surfaces). Variations in the surface involvement of restorations was one of the most commonly encountered inconsistencies between the ante-mortem and post-mortem records (n=9) (Table 1).

Figure 7. A tooth-coloured restoration on tooth #23 (FDI notation) (labelled) of Subject 2 that was initially omitted from the post-mortem record. From L-R: buccal view; incisal view; palatal view



The lack of tactile feedback when viewing the videos compared to performing a traditional post-mortem examination was an obvious disadvantage, as the videos offered a 2-dimensional representation of 3-dimensional structures. Radiographs could have assisted to resolve some of these challenges with charting. However, in this study the lack of radiographs did not hinder positive identifications from being established. Intra-oral videos have an advantage over intra-oral photographs (that are typically taken using mouth mirrors) in that the latter results in reversed images.[10] Therefore, with intra-oral photographs there is the danger that the dentist charting the post-mortem findings may confuse between the upper and lower jaws, and/or the right- and left-hand sides of the mouth.[10]

At this stage, it is not an aim for the authors to prove the superiority of this novel virtual dental autopsy approach (note: another group of researchers have coined the term “forensic tele-odontology”[6]) over a traditional post-mortem examination. However, this teledental approach has proved to be accurate and time-efficient (which were the aims of this study). The video of each individual surface (of which there were six per subject) lasted on average one minute or less and this was sufficient duration to capture the necessary information without inducing motion blur in the video. The average amount of time taken to create the intra-oral videos for each subject by a non-dental staff in this study was comparable with that of an earlier study (which took a dentist who was inexperienced with using that particular model of IOVC 4 minutes).[7] Although the time to interpret the video may be longer than the time taken to perform a visual only post-mortem dental examination, time is significantly saved in other ways, notably travel to the site and/or for the forensic odontologist to take time off his/her clinical schedule.

These are very encouraging results from a pilot study, and offers the promise of: (i) manpower savings, especially during disaster victim identification efforts,[7,12] in jurisdictions with limited number of forensic odontologists to allow them to work remotely, (ii) time savings without the need for the forensic odontologist to travel[7] to the mortuary or disaster site (particularly in remote areas), (iii) the ability to do a second independent examination remotely,[8,12] and (iv) the ability to undertake the dental identification in a relatively sterile environment without exposure to the typical sights, smells, and tactile sensations associated with traditional dental post-mortem examinations.[19] This last point is important because the dentist on the receiving end of these videos and images may not be forensically trained (for example, in jurisdictions without any forensic odontologists), and/or emotionally adept at handling the trauma of mass fatalities. In addition, such a virtual dental autopsy may be less invasive than a traditional post-mortem dental examination,[7] which is especially important for examining friable or fragile remains.[19] Another potential further development from this study, is the printing out of 3D printed models from IOVC images which can later be used, for example, to present in the courts to spare the lawyers, judges, and juries the trauma of viewing photos/videos of deceased persons.

Virtual dental autopsies can also be undertaken using computed tomography (CT) scans[12,26] where the DICOM (Digital Imaging and Communications in Medicine) files obtained from the scans can be shared with forensic odontologists. However, as a means of undertaking virtual dental autopsies, IOVCs possess some significant advantages over CT machines, which include but are not limited to: (i) From a cost-benefit analysis, intra-oral cameras and their associated software typically costs 8.5x to 16x less than a CT scan ecosystem such that resource constrained jurisdictions/settings may not be able to afford a single CT machine. (ii) There is often significant scatter arising from metallic objects (such as amalgam restorations and prostheses) in CT scans (i.e. “metallic artifacts”). (iii) Training is required to interpret and manipulate CT scans. For instance, some skill is required to “convert” CT scans (3-D) to an orthopantomogram (OPG) / dental panoramic tomography (DPT) (which are 2-D). (vi) Intra-oral cameras are safer from an occupational health perspective since they do not produce ionising radiation. (vii) intra-oral cameras are more accessible and familiar for dental staff to operate compared to CT machines; and (vii) intra-oral cameras are much more portable than CT machines, and this would matter in situations where virtual dental autopsies are called for. The main disadvantage of using intra-oral cameras compared to CT scans for undertaking virtual dental autopsy, is that the former is unable to produce radiographs. However, the lack of radiographs in this study did not hinder positive identifications from being established amongst the study population. Therefore, from the perspective of undertaking virtual dental autopsies, intra-oral cameras have considerably more advantages compared to CT scans. However, if a mortuary is able to afford both IOVC and CT machines, then intra-oral cameras and CT scans could be seen as complementary methods for undertaking virtual dental autopsies.

For this proposed virtual dental autopsy method to be functional and operational on a larger scale, the approach must prove itself to be cost-effective, efficient, and accurate. As such, further larger-scale studies involving, for example, multiple dentists and non-dental forensic staff and other IOVC brands and models are required before widespread application of this teledental approach to undertaking remote forensic dental identification. Should this teledental application of virtual dental autopsy be implemented, time and money would need to be invested in training the non-dental staff to gain experience and familiarity with operating IOVCs, and to a certain extent to make an independent assessment of whether the videos and images they had created would be acceptable for forensic dental identification or not. Dentists who are unfamiliar with the principals behind operating IOVCs and manipulating the videos/images would also require some training, but presumably the time to train dentists would be shorter than to train non-dental staff. It is envisioned that man-hour costs incurred in the training would be the main start-up cost for this virtual dental autopsy approach, as intra-oral cameras and their accompanying software are relatively inexpensive and readily available (compared to other digital dental technology) in developed countries.

The strength of this study lies in the novelty in undertaking remote forensic dental identification using intra-oral videos produced by a non-dental staff. The limitations of the study were (i) the inability to take radiographs which would have allowed the authors to resolve four charting differences, and (ii) the relatively small number of subjects, which were sufficient for the purposes of a pilot study but more subjects would be required for further larger-scale testing of this virtual dental autopsy approach.

CONCLUSION

This pilot study has demonstrated that (i) non-dental staff can be trained to operate an IOVC and (ii) this virtual dental autopsy approach holds potential for accurately identifying bodies by forensic odontologists in a location remote from the mortuary. As such, this virtual dental autopsy poses a viable alternative to a traditional post-mortem dental examination when there are time sensitivities and pressures for the reconciliation to be concluded, no or limited number of forensic odontologists available locally, and to minimize exposure to handling human remains for religious[5] and/or mental health reasons. In answer to the research question and pending verification of these findings by larger-scale studies: forensic dentists can undertake dental identifications remotely.

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Post-mortem Interval estimate based on dental pulp: A histomorphology approach

Ilenia Bianchi [1], Ana Rodrigues [2], Rui Santos [3], Diana Augusto [2], Martina Focardi [1], João Aquino [4], Isabel Fonseca [5], Cristiana Palmela Pereira [6]

[1] Laboratory of Personal Identification and Forensic Morphology, Department of Health Sciences, University of Florence, Florence, Italy. [2] Research Group in Forensic Dental Sciences FORENSEMED from the Research Unit UICOB, Faculty of Dental Medicine, University of Lisbon, Portugal. [3] School of Technology and Management, Polytechnic Institute of Leiria, Portugal; Centre of Statistic and its Application CEAUL from University of Lisbon, Portugal. [4] Faculty of Dental Medicine, University of Lisbon, Portugal. [5] Faculty of Medicine, Institute of Pathological Anatomy, University of Lisbon, Portugal. [6] FORENSEMED/UICOB from the Faculty of Dental Medicine, University of Lisbon; Centre of Statistic and its Application CEAUL from University of Lisbon; Faculty of Medicine University of Lisbon, Portugal

Corresponding author: ilenia.bianchi@unifi.it

The authors declare that they have no conflict of interest.

KEYWORDS

Post-mortem interval; Histological changes; Dental pulp; Haematoxylin and Eosin stain; High Resolution Microscopy

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ABSTRACT

Estimating the post-mortem interval (PMI) of human remains based on the histomorphology of dental pulp parameters is promising, but available evidence is scarce and sometimes contradictory without a scientific model.

The aim of the study is to characterise the histomorphological changes of dental pulp associated with the decomposition of human remains by a qualitative and quantitative approach. The main aim is to establish a correlation based on post-mortem (PM) dental pulp histomorphology and the PMI, and whether pulp degradation could be an available medico-legal tool for PMI estimation beyond the first week after death (late PMI).

The eligible sample consisted of 27 sound teeth from 16 healthy patients aged 16 to 72 years due to orthodontic or oral surgery treatment, to create PMI's simulating the death of the subject as the time elapsed from tooth avulsion. Data collected from patients (sex, date of birth, tooth position, date and hour of the avulsion, date and hour of pulp extraction) were anonymised in accordance with the requirements of Faculty of Dental Medicine of the University of Lisbon.

The sample was divided into 9 groups of 3 teeth according to different PMI sets from T0 (baseline) up to 2 weeks (T0, 7, 12, 24, 36, 48, and 72 hours, 1 and 2 weeks). All the dental samples were stored at room temperature up to the time of pulp extraction and then prepared with haematoxylin and eosin stain. High-resolution microscopy was performed to obtain histological images. An operator performed the qualitative evaluation of blood vessels, collagen fibres, and the extra-cellular matrix (ECM) in PM pulps and measured the variation in cells/nuclei density by counting 6 different ROIs (Regions of Interest) for each pulp manually and automatically (quantitative analysis).

Qualitative results showed that the degeneration of dental pulp appears 7 hours after death but histological changes in vessels, fibres, and ECM in PM dental pulp are characterised by high variability, consequently it is not possible to generalise the results for early PMIs. Quantitative measurements proved that cell count cannot be standardised due to the presence of superimposed layers of cells and nuclei fragmentation. Odontoblasts did not demonstrate evidence of cellular or nuclear lysis up to 14 PM suggesting their applicability in late PMIs.

Future research will focus on late PMIs and different techniques of tooth preparation.

INTRODUCTION

A reliable estimation of the post-mortem interval (PMI) is an important step in forensic investigations since criminal justice requires the charge to be proven beyond reasonable doubt. The most common tool for early PMI estimation (from 3 to 72 hours after death) is the algorithmic combination of different parameters (in particular, body-environmental temperatures) through the so-called "compound method". One crucial limitation of this technique is that it cannot be applied to longer PMIs, with measurement errors generally in months, since they are based on decomposition stages (fresh, early and advanced decomposition, skeletonisation, and extreme decomposition) [1]. In particular, it has been observed that macroscopic/morphological modifications of skeleton, teeth, or soft tissues provide less reliable PMI estimates due to highly variable individual influences, even under the same environmental conditions. Nevertheless, they are useful for other identification investigations, such as age estimation or sex classification [2-9].

Currently, alternative methods are focused on post-mortem (PM) biochemical or histological changes [10-23]. One of the most stable matrices for estimating the time elapsed since death from early to late PMIs appears to be dental pulp, which is protected from damaging factors (such as heat, bacteria, UV light, and moisture) by the hard tissues of the tooth [24-26]. Despite having great resistance, teeth undergo histomorphological changes due to physical-chemical factors related to the absence of physiological regulation of metabolic processes, ischemia, putrefaction, and bacterial and fungal proliferation [27-36].

It has been observed that as time elapses after death, odontoblasts cluster and detach from dentine, collagen fibres and nuclei of odontoblasts, fibroblasts, endothelial and immunity cells, and stromal cells decrease in density, and the extra-cellular matrix (ECM) degenerates into a vacuolated and contracted tissue [30-36]. Nevertheless, the rate of degeneration and its correlation with the PMI is not clearly defined. Some authors describe changes that occur in the first hours after death up to an almost complete disappearance of fibres and cells in a week PM [30, 32-33]; other studies report the persistence of collagen fibres and cells up to 6 months post-mortem and complete destruction of the ECM 2 years after death [34-36].

Previous studies on human teeth that verify dental pulp's histomorphological changes correlated with the time elapsed since death provide a rationale for developing a tool for estimating PMI based on teeth, however, it is challenging with limited results, and a scientific model is still missing. Therefore, the aim of the study is to verify which qualitative and quantitative dental pulp changes are significant for the correlation with PMI and whether pulp degradation could be a reliable forensic technique beyond the first week after death (late PMIs). Pilot research was developed to standardise the study method for both qualitative and quantitative PM dental pulp changes.

OBJECTIVES OF THE PILOT STUDY

Qualitative analysis

Qualitative analysis consisted of the identification and description of morphological structures of PM pulp to answer experimental hypotheses, noted in previous literature [30-36]:

1. The dilatation of vessels observed in the dental pulp as a dental element in the medico-legal estimation of the PMI:
   H0: The dilatation of vessels is not significantly different over time after death;
   H1: The dilatation of vessels is significantly different over time after death.
2. The rate of homogenisation of collagen fibres observed in the dental pulp is as follows:
   H0: The homogenisation of collagen fibres is not significantly different over time after death;
   H1: The homogenisation of collagen fibres is significantly different over time after death.
3. The vacuolation of the ECM of dental pulp is as follows:
   H0: The tissue vacuolation is not significantly different over time after death;
   H1: The tissue vacuolation is significantly different over time after death.

Quantitative analysis

Quantitative analysis consisted of the measurement of cell density as nuclei count/ROI area [30-36]. More specifically cell density was measured by the variation in the density of fibroblasts, immune and endothelial cells, and odontoblasts (odontoblast layer), as follows:

Ho: The cell density of the dental pulp is not significantly different over time after death;
H1: The cell density of the dental pulp is significantly different over time after death.

MATERIALS AND METHODS

Sample collection and storage

Sound teeth from different people who underwent dental surgery for clinical reasons (e.g. orthodontics, impacted tooth, etc) were collected from two medical centres: the Faculty of Dental Medicine and Faculty of Medicine of the University of Lisbon (ULisboa). The following inclusion/exclusion criteria were considered:
Inclusion criteria: permanent, complete, and sound tooth (both single- and multi-rooted).
Exclusion criteria: hard tissue pathology (e.g. caries), restorative treatment (e.g. filling, endodontic, prosthetic), periodontal pathology, severe wear, incomplete root formation, deciduous teeth.
A total of 27 sound teeth were collected from 16 healthy patients aged 16 to 72 years. Information about the sample and the time of tooth/pulp extraction was recorded in the *Dataset* figured in Table 1.

Table 1. Dataset of the sample and time of tooth/pulp extraction

Sample					**Time of death**		**Pulp fixed in formalin**		
ID Code	**Tooth**	**Sex**	**Date of birth**	**Age (year)**	**Date of tooth extraction**	**Hour of tooth extraction**	**Date of pulp extraction**	**Hour of pulp extraction**	**PMI**
1	35	M	22.06.1976	46	19.04.2023	12:00	20.04.2023	11:50	24 h
2	24	M	08.02.2000	23	20.04.2023	15:50	21.04.2023	16:12	24 h
3	34	M	08.02.2000	23	20.04.2023	16:05	21.04.2023	16:44	24 h
4	18	F	07.06.1998	24	26.04.2023	15:30	28.04.2023	16:05	48 h
5	25	M	29.04.2007	16	04.05.2023	11:45	04.05.2023	18:43	7 h
6	35	M	29.04.2007	16	04.05.2023	12:09	04.05.2023	19:09	7 h
7	18	F	08.02.2003	20	12.05.2023	11:30	12.05.2023	11:50	0 h
8	48	F	08.02.2023	20	12.05.2023	11.45	12.05.2023	12.05	0 h
9	45	M	29.04.2007	16	18.05.2023	12:10	25.05.2023	12:13	7 d
10	17	M	26.10.2001	21	24.05.2023	15:30	26.05.2023	16:10	48 h
11	25	F	09.04.2002	21	31.05.2023	9:20	03.06.2023	9:18	72 h
12	47	M	02.09.1962	61	02.06.2023	10:49	05.06.2023	11:00	72 h
13	15	M	29.04.2007	16	24.05.2023	11:16	07.06.2023	11:00	14 d
14	38	F	13.10.1952	72	31.05.2023	16:00	07.06.2023	16:15	72 h
15	35	M	25.02.2004	19	31.05.2023	9:38	14.06.2023	09:42	14 d
16	45	F	24.11.2004	18	21.06.2023	9:35	28.06.2023	9:40	7 d
17	15	F	24.11.2004	18	21.06.2023	10:03	28.06.2023	10:05	7 d
18	25	M	30.06.2001	21	21.06.2023	11:41	05.07.2023	11:41	14 d
19	18	M	20.03.2005	18	06.07.2023	09:32	07.07.2023	09:35	36 h
20	45	F	07.07.1998	25	12.07.2023	10:24	12.07.2023	17:25	7 h
21	15	F	07.07.1998	25	12.07.2023	9:47	12.07.2023	21:45	12 h
22	35	F	07.07.1998	25	13.07.2023	9:44	13.07.2023	21:44	12 h
23	14	F	26.07.2001	21	12.07.2023	11:45	14.07.2023	11:45	48 h
24	25	F	07.07.1998	25	13.07.2023	10:04	14.07.2023	22:04	36 h
25	38	M	06.04.2005	18	26.07.2023	9:45	26.07.2023	10:45	0 h
26	48	M	06.04.2005	18	26.07.2023	9:45	26.07.2023	21:45	12 h
27	18	M	06.04.2005	18	26.07.2023	9:45	27.07.2023	21:45	36 h

The period of sample storage included spring and summer seasons with an average recorded temperature of 22°C (range of 20-24°C) for the months of April and May, and of 27.5°C (range of 26-29°C) for the months of June and July.
The sample size considered 3 teeth in each PMI set, for a total of 9 PMI sets from T0 (baseline) up to 2 weeks.
All the data collected from the patients was processed in compliance with European Union law (General Data Protection Regulation - GDPR) and in accordance with the requirements of the Faculty of Dental Medicine of the University of Lisbon: a progressive numerical code was associated with each anonymous dental sample.
Tooth avulsion was assumed as the time of death of the patient since the extraction interrupts the blood perfusion of the dental pulp, and the PMI was considered as the time elapsed between tooth avulsion and the pulp extraction and fixation.
Teeth from clinical cases were selected, rather than those from forensic cases, to achieve known PMIs, as opposed to an estimated time of death.
Immediately after avulsion, teeth were placed in sterilisation pouches, labelled with sample data: the registered information for each patient included sex, date of birth, tooth position, date and hour of avulsion, and date and hour of pulp extraction (PMI). All samples were stored at room temperature recorded by a thermometer up to the established PMI to simulate real corpse conservation during time after death.

