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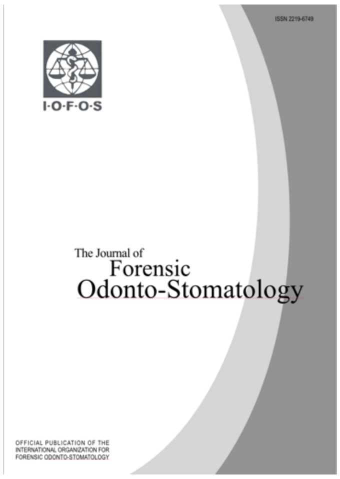

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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CONTENTS

Tooth crown mesiodistal measurements for the determination of sexual dimorphism across a range of populations: A systematic review and meta-analysis

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The authors declare that they have no conflict of interest.

KEYWORDS
Forensic Dentistry, Odontometry, Sexual Dimorphism, Tooth Crown, Permanent dentition, Crown dimension

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ABSTRACT

Objective: The aim of this study was to determine whether the tooth crown sexual dimorphism pattern reported in previous small studies can be generalized for a broader range of populations.
Literature review: A systematic literature review was performed by two independent examiners. The following databases were searched from October 2015 to July 2016: PubMed, Scopus, Lilacs, ScienceDirect, Medline, and Cochrane Reviews. No language restrictions were applied to the search.
Selection criteria: The inclusion criteria comprised original studies investigating mesiodistal permanent teeth that reported the sample population and standard deviation. All right-sided teeth, except the third molars, were measured and separated by sex in the included studies. Thirty-one studies were included in the quantitative data synthesis and meta-analysis. Studies of non-human teeth, skeletal remains, or an overly specific study population were excluded.
Main results: Thirty-one trials, involving 6481 participants, provided data for the meta-analysis of teeth. Sexual dimorphism in mesiodistal crowns was found in all teeth across a range of populations, principally in lower canines (5.73%) and maxillary canines (4.72%), followed by the lower second molars (3.54%) and upper second molars(3.20%), and finally in the lower first molars(3.14%) and upper first molars(2.64%).
Conclusions: A small degree of sexual dimorphism exists in all human teeth. Second molars and canines show the greatest sexual dimorphism. Additionally, smaller racial differences are present in mesiodistal crowns among groups living in different geographic areas; however, it is not possible to establish a single value applicable for all populations.

INTRODUCTION

Dental anatomy is an important factor in establishing an accurate diagnosis, and can be used in the assessment of treatment and control cases.[1] Dental crown dimensions and root dimensions are important in determining appropriate treatment[2] and achieving greater stability in orthodontic planning.[3] Dental parameters are also used for the identification of human remains by anthropologists, biologists, and forensic experts; such parameters enable post-mortem determination of sex and age, as teeth may be more likely to remain intact when other bony structures are destroyed.[4,5]

Within this context, human dentition and crown size are considered a useful aid in determining a subject's sex; most teeth are fully developed before skeletal maturation, and are thus valuable sex indicators.[6]
The two most commonly used measures of the tooth crown are mesiodistal and buccolingual diameters[7,8] notably, other measures have been developed: trigonid mesiodistal, trigonid buccolingual, talonid mesiodistal, and talonid buccolingual diameters.[9] Moreover, there are more sophisticated methods to measure the dental crown, as well as the whole tooth, with reduced discrepancy.[10,11] Prior studies have shown that the dental crown dimensions of permanent dentition tend to be larger in men than in women; the lower lateral incisors and canines are the most useful teeth for determining dental sexual dimorphism[12]. However, a study in Nepal[8] provided a contradictory conclusion, in that the mesiodistal measurements of the lower second premolars were larger in women than in men; this study indicated that the measurement variables had greater utility in sex assessment when using discriminant analyses. Thereafter, several studies attempted to identify the differences between sexes through measurements of human dental crowns in different populations, with inconsistent results.[1,4,13]
Considering the number of anatomical studies in the dental literature and the diversity of their outcomes, this observed variation in dental dimensions is of fundamental importance; the future of dental anatomy depends on the use of new methods to study anatomical variations.[14] The present study aimed to determine whether the pattern of dental sexual dimorphism found in a small number of samples by previous researchers is consistent when tested more extensively across a wider range of populations.

METHODS:

Protocol
The present study was performed in accordance with the guidelines of the PRISMA statement for conducting systematic reviews and meta-analyses. The review protocol can be accessed online (http://www.crd.york.ac.uk/PROSPERO. Registration number: CRD42015023373).

Search Strategy
A systematic review was performed. The electronic literature search was performed by two independent examiners to guarantee the quality of the data collected in the studies. The following databases were searched: PubMed, Scopus, Lilacs, Science direct, Medline, BBO and Cochrane Reviews. The search was performed during the period between October 2015 and July 2016. In addition to the peer-reviewed literature search, a grey literature and hand search was performed in USP web science (http://www.sibi.usp.br/bibliotecas/) and Banco de Theses CAPES. No language restrictions or dates were imposed; keyword searches used Boolean operators. The search terms included: Sex characteristics, Odontometric, Dental index, Dental index determination, Sex determination, Dental dimorphism, Sexual dimorphism, and Tooth crown; these terms were present in the title or abstract of identified studies.

Inclusion criteria
Original studies that were conducted in different population groups and investigated mesiodistal crowns in the permanent teeth were the targets of this analysis. Comparison groups were male and female populations. Outcomes included studies that measured all right-sided teeth, except the third molars. Analyses of mesiodistal crowns, odontometric population characteristics, and sexual dimorphism from cross-sectional studies were included.

Exclusion criteria
Studies using non-human teeth, skeletal remains, or a very specific study population were excluded. Studies that did not report the sample population and studies without full text accessibility were not reviewed.

Data items
Data were collected regarding the mesiodistal measurements of each tooth crown: population, sample, calibration, standard deviation, collection instrument, and detection of sexual dimorphism. In the event of any irregularities in the data, authors were contacted by email for supplementary information. If these were unavailable, the article was excluded.

Synthesis of results
Syntheses of the results are presented according to sex, specifically on the right side. Analyses did not include the right and left sides together.

Statistical analysis
Meta-analyses were performed using BioStat v. 5.3 (InstitutoMamirauá, Amazonas, Brazil) and STATA 13.0 (StataCorp LP, College Station, TX, USA) software for mean differences between sexes with respect to teeth. Dissimilarity indices between studies were determined by heterogeneity tests using both the chi-squared test and I-squared statistic. Percentage dimorphism was calculated as (median male/ median female -1) × 100.[15] A positive value indicated that the male tooth dimension was larger, whereas a negative value indicated that the female tooth dimension was larger. The total percentage dimorphism was the sum of all percentages / number of all teeth.[16]

RESULTS

Study selection:
Potential records identified through database searches were as follows: PubMed (n=3240), Scopus (n=172), Cochrane Library (n=49), Hand Searching (n=1), Lilacs (n=10), and ScienceDirect (n=195). Principal duplicates excluded were as follows: PubMed X Scopus (n=53), PubMed X ScienceDirect (n=22), and Scopus X ScienceDirect (n=19). According to pre-determined inclusion criteria, 82 abstracts were initially obtained. An article was read in full if one reviewer considered the abstract to be of potential relevance. If there was a lack of consensus regarding study credibility between the two reviewers, a third examiner performed the evaluation. In grey literature just one thesis[17] was found.
Full text articles that did not fulfill the inclusion criteria were excluded from further analysis (n=14). Two additional studies were excluded because the full article could not be obtained,[18,19] despite direct contact with the authors. Sixty-six full text articles were assessed for eligibility. Thirty-seven studies in this section were excluded for a variety of reasons: sex not separated (n=13); teeth not separated (n=11); missing data (without the median or standard deviation) (n=6); identical values in the tables for upper and lower teeth;[20] male data only;[21,22] duplicate data from the same population;[23] graphics only,[24] data were not for mesiodistal crowns;[25] only the values of the differences were available.[26] Therefore, two additional studies were identified by reference linkage.[27,28] Finally, 31 studies were selected for review and meta-analysis. The details and results of the search strategy are shown in Figure 1.

Figure 1: Flowchart describing the study selection process

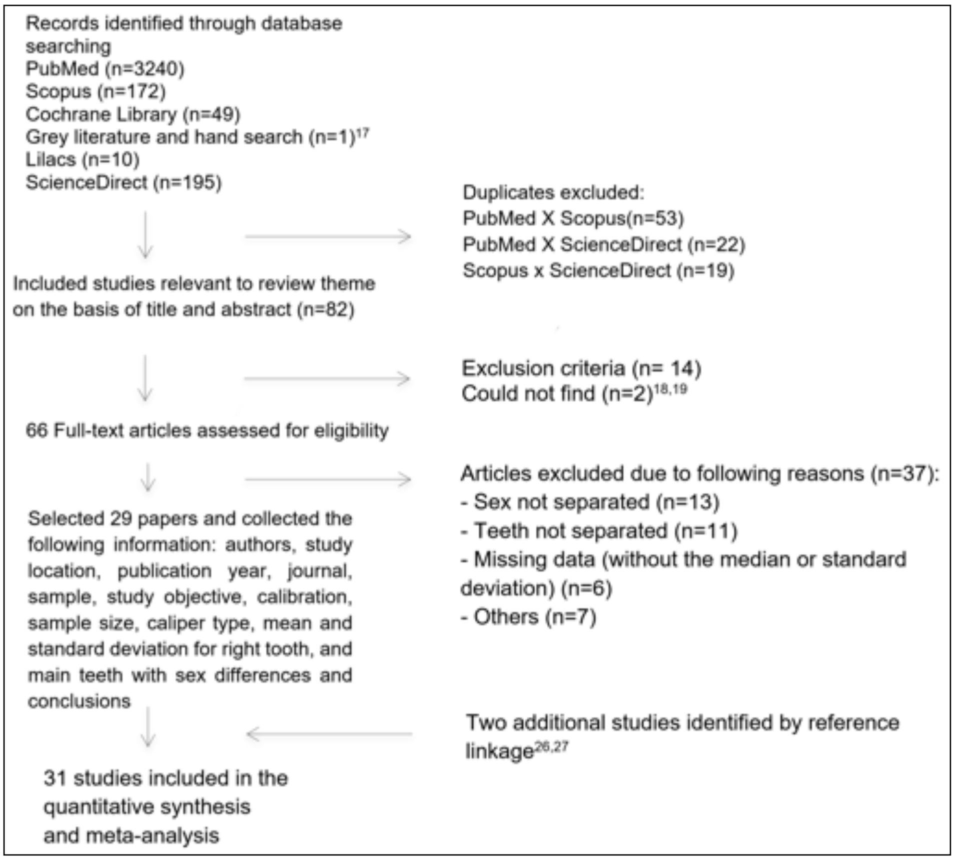


Table 1. The 31 selected articles including publication dates, sample, study population, and main objectives.