PMIs set and Pulp extraction
The sample was divided into 9 groups of 3 teeth according to the established PMI:

- Group 1: PMI T0 – pulp immediately after tooth extraction
- Group 2: PMI 7 hours – pulp 7 hours after tooth extraction - within which the intraocular pressure decreases drastically and stabilises after death [1] and histological examination of the skin shows no morphological changes [3]
- Group 3: PMI 12 hours - pulp 12 hours after tooth extraction - stability phase of rigor mortis [3-4]
- Group 4: PMI 24 hours - pulp 24 hours after tooth extraction - stability phase of rigor mortis [3-4]
- Group 5: PMI 36 hours - pulp 36 hours after tooth extraction - from the first day PM until the third day PM, the investigations are performed every 12 hours to study the gradual degradation of the pulp
- Group 6: PMI 48 hours - pulp 48 hours after tooth extraction
- Group 7: PMI 72 hours - pulp 72 hours after tooth extraction
- Group 8: PMI 7 days - pulp 7 days after tooth extraction - to verify the availability of PM dental factors for intermediate PMIs up to 1 and 2 weeks after death (3 teeth)
- Group 9: PMI 14 days - pulp 14 days after tooth extraction.

At the time of pulp extraction, each tooth was rinsed with a 70% alcohol solution and the aid of sterile gauze.
The tooth is then positioned with the crown securely stabilised inside a clamp and a horizontal cut is made to separate the crown from the root with a flame diamond bur under an abundant jet of water at the height of the furcation for multi-rooted teeth and about 2 mm below the cementum-enamel junction for single-rooted teeth.
Once the crown is separated from the root/roots, the floor of the pulp chamber is worn down until the pulp is completely exposed, with the diamond bur under abundant cold water. The coronal pulp was carefully separated from the chamber walls with a thin probe and then delicately pulled out from the coronal chamber with tweezers.
Only the coronal pulp was selected for this study. Once extracted, the entire chamber pulp fragment was immersed in the fixative liquid for standard histology (10% neutral buffered formalin - NBF).

Histological processing and stain of soft tissues [Haematoxilin/Eosin stain]
The histological preparation procedure for dental soft tissue followed the protocol applied by the Laboratory of Histomorphology from the Faculty of Dental Medicine of the University of Lisbon and consisted of the following phases: dehydration, clearance, fixation, impregnation, inclusion, microtomy, expansion, adhesion, staining, and assembly.

- The dehydration phase: sequential application of ethanol application to 70%, to 96%, and to 100%.

- The dehydration solution was replaced by a solvent for a means of inclusion (e.g. Xylol, Chloroform, Toluene, Benzene) since the specimens became transparent (clearance).
- The samples were included in paraffin and the inclusion was oriented according to the structure of pulp tissues (lengthwise), thus the major axis of the sample is parallel to the cut area of the paraffin block.
- Once solidified, cuts of 2/3 microns thick were made. Only sound sections were considered.
- The sections were then placed, first in cold water and then in hot water. This phase needed a black background to ease the visibility of the pulp sections and their positioning on the slides.
- The adhesion of the pulp sections to the slides took place in an incubator set at 58-59 °C to liquefy the paraffin and complete the adhesion.
- For histological staining, the procedure followed a de-paraffinisation/de-waxing with xylene, hydration with ethanol from 100%, to 96%, and to 70%, then a stain with Haematoxilina de Harris, a differentiation with hydrochloric acid at 1%, and a wash with water. Subsequently, the slides were coloured with eosin, dehydrated with ethanol at 96% and then at 100%. After a clarification with xylene, the assembly was performed with synthetic resin entellan which completes the adhesion of the section to the slide.

High Resolution Microscopy

Each slide was scanned and acquired with *Aperio GT 450 DX Digital Slide Scanner* (Leica Biosystems) at the Faculty of Medicine, Institute of Pathological Anatomy of the University of Lisbon.

High resolution images of the entire pulp section were used by the operator to:

- describe qualitative characteristic of PM pulp
- select two different kinds of ROIs (regions of interest), at the same magnification (40x) but with a larger area [249480 μm² area] and smaller [3180 μm² area], for each section's nuclei count

To find the most reliable and repeatable method to calculate cell density, 6 different ROIs at magnification of 40X for each slide were selected: 3 larger [249480 μm² area] and 3 smaller [3180 μm² area] including 1 region of the odontoblast layer and 2 regions of cell rich layer, fibres, vessels, and ECM. Larger and smaller ROIs were considered separately.

The nuclei count was conducted both manually by the operator and automatically using *Fiji ImageJ software* (free open source, accessible and downloadable from https://imagej.net/software/fiji/downloads).

To reduce the risk of measurement error within the same slide, the average nuclei count was calculated on 3 different ROIs (both on smaller and larger areas of selection). To reduce the risk of error within the same PMI set, cell density was obtained as the mean of the 3 teeth (PMI groups).

Statistical Analysis

Cohen's Kappa (ϰ) statistic was applied to calculate the intra-rater and inter-rater reliability of the quantitative analyses. 3 PMI groups (T0, 7 days, and 14 days) were selected for the intra-rater agreement, conducted by the same operator 15 days after the first measure, and for the inter-rater agreement, conducted blind by another operator.

RESULTS

Qualitative results

A summary of the most significant histological features at PMI T0 (baseline), 7h, 12h, 24 h, 36h, 48h, 72h, 7 days, 14 days is recorded in Table 2.

Quantitative results

According to Cohen's Kappa (ϰ) statistic, the intra-rater agreement values for both the smaller and larger ROIs were almost 1.

The inter-rater agreement values for the larger ROIs count and density measurement were 0.892 and 0.758 respectively, for the smaller ROIs count and density measurement were 0.931 and 0.957, respectively.

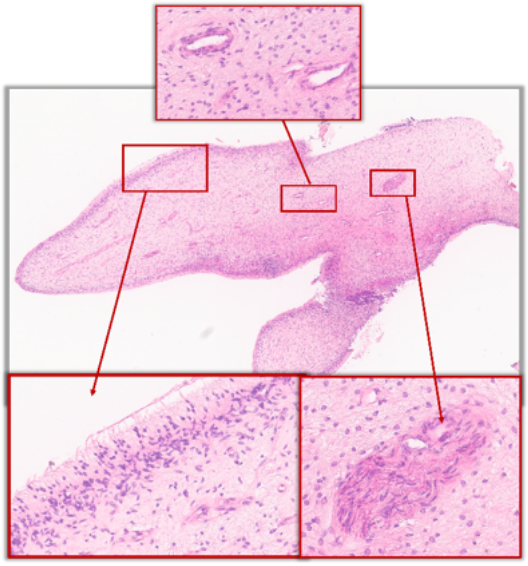
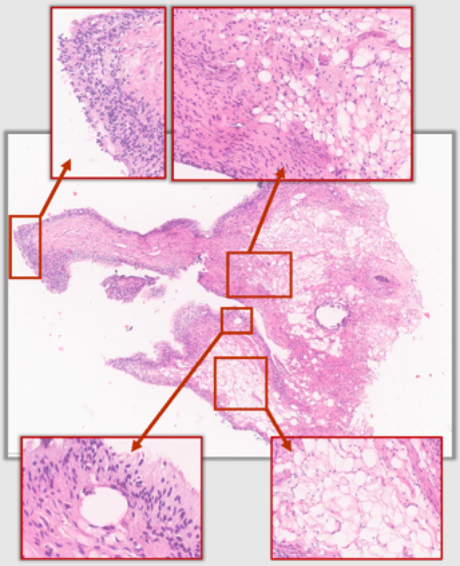
Manual cell counts and density measurements for each PMI calculated on the larger ROIs are recorded in Figures 1 and 2 respectively, on the smaller ROIs in Figures 3 and 4 respectively.

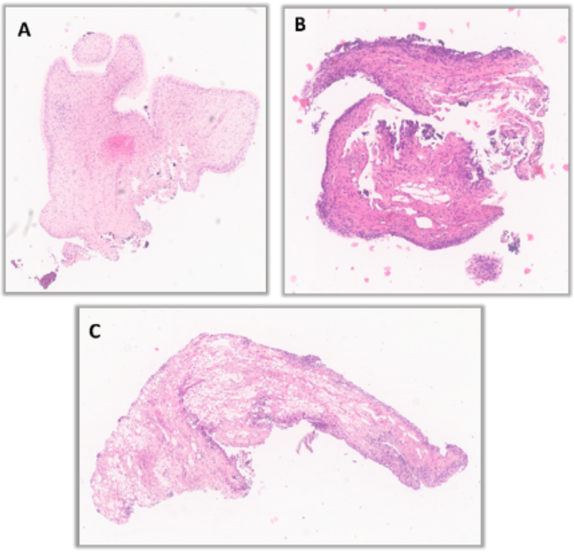
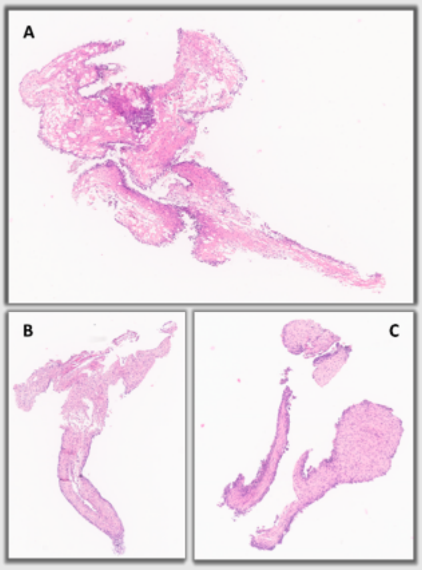
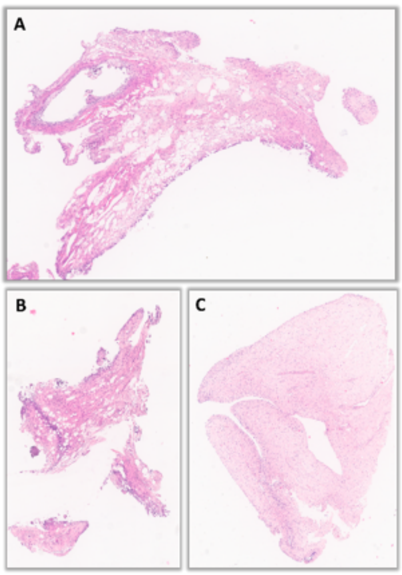
Automatic cell counts and density measurements calculated on the larger ROIs are shown in Figures 5 and 6 respectively, on the smaller ROIs in Figures 7 and 8 respectively.

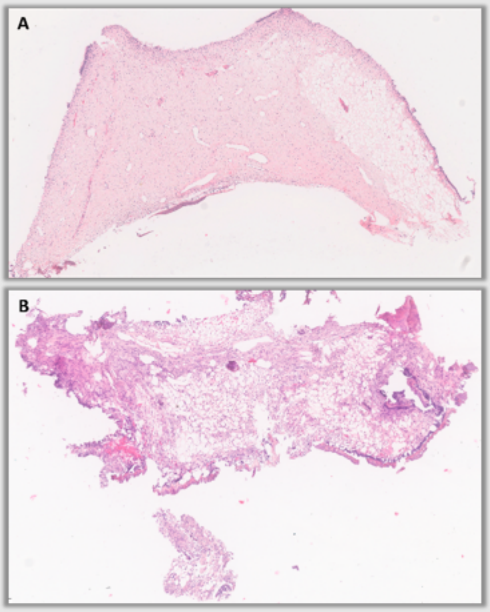
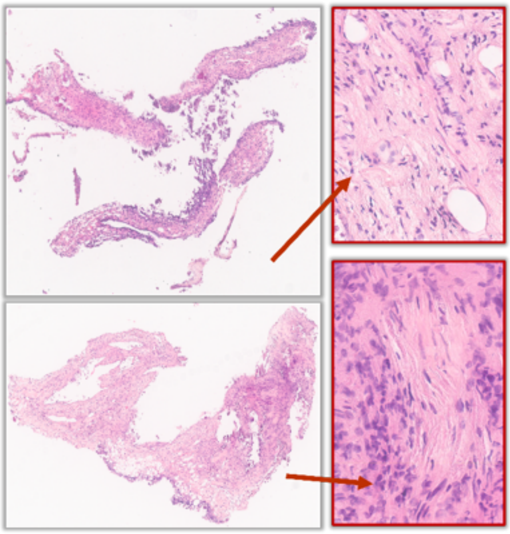
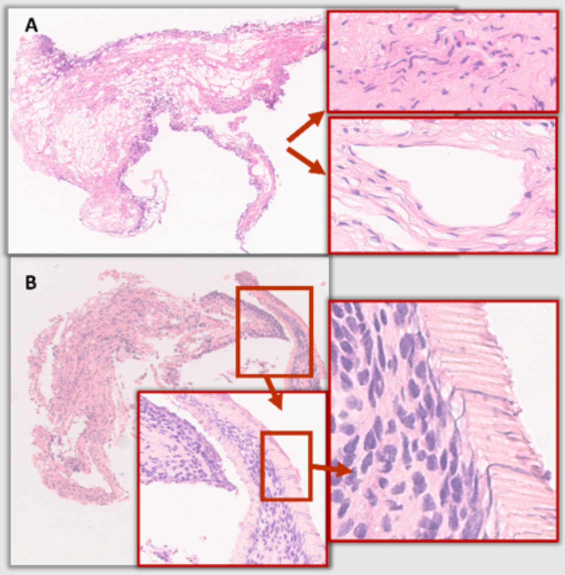
According to the different methods of count, nuclei density calculated manually considered as unit of measurement the Square Micrometer (μm²) and the area included was the same for each

ROI, whilst the method calculated by automatic software considered Pixels and the automatic setting (the standard proposed by the software as the best fitting) considered different areas for each ROI.

Table 2. Summary of the most significant histological characteristics of dental pulp at PMI T0 (baseline), 7h, 12h, 24 h, 36h, 48h, 72h, 7 days, 14 days

PMI	Stain Haematoxilin/Eosin	Histological features
T0 (baseline)		• Homogeneous distribution of extracellular matrix without vacuolisation; • Well represented odontoblasts with homogeneous distribution and stratification of nuclei within the odontoblast layer; • Well represented different cell types with circular, ovoid, and elongated shaped nuclei; • Well conserved endothelial cells of blood vessels; • Well conserved nerve cells.
7h		• Extracellular matrix disaggregated with vacuolisation areas; • Well conserved circular, ovoid, and elongated shaped nuclei, endothelial cells of blood vessels, and nerves distributed among the degenerative areas; • Well represented odontoblasts with homogeneous distribution and stratification of nuclei within the odontoblast layer.

12h		Different stages of extracellular matrix degeneration: • Panel A: homogeneous distribution of extracellular matrix without vacuolisation; • Panel B: extracellular matrix with localised disaggregated and vacuolised areas; • Panel C: extracellular matrix with massive disaggregated and vacuolised areas; • All three samples: well conserved circular, ovoid, and elongated shaped nuclei, endothelial cells of blood vessels, and nerves, and well represented odontoblasts with homogeneous distribution and stratification of nuclei within the odontoblast layer.
24h		Different stages of extracellular matrix degeneration: • Panel A: extracellular matrix with massive disaggregated and vacuolised areas; • Panel B-C: homogeneous distribution of extracellular matrix without vacuolisation; • All three samples: well conserved circular, ovoid, and elongated shaped nuclei, endothelial cells of blood vessels, and nerves, and well represented odontoblasts with homogeneous distribution and stratification of nuclei within the odontoblast layer.
36h		Different stages of extracellular matrix degeneration: • Panel A-B: extracellular matrix with massive disaggregated and vacuolised areas; • Panel C: homogeneous distribution of extracellular matrix without vacuolisation; • All three samples: well conserved circular, ovoid, and elongated shaped nuclei, endothelial cells of blood vessels, and nerves, and well represented odontoblasts with homogeneous distribution and stratification of nuclei within the odontoblast layer.

48h		Different stages of extracellular matrix degeneration but degenerative tissue is well represented in samples: • Panel A: extracellular matrix with localised disaggregated and vacuolised areas, conserved circular, ovoid, and elongated shaped nuclei, blood vessels, and nerves, and represented odontoblasts with homogeneous distribution and stratification of nuclei within the odontoblast layer; • Panel B: extracellular matrix with massive disaggregated and vacuolised areas. All structures (nuclei, odontoblast layer, vessels, and nerves) are visible.
72h		• Extracellular matrix with several disaggregated and vacuolised areas; • Still represented cells with not defined nuclei morphology; • Still represented odontoblasts with homogeneous distribution and stratification of nuclei within the odontoblast layer; • Still present and well recognisable blood vessels and nerves morphology.
7d		Different stages of extracellular matrix degeneration: • Panel A: extracellular matrix with massive disaggregated and vacuolised areas; • Panel B: homogeneous distribution of extracellular matrix without significant signs of vacuolisation; • All three samples: all structures (cells, vessels, and nerves) are visible but not defined nuclei morphology, irregular and wide vascular lumen, less defined morphology of nerve cells; • Still well represented odontoblasts with homogeneous distribution and stratification within the odontoblast layer and cytoplasmic extensions, but irregular morphology of nuclei.