Article	Male sample	Female sample	Population sample	Year	Objectives
Martins-Filho[17]	100	100	Brazilians	2013	Sexual dimorphism through dental measurements.
Khamis et al.[28]	200	200	Malaysians Chinese, Tamils and Malays	2014	Sex prediction model to Malaysians.
Mitsea et al.[29]	64	108	Greeks	2014	Sex assessment from tooth measurements
Angadi et al.[30]	294	306	Indians	2013	Develop a logistic regression for sex prediction.
Fernandes et al.[1]	50	50	Brazilians African ancestry, Caucasian ancestry and Japanese ancestry	2013	Sexual dimorphism in mesiodistal crown with normal occlusion and compared between these populations.
Thapar et al.[4]	96	104	Indians	2012	Correlation between tooth and skull size in sex determination.
Al-Gunaid et al.[31]	82	94	Yemeni Arabians	2012	Determination of mean mesiodistal crown and Bolton's ratios.
Castillo et al.[27]	39	27	Colombians	2011	Determination of the mean diameter of each tooth, sexual dimorphism, and bilateral symmetry.
Phabhu & Acharya.[32]	52	52	Indians	2009	Determination of odontometric standards and sexual dimorphism with statistical analyses.
Antoszewski et al.[33]	67	62	Polonies	2009	Odontometric characteristics of transsexual women in comparison of males and females.
Archarya & Mainali[8]	60	56	Nepalese	2007	Sexual dimorphism through dental measurements.
Ling & Wong [34]	264	148	Southern Chinese	2007	Sexual dimorphism through dental measurements.
Ngom et al.[35]	52	52	Moroccan and Senegalese	2007	Analysis of mesiodistal crowns.
Ates et al.[36]	50	50	Turks	2006	Sexual dimorphism through dental measurements and compared with others populations.
Singh & Goyal[37]	40	70	North Indians - Punjabis	2006	Analysis of mesiodistal crowns.
Hashim & Al-Ghamdi[38]	60	60	Saudi Arabians	2005	Comparison of mesiodistal crown between normal occlusion and malocclusion in both sexes.
Santoro et al.[39]	36	18	Dominican Americans	2000	Analysis of mesiodistal crowns.
Lund & Mörnstad[6]	28	28	Swedes	1999	Investigation of the accuracy with which sex can be differentiated by odontometric analyses.
Yuen & Tang[40]	60	49	Southern Chinese	1997	Analysis of mesiodistal crowns and compared with others populations.
Hattab et al.[41]	82	110	Jordanians	1996	Analysis of mesiodistal crowns

Hashim & Murshid[42]	60	60	Saudi Arabians	1993	Sexual dimorphism in mesiodistal crowns.
Lukacs & Hemphill[43]	344	237	Northwest India – Bhils, Rajput and Garasias	1993	Analysis of odontometric characteristics compared among these populations.
Bishara et al.[44]	91	80	Americans, Mexican and Egyptians	1989	Odontometric characteristics of normal occlusion and comparison among these populations.
Kieser et al.[45]	55	65	South Africa Caucasoid	1985	Odontometric characteristics of this population and comparison with other populations.
Axelsson & Kirveskari[46]	465*	482*	Icelanders	1983	Odontometric characteristics and Sexual dimorphism.
Potter et al.[47]	183	164	Filipinos	1981	Odontometric characteristics of this population and sexual dimorphism compared with other populations.
Ghose & Baghdady[48]	30	30	Iraqis, Bedouins and Yemenites	1979	Analysis of mesiodistal crowns.
Richardson & Malhotra[49]	158	160	Americans Negroes	1975	Analysis of mesiodistal crowns.
Garn et al.[50]	288	322	Americans - Southwest	1968	Sexual dimorphism in mesiodistal crowns.
Garn et al.[13]	204	258	Americans - Ohio	1967	Odontometric characteristics of this population and comparison with other populations.
Moorrees et al.[51]	85	87	Americans - Northeast	1957	Analysis of mesiodistal crowns: permanent and deciduous.

* Lower incisors

Table 2. All mesiodistal values of crowns for every tooth (mean and standard deviation) found in the 31 selected studies separated by male and female.

TOOTH	11	12	13	14	15	16	17	41	42	43	44	45	46	47
Populations	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**	**M F**
Brazilians (2013)	8.75 0.70	6.94 0.64	8.13 0.56	7.15 0.44	7.03 0.55	10.95 0.70	10.04 0.87	5.36 0.82	5.75 0.54	7.17 0.64	7.36 0.55	7.56 0.52	10.80 1.08	10.89 0.78
	8.52 0.69	6.70 0.62	7.80 0.54	6.91 0.55	6.75 0.58	10.45 0.82	9.75 0.85	5.18 0.52	5.62 0.63	6.83 0.50	7.10 0.56	7.28 0.57	10.63 0.62	10.49 0.72
Malaysian Chinese (2014)	8.89 0.45	7.35 0.52	8.33 0.47	7.73 0.40	7.32 0.44	10.68 0.49	10.32 0.45	5.60 0.31	6.14 0.35	7.23 0.40	7.54 0.35	7.54 0.45	11.67 0.47	10.81 0.49
	8.55 0.47	7.05 0.56	8.03 0.46	7.48 0.46	7.06 0.39	10.36 0.50	9.88 0.49	5.46 0.35	6.05 0.35	6.86 0.39	7.32 0.38	7.25 0.44	11.20 0.53	10.14 0.40
Malaysian Tamils (2014)	8.81 0.32	6.99 0.41	7.92 0.34	7.31 0.36	6.97 0.37	10.64 0.51	10.38 0.55	5.52 0.30	6.07 0.36	6.99 0.36	7.33 0.32	7.40 0.35	11.40 0.58	10.57 0.28
	8.52 0.46	6.89 0.51	7.68 0.37	7.17 0.37	6.81 0.31	10.37 0.50	10.01 0.55	5.41 0.31	5.89 0.34	6.62 0.29	7.19 0.38	7.23 0.45	11.07 0.52	10.29 0.51

Malays (2014)	8.66 0.46	7.07 0.54	8.25 0.38	7.47 0.45	7.03 0.40	10.62 0.48	10.13 0.48	5.54 0.34	6.13 0.34	7.19 0.41	7.39 0.49	7.34 0.47	11.60 0.53	10.55 0.64
	8.44 0.51	6.91 0.62	7.81 0.46	7.40 0.38	6.96 0.41	10.47 0.46	9.90 0.51	5.43 0.30	6.06 0.36	6.77 0.34	7.27 0.42	7.32 0.40	11.32 0.46	10.27 0.57
Greeks (2014)	8.93 0.82	6.49 1.67	8.04 0.51	6.16 2.31	6.86 0.45	10.06 2.00	- -	5.66 0.66	6.11 0.56	7.17 0.52	6.27 2.36	5.87 2.86	10.43 2.33	- -
	8.59 0.95	6.45 1.36	7.74 0.51	6.16 2.07	6.74 0.56	9.38 2.65	x x	5.45 0.49	5.95 0.71	6.83 0.48	6.55 1.66	6.24 2.40	9.84 2.91	x x
Indians (2013)	8.58 0.57	6.81 0.64	7.84 0.51	7.05 0.50	6.68 0.47	10.29 0.55	9.83 0.73	5.41 0.37	5.94 0.41	6.84 0.45	7.03 0.50	7.09 0.54	10.96 0.65	10.26 0.66
	8.38 0.59	6.64 0.64	7.51 0.48	6.89 0.48	6.58 0.57	10.09 0.57	9.49 0.75	5.35 0.43	5.82 0.39	6.47 0.40	6.96 0.46	6.97 0.50	10.70 0.61	9.92 0.62
Brazilian African ancestry (2013)	9.05 0.56	7.37 0.53	8.26 0.50	7.63 0.59	7.10 0.67	10.96 0.62	- -	5.61 0.40	6.33 0.45	7.44 0.57	7.70 0.46	7.63 0.57	11.66 0.49	- -
	8.63 0.57	7.03 0.68	7.73 0.54	7.31 0.64	6.84 0.52	10.22 0.48	x x	5.25 0.40	5.97 0.42	6.86 0.49	7.30 0.54	7.11 0.60	11.09 0.58	x x
Brazilian Caucasian ancestry (2013)	8.70 0.55	6.53 0.48	7.82 0.45	6.86 0.49	6.60 0.34	10.01 0.39	- -	5.29 0.29	5.81 0.31	6.84 0.33	7.05 0.43	7.04 0.38	11.01 0.77	- -
	8.40 0.36	6.51 0.50	7.54 0.46	6.89 0.42	6.56 0.31	9.80 0.55	x x	5.14 0.21	5.71 0.30	6.48 0.33	6.85 0.43	6.90 0.42	10.42 0.57	x x
Brazilian Japanese ancestry (2013)	8.54 0.40	7.16 0.36	7.95 0.44	7.35 0.52	6.82 0.40	10.36 0.57	- -	5.31 0.35	5.92 0.36	7.02 0.42	7.24 0.43	6.98 0.48	11.21 0.41	- -
	8.36 0.39	6.74 0.56	7.70 0.50	7.16 0.39	6.69 0.37	10.19 0.41	x x	5.07 0.22	5.62 0.32	6.61 0.48	7.11 0.47	7.01 0.33	11.05 0.51	x x
Indians (2012)	8.50 0.61	6.70 0.62	7.80 0.51	6.90 0.52	6.50 0.48	10.10 0.60	9.90 0.77	5.30 0.36	5.90 0.43	6.80 0.44	6.90 0.42	6.90 0.60	10.80 0.63	10.31 0.64
	8.40 0.73	6.60 0.68	7.50 0.97	6.70 0.58	6.40 0.63	9.97 0.74	9.60 0.80	5.40 0.39	5.80 0.44	6.50 0.36	7.00 0.49	6.80 0.53	10.60 0.66	9.90 0.70
Yemeni Arabians (2012)	8.57 0.56	6.50 0.52	7.57 0.41	6.68 0.50	6.33 0.53	9.95 0.65	- -	5.21 0.44	5.74 0.44	6.73 0.43	6.74 0.51	6.80 0.55	10.87 0.74	- -
	8.34 0.61	6.40 0.66	7.30 0.44	6.49 0.49	6.22 0.53	9.82 0.55	x x	5.12 0.48	5.62 0.48	6.42 0.38	6.61 0.50	6.67 0.49	10.54 0.62	x x
Colombi ans 2011)	8.65 0.62	6.89 0.57	7.91 0.58	7.26 0.78	7.00 0.72	10.16 1.26	- -	5.32 0.46	5.86 0.50	6.91 0.51	7.25 0.44	7.21 0.50	11.33 0.57	- -
	8.37 0.46	6.88 0.58	7.93 0.72	7.19 0.77	6.90 0.61	10.19 0.55	x x	5.30 0.38	5.94 0.46	6.83 0.62	7.22 0.44	7.11 0.63	11.03 0.64	x x
Indians (2009)	8.39 0.61	6.63 0.78	7.65 0.54	6.87 0.64	6.50 0.63	9.96 0.55	9.43 0.72	5.37 0.40	5.88 0.35	6.61 0.38	6.76 0.45	6.88 0.66	10.80 0.67	9.89 0.69
	8.29 0.57	6.50 0.59	7.44 0.40	6.77 0.45	6.59 0.83	9.79 0.49	9.24 0.75	5.23 0.37	5.75 0.33	6.45 0.41	6.78 0.46	6.74 0.48	10.54 0.56	9.83 0.64
Polonies (2009)	8.68 0.61	6.61 0.68	7.78 0.72	6.73 0.52	6.49 0.50	10.52 0.92	9.70 0.85	5.44 0.39	5.93 0.43	6.76 0.54	6.92 0.53	6.97 0.52	11.20 0.83	10.46 0.79
	8.54 0.67	6.47 0.63	7.48 0.81	6.80 0.59	6.53 0.60	10.16 0.82	9.54 0.91	5.30 0.43	5.87 0.35	6.49 0.54	6.90 0.53	6.76 0.61	10.69 0.82	10.20 0.83

Nepalese (2007)	8.79 0.62	6.87 0.67	7.94 0.45	7.00 0.42	6.61 0.37	10.61 0.56	9.76 0.66	5.45 0.38	6.05 0.40	6.96 0.39	7.08 0.40	6.96 0.42	11.10 0.58	10.50 0.67
	8.52 0.55	6.81 0.57	7.60 0.40	6.96 0.45	6.53 0.39	10.35 0.56	9.69 0.69	5.40 0.33	5.93 0.33	6.58 0.35	7.02 0.39	7.02 0.76	10.95 0.61	10.13 0.63
Southern Chinese (2006)	8.85 0.53	7.36 0.59	8.30 0.47	7.77 0.42	7.26 0.36	10.99 0.51	10.26 0.49	5.62 0.34	6.22 0.41	7.31 0.42	7.58 0.42	7.56 0.41	11.69 0.60	10.73 0.67
	8.69 0.47	7.18 0.61	7.92 0.37	7.57 0.35	7.10 0.34	10.67 0.50	9.95 0.65	5.57 0.33	6.14 0.31	6.89 0.34	7.36 0.34	7.35 0.37	11.29 0.51	10.37 0.55
Moroccan (2007)	9.08 0.60	7.71 0.58	8.11 0.53	7.29 0.47	6.90 0.45	10.69 0.54	- -	5.74 0.35	6.24 0.44	7.06 0.55	7.46 0.57	7.44 0.75	11.20 0.66	- -
	8.80 0.65	6.83 0.67	7.69 0.43	7.12 0.41	6.72 0.36	10.56 0.66	x x	5.48 0.42	5.96 0.46	6.70 0.37	7.14 0.45	7.09 0.42	11.02 0.58	x x
Senegalese (2007)	9.22 0.67	7.42 0.75	8.36 0.53	7.81 0.46	7.27 0.48	10.91 0.68	- -	5.58 0.43	6.23 0.48	7.54 0.55	8.01 0.64	7.89 0.64	11.1 0,65	- -
	8.98 0.49	7.37 0.68	7.99 0.52	7.73 0.42	7.03 0.42	10.70 0.51	x x	5.56 0.37	6.13 0.39	7.10 0.40	7.72 0.48	7.68 0.44	11.20 0.47	x x
Turks (2006)	8.51 0.49	6.75 0.55	7.89 0.45	6.97 0.49	6.67 0.60	10.24 0.51	10.03 0.72	5.37 0.36	5.88 0.43	6.95 0.48	7.02 0.51	7.13 0.47	10.98 0.61	10.46 0.76
	8.41 0.52	6.50 0.59	7.49 0.38	6.86 0.39	6.54 0.44	10.04 0.61	9.88 0.56	5.32 0.34	5.86 0.34	6.58 0.34	6.95 0.39	7.01 0.42	10.80 0.62	10.39 0.63
North Indians (2006)	9.05 3.00	7.07 2.66	8.16 2.85	7.35 2.71	7.10 2.66	10.35 3.21	9.95 3.15	5.68 2.38	6.31 2.50	7.26 2.69	7.42 2.72	7.55 2.73	11.23 3.35	10.33 3.22
	8.62 2.93	6.95 2.63	7.86 2.80	7.20 2.68	6.76 2.60	10.03 3.16	9.57 3.08	5.55 2.35	5.98 2.44	6.88 2.61	7.02 2.62	7.17 2.67	10.80 3.28	10.01 3.16
Saudi Arabia (2005)	8.78 0.60	6.80 0.58	7.95 0.48	6.98 0.41	6.48 0.42	10.14 0.57	- -	5.46 0.37	5.95 0.44	6.88 0.47	7.03 0.44	6.86 0.50	11.08 0.66	- -
	8.60 0.52	6.68 0.51	7.54 0.42	6.87 0.39	6.40 0.45	10.08 0.63	x x	5.34 0.36	5.81 0.39	6.53 0.44	6.91 0.42	6.96 0.65	10.71 0.58	x x
Dominican Americans (2000)	8.96 0.67	6.98 0.69	8.15 0.52	7.54 0.49	7.10 0.42	10.81 0.70	- -	5.56 0.36	6.16 0.42	7.12 0.55	7.48 0.52	7.53 0.56	11.32 0.60	- -
	8.72 0.56	6.99 0.56	7.84 0.48	7.37 0.44	6.97 0.49	10.51 0.66	x x	5.47 0.35	6.08 0.36	6.82 0.40	7.44 0.51	7.34 0.49	11.02 0.67	x x
Suécia (1999)	8.88 0.68	6.98 0.50	8.26 0.49	6.87 0.31	6.73 0.52	11.00 0.63	10.40 0.65	5.48 0.43	6.09 0.39	7.19 0.52	7.12 0.38	7.36 0.53	11.13 0.63	10.52 0.76
	8.48 0.60	6.65 0.55	7.61 0.48	6.76 0.39	6.65 0.53	10.58 0.72	9.94 0.61	5.32 0.48	5.90 0.41	6.56 0.39	6.98 0.47	6.92 0.38	10.80 0.60	10.22 0.57
Hong Kong (1997)	8.73 0.51	7.18 0.60	8.30 0.41	7.76 0.42	7.24 0.42	10.41 0.50	- -	5.48 0.33	6.10 0.33	7.29 0.37	7.58 0.36	7.44 0.38	11.30 0.54	- -
	8.66 0.46	7.12 0.50	8.02 0.40	7.54 0.43	7.07 0.47	10.11 0.45	x x	5.53 0.32	6.13 0.35	6.92 0.43	7.44 0.47	7.28 0.40	11.15 0.44	x x
Jordanians (1996)	8.99 0.61	6.99 0.66	8.10 0.59	7.19 0.49	6.99 0.43	10.57 0.53	- -	5.67 0.33	6.23 0.43	6.94 0.44	7.39 0.45	7.40 0.41	11.29 0.62	- -
	8.66 0.52	6.72 0.60	7.68 0.50	7.02 0.47	6.84 0.54	10.25 0.57	x x	5.54 0.39	6.09 0.52	6.61 0.45	7.03 0.39	7.16 0.48	10.84 0.66	x x

Saudi Arabians (1993)	8.60 0.67	6.77 0.58	7.80 0.50	7.00 0.56	6.59 0.43	10.59 0.59	- -	5.33 0.36	5.99 0.59	6.80 0.40	6.88 0.65	7.10 0.49	11.05 0.69	- -
	8.63 0.46	6.58 0.41	7.37 0.46	6.84 0.31	6.58 0.32	10.48 0.42	x x	5.37 0.29	6.00 0.40	6.50 0.43	7.03 0.39	7.07 0.31	10.89 0.54	x x
Northwest India – Bhils (1993)	8.57 0.52	6.76 0.55	7.79 0.44	7.02 0.44	6.52 0.46	10.37 0.54	9.58 0.67	5.37 0.36	5.96 0.42	6.89 0.38	7.00 0.47	7.07 0.48	11.22 0.62	10.36 0.52
	8.21 0.62	6.43 0.60	7.40 0.42	6.90 0.43	6.41 0.43	10.01 0.48	9.19 0.70	5.28 0.35	5.81 0.38	6.43 0.38	6.87 0.42	6.93 0.47	10.89 0.65	10.11 0.65
Northwest India – Rajputs (1993)	8.62 0.56	6.70 0.52	7.64 0.48	6.77 0.47	6.45 0.46	10.35 0.57	9.71 0.84	5.30 0.40	5.87 0.44	6.86 0.46	6.80 0.44	6.88 0.50	11.02 0.60	9.89 0.76
	8.28 0.67	6.47 0.51	7.32 0.41	6.64 0.53	6.30 0.62	10.00 0.57	9.10 0.70	5.18 0.38	5.68 0.33	6.42 0.35	6.65 0.50	6.62 0.55	10.54 0.67	9.51 0.63
Northwest India – Garasias (1993)	8.53 0.61	6.73 0.65	7.67 0.48	6.97 0.58	6.49 0.71	10.53 0.59	9.47 0.84	5.30 0.43	5.94 0.44	6.84 0.48	6.91 0.50	6.94 0.53	10.89 0.56	10.22 0.67
	8.33 0.54	6.47 0.67	7.28 0.44	6.71 0.45	6.29 0.57	10.23 0.58	9.20 0.84	5.19 0.39	5.76 0.41	6.38 0.39	6.77 0.44	6.81 0.50	10.56 0.52	9.74 0.71
Americans (1989)	8.60 0.50	6.70 0.40	7.80 0.50	6.90 0.40	6.70 0.4	10.5 0.6	- -	5.4 0.40	5.90 0.40	6.80 0.40	6.9 0.40	7.0 0.4	11.00 0.70	- -
	8.50 0.70	6.60 0.60	7.50 0.40	6.70 0.40	6.50 0.40	10.10 0.50	x x	5.20 0.40	5.75 0.40	6.40 0.40	6.80 0.40	6.80 0.30	10.40 0.60	x x
Mexican (1989)	8.40 0.60	6.60 0.60	7.90 0.60	6.90 0.305	7.00 0.50	10.55 0.50	- -	5.50 0.40	6.00 0.40	6.90 0.30	7.00 0.40	7.30 0.40	10.90 0.60	- -
	8.20 0.50	6.50 0.60	7.60 0.50	6.60 0.40	6.60 0.40	10.20 0.70	x x	5.40 0.40	5.80 0.40	6.45 0.40	6.70 0.50	7.00 0.60	10.50 0.50	x x
Egyptians (1989)	8.90 0.50	6.90 0.50	7.90 0.50	7.10 0.40	6.80 0.30	10.40 0.50	- -	5.40 0.30	5.90 0.40	6.90 0.40	7.10 0.40	7.20 0.40	11.0 0.75	- -
	8.90 0.50	6.80 0.60	7.50 0.40	7.10 0.40	6.70 0.30	10.25 0.50	x x	5.55 0.50	6.00 0.30	6.60 0.40	7.10 0.35	7.20 0.40	11.00 0.60	x x
South Africa Caucasoid (1985)	8.94 0.70	7.08 0.54	8.43 0.59	7.53 0.51	7.49 0.63	11.22 0.65	10.71 0.67	5.54 0.32	6.20 0.43	7.34 0.48	7.68 0.50	7.81 0.51	11.56 0.58	10.80 0.62
	8.40 0.66	6.56 0.57	7.74 0.42	7.24 0.45	7.04 0.41	10.74 0.50	10.00 0.49	5.33 0.37	6.01 0.46	6.79 0.36	7.30 0.53	7.38 0.44	10.88 0.55	10.20 0.59
Icelanders (1983)	8.99 0.54	6.95 0.54	8.14 0.42	7.22 0.41	6.89 0.43	10.98 0.57	10.08 0.58	5.59 0.35	6.20 0.36	7.13 0.41	7.30 0.41	7.45 0.46	11.45 0.58	10.85 0.60
	8.75 0.52	6.83 0.51	7.79 0.40	7.07 0.42	6.84 0.42	10.70 0.57	9.78 0.53	5.48 0.34	6.02 0.37	6.80 0.35	7.12 0.42	7.27 0.44	11.12 0.60	10.49 0.64
Filipinos (1991)	8.33 0.49	6.76 0.63	7.75 0.51	6.89 0.44	6.56 0.50	10.02 0.67	9.24 0.65	5.08 0.34	5.74 0.39	6.77 0.47	6.77 0.44	6.75 0.47	10.73 0.68	10.24 0.78
	8.03 0.48	6.44 0.66	7.45 0.45	6.82 0.47	6.41 0.41	9.77 0.50	9.65 0.56	4.98 0.34	5.58 0.43	6.37 0.41	6.66 0.50	6.64 0.54	10.48 0.64	9.92 0.72
Iraqis (1979)	9.03 0.64	6.95 0.75	8.06 0.60	7.17 0.53	6.94 0.50	10.70 0.60	- -	5.61 0.44	6.20 0.50	6.97 0.44	7.12 0.48	7.36 0.57	11.26 0.69	- -
	8.84 0.60	6.87 0.69	7.84 0.53	7.06 0.55	6.92 0.53	10.62 0.67	x x	5.66 0.45	6.19 0.45	6.78 0.52	7.04 0.61	7.28 0.52	11.03 0.64	x x