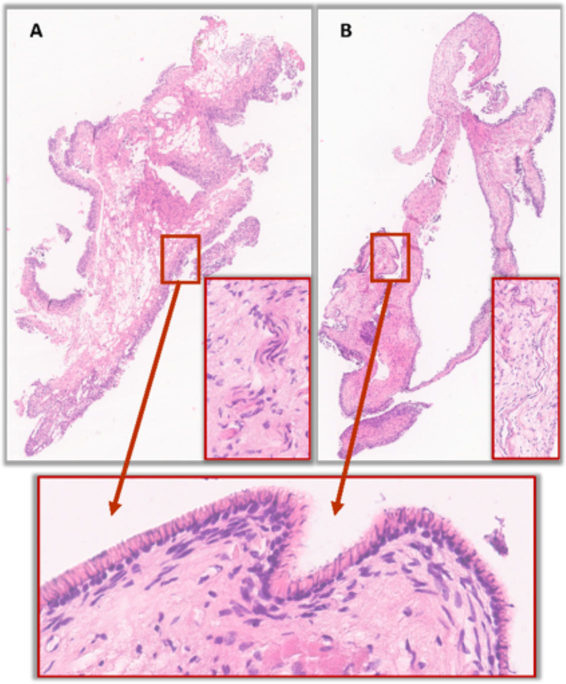
14d		Different stages of extracellular matrix degeneration: • Panel A: extracellular matrix with massive disaggregated and vacuolised areas; • Panel B: homogeneous distribution of extracellular matrix without significant signs of vacuolisation; • All three samples: all structures (cells, vessels, and nerves) are visible but not defined nuclei morphology, irregular and wide vascular lumen, less defined morphology of nerve cells; • Still well represented odontoblasts with homogeneous distribution within the odontoblast layer and cytoplasmic extensions, but thinner stratification and irregular morphology of nuclei.

Figure 1. Manual cell count calculated as the average of the 3 larger ROIs selected for each tooth of the PMI set

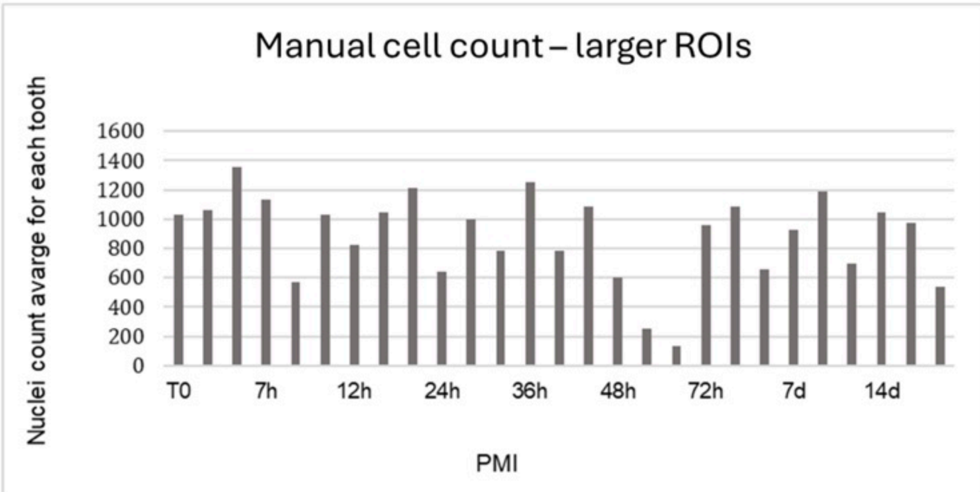


Figure 2. Manual cell density measurement calculated as the average of the 3 teeth for each PMI set, based on larger ROIs

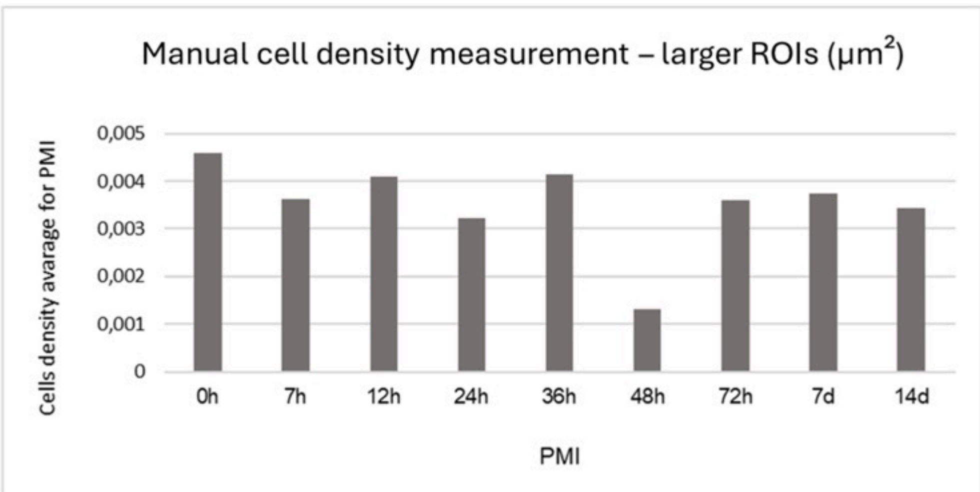


Figure 3. Manual cell count calculated as the average of the 3 smaller ROIs selected for each tooth of the PMI set

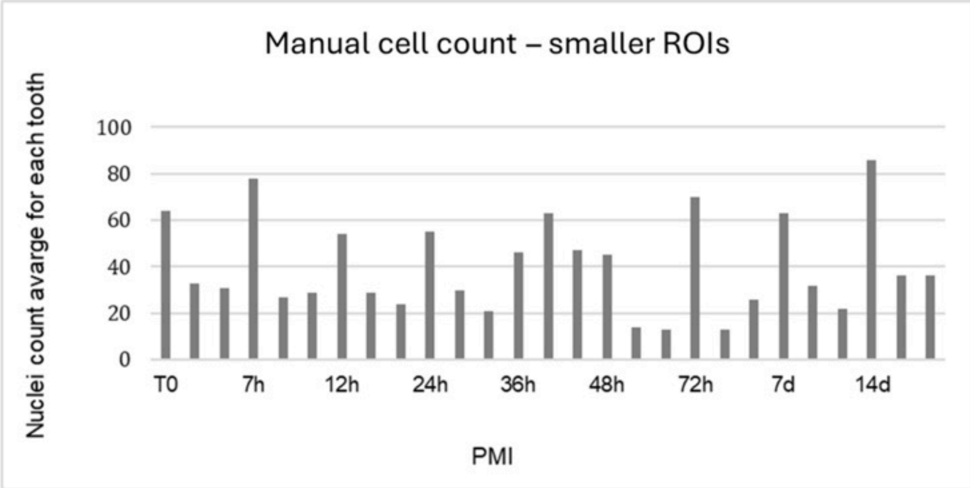


Figure 4. Manual cell density measurement calculated as the average of the 3 teeth for each PMI set, based on smaller ROIs

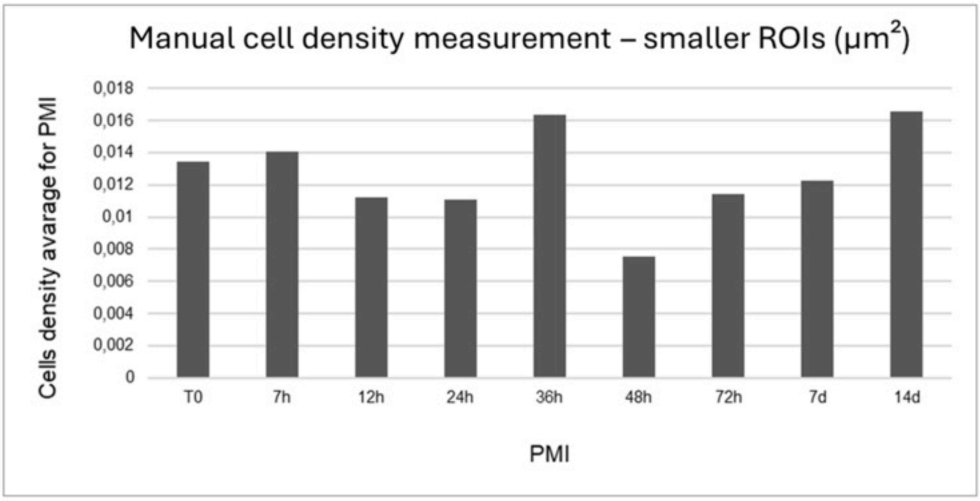


Figure 5. Automatic cell count calculated as the average of the 3 larger ROIs selected for each tooth of the PMI set (in pixels)

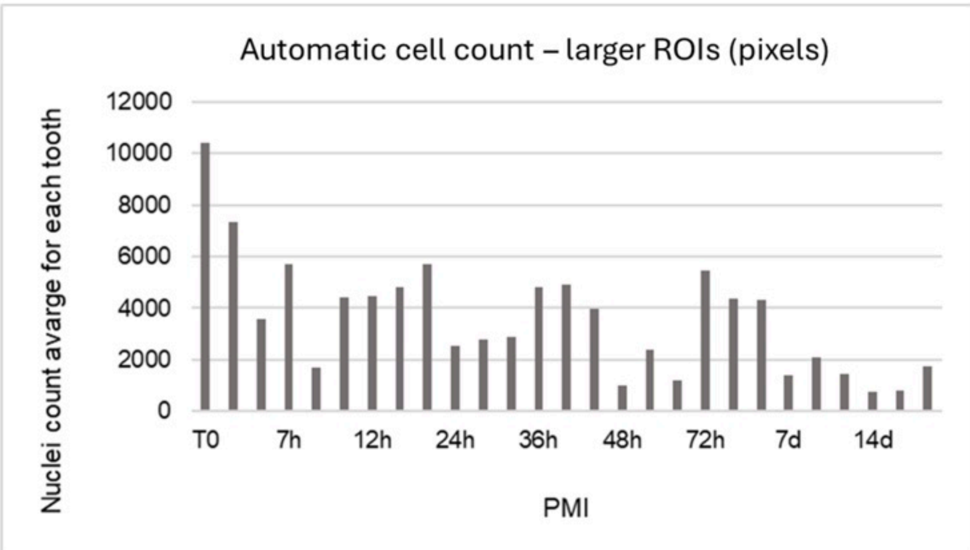


Figure 6. Automatic cell density measurement calculated as the average of the 3 teeth for each PMI set, based on larger ROIs (in pixels)

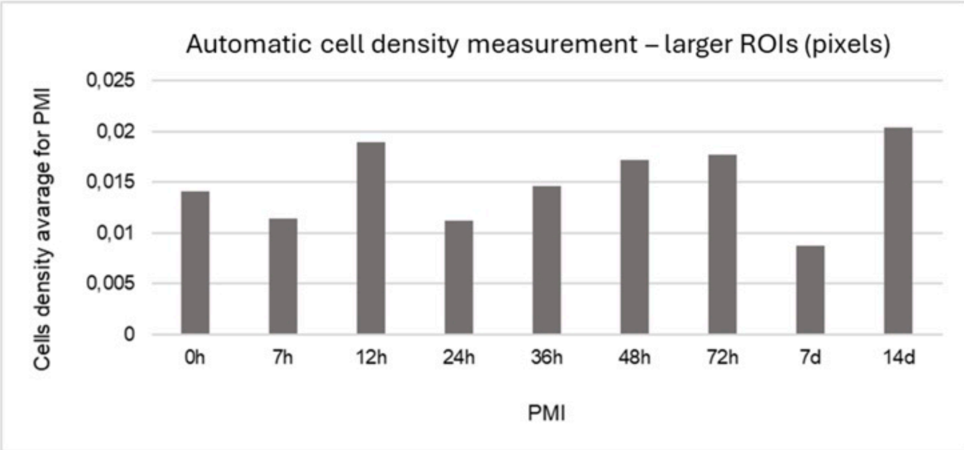


Figure 7. Automatic cell count calculated as the average of the 3 smaller ROIs selected for each tooth of the PMI set (in pixels)

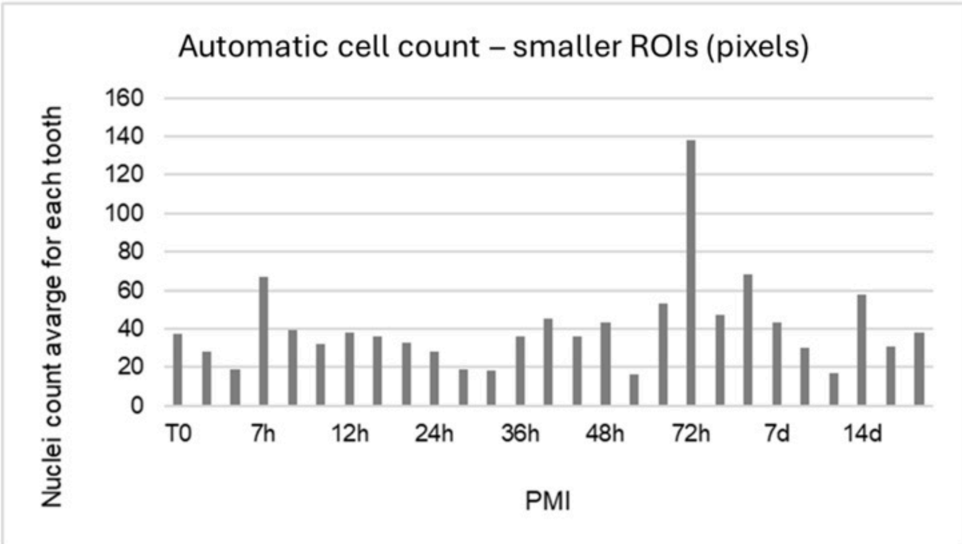
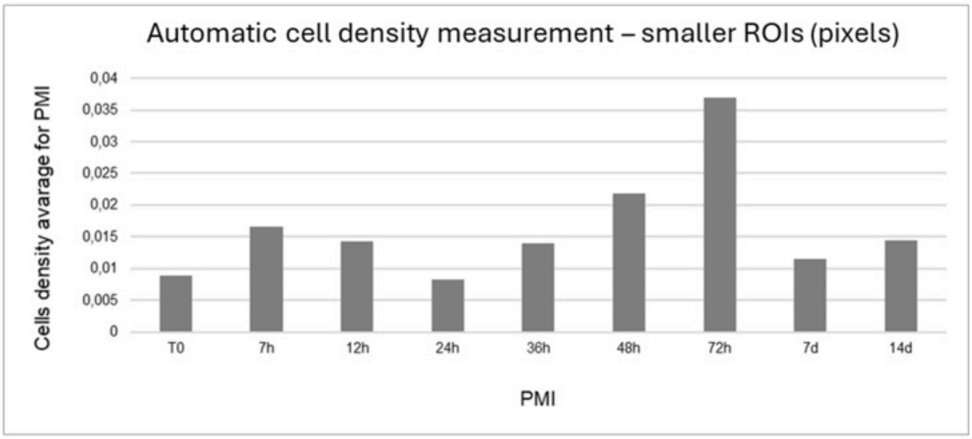


Figure 8. Automatic cell density measurement calculated as the average of the 3 teeth for each PMI set, based on smaller ROIs (in pixels)



DISCUSSION

Estimating the PMI is one of the challenging issues in forensic pathology especially when the corpse is found after the first week following death (late PMI). Teeth can be considered a stable matrix for estimating the time elapsed since death from early to late PMIs since they have great resistance to external damaging factors (such as heat, bacteria, UV light, and moisture) [37-39], and the pulp undergoes morphological, histological and biochemical changes after the interruption of the blood supply [27-36]. Previous studies were promising, but they were not able, either to yield evidence about the timing of the most significant post-mortem (PM) alterations of dental pulp or to indicate definitive hypotheses regarding the occurrence of these alterations [40]. The tissue characteristically consists of four main areas from the outer surface towards the inner: a layer of specialised cells (odontoblast layer) in close relationship with the dentine, an acellular zone formed by fibrils of connective tissue (Weil's basal layer), an area of high cell density composed of differentiated and undifferentiated cells (fibroblasts, macrophages, lymphocytes, inflammatory cells, and young mesenchymal cells) immersed in ECM, and neuro-vascular complex. All these structures seem to demonstrate different stages of degeneration according to the anaerobic metabolism of the various areas of dental pulp [41-42] and the effect of enzymatic activity after death [43-44].

The absence of significant histological changes at T0 (Tab. 2) was expected, and the results confirmed that the dental pulp samples were healthy, and the pulp extraction technique (bur cutting with careful heating control) was conservative and caused no alterations in the pulp tissue.

All samples analysed 7 hours after death (Tab. 2) showed extensive tissue degeneration, characterised by vacuolisation of the ECM but preservation of the other structures (vessels, nerves, nuclear morphology). The results seem to partially confirm the findings of Caballin et al [30] and Bhuyan et al [35] who noted the presence of ECM disaggregation as the main change in the PM pulp within the first 24-72 hrs. Caballin et al [30] attributed this phenomenon to the detachment of the odontoblasts from the dentinal wall. However, a different technique was used to prepare the sample (decalcification of the tooth and microtome cutting) and analysed the whole tooth, consequently, it is not possible to regard their hypothesis as valid. Bhuyan et al [35] reported the initial, localised presence of vacuoles in the ECM due to the release of intra-cellular hydrolytic enzymes and the production of gases by gram-positive anaerobic putrefying bacteria (staphylococci and streptococci) found in the pulp. Since no microbiological analyses was conducted in this study, it is not possible to confirm bacterial involvement and that the ongoing putrefactive process contributed to the late onset (from 24-72 hours PM) of pulp degeneration. However, the obtained results highlight an extensive and early occurrence (within 7 hours of death - Tab. 2) of ECM disaggregation which seems to be in complete contrast with Gawande et al [31] and Carrasco et al [36] who observed a well-preserved nature of pulp tissue with homogeneous distribution of ECM without vacuolization up to 24-72 hours PM.

This significant variability in the rate and extension of the degeneration of the ECM is demonstrated by results obtained for all subsequent PMIs up to 14 days PM, as shown in Table 2, panels A-B-C. It emerged that from 7 hours to two weeks after death it is not possible to correlate the alterations detected in the pulp with the PMI. In fact, samples with almost intact pulp have been observed even for up to 14 days. This finding is consistent with the results obtained by Karthikeyan et al [45] on 25 teeth buried together with fragments of organic tissue (flesh) and examined up to 12 days PM. They reported that most samples analysed at the same PMI showed a similar degree of rate of degradation, but with relevant exceptions that conflicted with the generalisation of histomorphology results for all teeth and seasons [45]. In this study, it is not possible to attribute the variability of results to the putrefactive process of organic tissues around teeth nor to the different characteristics of soil, since the sample teeth were stored outside alveolar bone at room temperature. The study can conclude that the variability observed does not seem to be influenced by the sex of the subject or the type of tooth, being homogeneously distributed between the different PMIs as reported in Tab. 1. Furthermore, not even the temperature variation seemed to influence the rate and extent of the degeneration considering that recorded temperatures showed slight differences as the study was carried out only in the hottest seasons (spring and summer). In fact, the parameter that seemed to influence the variability of the pulp's response to the PM degenerative phenomena was the age of the subject and of the tooth's physiological age of root maturation, and therefore of the residual dentinogenic activity. In particular, samples 25-26-27 (Tab. 1) belonged to the same 18 year old male subject and were all third molars. Despite being analysed at different PMIs (T0, 12 h, and 36 h, Tab. 2 panels A and C), they never showed signs of degeneration, as did samples 2 and 3 (Tab. 1, Tab. 2 panels B and C) extracted from the same 23 year old male subject at PM 24h. Considering that third molars in males erupt around 17-19 years and complete maturation at 22-23 years of age, these dental elements, although complete, were barely mature with residual dentinogenic activity and not yet subjected to continuous masticatory stress. In fact, samples 2 and 3 showed sound pulp at the same PMI as sample 1, tooth 35 in a 46 year old male subject (Table 1) who on the contrary showed advanced signs of degeneration of the ECM 24 hours after death (Tab. 2, panel A). A similar observation can be made for samples 20-21-22-24 (Tab. 1), all second premolars from a 25 year old female subject. These teeth usually have erupted and completed root calcification around 12-13 in females, therefore they have completed mineralisation at least 10 years before this examination, with no residual primary dentinogenic activity and under masticatory stress and repeated insults. Analysed at three different PMIs (7h, 12h, and 36h, Tab. 1), teeth always showed extensive signs of degeneration (Tab. 2, panels B-C for PMI 12h and A for PMI 36h). Hence in the examined sample the younger the subject, the shorter the time from root completion and the fewer the degenerative alterations of the pulp.

In this sense, a 2015 study [41] on the mechanism of the anaerobic glycolysis in PM bovine dental pulp under oxygen-free conditions applied *in vitro* showed that metabolic exhaustion with added glucose occurred after 80-95 minutes of anaerobic activity, depending on the degree of residual activity of the pulp in the process of dentine deposition. This finding demonstrated that the pulp could cope with the absence of blood supply through the activation of alternative energy mechanisms for a variable time after death according to the maturity stage of the tooth. Therefore, the pulp of subjects of different ages could have different resistance to PM degenerative phenomena, supporting the hypothesis that ageing and the time span since the tooth erupted and completed mineralisation can affect the post-mortem resistance of the pulp and the onset and progression of pulp degeneration after death.

On the contrary, both Bhuyan [35] and Carrasco [36] detected increasing vacuolations of the ECM with time [48h, 72h, 1 month, 3 months, over 6 months], thus suggesting that the variability decreases as the ranges of PMIs considered increase and therefore that histomorphological changes of dental pulp could be a more reliable instrument in late PMIs.

For quantitative measurement of pulp alteration after death, Vavpotič et al [32] found that the estimated time for odontoblast's presence, with a 95% confidence interval, is about 5 days after death with an average drop in the density of 130 odontoblasts/ square millimeter per hour at room temperature. That study is quite different compared to the present research, since Vavpotič et al considered PMIs limited to 5 days after death and the technique required a lengthy preparation of samples with decalcification of tooth, that could have affected results. A faster and simpler technique has been developed here, but both manual and automatic software counting, despite the use of two different units of measurement (μm² and pixels), revealed that the density of cells in such a narrow PMI (up to 14 days after death) is not significant (Fig. 2-4-6-8). In the manual measurement of larger ROIs this study obtained a wave-like pattern with density peaks equal to 0.004-0.005 μm² for T0, 12h and 36 h PM and a slight constant decrease in density from 72h to 14 days PM (0.003-0.004 μm²) with a very low peak at 48h after death (0.001-0.002 μm²). On the contrary, for the measurement of smaller ROIs, the minor peak at 48h is confirmed (0.006-0.008 μm²) but from 72h to 14 days after death there is even an increase in density (from 0.01-0.012 μm² to 0.016-0.018 μm²) with a recorded density even greater at 14 days PM compared to T0 (0.012-0.014 μm²). The density measurement with software also showed great variability between the two ROIs, highlighting for larger ROIs a higher density at 14 days PM (0.02 pixels) compared to T0 (0.014 pixels); while for smaller ROIs, a density of 0.09 pixels at T0, with a peak at 72h PM (0.035-0.04 pixels) and a significant decrease at 14 days after death (0.015 pixels) still greater than the value recorded at T0.

These results are partially consistent with those of Carrasco et al [36] who reported the presence of nuclei of all the different cell types of dental pulp up to 6 months, but they revealed a progressive decrease in cell density between 24 hours, 1 month, 3 months and 6 months after death.

The progression of the research is ongoing from the present findings and quite interesting previous reports about the onset and time progression of DNA alteration in dental pulp after death [24]. Caviedes-Bucheli et al [43] demonstrated that in dental pulp, cell activity and DNA expression of lactate-dehydrogenase (LDH) decrease progressively from 89, to 68, up to 41% measured 6, 12, and 24 hours PM, respectively. Boy et al [44] demonstrated that DNA denaturation in dental pulp is continuous for about 72 hours after death and then undergoes minimal progression of degradation by 144 hours, thereby suggesting the usefulness of dental DNA for estimating late rather than early PMIs. This study's results, Tab. 2 - PMI T0, 7 d, and 14 d, demonstrated the presence and activity of odontolblasts after death, suggesting their application in late PMIs (beyond the 2 weeks after death).

Hence late PMIs are under investigation by using easy, quick, and inexpensive histological methods that apply specific colouration for DNA integrity (e.g. Feulgen stain).

The research is limited to two weeks after death to observe the alterations and presence of pulp characteristics in an intermediate PMI. Further studies are already ongoing to extend the PM window of analyses, given the significance of the results obtained.

One of the main limits is the method of extraction and fixation of the pulp, that is an easier and faster method compared to those requiring the tooth decalcification, but can cause an overlap of layers and do not allow a topographic definition of the structures. Moreover, the superposition of nuclei images does not allow repeatable and reliable nuclei counting either manually or via software. Further research will aim at verifying whether the pulp preparation technique with decalcification and cutting is more reliable for standardising the quantitative analysis method on cell density.

CONCLUSION

A pilot study was carried out to analyse the histomorphological changes of 27 PM dental pulps as a possible tool for estimating the PMI up to 14 days after death (intermediate PMI for which the estimate remains complex).

The study demonstrated that dental pulp degeneration appears 7 hours after death, but qualitative histological changes in PM dental pulp are endowed with high variability so it is not possible to generalise the results for early PMIs. ECM, cells, vessels, and fibres are still present 14 days after death even if they show different and uneven degenerative stages. No patterns of degenerative alterations of cells, fibres or nuclei emerged that correlated to different PMIs.

The ageing of the subject and the time span since the tooth erupted and completed mineralisation can affect the post-mortem resistance of the pulp and the onset and progression of pulp degeneration after death.

The quantitative measurements of cell density were not reliable because the cell counts were not standardisable, neither manually nor automatically, due to overlapping layers and nuclei fragmentation. Nevertheless, the presence and activity of odontoblast cells up to 2 weeks after death suggested their application to the study of late PMIs.

Future research should focus on a wider sample analysed at late PMIs (more than 2 weeks after death) and both with pulp extraction-fixation technique and tooth decalcification-cutting method to compare results.

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Bitemark analysis comparing the use of digital scans and 3D resin casts

Andrea Di Palma [1], Ilenia Bianchi [2], Martina Focardi [2], Chiara Cioffi [1], Stefano Bonetti [1], Domenico Dalessandri [1]

[1] Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy. [2] Laboratory of Personal Identification and Forensic Morphology, Department of Health Sciences, University of Florence, Florence, Italy.

Corresponding author: ilenia.bianchi@unifi.it

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ABSTRACT

Although dental patterns are unique, the use of bitemark analysis in personal identification remains controversial. To accurately reproduce and compare three-dimensional models of bitemarks and dental arches, intraoral three-dimensional scans, commonly utilized in clinical dental practice for precise and stable digital impressions, are recommended. This study aims to compare two different techniques for bitemark analysis: a digital method based on the superimposition of digital scans of dental patterns and lesions, and a visual method based on the physical superimposition of impressions and resin casts produced by 3D printing.

A sample of 12 volunteers (6 males and 6 females) with a mean age of 26 years was collected as biters. Each subject was asked to bite on custom supports made from semi-rigid water bottles covered with imprintable dental wax. The dental arches and bitemarks were then recorded using an intraoral scanner and dental impressions. Scan superimposition analysis was conducted using CloudCompare software, while resin casts were printed using a 3D printer and physically superimposed on the bitemark impressions by a blind operator, who was not involved in sample collection, bite test execution, prior cast acquisition, or CloudCompare analysis. Both superimposition techniques relied on the selection of 10 corresponding landmarks (on canines and central and lateral incisors of the upper and lower arches) between the dental arches and impressions.

The digital superimposition showed an average concordance of 92.5% for the upper arch landmarks and 85% for the lower arch landmarks, with an overall average concordance of 88.8% for both arches combined. In contrast, the visual analysis of resin casts showed an average concordance of 77.5% for the upper arch and 76.7% for the lower arch, with an overall average of 77.1% for both arches combined. In the analysis performed using CloudCompare, the maxillary arch demonstrated the best superimposition, with 4 landmarks (R0, R1, R2, R5) consistently overlapping. The digital analysis outperformed the visual analysis in all four quadrants, particularly in the upper right arch compared to the lower left arch, thereby supporting the integration of digital techniques in forensic applications.

Further studies are necessary to validate the digital technique on a larger sample, including subjects with different dental characteristics, bite dynamics, and varying types of supports and substrates.

INTRODUCTION

Although dental patterns are unique, the use of bitemark analysis in personal identification remains controversial. The longstanding debate between the scientific validity of bitemark evidence and its judicial value in court highlights the role of forensic odontologists in determining the degree of concordance or exclusion between different dental patterns. This determination is based on the objective collection and analysis of marks, along with scientific rigor in drawing conclusions, while leaving the determination of guilt to the judicial system. According to the 2023 review report by the National Institute of Standards and Technology (NIST), forensic bitemark analysis still lacks sufficient scientific support for reliably recognizing complete dental patterns transferred onto human skin or objects, accurately registering identifying characteristics, and using appropriate techniques to compare different dentitions and draw conclusions regarding the exclusion or inclusion of individuals as potential perpetrators of bites.

To minimize errors and subjectivity, odontologists are advised to follow established guidelines, recommendations, and standards for bitemark analysis procedures, as well as to engage in rigorous scientific research assessing the validity and reliability of both metric and non-metric methods .

Bitemark records can be created using two-dimensional methods, which involve photographic analysis performed with specific standards (e.g., ABFO No. 2 reference scale) for both wounds presumed to be bites and dental impressions/casts of potential biters' arches. These are then compared through image superimposition, using techniques such as hand tracing from study casts, hand tracing from wax impressions, xerographic methods, radiopaque impression methods, and 2D computer-based methods. However, these techniques carry a risk of error, as they attempt to represent three-dimensional models in a two-dimensional format, leading to inevitable distortions and alterations in both qualitative and metric aspects.

To address these limitations, physical three-dimensional techniques have been developed, where the model of the suspected biter's dental arch is superimposed onto the model of the bite obtained through impressions (e.g., in alginate or silicone), either directly on the object or on the skin . These methods also have significant limitations, particularly due to the challenges in obtaining clear and accurate wound models using traditional dental impression materials. Furthermore, there is a distinction between bitemarks left on different types of objects, especially food, and those left on skin. The variability in bitemark characteristics on human skin is partly due to the nonspecificity of some marks, which often manifest as superficial bruises with imperfections and abrasions, as well as distortions caused by the biological processes of injury healing, tooth wear, and the skin's malleability and deformation. These factors are influenced by the body part affected, the dynamics of the event, the force applied, and any movement by the victim.

To accurately reproduce and compare three-dimensional models of bitemarks and dental arches, three-dimensional scanning technology is required. Intraoral scanners are now commonly used in clinical dental practice, providing digital impressions that are accurate, stable, and can be analyzed using specialized software that is continually advancing.

This study aims to compare the results obtained from two different bitemark analysis techniques: the direct comparison of digital scans and the physical superimposition of impressions and resin casts produced by 3D printers.

MATERIALS AND METHODS

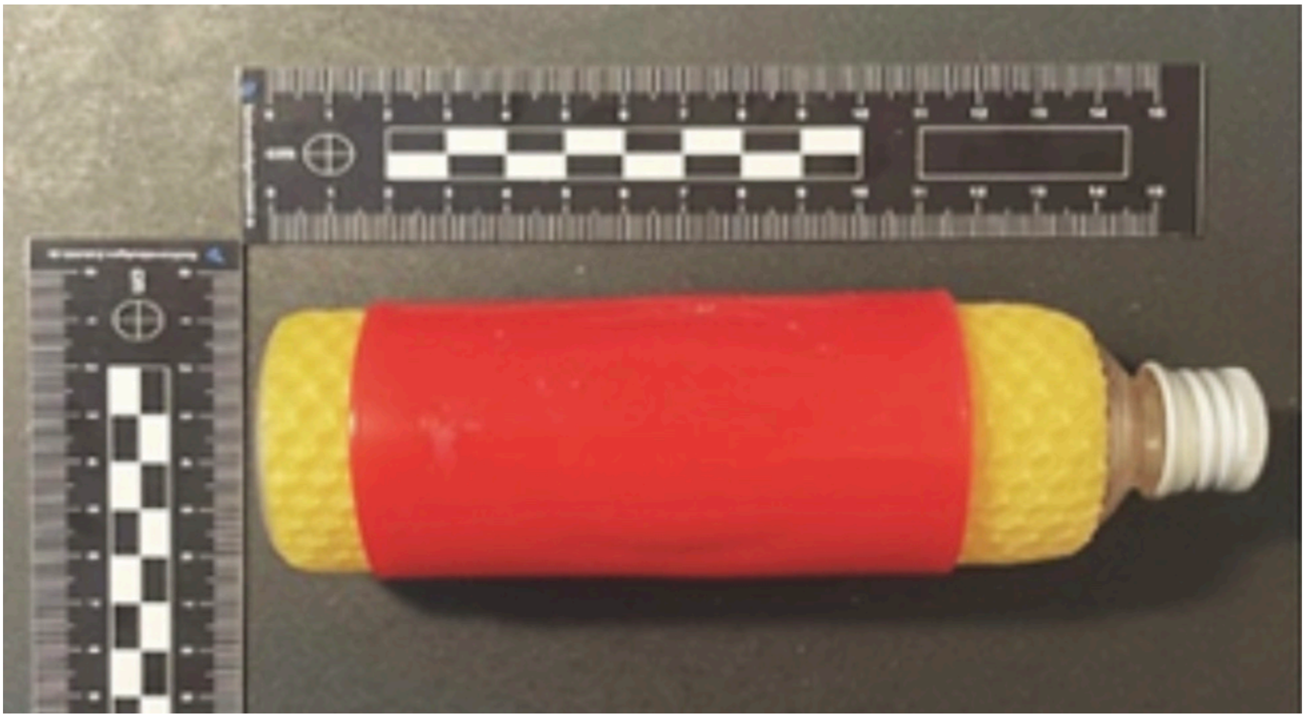
Sample collection - Biters

Volunteer subjects were recruited from among the students and assistants of the Master's Course in Dentistry at the University of Brescia, Italy. The inclusion criteria were male and female subjects without specific dental anomalies, such as dental malposition, restorative or prosthetic treatments, dental agenesis, severe tooth wear, or orthodontic brackets. Subjects who did not meet one or more of the inclusion criteria were excluded from the study.

Sample development – Test bite supports

Special supports were designed to closely replicate the shape, bone rigidity, and deformable surface of a female subject's wrist with a circumference between 14-18 cm. Semi-rigid plastic bottles with a diameter of 4.5 cm and a height of 15 cm were used, coated with beeswax and dental wax. To create the test bite supports, each plastic bottle was made semi-rigid by filling at least two-thirds of its volume with water to simulate bone support. The bottles were then coated with an initial layer of beeswax, which was manually modeled to adapt to the smooth surface of the support, mimicking the deformability of the deeper layers of the skin (dermis and hypodermis). An additional layer of dental wax was applied on the outer surface, simulating the deformability of the epidermis and allowing for the recording of bite marks (Fig. 1).

Figure 1. Support for bite tests: semi-rigid plastic bottle coated with two layers of wax to simulate the superficial deformability of the skin and record bite marks.