Bedouins (1979)	8.76 0.55	6.89 0.48	7.80 0.49	7.02 0.46	6.75 0.47	10.63 0.58	- -	5.45 0.55	6.09 0.48	7.03 0.49	7.03 0.46	7.21 0.47	11.27 0.58	- -
	8.49 0.59	6.79 0.63	7.49 0.49	6.72 0.39	6.53 0.59	10.22 0.62	x x	5.23 0.33	5.88 0.44	6.52 0.44	6.78 0.40	6.92 0.44	10.76 0.64	x x
Yemenites (1979)	8.06 0.54	6.32 0.37	7.51 0.66	6.88 0.41	6.49 0.64	10.12 0.63	- -	5.61 0.28	6.17 0.41	6.49 0.24	6.70 0.45	6.97 0.64	10.83 0.75	- -
	8.42 0.69	6.26 0.85	7.28 0.56	6.78 0.55	6.61 0.52	10.44 0.67	x x	5.23 0.43	5.62 0.48	6.43 0.40	6.59 0.64	6.98 0.48	10.75 0.34	x x
American Negroes (1975)	9.12 0.67	7.26 0.64	8.19 0.53	7.66 0.49	7.25 0.49	11.04 0.64	10.74 0.63	5.53 0.39	6.13 0.44	7.37 0.57	7.76 0.51	7.85 0.55	11.76 0.72	11.53 0.86
	8.72 0.58	7.08 0.56	7.74 0.38	7.37 0.43	6.94 0.39	10.57 0.52	10.35 0.73	5.38 0.39	5.99 0.46	6.86 0.42	7.41 0.50	7.61 0.50	11.28 0.62	10.94 0.73
Americans Southwest (1968)	8.83 0.58	6.73 0.57	7.99 0.44	7.09 0.44	6.78 0.43	10.14 0.49	9.99 0.60	5.44 0.38	6.03 0.41	6.97 0.38	7.21 0.47	7.24 0.43	11.39 0.63	10.69 0.67
	8.58 0.55	6.61 0.64	7.65 0.42	6.93 0.45	6.64 0.47	9.89 0.54	9.69 0.60	5.38 0.38	5.91 0.39	6.59 0.39	7.02 0.42	7.09 0.51	10.96 0.68	10.41 0.66
Americans Ohio (1967)	8.78 0.57	6.71 0.58	7.95 0.45	7.14 0.47	6.84 0.46	10.17 0.49	10.05 0.58	5.38 0.38	6.02 0.42	6.98 0.40	7.27 0.47	7.26 0.46	11.38 0.55	10.63 0.62
	8.50 0.57	6.47 0.67	7.51 0.44	6.90 0.40	6.60 0.41	9.81 0.60	9.63 0.61	5.31 0.39	5.86 0.40	6.56 0.41	6.99 0.40	7.03 0.40	10.86 0.67	10.18 0.60
Americans Northeast (1957)	8.78 0.46	6.64 0.63	7.95 0.42	7.01 0.38	6.82 0.37	10.81 0.56	10.35 0.63	5.42 0.31	5.95 0.38	6.96 0.36	7.07 0.35	7.29 0.52	11.18 0.47	10.76 0.71
	8.40 0.53	6.47 0.62	7.53 0.37	6.85 0.42	6.62 0.43	10.52 0.51	9.81 0.49	5.25 0.36	5.78 0.38	6.47 0.32	6.87 0.38	7.02 0.40	10.74 0.56	10.34 0.62
TOOTH	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**41**	**42**	**43**	**44**	**45**	**46**	**47**
Sex	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F
Median Total	8.75	6.89	7.98	7.17	6.83	10.50	10.00	5.46	6.03	7.01	7.17	7.22	11.17	10.53
	8.51	6.71	7.62	7.01	6.69	10.23	9.69	5.35	5.89	6.63	7.02	7.05	10.83	10.17
% Sexual dimorphism	2.82	2.68	4.72	2.28	2.09	2.64	3.20	2.06	2.38	5.73	2.14	2.41	3.14	3.54

Table 3. Percentage dimorphism for every tooth and population.

Tooth Article	11	12	13	14	15	16	17	41	42	43	44	45	46	47	Total	Ranking	Population
Martins-Filho[17]	2.70	3.58	4.23	3.47	4.15	4.78	2.97	3.47	2.31	4.98	3.66	3.85	1.60	3.81	3541	14	Brazilians
Khamis et al.[28]	3.98	4.26	3.74	3.34	3.68	3.09	4.45	2.56	1.49	5.39	3.01	4.00	4.20	6.61	3842	12	Malaysian Chinese
Khamis et al.[28]	3.40	1.45	3.13	1.95	2.35	2.60	3.70	2.03	3.06	5.59	1.95	2.35	2.98	2.72	2804	21	Malaysian Tamils

Khamis et al.[28]	2.61	2.32	5.63	0.95	1.01	1.43	2.32	2.03	1.16	6.20	1.65	0.27	2.47	2.73	2.341	**28**	Malays
Mitsea et al.[29]	3.96	0.62	3.88	0.00	1.78	7.25	x	3.85	2.69	4.98	-4.27	-5.93	6.00	x	2.066	**33**	Greeks
Angadi et al.[30]	2.39	2.56	4.39	2.32	1.52	1.98	3.58	1.12	2.06	5.72	1.01	1.72	2.43	3.43	2.588	**23**	Indians
Fernandes et al.[1]	4.87	4.84	6.86	4.38	3.80	7.24	x	6.86	6.03	8.45	5.48	7.31	5.14	x	5.938	**2**	Brazilian African ancestry
Fernandes et al.[1]	3.57	0.31	3.71	-0.44	0.61	2.14	x	2.92	1.75	5.56	2.92	2.03	5.66	x	2.562	**24**	Brazilian Caucasian ancestry
Fernandes et al.[1]	2.15	6.23	3.25	2.65	1.94	1.67	x	4.73	5.34	6.20	1.83	-0.43	1.45	x	3.085	**18**	Brazilian Japanese ancestry
Thapar et al.[4]	1.19	1.52	4.00	2.99	1.56	1.30	3.13	-1.85	1.72	4.62	-1.43	1.47	1.89	4.14	1.874	**36**	Indians
Al-Gunaid et al.[31]	2.76	1.56	3.70	2.93	1.77	1.32	x	1.76	2.14	4.83	1.97	1.95	3.13	x	2.484	**25**	Yemeni Arabians
Castillo et al.[27]	3.35	0.15	-0.25	0.97	1.45	-0.29	x	0.38	-1.35	1.17	0.42	1.41	2.72	x	0.843	**41**	Colombians
Phabhu & Acharya.[32]	1.21	2.00	2.82	1.48	-1.37	1.74	2.06	2.68	2.26	2.48	-0.29	2.08	2.47	0.61	1.586	**37**	Indians
Antoszewski et al.[33]	1.64	2.16	4.01	-1.03	-0.61	3.54	1.68	2.64	1.02	4.16	0.29	3.11	4.77	2.55	2.138	**31**	Polonies
Archarya & Mainali[8]	3.17	0.88	4.47	0.57	1.23	2.51	0.72	0.93	2.02	5.78	0.85	-0.85	1.37	3.65	1.950	**34**	Nepalese
Ling & Wong [34]	1.84	2.51	4.80	2.64	2.25	3.00	3.12	0.90	1.30	6.10	2.99	2.86	3.54	3.47	2.951	**19**	Southern Chinese
Ngom et al.[35]	3.18	12.88	5.46	2.39	2.68	1.23	x	4.74	4.70	5.37	4.48	4.94	1.63	x	4.474	**3**	Moroccan
Ngom et al.[35]	2.67	0.68	4.63	1.03	3.41	1.96	x	0.36	1.63	6.20	3.76	2.73	-0.89	x	2.348	**27**	Senegalese
Ates et al.[36]	1.19	3.85	5.34	1.60	1.99	1.99	1.52	0.94	0.34	5.62	1.01	1.71	1.67	0.67	2.103	**32**	Turks
Singh & Goyal[37]	4.99	1.73	3.82	2.08	5.03	3.19	3.97	2.34	5.52	5.52	5.70	5.30	3.98	3.20	4.026	**7**	North Indians
Hashim & Al-Ghamdi[38]	2.09	1.80	5.44	1.60	1.25	0.60	x	2.25	2.41	5.36	1.74	-1.44	3.45	x	2.212	**30**	Saudi Arabia
Santoro et al.[39]	2.75	-0.14	3.95	2.31	1.87	2.85	x	1.65	1.32	4.40	0.54	2.59	2.72	x	2.233	**29**	Dominican Americans
Lund & Mörnstad[6]	4.72	4.96	8.54	1.63	1.20	3.97	4.63	3.01	3.22	9.60	2.01	6.36	3.06	2.94	4.274	**4**	Suécia
Yuen & Tang[40]	0.81	0.84	3.49	2.92	2.40	2.97	x	-0.90	-0.49	5.35	1.88	2.20	1.35	x	1.901	**35**	Hong Kong

Hattab et al.[41]	3.81	4.02	5.47	2.42	2.19	3.12	x	2.35	2.30	4.99	5.12	3.35	4.15	x	3.608	**13**	Jordanians
Hashim & Murshid[42]	-0.35	2.89	5.83	2.34	0.15	1.05	x	-0.74	-0.17	4.62	-2.13	0.42	1.47	x	1.282	**39**	Saudi Arabians
Lukacs & Hemphill[43]	4.38	5.13	5.27	1.74	1.72	3.60	4.24	1.70	2.58	7.15	1.89	2.02	3.03	2.47	3.353	**16**	Northwest India/Bhils
Lukacs & Hemphill[43]	4.11	3.55	4.37	1.96	2.38	3.50	6.70	2.32	3.35	6.85	2.26	3.93	4.55	4.00	3.845	**11**	Northwest India/ Rajputs
Lukacs & Hemphill[43]	2.40	4.02	5.36	3.87	3.18	2.93	2.93	2.12	3.13	7.21	2.07	1.91	3.13	4.93	3.513	**15**	Northwest India/ Garasias
Bishara et al.[44]	1.18	1.52	4.00	2.99	3.08	3.96	x	3.85	2.61	6.25	1.47	2.94	5.77	x	3.300	**17**	Americans
Bishara et al.[44]	2.44	1.54	3.95	4.55	6.06	3.43	x	1.85	3.45	6.98	4.48	4.29	3.81	x	3.901	**10**	Mexican
Bishara et al.[44]	0.00	1.47	5.33	0.00	1.49	1.46	x	-2.70	-1.67	4.55	0.00	0.00	0.00	x	0.828	**42**	Egyptians
Kieser et al.[45]	6.43	7.93	8.91	4.01	6.39	4.47	7.10	3.94	3.16	8.10	5.21	5.83	6.25	5.88	5.972	**1**	South Africa Caucasoid
Axelsson& Kirveskari[46]	2.74	1.76	4.49	2.12	0.73	2.62	3.07	2.01	2.99	4.85	2.53	2.48	2.97	3.43	2.770	**22**	Icelanders
Potter et al.[47]	3.74	4.97	4.03	1.03	2.34	2.56	-4.25	2.01	2.87	6.28	1.65	1.66	2.39	3.23	2.463	**26**	Filipinos
Ghose& Baghdady[48]	2.15	1.16	2.81	1.56	0.29	0.75	x	-0.88	0.16	2.80	1.14	1.10	2.09	x	1.260	**40**	Iraqis
Ghose& Baghdady[48]	3.18	1.47	4.14	4.46	3.37	4.01	x	4.21	3.57	7.82	3.69	4.19	4.74	x	4.071	**6**	Bedouins
Ghose& Baghdady[48]	-4.28	0.96	3.16	1.47	-1.82	-3.07	x	7.27	9.79	0.93	1.67	-0.14	0.74	x	1.391	**38**	Yemenites
Richardson & Malhotra[49]	4.59	2.54	5.81	3.93	4.47	4.45	3.77	2.79	2.34	7.43	4.72	3.15	4.26	5.39	4.260	**5**	American Negroes
Garn et al.[50]	2.91	1.82	4.44	2.31	2.11	2.53	3.10	1.12	2.03	5.77	2.71	2.12	3.92	2.69	2.826	**20**	Americans /Southwest
Garn et al.[13]	3.29	3.71	5.86	3.48	3.64	3.67	4.36	1.32	2.73	6.40	4.01	3.27	4.79	4.42	3.925	**9**	Americans /Ohio
Moorrees et al.[51]	4.52	2.63	5.58	2.34	3.02	2.76	5.50	3.24	2.94	7.57	2.91	3.85	4.10	4.06	3.930	**8**	Americans /Northeast
Total	**2.74**	**2.71**	**4.64**	**2.3**	**2.15**	**2.64**	**3.22**	**2.04**	**2.45**	**5.64**	**2.14**	**2.37**	**3.09**	**3.51**	**2.74**		
Ranking	**6**	**7**	**2**	**11**	**12**	**8**	**4**	**14**	**10**	**1**	**13**	**9**	**5**	**3**	**total**		

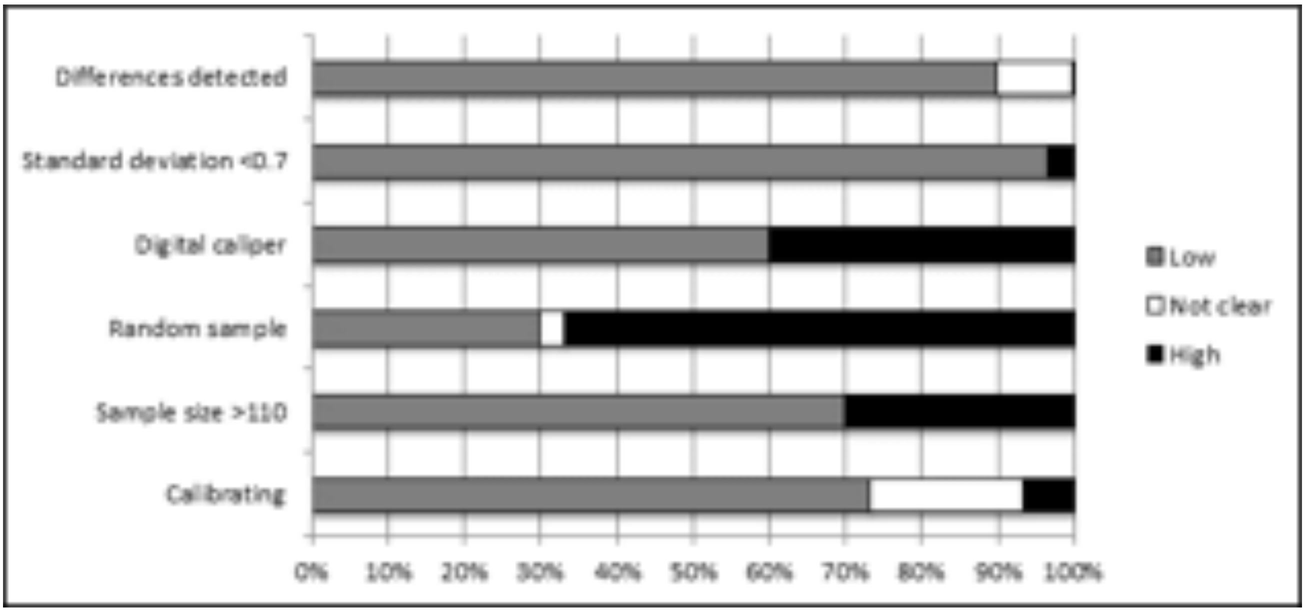
Risk of bias results

The risk of individual bias is shown in Table 4 and Figure 2, which compare the selected studies and their relative contribution to the final results of the systematic review.

Table 4. Risk of bias: review authors' judgments about each risk of bias item presented in all 31 included studies.

Articles	Khamis et al.[28], Angadi et al.[30], Ling & Wong[34], Yuen & Tang[40]	Thapar et al.[4], Martins-Filho [17], Al-Gunaid et al. [31], Phabhu & Acharya.[32], Hashim & Al-Ghamdi.[38], Hattab et al.[41]	Kieser et al.[45], Richardson & Malhotra [49], Moorrees et al.[51]	Lukacs & Hemphill.[43], Bishara et al.[44], Axelsson & Kirveskari.[46]	Archarya & Mainali[8], Ngom et al. [35], Ates et al.[36]	Fernandes et al.[1]	Antoszewski et al.[33]	Santoro et al.[39]	Hashim & Murshid[8]	Singh & Goyal[37]	Mitsea et al. [29]	Castillo et al. [27]	Lund & Mörnstad[6]	Ghose & Baghdady[48], Garn et al.[50], Potter et al.[47]
Calibrating	Yes	Yes	Yes	Yes	Yes	Yes	NO	Not clear	Not clear	No	Yes	Yes	No	Not clear
Sample size >110	Yes	Yes	Yes	Yes	No	NO	NO	NO	NO	NO	Yes	NO	NO	Yes
Random sample	Yes	No	No	Yes	No	Not clear	Yes	No	No	No	Yes	No	No	No
Digital caliper	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	No
Standard deviation <0.7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Differences detected	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Not clear	Not clear	Not clear	Yes	No	Yes	Yes

Figure 2. Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all 31 included studies.



Some studies had an insufficient sample size (n<110) for in-depth analysis regarding dental sexual dimorphism, especially if the purpose of the study was to analyze differences between the sexes.[1,6,27,33,35-39] Therefore, we considered that these studies presented a high risk of bias for the analysis of differences in the dimensions of dental crowns. However, these smaller sample studies would have had less influence on our meta-analysis.
Only two studies[29,37] presented a standard deviation above 0.7; although this value did not apply to all teeth, this may indicate methodological flaws, and was considered a risk of bias. In the specific case of a North Indian study,[37] no examiner calibration was reported. In another study,[29] the sample was considered insufficient to detect a high standard deviation.
Potential problems regarding tooth measurement method were evaluated; however, a significant difference between manual and digital methods was not found, indicating that these are interchangeable[52]. Despite this indication, we considered manual measurements[37,39,43-46,48-50] as a risk of bias in the present review, due to their reduced accuracy with respect to digital calipers. However, it should be noted that some studies were published before the advent of digital calipers.[44-46, 49-5] In addition, non-specification of the calipers used[13,27] was considered a risk of bias in the present study,
In one study,[33] the odontometric characteristics of transsexual women were evaluated in comparison with male and female subjects; this review collected male and female participants. One study that did not find sexual dimorphism[27] was considered a type of bias, because our initial hypothesis was that there is difference in mesiodistal measurements in all teeth. In some studies[37-39], the differences between single teeth were unclear; therefore, no statistical proof could be considered.
Three studies[6,33,37] did not describe the calibration process and were therefore considered to have a risk of bias. In other studies,[13,38,39,47,48,50] the risk of bias was unclear due to poorly defined calibration methodologies or a lack of information.
Regarding the type of sample, we considered whether the sample was representative of the population, on the basis of being randomized or specifically selected.
Most selected studies[6,8,13,17,19,31,33,35,36,38,39,41,45,47,48-50] used a sample of convenience and were therefore considered to have a high risk of bias. However, we must consider that in some of studies, the aim was not to determine dental sexual differences. The risk of bias in one study[1] was unclear, because, despite the authors' concern for dividing the sample by racial origin, the sample size per group was small.
The use of the right side only as a reference does not influence the results, because the differences due to asymmetry in humans are not significant.[15,17,27,40,31,38,53]
We did not separate the studies based on whether they measured plaster casts or human teeth. It has been shown that human teeth and plaster measurements can produce similar results;[53] therefore, this was not considered a risk of bias.
We also excluded skeletal remains in this review, because the mesiodistal diameter of the crown is typically not preserved;[54] similarly, very specific populations exhibit particular intrinsic (genetic) and extrinsic (environmental) variables.[13,16] Therefore, our results focused on young adults with permanent teeth.

Meta-analysis by individual tooth
The meta-analysis graphs were generated by teeth (Figures 3 to 16). The mean differences between male and female subjects are in Table 5. The I-squared statistic should be interpreted as follows: 0% to 40% might not be important; 30% to 50% may represent moderate heterogeneity; 50% to 90% may represent substantial heterogeneity; and 75% to 100% may represent considerable heterogeneity.[55] The results of Egger's test for small-study effects were not significant and did not indicate publication bias.

Table 5. Meta-analysis by individual tooth: mean differences between male and female subjects.