Impression materials and intraoral scanner
Bitemark impressions on the test bite supports were made using a light-consistency polyvinylsiloxane (PVS). Scans of the PVS impressions and the direct dental arches of the biters were conducted using the Carestream CS3600 intraoral scanner.

3D printing
Resin casts of the bitemark impressions were produced using the Anycubic Photon M3 benchtop printer, along with the Anycubic Wash & Cure Machine 2.0 benchtop washing and curing system.

Software for images processing and comparison
The CS ScanFlow software, installed on Carestream systems, was used to process the STL files generated by the CS3600 scanner. Meshmixer software was employed to eliminate scanning defects in the native STL files and to create virtual bases for 3D printing. The Anycubic Photon Workshop software, provided with the Anycubic printer, was used to generate the necessary supports for the 3D printer to recreate impressions in resin and to slice the STL files. CloudCompare, an open-source software, was used to process the acquired 3D images into point clouds (reference and alignment clouds: point-to-point analysis) for the purpose of superimposing bitemark impression scans onto the dental arches of the biters.

Study design
- Test bites and arches scans. Each subject bit one of the developed supports, applying a medium force sufficient to leave clear dental marks in the dental wax without deforming the bottle. Scans of the arches were then performed using an intraoral scanner and saved as STL files.
- Impressions of test bites and impressions scans. Test bite impressions were made using a double-layer technique with light-consistency polyvinylsiloxane (PVS) to ensure the durability and stability of the bite impressions. These

impressions were then scanned with the intraoral scanner and saved as STL files.

- Scans superimposition analysis. Using CloudCompare software, each scan was digitally transformed into a cloud of points (reference clouds for arch scans and alignment clouds for bitemark scans) to superimpose corresponding reference areas for each pair of arch-bite scans. Following the method proposed by Fournier et al. [25], at least three landmarks must be selected for alignment calculations in CloudCompare and must be identifiable on each arch and corresponding bite. The more landmarks identified, the lower the likelihood of computational errors.
- 3D printing resin casts. The STL files were processed using Meshmixer software to eliminate scanning defects, prepared for slicing, and then printed with the Anycubic Photon M printer using photopolymerizable resin that is washable in water. The processing time was approximately 90-110 minutes per cast, with the impressions of the arches printed in light blue and the bitemark impressions printed in grey to distinguish between them.
- Resin casts overlap analysis. An operator, who was not involved in the sample collection or execution of the bite tests, performed the physical matching of the resin casts of the maxillary and mandibular arches with the corresponding bitemark impressions. This operator was blind to the prior acquisition of casts and the CloudCompare results. Each matched arch-bite pair was then analyzed by a second operator, who visually identified landmarks with no or minimal overlap, marked them in red, and photographed them to allow for direct comparison with the overlays generated by the software (Fig. 3).

RESULTS

A sample of 12 volunteers, consisting of 6 males and 6 females with a mean age of 26 years, was collected as biters.

The reference areas were based on the positions of 10 landmarks (Fig. 2), chosen for their anatomical consistency and ease of identification in replicable positions on antagonistic teeth, as indicated in Table 1.

Figure 3 presents an example of superimposition analysis performed using both digital and manual techniques.

Figure 2. Panel A: a scan of a maxilla with the landmarks positioned from R0 to R9; Panel B: a mandibular scan with respectively landmarks from R0 to R9.

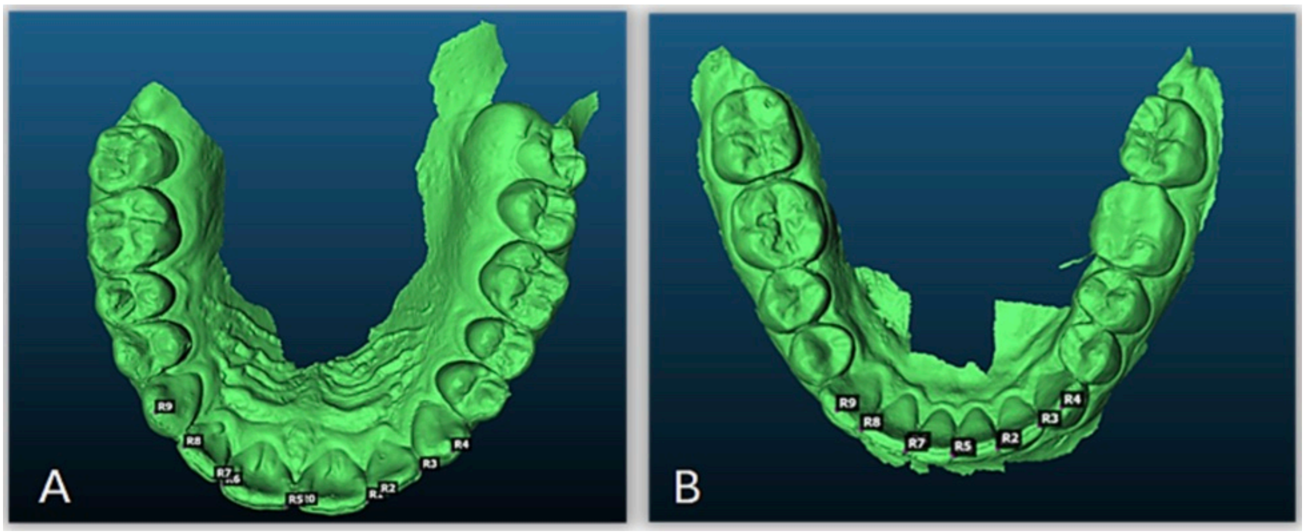


Table 1. Selected reference areas for superimposition based on the position of 10 landmarks from R0 up to R9.

Landmarks	Anatomical areas on antagonists teeth *
R0	Mesial angle of incisal edge of dental elements 1.1 and 3.1
R1	Distal angle of incisal edge of dental elements 1.1 and 3.1

R2	Mesial angle of incisal edge of dental elements 1.2 and 3.2
R3	Distal angle of incisal edge of dental elements 1.2 and 3.2
R4	Cusp tip of dental elements 1.3 and 3.3
R5	Mesial angle of incisal edge of dental elements 2.1 and 4.1
R6	Distal angle of incisal edge of dental elements 2.1 and 4.1
R7	Mesial angle of incisal edge of dental elements 2.2 and 4.2
R8	Distal angle of incisal edge of dental elements 2.2 and 4.2
R9	Cusp tip of dental elements 2.3 and 4.3

* According to the Fédération Dentaire Internationale (FDI) dental numbering system [26]

Figure 3. Example of superimposition analysis. Panel A: maxillary (upper image) and mandibular (lower image) scans overlapped on bite scans carried out with *CloudCompare*; Panel B: overlapping of the maxillary (upper image) and mandibular (lower image) resin casts on bite casts carried out manually. Non-overlapped landmarks are circled in red.

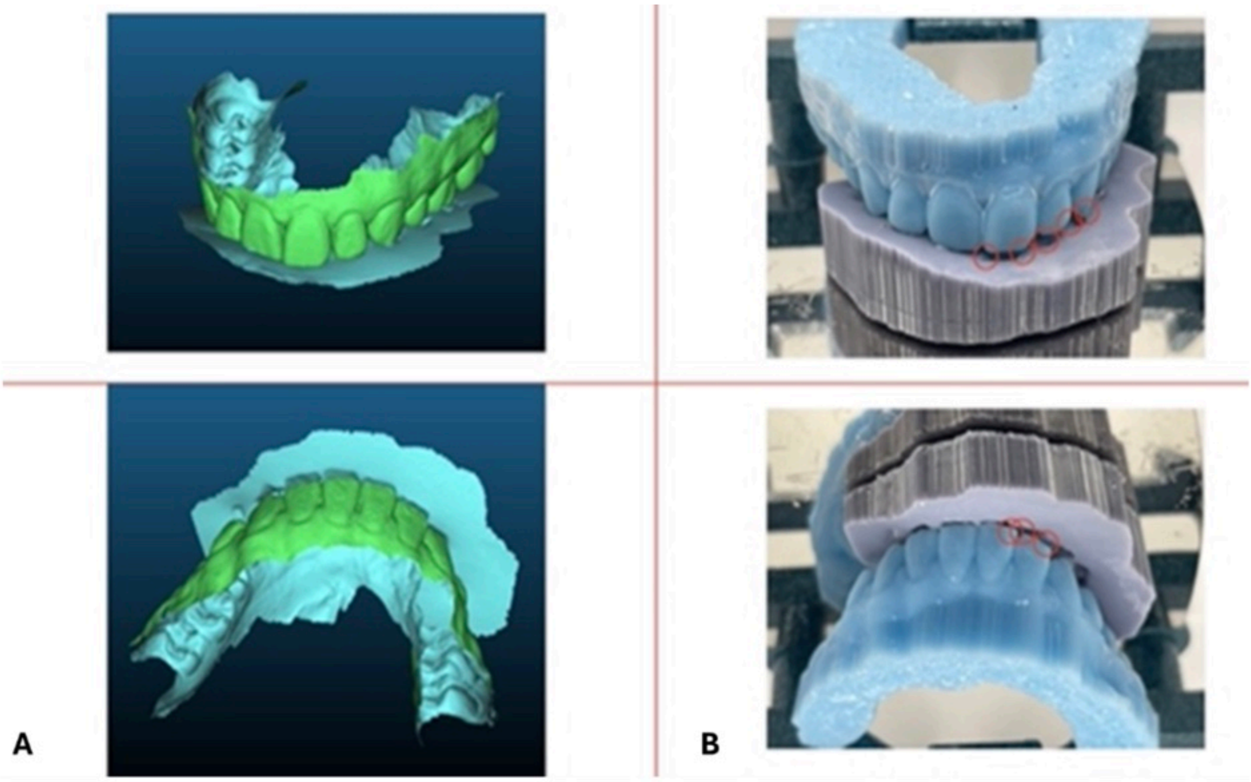


Table 2 shows the superimposition results according to the number of correctly overlapped landmarks for both the digital analysis using CloudCompare software and the visual analysis of the resin casts. The digital superimposition showed an average concordance of 92.5% for the upper arch landmarks and 85% for the lower arch, with an overall average of 88.8% when both arches were considered together. In contrast, the visual analysis of the resin casts showed an average concordance of 77.5% for the upper arch landmarks and 76.7% for the lower arch, with an overall average of 77.1% when both arches were considered together.

Table 3 reports the incidence of errors in landmark overlapping. In the analysis performed with CloudCompare, the maxillary arch demonstrated better superimposition, with 4 landmarks (R0, R1, R2, R5) overlapping in every case, whereas the mandibular arch had at least one instance of non-overlapping landmarks. Conversely, in the analysis of the resin casts, two landmarks (R6, R8) failed to overlap in 4 out of 12 cases for the maxillary arch, and there was at least one instance of non-overlap for each landmark. Additionally, R1 failed to overlap in 5 cases and R3 in 6 cases for the mandibular arch, showing the highest frequency of error.

Table 4 classifies the results by quadrants, showing the percentage of cases with non-concordant overlaps based on the distribution of landmarks between the left and right sides of both the maxillary and mandibular arches, comparing digital and visual analyses. The digital analysis outperformed the visual analysis in all four quadrants, and the upper arch showed better results compared to the lower arch.

Table 2. Number of concordant landmarks between maxillary/mandibular arches and digital scans or resin casts: comparison between digital and visual analysis.

ID case	Maxillary digital analysis	Mandibular digital analysis	Both arches digital analysis	Digital analysis concordance percentage	Maxillary visual analysis	Mandibular visual analysis	Both arches visual analysis	Visual analysis concordance percentage
1	10/10	9/10	19/20	95%	10/10	7/10	17/20	85%
2	9/10	10/10	19/20	95%	4/10	9/10	13/20	65%
3	10/10	3/10	13/20	65%	10/10	4/10	14/20	70%
4	10/10	10/10	20/20	100%	5/10	7/10	12/20	60%
5	9/10	9/10	19/20	95%	7/10	7/10	14/20	70%
6	9/10	9/10	18/20	90%	9/10	8/10	17/20	85%
7	10/10	10/10	20/20	100%	9/10	10/10	19/20	95%
8	10/10	10/10	20/20	100%	10/10	9/10	19/20	95%
9	10/10	9/10	19/20	95%	7/10	9/10	16/20	80%
10	7/10	4/10	11/20	55%	7/10	5/10	12/20	60%
11	9/10	9/10	18/20	90%	8/10	10/10	18/20	90%
12	8/10	10/10	18/20	90%	7/10	7/10	14/20	70%
Total	111/120	102/120	213/240	-	93/120	92/120	185/240	-
Tot %	92,5%	85%	88,8%	-	77,5%	76,7%	77,1%	-

Table 3. Incidence of errors in superimposition, according to the kind of landmarks, the maxillary/mandibular arch, and digital or visual analysis.

Landmarks	Maxillary digital analysis	Mandibular digital analysis	Maxillary visual analysis	Mandibular visual analysis
R0	0/12	2/12	1/12	3/12
R1	0/12	2/12	2/12	5/12
R2	0/12	2/12	1/12	2/12
R3	1/12	1/12	2/12	6/12
R4	2/12	1/12	3/12	1/12
R5	0/12	2/12	3/12	3/12
R6	2/12	2/12	4/12	3/12
R7	1/12	1/12	3/12	3/12
R8	2/12	1/12	4/12	2/12
R9	1/12	2/12	1/12	0/12

Table 4. Percentage of cases with non-concordant overlap according to landmarks distribution between left and right of both maxillary and mandibular arch, comparing digital and visual analyses.

Quadrant	Digital analysis	Visual analysis
I	5%	15%
II	10%	25%
III	13,3%	28,3%
IV	13,3%	18,3%

DISCUSSION

The increasing development of digital tools in clinical practice has also opened new possibilities in the forensic field, particularly in personal identification. [27-29] Numerous studies have demonstrated the usefulness of 2D and 3D radiography, intraoral scanners, and facial scanners for recording the morphological and morphometric characteristics of skeletal and dental structures, which are valuable for identification purposes. [30-37]

Bitemark analysis has long been a primary application in forensic odontology. However, the reliability of techniques for identifying dental patterns remains debated, and the validity of recording and comparing bitemarks is still controversial. [38] Biting is a dynamic phenomenon, and the physical "marks" left by dental elements on the skin are often unevenly distorted due to factors such as the victim's position, skin characteristics, dental arch features, and the strength and movements of both the biter and the victim. [39-40] Therefore, it is crucial to have reliable methods for recording and comparing bitemarks that can accurately reconstruct the three-dimensional structure of both the arches and the bitemarks, preserving critical information for pattern overlap analysis. [41]

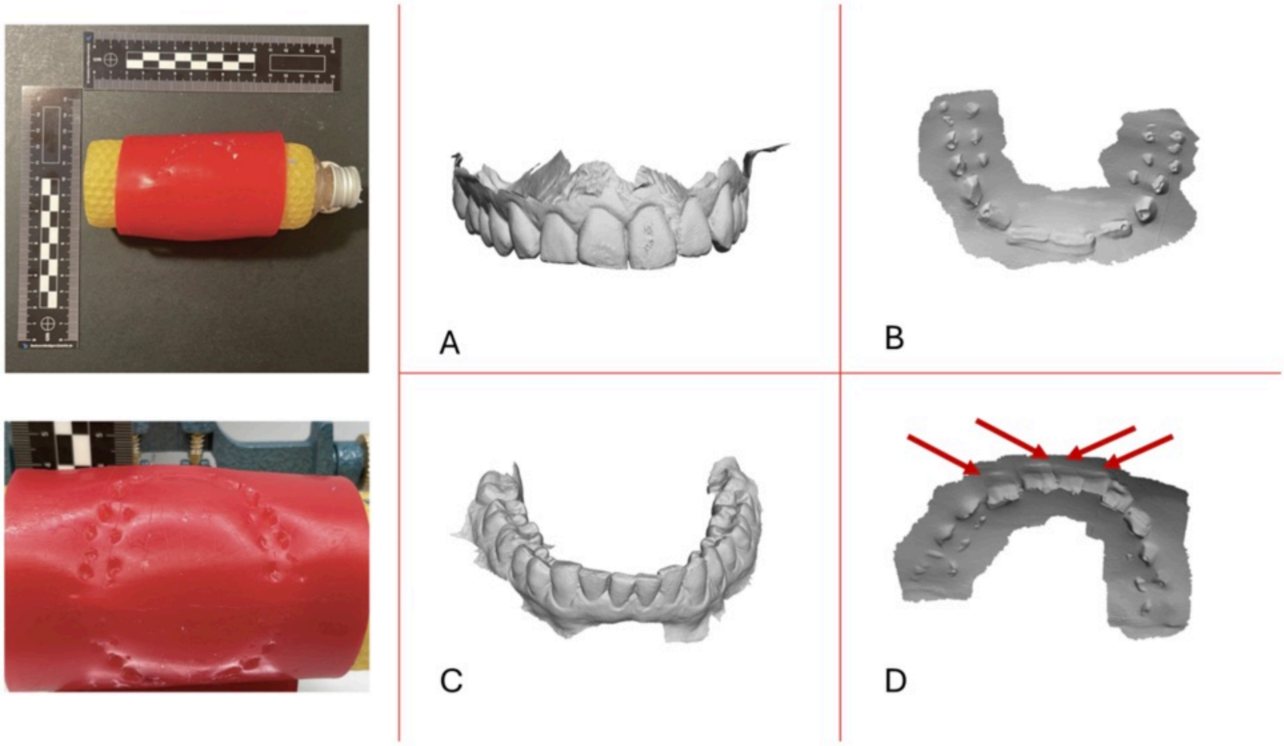
This study investigated and compared the results obtained from two different methods of recording and analyzing bitemarks: one entirely digital, based on intraoral scans of dental arches and bitemarks, and the other based on the physical comparison of resin casts and silicone impressions.