Tooth	**11**	**12**	**13**	**14**	**15**	**16**	**17[#]**	**41**	**42**	**43**	**44**	**45**	**46**	**47[#]**
Mean (SD)	0.242 (0.013)	0.174 (0.014)	0.359 (0.011)	0.170 (0.011)	0.145 (0.011)	0.274 (0.013)	0.306 (0.020)	0.091 (0.008)	0.135 (0.009)	0.378 (0.010)	0.165 (0.010)	0.171 (0.012)	0.348 (0.015)	0.365 (0.020)
95% CI	0.2167 0.26 75 0.1478 a 0.2030 0.3369 a 0.3815 0.1490 a 0.1922 0.1227 a 0.1667 0.2476 a 0.3009 0.2648 a 0.3441	0.1469 0.2018	0.3368 0.3814	0.1492 0.1924	0.1238 0.1676	0.2480 0.3011	0.2667 0.3452	0.0743 0.1083	0.1169 0.1539	0.3585 0.3974	0.1445 0.1869	0.1481 0.1951	0.3188 0.3778	0.3259 0.4041
***P*=**	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Heterogeneity test I-squared	**34.4%**	**34.4 %**	**23.7%**	**10.2%**	**36.4%**	**44.5%**	**78.1%**	**44.2%**	**24.3%**	**44.0%**	**57.7%**	**36.6%**	**42.9%**	**47.3%**
	P=0.017	*P=0.017*	*P=0.087*	*P=0.284*	*P=0.011*	*P=0.001*	*P=0.000*	*P=0.001*	*P=0.082*	*P=0.001*	*P=0.000*	*P=0.011*	*P=0.001*	*P=0.007*
Publication bias (Egger's test)	*P= 0.424*	*P= 0.378*	*P= 0.549*	*P= 0.490*	*P= 0.886*	*P= 0.391*	*P= 0.811*	*P= 0.220*	*P= 0.603*	*P= 0.437*	*P= 0.423*	*P= 0.726*	*P= 0.279*	*P= 0.772*

#n=23 SD= Standard deviation CI= Confidence interval

DISCUSSION

The primary question addressed by this review constituted whether the tooth crown sexual dimorphism pattern previously reported in limited samples could be verified when tested more extensively across a range of populations. Over 6700 males and females participated in the 31 included trials.

Tables 1 and 2 present the 31 selected articles, publication dates, study population, sample population and main objectives. Of these published studies, only one[11] failed to find sexual dimorphism through mesiodistal measurements, while another[30] found it only in canines. However, due to the risk of bias, only 4 articles (Table 3) fulfilled the requirements for all considered items, which makes this study very vulnerable to bias.

The selected studies generally focused on young adults; deterioration factors such as tooth wear, mainly for mesiodistal diameters of the crown, had a very important effect that reduced the number of teeth recorded, affecting >50% of the measures for some teeth.[53]

The greater dimensions of masculine canines are the consequences of differing enamel thickness, due to the longer period of amelogenesis in males.[4] Our results confirm that canines reflect the greatest sexual dimorphism: approximately 0.3585-0.3974 mm for lower canines and 0.3368-0.3814 mm

for upper canines (Table 4); however, the second molars were not reported by other specific studies in this area.[30,32,36,33,37,50] Notably, we found great differences in values: 0.3259-0.4041 mm for lower second molar and 0.2667-0.3452 mm for upper second molar (Table 4). Of the studies selected for this systematic review, only 23 (Figure 9 and 16) measured second molars, which indicates that the authors did not focus on the analysis of these teeth.
In the data ranking (Table 4), the teeth with the greatest mesiodistal crown sexual dimorphism were the lower canines, followed in order by the lower second molars, upper canines, and upper second molars. Several studies reported canines and first molars to be the most diffuse teeth with the greatest variation in morphology between male and female subjects.[32,33,35,38,44,48,50] Other studies found significant differences in central and/or lateral teeth.[6,1,27-30,38] We found it in fifth and sixth ranking. Some studies reported that the premolars exhibited sexual dimorphism,[6,30,31,35,37,40,43,44,47,49,51] while some studies reported the opposite.[1,8,29,32,33,35,48] Our results did not verify findings of sexual dimorphism. In both upper premolars and the first lower premolar, the differences were <2.3%.
Anatomical variation among populations is normal;[14,55] however, we cannot explain why only upper second molars (Figure 9) and lower first premolars (Figure 13) exhibited substantial heterogeneity (Table 5), although Egger's test showed no publication bias.
The crowns of premolar teeth showed the smallest dimorphism between males and females throughout the population studied (Figures 6, 7, 12, and 13). Linear measurement with extractions of premolar permanent teeth will be very similar between men and women (Table 2). However, measurements of the crowns of the remaining canines and molars should serve to determine an appropriate treatment plan and achieve greater stability in orthodontic planning, in addition to calculating these different forces in relation to the dental crown.[2] This implies important considerations of this study for this aspect. The measurement of the dental crowns is important for orthodontic forces and anchorage;[1,2] notably, the movement of the tooth as a whole will be realized.[11] Use of the second molar, when possible, for anchorage is a viable alternative to conventional molar anchorage.[56]
The differences between populations in the mesiodistal crowns are very large, even when considering all teeth (Table 4), which shows the fragility of this measurement with respect to sexual dimorphism. The total amounts range from 5.97% to 0.82% for all teeth. This cannot be fully explained by anatomical variables; factors such as genetics and environmental are closely related.[29,54] Some researchers suspect that the analyses for mesiodistal measurements should discriminate sex better than those for buccolingual dimensions; however, these measurements are lower than those derived by combining both dimensions.[8,57] However, our results showed the opposite; the difference between the sexes in all populations varied greatly, but this variation was insufficient to be considered a single method of differentiation between the sexes, with respect to teeth.
Most current articles regarding dental anatomy work with 3D technologies,[5,10,11] exceeding the accuracy of the results attained in our systematic review. However, the idea of working solely with mesiodistal measures was to provide an easy technique that could be reproduced in practice without sophisticated equipment.[29,53] For forensic experts, our study shows that using measurements of canine crowns and second molars will help in the identification of human remains to determine sex and age, even in cases where skeletal remains are damaged or destroyed.[4] However, mesiodistal crowns do not exhibit sufficient evidence of sexual dimorphism among the populations and probably should not be the sole method used.

LIMITATION OF STUDY:

The study was unable to identify differences between the populations; the data collected do not allow this inference.

Most current articles work with 3D technologies, thus exceeding the accuracy of the results attained in our systematic review.
The studies concentrated on either mesiodistal or buccolingual dimensions, or both. We focused on mesiodistal measurements; some important information may have been lost as a result.
Finally, we included studies conducted in different areas, which utilized different data collection techniques. Nevertheless, similar results were recorded across these studies.

CONCLUSION

This study shows that a small degree of sexual dimorphism exists in all human teeth. The second molars and canines show the greatest sexual dimorphism. Our results also indicate that this dental dimorphism occurs among different racial groups living in different geographic areas; however, it is not possible to establish a single value applicable to all populations.

CLINICAL RELEVANCE

Scientific rational for study
For forensic dentistry, this study supports other studies in the area using canines and second molars, as well as other cranial measurements for the post-mortem detection of sex; it supports the establishment of patterns that can be used across populations.

Practical implications
For forensic experts, our study shows that using measurements of canine crowns and second molars may help in the identification of remains for post-mortem determination of sex and age, along with other cranial measurements.
For orthodontics, premolars exhibit little mesiodistal difference between the sexes; however, orthodontic forces differ between males and females.
For prosthetics, use of the mesiodistal measurements of crowns for making prostheses (implant supported or not) that follow the mean values found in the populations.

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Physical violence against children and adolescents in Recife: a 5-year retrospective study

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KEYWORDS

Child abuse;
Physical abuse;
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ABSTRACT

The aim of the present study was to analyze the prevalence results of physical violence against children and adolescents in a 5-year period in Recife, Brazil. Inter-personal violence is one of the most recognizable forms of child aggression and has become as an imperative public health issue. All violence related forensic reports performed between 2009 and 2013 in the clinical services of the Institute of Legal Medicine Antônio Percivo Cunha were analyzed. Victims were classified according to sex, age, relationship with perpetrator, injuries and year of occurrence. Statistical analysis was performed using the SPSS (version 22.0). Continuous variables were described and when appropriate, frequencies were displayed and compared. The association between variables was evaluated using chi-square and Fisher's exact test. The margin of error for the statistical tests was 5.0%. A total of 9783 occurrences were evaluated, involving mainly male subjects (n=5447, 55.7%). Victims' mean age was 13.9 years, the most common perpetrators were victims' acquaintances (n=2538, 25.9%). Facial injuries were the most frequent affecting a little over a fifth of the total sample (n=3673, 20.1%). These findings support the important role dentists can play in identifying and reporting physical violence against children and adolescents.

INTRODUCTION

Physical violence against children and adolescents (PVCA) is related to inter-personal violence, where there may not be any personal relationship between the abuser and the victim.[1] It differs from child abuse, which refers only to any act of commission or omission by a parent or care giver that results in harm, potential harm, or threat to a child.[2] PVCA is the most recognizable form of child aggression,[3] and includes child abuse and other situations, such as dating violence,[4] violence against children from school staff,[5] bullying, children who sexually abuse other children, among others.[6] PVCA occurs when a child or adolescent is victim or is at risk of being harmed by relatives, care givers, known or unknown persons.[7]

Although the PVCA is studied worldwide, it is difficult to know its real prevalence, since the cultural, legal, conceptual and methodological differences of the regional studies, together with the under reporting of cases, make it difficult to consolidate the data on the subject.[8]

A study on the prevalence of physical violence against children and adolescents refers that, from January 2008 to October

2012, 39798 cases of aggression to children and adolescents were reported[9]. Yet, it has been stated that, in Brazil, for each case of physical abuse, 10 to 20 are not registered[10], which confirms the under reporting tendency reported in literature[11], also described in other countries, like Portugal, Canada and Australia, where the prevalence of the problem is also underestimated [2,12]. Problems associated with short observation period studies and small sample sizes, make it difficult to know the real proportion of the problem and are cited as likely reasons for the under reporting [3]. However, not knowing the real prevalence of this phenomenon impairs the development of preventive strategies aiming at PVCA reduction. Moreover, the forensic community has been increasingly confronted with cases in which a differential diagnosis between accidental and deliberately inflicted trauma is critical. Thus, knowledge about the whole PVCA phenomena may be crucial.
The aim of this study was to analyze the prevalence of PVCA in a 5-year period in Recife, Brazil, characterizing the subjects involved, the nature of the injuries suffered, and the victim actions after the occurrence.

MATERIALS AND METHODS:

All forensic reports of PVCA performed at the Institute of Legal Medicine Antônio Percivo Cunha (IMLAPC), in Recife, Brazil, in a five-year period were analysed. Inclusion criteria were confirmed cases of physical aggression registered at the IMPLAC, being the victims' age up to 18 years and occurrence taking place from January 2009 to December 2013. Cases of sexual violence, neglect, and psychological violence were excluded. Collected data concerned victim's sex and age, year of occurrence, time elapsed between the aggression and the examination, place of occurrence, injury site, alleged offender, type of medical care sought, history of past violent episodes and the nexus between the complaint and the injury type (causation). Body regions were divided into: head, face, neck; collar bone/spine; chest; abdomen; pelvis; right arm; left arm; right leg; left leg. Data was collected from the files by a single researcher, considering the studied variables.
Data was analysed using SPSS (Social Package for Social Sciences), version 22.0. Frequencies were displayed and continuous variables were described using mean, standard deviation (SD), minimum, and maximum values. The association between variables was evaluated using the chi-square or Fisher's exact test. The margin of error for the statistical tests was 5.0%.

RESULTS

A total of 9783 cases fitted the defined inclusion criteria. Cases distribution remained fairly constant between the studied years, occurring only a more pronounced increase between 2009 and 2010, and a slight and continuous decrease from 2010 to 2013 (Table 1).