An ad hoc test model (bite tests) was developed to reproduce the three-dimensional patterns of dental arches on an elastic and compressible surface, simulating human skin. This model allowed for the evaluation of a larger scale of tests in vitro, even though it is challenging to obtain real samples of skin or cadaveric limbs for research purposes. It is also important to note that cadaveric skin or limbs do not fully represent the characteristics of living tissue, as they lack the typical signs of lesion vitality. [42-45]

To compare the two methods, we selected and applied the same 10 reference points (landmarks R0 to R9, Table 1) on the canines and central and lateral incisors of both the upper and lower arches. [46] A sample of male and female subjects without specific dental features (such as dental malposition, conservative or prosthetic treatments, dental agenesis, severe tooth wear, orthodontic brackets) was collected to verify the reproducibility of comparisons based on specific natural landmarks (dental morphology, position, and distances). Although the small number of tests does not satisfy a sample size suitable for statistical analysis, it was considered valid for observational purposes to identify discrepancies between the two methods and serve as a foundation for future, larger studies.

When considering all landmarks for both arches (Table 2), the digital analysis conducted using an intraoral scanner and CloudCompare software achieved a concordance between arches and bitemarks of approximately 90%, with better results for the upper arch (92.5%) compared to the lower arch (85%). For the maxillary arches, 6 out of 12 cases showed concordance across all selected landmarks, 4 cases had 9 out of 10 concordant points, and only 2 cases had fewer than 9 concordant points. In the mandibular arches, full concordance of all landmarks occurred in 5 out of 12 cases, while 5 other cases had 9 out of 10 concordant points. This discrepancy could be attributed to the different dynamics between upper and lower arches, even when impressed simultaneously. The teeth's impact during the bite test likely involved progressively increasing pressure and direction adjustment, especially for the mandibular arches (Figure 4), as the nervous system adjusted to the action and the characteristics of the support being bitten (e.g., size, consistency, flavor). The subsequent closure of the bite by the lower arch may have caused slight tooth movements in the wax, leading to inaccuracies in some reference points. [22, 47]

Figure 4. Case 3 (referring to Tab. 2). Panel A-B: maxillary arch and bitemark impressed; Panel C-D: mandibular arch and bitemark impressed with signs of anterior dragging of the incisor teeth (red arrows).



The visual analysis of the resin cast superimpositions showed a concordance of 77.5% for the upper arch reference points and 76.7% for the lower arch, with an overall average of 77.1%. Full concordance of landmarks was observed in 3 out of 12 cases for the maxillary arch and 2 out of 12 cases for the mandibular arch. In both the upper and lower arches, there was only one case with 4 out of 10 visually superimposable points, representing the minimum registered match (Table 2). Notably, case 3 and case 10 demonstrated the worst performance for the lower arch (4 and 5 concordant landmarks, respectively), with better concordance for the maxillary arch, suggesting mandibular distortion due to inferior dragging and a subsequent greater error in recognizing and superimposing the reference landmarks.

Additionally, case 3 and case 10 showed slightly worse performance in the visual analysis (70% and 60% concordance, respectively) compared to the digital analysis (65% and 55%). This suggests that cases with significant distortion, particularly in the mandibular arches, are better recorded by physical casts than by a 3D digital scanner (Table 2). Overall, the digital analysis proved to be more effective in overlapping landmarks, but its success seems to be closely tied to the quality of the scans, particularly in cases of distortion that cannot be modified or corrected using software. [37]

The landmarks most frequently involved in overlapping errors (Table 3) were identified as 3 points for the upper arch (R4, R6, R8 – canines and distal angles of the incisal edge on the left side) and 6 points for the lower arch (R0, R1, R2, R5, R6, R9 – mesial and distal angles of the incisal edge on both the left and right sides, and canines on the left side) in the digital analysis. Conversely, 4 landmarks (R0, R1, R2, R4) of the maxillary arch achieved full concordance in all samples, unlike any case involving the mandibular arch. In the visual analysis of resin casts, landmarks R6 and R8 of the maxillary arch showed the highest frequency of non-overlap, along with landmark R3 for the mandibular arch. No landmarks achieved full concordance for the upper arch, while only R9 (left canine) did so for the mandibular arch (Table 3). These results confirm that the lower arch is the weaker link in both methods, likely due to the smaller size of the teeth and the greater variability in force applied during the biting process, making it more challenging to distinguish very close landmarks, as selected in this study. [13, 24-25, 48]

As shown in Table 4, there is a greater discrepancy in the mandibular arches across all quadrants (II-III on the left and I-IV on the right) in the visual analysis compared to the digital analysis, with the highest number of errors occurring on the left side in both analyses. This could be explained by the fact that the sample predominantly consisted of right-handed individuals (90% versus 10% left-handed), leading to an asymmetric distribution of force between the left and right sides. A study focused on left-handed subjects would be necessary to better support this hypothesis.

The main limitation of this study is the small sample size, which precludes statistical inference from the results obtained. However, since few studies are available on the use of intraoral scanners for bitemark analysis, the preliminary results suggest that digital analysis improves the comparison between dental patterns and bitemarks on an elastic and deformable support compared to the direct superimposition of resin casts and dental impressions. Therefore, this study supports the limited literature that highlights the usefulness and reliability of digital techniques for bitemark analysis, but further research on larger samples, different supports, and various types of scanners is needed.

CONCLUSION

The superior performance of digital approaches in bitemark analysis for both maxillary and mandibular arches suggests that digital techniques should be increasingly adopted in forensic fields. However, the traditional method of directly comparing resin casts with dental impressions still demonstrates excellent reliability, particularly in cases with significant distortion, especially for the lower arch, due to the varying dynamics of the bite and the type of substrate.

Moreover, the digital technique simplifies the recording and comparison of dental patterns, making it more accessible for less experienced operators and reducing visual subjectivity in identifying landmark concordance.

Further studies are needed to validate the digital technique with larger sample sizes, diverse dental characteristics, varying bite dynamics, and different types of supports and substrates.

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A scoping review of websites for forensic odontology training programs

Ranya Al Ghazi [1], Alexander Gardner [2], Peter Mossey [3], Doha Abualhija [4], Scott McGregor [5], Scheila Mânica [6]

[1] Centre for Forensic and Legal Medicine and Dentistry, University of Dundee, UK (ORCID: 0000-0002-8987-2993). [2] Department of Restorative Dentistry, University of Dundee, Department of Anatomy, University of St. Andrews, UK (ORCID: 0000-0001-8618-9622). [3] Department of Orthodontics, University of Dundee, UK(ORCID: 0000-0002-9914-6901). [4] Centre for Forensic and Legal Medicine and Dentistry, University of Dundee, UK (ORCID: 0000-0002-5644-9339). [5] Academic Librarian, Library and Learning and Culture and Information, University of Dundee, UK. [6] Centre for Forensic and Legal Medicine and Dentistry, University of Dundee, UK (https://orcid.org/0000-0002-8352-2888).

Corresponding author: alghazi.ranya@gmail.com

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KEYWORDS

Forensic Odontology, Dentistry, Qualification, Education, Standards, Training

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ABSTRACT

Background: Forensic Odontology developed as a discipline in 1898 with the publication of its first textbook; however, 126 years later, a standardised core curriculum for formal training in Forensic Odontology has yet to be decided. This website scoping review aims to provide information on three main distinct categories (and 17 subcategories): Availability, Content, and Quality of Forensic Odontology education globally. The information on formal education programmes was gathered by conducting a web search using 'Google Search' in English, Arabic, Spanish, and French languages between June and July 2022.

Highlights**:** Fifty-six programmes from 18 countries met the inclusion criteria. Of the 14 master's and 42 diploma programmes, only 7.14% provided information on their websites from all the subcategories investigated. The highest numbers of master's and postgraduate diploma programmes were identified in Europe (18 programmes), South America (15), North America (11) and Asia (7). A practical aspect was included by 10 master's and 12 diploma programmes' websites. Research integration in various forms was included by 11 master's and seven diploma programmes.

Conclusion: Programme providers and applicants must critically consider the quality of the programme being designed or applied to as there are currently no international training standards in Forensic Odontology. This study has developed an 'International Database of Forensic Odontology Programmes' (IDFOP).

INTRODUCTION

In 1968 Keiser Neilsen described Forensic Odontology as, '*that branch of Odontology, which in the interests of justice, deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of dental findings*'.[1] Forensic Odontology is a Dental speciality and a subspecialty of Forensic Science that initially emerged to study the problems related to deaths with unidentified bodies.[2] However, the discipline soon expanded to include Age estimation, Bitemarks, Disaster Victim Identification, Malpractice and Jurisprudence, Abuse and Neglect in children and seniors, Cheiloscopy, Rugoscopy, and establishing an Odontobiography (sex; race; ancestry; general, oral, and psychological health; habits; profession; habits; socio-economic status).[3,4]

Education and training in Forensic Odontology (FO) include significant challenges: availability, content, and quality. Although the need for training programmes was acknowledged more than half a century ago, there are few qualifying postgraduate FO degree programmes available worldwide despite the demand. Dentists and other professionals are offered many unregulated programmes such as workshops, online certificates, and similar didactic and cursory programmes on the subject are numerous;[5-8] Moreover, such programmes do not qualify one to practice or teach the subject, and this is not made clear in advance by the programme administrators.[9-11] There is also a sparsity of undergraduate Dental programmes that consider the subject in the curriculum even when teaching expertise is available via their postgraduate programmes.[12]

In 1978, Herschaft and Rasmussen commented on the lack of depth in the FO curriculum.[13] An ideal training programme would include a theoretical, a practical, and a research aspect, as suggested by several other authors.[6,8,14] Forensics and Dentistry, being parts of science, present a kinaesthetic-tactile learning nature so they are constantly evolving, with techniques regularly being updated. The development of those fields depends on research, making research a highly desirable aspect of any training programme.[15] The available programmes are varied in all aspects, including the curriculum, student eligibility criteria, and teaching faculty. Undergraduate students who have not been taught the topic are at the disadvantage of not knowing what to look for in a postgraduate programme.

This website scoping review aims to investigate information on the availability, content, and quality of FO education globally. The study objectives are: (i) to conduct a website scoping review of FO training programmes and (ii) to review the range of information provided by FO programme websites.

METHODS

The '*Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist*' (https://www.prisma-statement.org/) was used with modifications as a guide to conduct and for reporting this unconventional website scoping review.[16] The method included the development of the review question; search; eligibility criteria; selection; extraction; result charting of the included websites; and synthesis of results.[17] Registration of a scoping review protocol was not possible with PROSPERO.

Information sources, search and sreening:

The web search engine Google Search was used to conduct this scoping review of websites as it was rated the best search engine for its speed and relevancy.[18] The search strings in each language with rationale are illustrated in Table 1. The first five pages of the Search Engine Results Pages (SERP) for each search term were included in the review. The search was conducted between June 2022 to July 2022 without any filters using Google Search's default setting that lists results based on relevancy and to minimise duplicates.[17]

Eligibility criteria:

The inclusion criteria were master's and postgraduate diploma programmes to focus on the gold standard of postgraduate education in Dentistry.[19] The search strings were restricted to the four INTERPOL languages (English, Arabic, Spanish, and French), however, universities originally from other languages were considered in the results within the first five pages due to existing translations from the web.[20] The exclusion criteria were undergraduate programmes, lectures, workshops, shadowing and fellowship programmes, short courses, etc., due to the insufficient training in such programmes. The screened results were individually exported onto Microsoft Excel's worksheet (version 16.64) following six steps, as seen in Table 2.

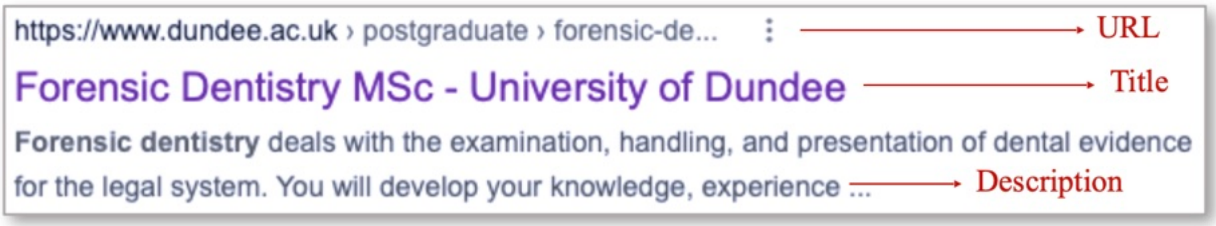
Table 1. Search strings used on 'Google Search' in each language with rationale

Language	Search string	Rationale
English	(i) Forensic Odontology Master (ii) Forensic Odontology Diploma (iii) Forensic Dentistry Master (iv) Forensic Dentistry Diploma	The words 'Odontology' and 'Dentistry' were used in the search as they are interchangeably used.
Arabic	(i) ماجستير في طب الأسنان الشرعي (ii) دبلوم في طب الأسنان الشرعي	In the Arabic language, the English words 'Dentistry' and 'Odontology' both translate to the term 'طب الأسنان', on 'Google Translate', ergo was the only term used.
Spanish	(i) *Maestría en Odontología Forense* (ii) *Diploma en Odontología Forense*	The words 'Dentistry' and 'Odontology' translated to 'Odontología' in Spanish on 'Google Translate', ergo was the only term used in the search.
French	(i) *Master en odontologie médico-légale* (ii) *Diplôme d'odontologie médico-légale* (iii) *Master en médecine dentaire légale* (iv) *Diplôme de médecine dentaire légale*	The English words 'Dentistry' and 'Odontology' are translated to '*Dentisterie*' and '*Odontologie*' respectively. Both words were used interchangeably, ergo included in the search.

Table 2. Steps involved in the recording of the results from the screening process

Step	Description
1. Date	The day the search was conducted.
2. Search term	The term used on the search engine to search relevant data
3. Result Page and number	The page number of the Search Engine Results Page (SERP). The result number was counted manually with the count starting from '1' on each page.
4. Result's web address	The Uniform Resource Locator (URL)/website link of each result - This was obtained by right-clicking on the result and selecting 'copy link'.
5. Result Screening (Title/Description)	When a search is conducted on '*Google Search*', the results are displayed in the form of snippets. Figure 1 is an example of one snippet when the term 'Forensic Odontology Master' was searched on Google Search. This snippet consists of three parts, (i) URL, (ii) Title, and (iii) Description. Each result was labelled under 'Included' or 'Excluded' depending on the eligibility criteria based on the title and/or description of the result. When classed under 'Excluded', a reason was specified.
6. Result Screening (full website)	The results classed under 'Included' from Step 5 were screened by examining the entire website. The websites were accessed by clicking on the data stored under 'Result web address' on the Microsoft Excel worksheet from Step 4. The results were again classed under 'Included' and 'Excluded' (with reason specified) depending on the eligibility criteria based on the entire website.

Figure 1. Example of a result snippet on Google's search engine with parts labelled



Programme detail:
The included results from Step 6 of the 'Result Screening Chart' were investigated for programme details and charted using three categories: 1) Presentation, 2) Accessibility and 3) Educational aspects. Each category was investigated using different topics associated with each category, as shown in Table 3. When the information provided by a programme website was incomplete, the programme administrator was emailed for more details. All data were recorded and analysed using graphs, charts, and maps default on Microsoft Excel's worksheet (version 16.64).

Table 3. Result screening (full website) categories and subcategories

Data	Description		
1. Provider	Institution, university, organization, etc...		
2. Location	City and country		
3. Programme	Course title		
4. Duration/Study mode	Length of programme (months/years); full-time or part-time		
5. Language	Language the programme is delivered in		
6. Learning mode	Online | face-to-face | blended | hybrid | distance		
7. Eligibility Criteria	Requirements from students for application		
8. Teaching Faculty	Name and qualifications of the instructors		
9. Course content	Modules covered in the programme		
10. Forensic Odontology curriculum	Topics covered under Forensic Odontology		
11. Availability of Practical sessions	Yes or no. Investigation would include information on access to mortuary | cadaver facility | wet lab; dental lab; dental and tooth specimens; radiography and photography equipment; diverse radiographs (OPGs) for age estimation; bitemark analysis and comparison on a photo manipulation software; mock courts, report writing, mock disaster victim identification, etc		
12. Availability of Research	Yes or no. Investigation would include information on dissertation/thesis; research project; research article; journal publication, etc.		
13. Handbook	Digital student handbook or overview of the programme.		
14. How to apply	Application form or instructions for application to the programme.		
15. Fee & Funding	Tuition fee for each academic year; funding opportunities.		
16. Contact	Contact information for programme administration/faculty		
17. Status	Active | Inactive | Discontinued | Under development		
Rationale (Category)	**Presentation**	**Accessibility**	**Educational aspects**

RESULTS

The search revealed 56 postgraduate programmes (14 master's and 42 diplomas) available worldwide, as seen in Fig 2. Out of 195 countries, only 18 countries offered postgraduate programmes in FO, with the majority from Central and South America and Europe, as illustrated in Table 4. Amongst the 52 providers were one medico-legal association, one online course provider, one national development agency, and 49 training institutes.

Figure 2. Adapted PRISMA Flowchart presenting the website selection process.

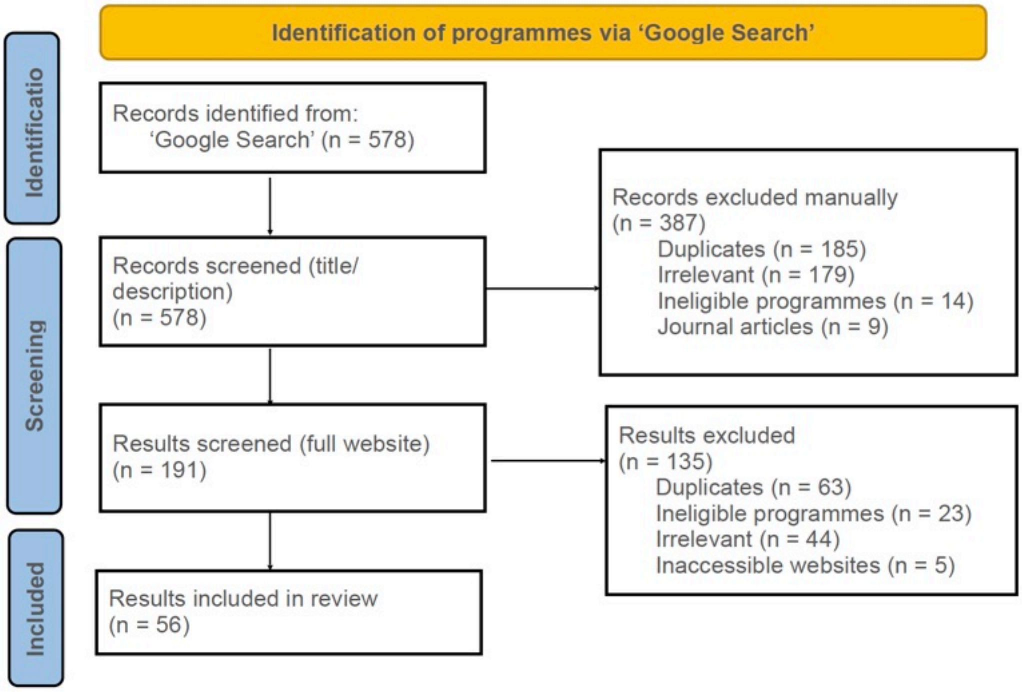


Table 4. Forensic Odontology Programmes providers identified

Provider	No. of	Location
Universidad de Londres	1	Mexico (10)
Universidad Del Valle De Toluca	1	
Instituto Tecnológico Superior de Sinaloa (ITESUS)	1	
Colegio Nacional de Estomatólogos EH Angle (CNEAC)	1	
Universidad Autónoma de Coahuila	1	
Universidad Autónoma Metropolitana	1	
Centro Universitario de Ciudad de Juárez	1	
Instituto Para El Desarrollo Y Actualización de Profesionale	1	
Academia Mexicana de Ciencias Periciales	1	
ULA - Latin American University	1	
Universidad Científica del Sur (UCSUR)	1	Peru (7)
Instituto de Criminalistica y Seguridad	1	
Universidad Nacional de San Martin Tarapoto	1	
Instituto de Desarrollo y Capacitación	1	
Instituto de Desarrollo Gerencial -IDG	1	
Universidad Peruna	1	
Instituto de Salud Oral (ISO)	1	
Odontocat	1	Spain (6)
Universidad Nacional Autónoma de México	1	
Escuela Internacional de Negocios - España	1	
Universitat Internacional de Catalunya (UIC Barcelona)	1	
Universidad Complutense Madrid	1	

Universidad de Sevilla	1	
Université de Lorraine	1	France (5)
Compagnie des Experts de Justice de Lyon	1	
Université de Bordeaux	1	
Université de Montpellier	2	
University of Dundee	3	UK (4)
University of Glamorgan (now University of South Wales)	1	
Universidad de Buenos Ares	1	Argentina (3)
Universidad del Salvador	1	
Universidad Nacional de Rosario	1	
University of Adelaide	1	Australia (3)
University of Western Australia	1	
University of Melbourne	1	
Associasón Colombiaba de Medicina Legal y Ciencias Forenses	1	Colombia (3)
Universidad Cooperativa de Colombia	1	
Pontificia Universidad Javeriana	1	
Bharat Sevak Samaj	1	India (3)
National Forensic Sciences University	1	
JSS Academy of Higher Education and Research	1	
Universidad de la Frontera	1	Chile (2)
Universidad de Chile	1	
University of Pretoria	2	South Africa
Katholieke Universiteit Leuven (KU Leuven)	1	Belgium
Istituto Stomatologico Toscano	1	Italy
Lebanese University	1	Lebanon
Institute of Advanced Dental Sciences & Research & Health Services	1	Pakistan
University of Lisbon	1	Portugal
University of Peradeniya	1	Sri Lanka
University of Sharjah	1	UAE
University of Tennessee	1	USA

The usual disclaimers of the faults in the information provided by the source apply in this study. First, the limitation of the selected languages may have excluded relevant results a priori. Second, the confirmation that some courses were found in other languages other than English, Arabic, Spanish, and French. For instance, a university was found in Italy even though the *Università degli Studi di Firenze* located in Florence, has been offering a master's programme for several years and it was not shown in the search. Similarly, a programme in Portuguese from the University of Lisbon, Portugal was shown as a result but other well-known programmes in Portuguese from Brazil such as from the School of Dentistry of *Ribeirão Preto* (USP - University of *São Paulo*) and younger faculty of *São Leopoldo* Mandic were not included. In addition, no programmes have been removed from the results.

Information on the duration of programmes was made available by 11 master's and 30 diploma programmes (Fig 3) whilst the programme language was made available by 11 master's and 10 diploma programmes (Fig 4). Programmes from Sri Lanka, UAE, Pakistan, Colombia, France, and Lebanon did not provide language information.

Figure 3. Duration distribution of Master's (blue) and Diploma (orange) programmes in months

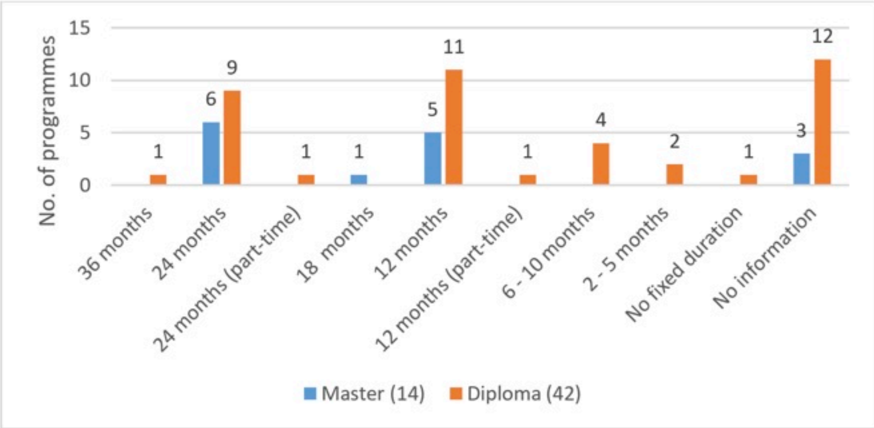
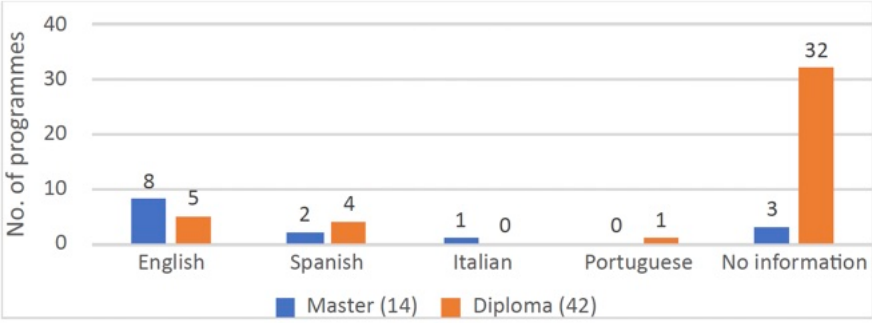


Figure 4. Language distribution of Forensic Odontology programmes



The learning mode was made available by 11 master's programmes, with 'Face–to–face' being the most offered mode, followed by 'blended', and the least in 'online' and 'hybrid' methods. Out of 19 diploma programmes, 'face-to-face' and 'blended' modes were most offered, followed by 'online' and 'distance', and the least in 'hybrid' as seen in Fig 5. The admission requirements for 13 master's programmes included professionals from 'medicine/surgery', 'law/police', 'biology/biotechnology', and stomatology, with all 13 programmes including 'dental surgery'. Out of 23 diploma programmes, the majority of professionals included 'dental surgery', followed by 'medicine/surgery', 'dentistry', 'dental technology/hygiene', 'forensic/criminology', 'law/police', 'no prerequisites', 'biology/biotechnology', 'anthropology', 'medico-legal interns', 'biochemistry/chemistry', 'psychology', 'nursing', and 'stomatology' as shown in Fig 6.

Figure 5. Learning mode distribution of Forensic Odontology

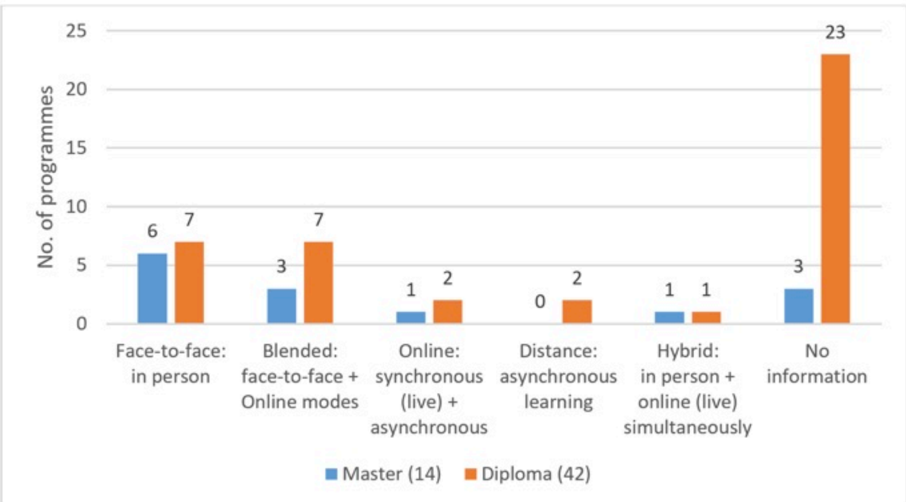
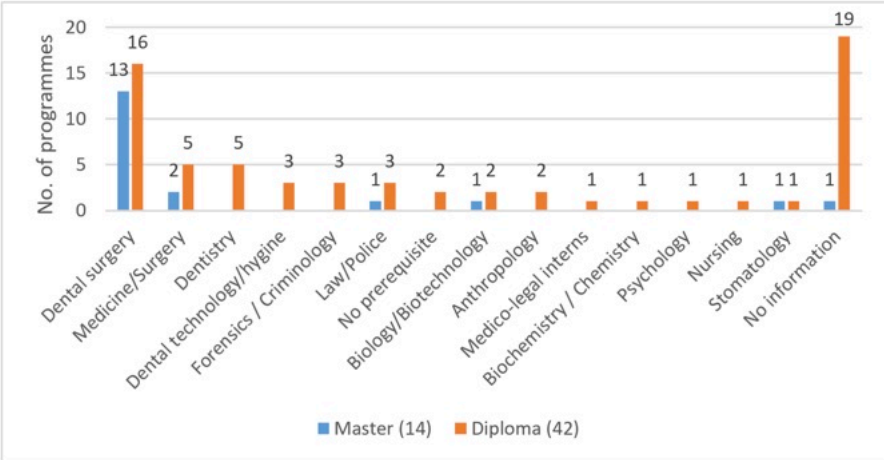


Figure 6. Acceptable professional backgrounds under Admission Requirements for Forensic Odontology Programmes



Amongst the eight master's programmes that provided information on teaching faculty, five included professional qualifications with at least one forensic odontologist (FOst) each. Out of 21 diploma programmes, seven provided information on teaching faculty, and six programmes had at least one FOst, as illustrated in Fig 7. All 14 master's programmes included their modules, of which 11 programmes elaborated on their curriculum with 'dental identification', 'age estimation', 'bitemarks', and 'law' included in all programmes apart from the one programme excluding 'bitemarks'. Of the 26 diploma programmes that presented their modules, 'law' was included in many of them, followed by 'dental identification' and 'age estimation', with the least in 'bitemarks' as seen in Fig 8.

Figure 7. Availability of information on Forensic Odontology programme faculty

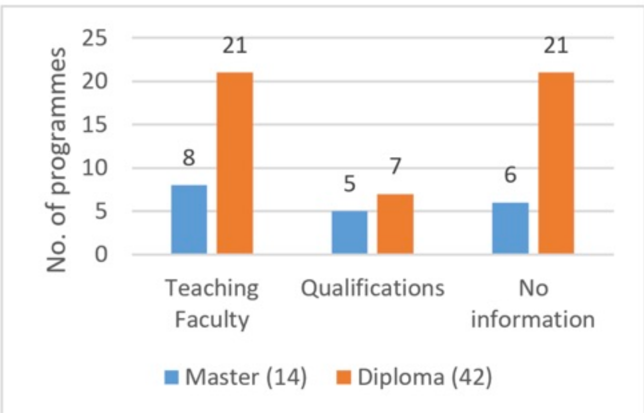
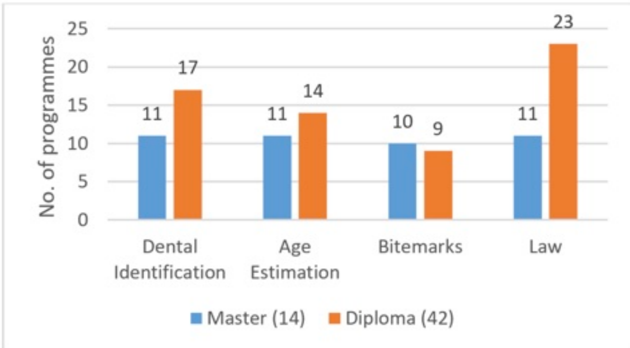


Figure 8. Distribution of basic topics in Forensic Odontology



Of the 11 master's and 14 diploma programmes that shared their practical aspect, only six master's and five diploma programmes listed the specifics. The practical activities relevant to FO included 'mortuary/cadaver facility', 'mock DVI/Plassdata', 'mock trial', 'age estimation', 'bitemarks', 'reports', 'photography', 'radiography', and 'anthropology'. One master's programme and two diploma programmes were theoretical, as shown in Fig 9. Information on research opportunities was made available by 11 master's programmes, except one including 'thesis/dissertation', followed by 'publication', 'research project', and at the least, an 'internship'.

Of nine diploma programmes that included information on research opportunities, most included 'research project', followed by 'thesis/ dissertation', and at the most minor 'assignments', with one programme making research optional with a research facility available as illustrated in Fig 10.

Figure 9. Distribution of offers on practical experience practical aspect of programmes

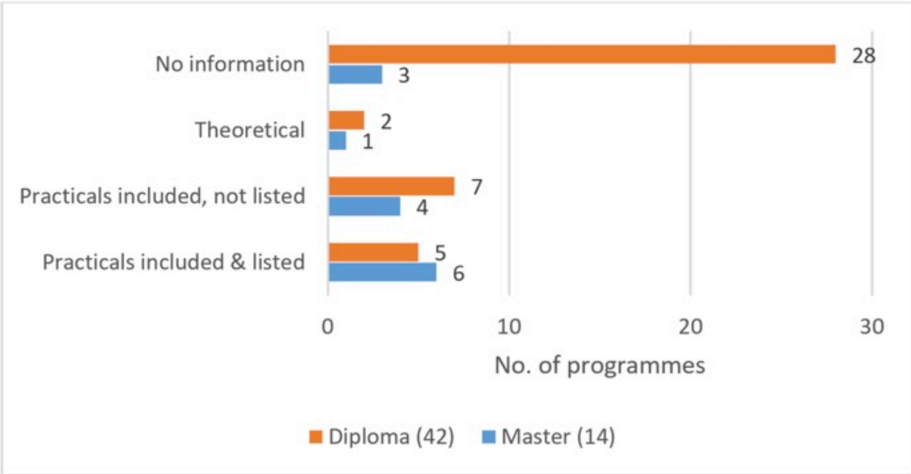
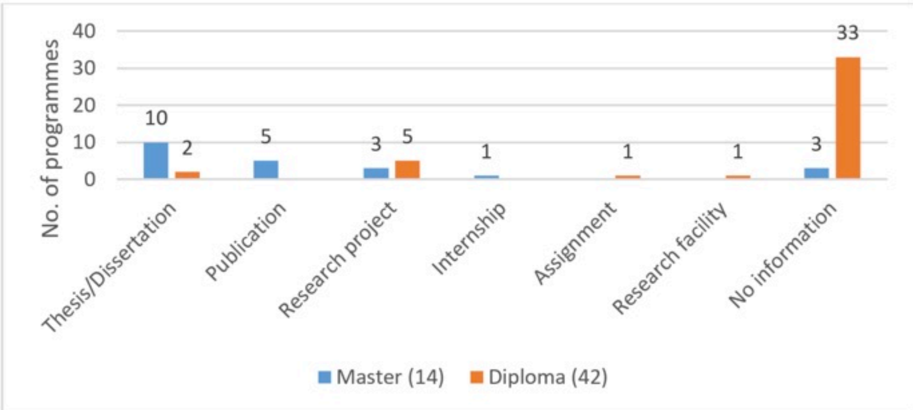
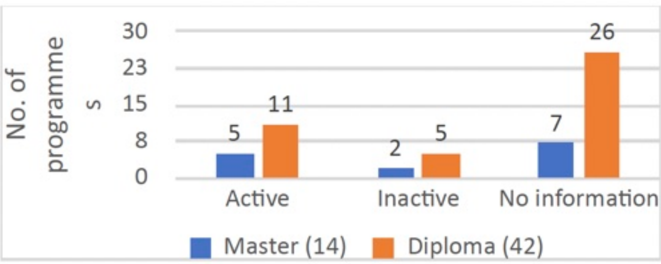


Figure 10. Distribution of programme research options



Student handbooks/programmes were made available by six master's and three diploma programmes; the application process was made clear by five master's and nine diploma programmes, and the fee and funding status was shared by 11 master's and 23 diploma programmes. As of July 2022, according to the programme websites, five master's and 11 diploma programmes appeared 'active' (currently running), with two master's and five diplomas appearing 'inactive' (discontinued / on hold), as shown in Fig 11.