Table 1: Occurrence of PVCA in the 5-year period

Year	Total cases: 9783	
	n	%
2009	1886	19.3%
2010	2213	22.6%
2011	1972	20.2%
2012	1893	19.3%
2013	1819	18.6%
Mean±SD	1956.6±153.25	

Mean victims' age was 13.9 ± 3.78 (minimum age=0 and maximum age=17). Table 2 displays victims' age distribution according sex. Most occurrences happened in ages 16 and 17 (50.9% in males and 46.5% in females) with significant differences between age groups and sex ($p \leq 0.001$) (Table 2). Males were most affected by violence (n=5447) in every age group except 12 to 14 years old. The most frequent offender overall were victims' acquaintances, but there were differences according the victim's sex ($p \leq 0.001$), with police officers being the most common aggressor in males (35.9%), and acquaintances in females (32.4%) (Table 3).
Public places were the most common locations where the events took place, for both sexes, representing 52.4% of all occurrences, with significant differences in the percentage of occurrences between sexes ($p \leq 0.001$) (Table 4).
A significant proportion of the victims did not receive medical care (84.8%), and the large majority (98.0%) reported it as being the first occurrence.
The region of the body most affected was the face (20.1%), and no statistical significant differences between sexes were found ($p \leq 0.001$) (Tables 5).

Table 2: Victims' age distribution according sex, n (%)

Age groups (in years)	Male	Female	Total
0	22(0.4)	16(0.4)	38(0.4)
1	47(0.9)	46(1.1)	93(1.0)
2	50(0.9)	44(1.0)	94(1.0)
3	63(1.2)	48(1.1)	111(1.1)
4	50(0.9)	48(1.1)	98(1.0)
5	59(1.1)	44(1.0)	103(1.1)
6	84(1.5)	43(1.0)	127(1.3)
7	75(1.4)	60(1.4)	135(1.4)
8	95(1.7)	55(1.3)	150(1.5)
9	109(2.0)	64(1.5)	173(1.8)
10	136(2.5)	101(2.3)	237(2.4)
11	157(2.9)	122(2.8)	279(2.9)
12	218(4.0)	240(5.5)	458(4.7)
13	302(5.5)	395(9.1)	697(7.1)
14	454(8.3)	541(12.4)	995(10.2)
15	752(13.8)	692(15.9)	1444(14.8)
16	1253(23.0)	880(20.2)	2133(21.8)
17	1521(27.9)	897(20.6)	2418(24.7)
TOTAL	5447(55.7)	4336(44.3)	9783(100)

Table 4: Place of occurrence according with sex, n (%)

Location	Male	Female	TOTAL
Public place	3406(62.5)	1725(39.8)	5131(52.4)
Home	782(14.4)	1216(28.0)	1998(20.4)
Not disclosed	672(12.3)	1035(23.9)	1707(17.4)
Institution	351(6.4)	119(2.7)	470(4.8)
School	236(4.3)	241(5.6)	477(4.9)
TOTAL	5447(55.7)	4336(44.3)	9783(100)

Table 3: Alleged offenders' distribution according sex of the victim, n (%)

Offenders	Male	Female	TOTAL
Acquaintance	1135(20.8)	1403(32.4)	2538(25.9)
Police officers	1956(35.9)	163(3.8)	2119(21.7)
Not disclosed	771(14.2)	1070(24.7)	1841(18.8)
Unknown to the victim	745(13.7)	408(9.4)	1153(11.8)
Mother	268(4.9)	280(6.5)	548(5.6)
Other family member	169(3.1)	303(7.0)	472(4.8)
Father	207(3.8)	201(4.6)	408(4.2)
Spouse	9(0.2)	215(5.0)	224(2.3)
Stepfather	89(1.6)	83(1.9)	172(1.8)
Boyfriend/girlfriend	14(0.3)	141(3.3)	155(1.6)
Stepmother	25(0.5)	36(0.8)	61(0.6)
Professor (teacher)	17(0.3)	21(0.5)	38(0.4)
Private security	30(0.6)	4(0.1)	34(0.3)
Other care giver	12(0.2)	6(0.1)	18(0.2)
Self-inflicted	0(0)	2(0)	2(0)
TOTAL	5447(55.7)	4336(44.3)	9783(100)

Table 5: Place of injury according with the victims' sex, n (%)

Injury site	Male	Female	TOTAL
Skull	912(9.5)	747(8.5)	1659(9.1)
Face	1698(17.7)	1975(22.5)	3673(20.1)
Neck	496(5.2)	662(7.6)	1158(6.3)
Shoulder	1047(10.9)	761(8.7)	1808(9.9)
Chest	812(8.5)	506(5.8)	1318(7.2)
Abdomen	336(3.5)	113(1.3)	449(2.5)
Pelvis	623(6.5)	251(2.9)	874(4.8)
Right upper limb	1157(12.1)	1238(14.2)	2395(13.1)
Left upper limb	1128(11.8)	1271(14.5)	2399(13.1)
Right lower limb	687(7.2)	584(6.7)	1271(6.9)
Left lower limb	671(7.0)	629(7.2)	1300(7.1)
TOTAL	9567(52.3)	8737(47.7)	18304(100)

DISCUSSION

The incidence of PVCA confirmed cases reported to the authorities increases every year[11]. For PVCA prevention, it is crucial to establish common parameters based on epidemiological information and scientific data. The availability of reliable information is an initial stage to develop or suggest changes to existing protection programmes[13].

PVCA is a real problem in today's society and reaches across different cultural, socio-economic, ethnic, and social groups. In Brazil, despite the increasing social awareness, the biggest challenge is still the full knowledge of this problem[14].

This study explores a series of reports of PVCA, aged 0–18 living in Refice, Brazil, recorded by the IMLAPC between 2009 and 2013.

It was noted that the prevalence of PVCA cases has had a very mild decrease, despite the slight increase observed in 2010, suggesting that the institutions connected to the PVCA have reported a similar number of cases, indicating that preventive measures are needed.

Males were more frequent victims, fact corroborated by several authors that state that temper and social exposure may explain these differences.[15,20] Moreover, in our study, victims were mainly male because a large number of cases involved assault by police officers, resulting from interventions and arrests, and young law offenders are, more frequently, male.[21] We acknowledge that some particular kinds of violence are female-orientated (e.g. sex crimes), but in physical violence, such a trend does not appear to exist.[15,16] The fact that our study concerned PVCA regardless of the context may explain our results.

The age of the children and adolescents' victims of PVCA grew in direct relation to the number of occurrences. Research has shown that rates of violence begin to increase in pre-adolescence or early adolescence.[19,22] Children are more dependent on their parents, while adolescents have greater autonomy and are more exposed to violence.[23]

In our study, victims' acquaintances were the most common offenders, followed by police officers. Our data agrees with other researchers. For instance, Souto et al. stated that in PVCA, regarding the offender, the highest frequency of reports involved friends.[24] It is admissible that after a violent event the involved, if friends in the past, call themselves acquaintances after the event.

Police officers were the most frequent offender of male victims, which may reflect temper and social exposure, for sex differences.[15,17,20]

Public places were the most common locations, representing 52.4% of the total number of occurrences, while the home environment was recorded as the second most common place. Mascarenhas et al. referred the home environment as the place where much of the violent events take place because, by staying longer in their homes, children end up being more often abused in these sites[25]. Yet, in this study only children younger than 10 years of age were involved, which may explain why the home environment was the most common place. In fact, recent research has shown that the largest number of aggressions in children under 12 years occurred at home and in adolescents above 13 years of age at public places.[11, 22 26,27]

Furthermore, it has been pointed out the public spaces (streets, and other public places) as important scenario for the occurrence of violent events involving males, while the majority of the violence against girls happened at home.[28] Our sample concerned mostly males, which can explain our results.

By seeking health care after physical aggression, victims have the documentary evidence of an assault, medical care being valuable proof. In our study, the percentage of PVCA victims who sought medical care was extremely low, in spite of the ease of access to health care provided by the Family Health Care program that provides one or more health units for each neighbourhood of the city.[29]

When a victim makes a complaint of aggression it is important to know if it was a first-time occurrence, as previous cases may draw attention to a continuous pattern of aggression by a perpetrator close to the victim.[16] Yet, it has been stated that in cases of domestic violence, usually there is no formal complaint [30]; furthermore, these cases are not reported because close parents, relatives or acquaintances, make it difficult for victims to report such violence.[31]

Our data concerning injuries agrees with that from previous reports, which state that the head (skull and face) and neck region were commonly affected by attacks.[15,20,32-34] Brink (2009)[32] estimates that 85% of acts of physical aggression against children affect the face, head, and neck.

Darche et al.[33] report a 78% prevalence rate of injuries involving the face and skull. According to Carvalho et al.,[34] Valente et al.,[15] and Cavalcanti[35] the face is the most exposed and the least protected part of the body and the region most associated with a variety of injuries. In our study, the face was the most affected area (20.1%). The face is most commonly struck due to the process of subjugation and humiliation inherent in the character of aggression while injuries to the arms, the second most frequent site of injury, are associated with the victims´ attempts to defend themselves from acts of aggression.[32] Dentists in their daily activity, play an important role in detecting violence, as injuries resulting from aggressions often reach their area of expertise (head, face and neck), these professionals are strategically placed to identify and report these cases.[33]
Some limitations can be expected due to problems of under reporting or data categorization. It would be useful to know in detail the type of face injuries involved, and eventually link them to a specific context, as it would be a valuable tool for differential diagnosis. Yet, the reports did not have that information, pointing out the need of having professionals with dental expertise to perform these examinations.

CONCLUSION

This 5-year study of PVCA reported in Recife, Brazil, from 2009 to 2014 showed that the majority of victims were males, and acquaintances were the main identified aggressors. Events took place mainly in public places and the body region most commonly was the face. The data points out the need to target public places as an arena for preventive and protective measures. Furthermore, it was a recognizable limitation of this study the lack of suitable information considering face injuries. Nonetheless, it is quite clear that policies towards PVCA prevention, diagnose and reporting must include dental professionals.

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The tongue protrusion in post-mortem fire

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ABSTRACT
Burned bodies raise relevant issues for forensic patologist and odontologist both for the identification and the cause of death and possible vital burning..
The tongue protrusion is regularly investigated for the death caused by strangulation or hanging, whilst seems to be overlooked in case of charred remains as the significance of this sign is still discussed.
Different mechanims are hypothized in literature to explain the toungue protrusion both for vital and non-vital burning. This paper retrospectively evaluates some cases of carbonized corpses examined at the Forensic Pathology service of the University of Florence. The tongue protrusion shows a high occurrence both in vital (100%) and non-vital fires (66%). The involvement of a forensic odontologist in the cadaver examination result to be limited to one third of the cases. In two non-vital cases the tongue was described as protruded and clenched between the dental arches. The rigor of the genioglossus induced by the heat could explain the phenomenon. Further research on fire fatalities is required to analyze the tongue bleeding as a possible parameter to discriminate the vital by the non-vital tongue protrusion. Moreover, the mechanism at the origin of vital and non-vital tongue protrusion, the different position of the tongue (protruded from an open mouth, protruded and clenched between the dental arches, etc.) in different death circumstances, should be furtherly investigated with a meaningful collaboration between forensic pathologists and odontologists for a complete registration and interpretation of all the mouth originated evidence.