Figure 11. Distribution of programme availability



The review of programme details indicated that out of the 56 programmes, only 4 provided information on all 17 categories from Table 2. The remaining programmes were contacted via email for unavailable details, and responses were received from 8 programmes with further information. Only 3 provided complete information. All communications were made in English.

DISCUSSION

Programme identification:

The International Organization for Forensic Odonto-Stomatology (IOFOS) conducted two surveys on FO education, with the first in 1984 and the second in 2021, and countries with postgraduate programmes included Denmark, Hungary, Japan, Sweden, West Germany, Norway, Brazil, South Korea, and Iceland.[21,22] However, programmes from the aforementioned countries were not identified by 'Google Search'. A possible explanation for this could be that the programmes are either not presented on websites, have been discontinued, or are in a language outside the limitations of this review.[23] The initiation of a Forensic Odontology programme can be decided by relevant civil and criminal cases documented within the country. An unpublished survey conducted by the authors of this review in 2020 received responses from trained FOsts from countries including New Zealand, Saudi Arabia, Switzerland, Syria, Oman, Nepal, Norway, Sweden, Malaysia, Canada, Indonesia, Brazil, Hong Kong, Finland, The Netherlands, Japan, Egypt, Panamá, and Denmark, clearly indicating a demand for it.[19] The countries listed in Table 5 need trained personnel given their high numbers of missing persons, risk of natural disasters, rape cases, and refugees.[24-27]

Table 5. Countries shown in green boxes have been identified to run at least one postgraduate FO programme (Highest to lowest from left to right).

Category	Country				
Missing Persons (2022)	India	UK	Syria	Colombia	Mexico
Natural Disaster Risk (2021)	Vanuatu	Solomon Islands	Tonga	Dominica	Antigua and Barbuda
Rape (2022)	Botswana	Lesotho	South Africa	Bermuda	Sweden
Refugee host (2022)	*Türkiye*	Colombia	Uganda	Pakistan	Germany

Programme standards:

Duration:

Generally, credit assigned to programmes is based on the approximate number of hours a learner is expected to spend learning to achieve the learning outcomes. The UK Quality Code for Higher Education requires a minimum of 180 credits (approximately 12 months) for a Taught Master's programme (1,800 hours of learning = number of credits x 10). For example, the master's programme offered by *Istituto Stomatologico Toscano* seems to cover the duration required for 180 credits. A typical Postgraduate Diploma programme requires a minimum of 120 credits taking approximately six months (two academic terms) to complete in a full-time programme.[28,29] For instance, the diploma programme offered by the Institute of Advanced Dental Sciences and Research and Health Services Academy, Pakistan has covered sufficient duration for the required 120 credits. This study, however, revealed diploma programmes less than six months in duration.

Admission Requirements:

All master's programmes required the applicant to be a Dental surgeon, the University of Peradeniya additionally required Dentist applicants to possess one year's work experience. Some diploma programme providers like *Universidad Nacional de Rosario, Universidad de Chile, Pontificia Universidad Javeriana*, University of Adelaide, CNEAC, and more only accepted Dental Surgeons. The diploma programme by the University of Western Australia additionally required two years of work experience. As Neilsen stated, FO is a branch of Dentistry that examines Dental findings, ergo, education providers must make it clear to applicants that only Dentists will be qualified to practice FO as a profession after completion.[1]

Faculty/Staff Profile and Employment Prospects:

Some programmes from this review provided faculty names, qualifications, titles, and even biography in contrast to programme administrators who even upon request responded with phrases like '*professionals experienced in the field*' when asked about their teaching faculty. It is undetermined if the faculty with the title 'Forensic Odontologist' underwent formal training. For instance, the programmes at *Universidad de Londres*, UCSUR, *Montpellier, Universidad Peruna*, and more, included their faculty members' names. The Universities of Adelaide, Sharjah, etc, additionally included faculty qualifications. The programmes by *Associasón Colombiana de Medicina Legal y Ciencias Forenses*, and *Universidad de Sevilla* further included faculty member's speciality and *Universidad de Chile* even included each member's title, for example, professor, demonstrator, etc.

The responsibility of teaching the subject frequently falls on Oral Pathologists, who are often not qualified FOsts.[13] With the first chapters on forensic odontology published in 1862 and 1882, the first textbook published in 1898, the first lecture delivered in 1899, and the 2004 Sumatra-Andaman earthquake resulting in a sudden boom in the number of programmes, is it still acceptable for this discipline to be taught by professionals who are not formally trained?[12,23,30,31]
In Syria, dental surgeons can get certified to practice Forensic Odontology upon completing an internship offered by the Ministry of Health; however, only a dental surgeon with formal postgraduate training in the discipline is permitted to teach. Designing a programme here requires a professor (10 years of experience) and an assistant professor (five years of experience) specialized in the discipline with a master's/PhD degree (WM Rihawi, personal communication, August 30, 2022). Another example, the Botswana police service is developing a state-of-the-art forensic laboratory; however, due to the lack of awareness of the discipline amongst dental practitioners, it employs no FOsts to date (C Kgabi, personal communication, September 29, 2022).

Curriculum:
This study considered (i) Dental Identification/ Disaster Victim Identification, (ii) Age estimation/Dental Anthropology, (iii) Bitemarks, and (iv) Law (including Dental Jurisprudence and Malpractice) as the primary topics to analyse the programme content all though there is no clear understanding on what the primary and secondary topics in the field are according to the rank of importance.[32,33] From the results, it is undetermined whether the lack of information available from the programme websites is due to incomplete information on their websites or if the topics are not taught. For example, the diploma programme at the *Universitat Internacional de Catalunya* (UIC Barcelona) listed the programme modules in addition to the areas covered under the FO module. A comparison of the Diploma programmes under 'Forensic Odontology Curriculum' and 'Duration' showed that programmes with all four primary topics were at least six months long indicating a minimum of six months is required to teach all four primary topics.

Applied and Collaborative Training:
Gradual introduction to graphic imagery is suggested in training as this may pose a psychological challenge. The practical part of the discipline is appreciated across several topics including dental Identification of the deceased either in the mortuary or a wet lab/cadaver centre, age estimation on radiographs, dental anthropology on human remains, comparative dental anatomy on human and animal dentition, dental profiling on dental remains, post-mortem photography and radiography, interpretation of bitemarks on photo manipulation software, mock DVI including chemical, biological, radiological, nuclear, explosive (CBRNE) disasters, mock trials, report writing, and more.[10,12] Formal training programmes should provide facilities for such exercises.[34] For instance, results of a study that evaluated the ability of dental students to match simulated antemortem and post-mortem dental radiographs from human skulls compared to experts showed that dental students needed more post-mortem images before deciding on a match than did experts. They also scored false positives proving the importance of training.[35]
Another study on the accuracy of dental registrations in forensic odontology among dental students proved several basic errors such as the incorrect judgement of the extent of single restorations and the confusion in naming groups of teeth such as mandibular premolars and molars.[36]
To highlight some of the applied training opportunities, the masters programme at the University of Tennessee has mentioned the various facilities available for hands-on training including dry and wet labs, decomposition processing facility, and clandestine graves; *Universidad de Londres* (master's) included ballistic, anthropology, and forensic photography; CNEAC (diploma) included report writing, ear print, and facial reconstruction amongst many others; *Université de Lorraine* (diploma) included an internship. Forensic Odontologists may work as a part of a team within and outside the forensic unit, ergo collaborative exercises with Law, Policing, Forensic Pathology, Forensic Anthropology, and other relevant fields will help build communication skills that play a crucial role in Forensics.[32,33] From reviewing the websites of three master's and the 33 diploma programmes it is unclear if they do not offer practical exercises

or simply have not mentioned it on their websites. However, no response was received upon request via email either. [32,33]

Teaching Model:
Another finding from this study was the two purely theoretical diploma programmes, although diploma programmes are meant to be vocational, involving more hands-on activity. There are four types of teaching models commonly used, including (i) face-to-face, (ii) remote (asynchronous and/or synchronous), (iii) dual mode/hybrid (face-to-face + synchronous + asynchronous), and (iv) blended (face-to-face and/or synchronous and/or asynchronous). However, the ideal approach to delivering formal training in FO would be a model that accommodates some element of face-to-face practical experience on all aspects of forensic odontology, allowing learner-learner interaction.[6,8,14,37] The review revealed that most master's and diploma programmes were either face-to-face or blended. The diploma programme at CNEAC mentioned they were delivered online (synchronously), however included practical activities including visits to a forensic institution. Conversely, the master's programme offered by KU Leuven is theoretical although delivered face-to-face.

Research Integration:
Without research, investigative methods would become obsolete rather than evolve. For instance, Systematic research is needed to record the evolution of methods with the development of research and technology, and they should be incentivised. A variety of methods should be created, and current ones should be tested in different populations. In addition, the work of a FOst involves rapid problem-solving and decision-making. For example, in a DVI operation (temporary mortuary), all necessary/conventional equipment may not be accessible in contrast to a permanent mortuary so the professionals will have to improvise with what is available. Being familiar with past research equips one with the various methods that have and can be used in different scenarios, easing the development of ad-hoc solutions.[7,38]
Almost every master's programme included research in some form including a thesis dissertation, and sometimes publication in journals. While it is not a requisite for diploma programmes to incorporate research, the Universities of Adelaide, Western Australia, Lorraine, Buenos Ares, and Salvador, Institute of Advanced Dental Sciences and Research and Health Services Academy, and *Pontificia Universidad Javeriana* included research, and the University of Lisbon made research optional.

Challenges in Forensic Odontology Training:
(i) Undergraduate Curriculum:
A critical factor in training is an applicant's compatibility with the discipline's scope. Unlike other specialisations in Dentistry, Forensic Odontology is not traditionally included as a module in the undergraduate Dental school curriculum.[6,8,15,39] Dentists are often unaware of what the discipline entails, and this has resulted in individuals experiencing discomfort and unfortunate incidents/accidents, after enrolling on a postgraduate programme. This lack of awareness can be addressed by including FO in the undergraduate Dental curriculum. Given the factors mentioned prior, would it be deemed appropriate for an individual who is not a Dental Surgeon but received FO training, or is a dental surgeon with only theoretical FO training, or has been trained by individuals with no field experience in FO to identify victims in a complex mass fatality operation or give an expert opinion in a court of law which directly influences the court's decision? There is a handful of model curricula available in Forensic Odontology, albeit lacking teaching guidelines.[5,6,32,33,40]
(ii) Standardised Postgraduate Curriculum:
This review has brought to light that most FO training programmes are similar only in differences, for example, the durations are diverse, teaching faculty are not all qualified Forensic Odontologists, admission requirements include students and professionals from varying disciplines, etc. However, there were also positive similarities, for example, Human Identification, Age Estimation, and Dental Jurisprudence and Malpractice seem to be included in several programmes, and most master's programmes included practical activities and a research component. There is a general lack of standardisation of programme structure and curriculum amongst all master's and diploma programmes in the core and essential aspects

that affect teaching and learning quality, which in turn affect the quality of the work produced in practice. Education providers must set minimum requirements for FO faculty such as postgraduate qualification in FO from a recognised programme, or registration with the local/national FO body, etc. to maintain the standards of training and quality of knowledge disseminated.

(iii) Awareness:
The challenge qualified Forensic Odontologists face in employment is often due to the lack of awareness of the scope, availability, and expertise of Forensic Odontology amongst Law Enforcement officers, Lawyers, Judges / Magistrates / Procurator Fiscals, Fire Fighters, First Responders, Forensic professionals, Refugee and Asylum agencies, and Cross-border medical aid providers. This issue can potentially be addressed by FO organisations or qualified FOs by developing and delivering awareness programmes. Some institutions provide sanctuary to refugees and asylum seekers in higher education which ties in with the humanitarian cause of Forensic Odontology. Centres for education must provide staff and students with a safe space and practice 'Equality, Diversity, and Inclusion' by regularly training staff members and conducting impact assessments.[41]

The following is a suggested template based on the information gathered from this review and the Course Page Guide for New Websites by the University of Derby that could be used by education providers when developing or updating their FO programme's website information.[42]

Table 6. Template of Essential Information for Forensic Odontology Programme Websites

Category	Description
Programme title	full name of the programme with degree abbreviation received upon completion.
Programme Level	Master's; Postgraduate Diploma; Mentorship
Programme Overview	detailed overview of the programme with aims and outcomes; programme accreditation and ranking.
Curriculum	programme structure including module titles and areas covered within the FO module; aims and outcomes for each module; practical activities; facilities (library, laboratory, cadaver centre/ mortuary access, study specimens, moot court, IT suite, etc); research options; collaborative exercises (with police departments, mortuaries, other programmes within and outside the training institution, etc).
Faculty Profile	name and title; biography; educational background; research interests; publication with link to research profile (ORCID, ResearcherID, Scopus Author ID, etc); teaching experience; professional affiliations; awards.
Admission requirements	programme eligibility criteria; prerequisites; additional requirements
Language	teaching language/s
Location	address of the education provider and the training location
Duration	full-time duration; part-time duration if available; start and end dates; holiday periods
Teaching model	face-face | remote | dual mode | hybrid | blended with a description of provided models
Assessment	engagement; exams; assignments; research projects; presentations; viva voce; thesis/dissertation submission process; degree completion criteria; certification
Employment Opportunities	enlisting on the local and national register of qualified Forensic Odontologists available to the court, law enforcement professionals, and first responders; list local and national mortuaries, DVI teams, Refugee and asylum providing agencies, museums with human dental remains, mentorship opportunities, networking opportunities, and other relevant options available.

Student services	academic skills support; referencing training and support; research ethics and integrity training; publishing advice; equipment loans; Equality, Diversity, and Inclusion training, career services; counselling; academic advisory service, etc.
Tuition and Financial Aid	tuition fee for national and international students, payment options, available scholarships or grants
Application Process	step-by-step guide for national and international applicants; deadlines; required documents; and fees.
Contact Information	programme coordinator; admissions office; and other relevant personnel.
Dates	official start date of the programme; orientation/induction; registration; academic calendar; deadlines
Programme Status	active | on-hold | inactive | discontinued.
Collaboration	Police Department; Mortuary; Procurator Fiscal/Coroner/Medical examiner; other education providers or FO programmes; Refugee and Asylum organisations; FO organisation and associations; Forensic Laboratories
Additional information	Programme highlights; testimonials; accreditation; Networking opportunities; exchange programmes for students and staff;

Strenghts and limitations:

The 'International Database of Forensic Odontology Programmes' (IDFOP) intends to be a source to provide potential applicants with all the information they must know before making a career decision.[43] The database will also serve as a guide to providers planning on designing a postgraduate programme and it will be available for download in Portable Document Format (PDF) format, allowing the viewer to search for specific terms or words on the document. The search was conducted in four languages which excluded programmes from more countries.[25,37] Unlike a bibliographic systematic review, the quality assurance measures were limited by the significant number of results with reduced filter options, the small number of Search Engine Results Pages (SERP) included, and the varied reading and navigating approach within each result.[17] However, the results may not accurately reflect the current state of the programmes, as websites are not always promptly updated, and some programmes do not disclose certain information. Irrespective of this, the juxtaposition of the various programmes can be challenging given they are from different countries and academic systems.

Considering the programmes that included information on curricular, practical, and research aspects, there were only six programmes (excluding inactive programmes) that provided research facilities, and theoretical and practical training, on all four primary topics of dental identification, age estimation, bite marks, and the legal aspects that involve dental malpractice and evaluation of dental damage. The six programmes are as follows: (i) Master 2nd level in Legal and Forensic Dentistry at *Istituto Stomatologico Toscano*, (ii) Master of Forensic Odontology (MFOdont) and (iii) Master of Science in Forensic Dentistry (MSc) at the University of Dundee, (iv) Graduate Diploma in Forensic Odontology at the University of Adelaide, (v) Specialisation in Forensic Odontology (diploma) at the University of Lisbon, and (vi) Specialisation in Legal and Forensic Odontology (*Especialización en Odontología Legal y Forense*) at *Pontificia Universidad Javeriana*.

Future Perspectives:

The gaps in information on the IDFOP can be addressed by collecting information using methods other than a website scoping review. Considerations should be taken to improve possible future scoping or systematic review to include more languages and undergraduate programmes. The proposed 'International Forensic Odontology Programme Database' should be updated yearly and could be used as a basis for discussion about the quality and availability of education in Forensic Odontology. A future liaison between the programme leads and the Forensic Odontology Organization is suggested.

CONCLUSION

This scoping review gathered the number and content of programmes and concluded that Forensic Odontology has no international training standards. As postgraduate FO training programmes become increasingly popular, it is critical to take note of the quality of training, which could be indicated by (i) the recognition of the programme by the national dental council, Forensic Odontology associations or organisations, (ii) the inclusion of theory, practical, and research aspects, and (iii) the limitations of methods taught in the discipline. This scoping review gathered the number and content of programmes and concluded that there are no international training standards in FO.
As a result of this scoping review, an international database of postgraduate Forensic Odontology programmes has been created to facilitate the task of deciding on an appropriate training programme for future Forensic Odontologists.

ETHICAL STATEMENT

This study reviews the existing Forensic Odontology programmes' websites and does not involve any experimentation on specimens or subjects. Thus, ethical approval was not required.

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Dr Hemlata Pandey
Forensic Odontology Lecturer, Centre for Forensic and Legal Medicine and Dentistry, University of Dundee (ORCID: https://orcid.org/0000-0002-7554-1595)
Dr Wassim Mohamed Rihawi (Syria)
Forensic Dentist
Candy Kgabi (Botswana)
Forensic Odontology master's student, University of Dundee

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