INTRODUCTION

Burned bodies are a real challenge for forensic patologist and odontologist charged of the answer to the crucial question of the identification of the cause of death and possible ante-mortem (AM) exposition to the fire.[1,2]
According to previous literature, the most reliable signs of vitality include soot deposits in the respiratory or digestive tract, a blood level of carboxi-hemoglobin (COHb) or HCN.[3-6]
An unclear or inconclusive evidence of vitality is present in some cases due to the specific dynamic of fire (e.g. "flashfire").
The tongue protrusion is regularly investigated in case of death caused by strangulation or hanging, but tends to be overlooked in case of charred remains as the significance of

this sign in the determination of the cause of death or burning vitality is still discussed. Bernitz reported a significant prevalence of tongue protrusion in cases of vital burning and concluded for the evidence of a correlation between the survival time and a protruded tongue.[7] Hejna and Janik hypothesized the occurrence of a vital bleeding of the tongue[8], actually described in different kind of death (hanging, strangulation and burning), as a possible explanation for the protrusion. Bohnert and Hejna discussed cases of protruded tongue in non-vital fire and outlined alternative mechanisms for the vital and post-mortem tongue protrusion following fire exposition.[9]This paper retrospectively evaluates cases of charred bodies with tongue protrusion in case of vital or non-vital fire examined at the Forensic Pathology service of the University of Florence, Italy.

MATERIALS AND METHODS:

The sample consisted of thirty-four reports written by forensic pathologists charged by the Public Prosecutor to perform a judicial autopsy addressed to establish the cause of death and sometimes the identity of the subjects. No further documentation with the exception of the anonymous reports and the attached pictures was available to the authors and no further examinations were possibly obtained and conducted on the bodies other than those performed by the charged pathologists. The following data were collected from the reports examined:

1- age and gender of the individual
2- the extent of the burns (Table 1) that was assessed according to the Crow-Glassman Scale (CGS)
3- blood values of COHb and HCN
4- soot deposits in the respiratory or digestive tract
5- the involvement of a forensic odontologist in the case
6- the tongue protrusion

RESULTS

The age of the sample ranged from 5 to 90 years; the females individuals were 7 and the males 27. Of the 34 examined cases, only 11 reports included a detailed description of the tongue, whilst in 25 cases the tongue position was probably not detectable due to the severe destruction of the jaws or the tongue itself (e.g. charred bodies with open mouth).

The Tables 2-4 show the blood values of COHb, HCN and soot deposits in the digestive or respiratory tract in the cases with protruded tongue. Only in the 38% of the cases a forensic odontologist was involved in the examination. Even if the number of cases in which the position of the tongue was detectable or reported by the pathologist is small, the protrusion of the tongue was present in all the vital burnings; two in three post-mortem fire cases presented tongue protrusion. Hence, the tongue protrusion shows a high occurrence both in vital (100%) and non-vital fires (66%) in our limited sample.

Two badly burned cadavers were found in the ground after a post-mortem fire caused by an helicopter accident. The male bodies were identified as the pilot and passenger, respectively 61 and 54 years old. During the autopsy the forensic pathologist found severe traumatic lesions in both bodies (fractures of vertebrae, ribs, skull base, long bones, etc), and the tear of the aorta thoracic and abdominal tract respectively. The forensic pathologist concluded for immediate death following the precipitation. In both cases the tongue was described as protruded and clenched between the dental arches . The values of COHb and HCN were negative and no soots deposit were found in the respiratory or digestive tract. As shown in Table 1, both cadavers presented extended burns (CGS 4).

Table 1: Crow-Glassman Scale (CGS)

CGS-1	Death by inhalation of toxic fumes. First and second degree burns on the body. Visual identification is still possible.
CGS-2	Significant carbonization of the body. Possible mutilation of small bones of the hands and feet. Identification can be performed through dental records and /or DNA .
CGS-3	Significant carbonization of the body, with the skull still intact. Possible mutilation also of the limb bones. Possible identification through dental records and /or DNA.
CGS-4	Total fragmentation of the skull and further mutilation of the limb bones. Possible identification through dental records and /or DNA.
CGS-5	Skeletal remains. There are no remaining soft tissues and any remaining skeletal component is fragmented. The identification of the remains is highly problematic.

Table 2: Frequence of the tongue, vital signs, CGS and the intervention of a FOd

	Tongue protrusion	**No tongue protrusion**	**Tongue position not detectable**
Soot deposits	6	/	16
No soot deposits	4	1	5
COHb <10%	5	1	16
COHb >10%	5	/	7
CGS 1-2	2	1	9
CGS 3-5	8	/	14
Intervention of the forensic odontologist (FOd)	4	/	9

Table 3: Fire-fatalities. Correlation between tongue protrusion and cause of death

Death due to:	Tongue protrusion	No tongue protrusion	Tongue position not detectable
COHb - open place - closed place	 / 2	 / /	 / 11
HCN - open place - closed place	 / 1	 / /	 / 1
COHb and HCN	1	/	2
Fire- high temperature	4	/	4
Multiple factors	2	1	5

DISCUSSION

The tongue protrusion is a common finding in some deaths for asphyxiation (e.g., hanging, incomplete strangulation), and the compression of neck tissues and vessels is considered the cause of the phenomenon. A protruded tongue, clenched between the dental arches, can be frequently observed also in carbonized cadavers and the question if this sign could be considered an useful hint for the discrimination of vital and non-vital burning is still an unresolved matter of discussion in the Literature.[7-11] Different mechanisms have been hypothesized as the cause for the lingual protrusion in vital burning and post-mortem fire cases.

Bernitz found a correlation between the tongue protrusion and the occurrence of a vital burning.[7] According to his causal hypothesis, the protrusion is attributed to the laryngospam caused by a temperature higher than 150 °C and hyperventilation. Nikolic criticised Bernitz's conclusions saying that they were based on an incorrect statistical analysis and found a tongue protrusion in two post-mortem burning cases.

Bohnert and Hejna found that the tongue protrusion is not correlated to vital burning or heat-induced shrinkage of the cervical soft tissue.[9]

Different mechanics have been proposed to explain tongue protrusion in fire fatalities, among which the heat-induced shrinkage of cervical tissues, the heat-related vapor pressure,[10] focal hemorrhage and interstitial edema of the lingual tissues, [8] or the rigor of the genioglossus muscle caused by the high temperature.[11]

Some authors [Ishikawa et al 2018, Hashimoto et al 2003 and Quan et al 2003] focused their attention on the intramuscolar bleeding at the tongue base of charred cadavers [ImBT]: the proposed mechanic is linked to the cervical compression caused by an incomplete occlusion of the carotids, cranial venous stasis, spasm of the lingual muscles and hypertensive agony that would cause the intramuscolar bleeding of the tongue, similarly to what happens in some asphyctic deaths (e.g., incomplete, manual and ligature strangulation). Hashimoto and Ishikawa reported intramuscolar bleeding of the tongue in vital fire cases with low levels of COHb and severe charring of the body. In cases with insufficient blood level of COHb the proposed cause of death was the extensive carbonization of the cervical tissues that dramatically harden and compress the neck quite similarly to what happens in the abovementioned asphyctic deaths (e.g., strangulation). This could explain the occurrence of the intramuscolar bleeding reported in some vital carbonization cases. [12-14]

Because of the retrospective nature of the present study, the report is limited to 10 cases of tongue protrusion. Despite the small number of our sample, the occurrence of tongue protrusion was observed in all the cases of vital burning and in two of three cases of post-mortem fire. Unfortunately, in some of our cases the position of the tongue was not reported by the forensic

pathologist in charge of the autopsy and the involvement of a forensic odontologist is limited to very few cases. A collaboration between the forensic pathologist and an odontologist would have perhaps resulted in a most appropriate attention to the mouth not only for identification issues [15,16] but also for the possible contribution that the oral findings can give to the ascertainment of the cause of the death.
In the two cases of non-vital fire, the levels of COHb (less than 2%) and HCN were negative, no soot deposits were found and the presence of aortic and skull lesions, led to conclude for a death immediately following the helicopter crash. The carbonization of two bodies was extensive, the facial and neck tissue were severely destroyed by the fire, the tongue hemorrhage was not investigated during autopsy. Anyway, we can suppose that it would be likely absent, since the tongue bleeding requires an hypertensive agony and a vital burning that were excluded in the described cases. Among the different mechanic described in the literature as a cause of tongue protrusion in the perimortem period, mostly related to asphyctic deaths or vital burning, only the heat-induced retraction of the cervical tissues and the lowering of the mandible, seem to be considered in these two non-vital burning cases. Nikolic, in two post-mortem burning cases, described [11] a heat rigor in the cervical region as it occurs in the rest of the body due to the shortening of muscles and tendons, that causes relevant modifications of the position of the body (e.g., pugilistic position). The protrusion of the tongue is considered as a sort of lingual rigor due to the heat that causes a shortening of the genioglossus which, in a condition similar to a physiological activation, produce the thrust of the tongue out of the mouth.

CONCLUSION

A tongue protrusion is observed and reported in different types of death. Generally speaking, the position and modifications (especially bleeding) of the tongue are deeply investigated in asphyctic deaths (hanging, strangulation, etc), whilst these conditions are not so considered in fire casualties. A long discussion stands in the literature between authors that believe that the protrusion of the tongue can be considered an useful sign of vitality during a fire and those who criticized this assumption.
Our study is based on a limited number of cases, but reveals the occurrence of a protruded tongue in all the cases of vital fire, but also in two thirds of non vital fire, emerging as an aspecific sign possibly correlated with exposition to high heat regardless of its ante or post-mortem occurance. The mechanics of the lingual protrusion in vital and non-vital burning is still a matter of discussion. In cases of tongue protrusion in vital burning with lower COH levels similar mechanisms as in some types of asphyctic deaths are hypothesized to occur. In cases of non vital tongue protrusion a heat induced rigor of the genioglossus could explain the phenomenon. Further research on fire fatalities is required to analyze the tongue bleeding as a possible parameter to discriminate the vital by the non-vital tongue protrusion[12]. Moreover, the mechanism at the origin of vital and non-vital tongue protrusion, the different position of the tongue (protruded from an open mouth, protruded and clenched between the dental arches, etc.) in different death circumstances, should be furtherly investigated with a meaningful collaboration between forensic pathologists and odontologists for a complete registration and interpretation of all the mouth originated evidence.

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Predictive accuracy of Demirjian's, Modified Demirjian's and India specific dental age estimation methods in Odisha (Eastern Indian) population

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KEYWORDS

Chronological Age; Orthopantomograph; Demirjian's method; modified Demirjian's method; Acharya's Indian Formula; Age estimation

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ABSTRACT
This study is aimed at finding the predictive accuracy of Demirjian's (D), modified Demirjian's (MD) and India specific age estimation methods (AA) Indian specific age estimation methods in 522 healthy children of Odisha population among 3-18 years. Correlations between chronological age (CA) and derived age (DA) by above mentioned methods were evaluated by Wilcoxon signed rank test and Pearson's correlation analysis. Analysis of mean absolute error concluded that D and MD predicted the CA with fair accuracy, whereas, AA had lower accuracy in Odisha children. Odisha specific polynomial regression formula, derived in this study is showing a strong correlation with CA (r=0.84). Comparison of mean absolute error of D, MD, AA and Odisha specific method indicated a better predictive accuracy of Odisha specific method.

INTRODUCTION
Age is a significant intellectual, religious and social event. Chronological age (CA) of an individual should be derived from date of birth, which is conventionally confirmed by documents of birth registration. Surprisingly, birth registration is not followed stringently in many parts of the world. Globally, largest number of unregistered children belong to South Asia accounting up to 22.5 million which is more than 40% of the whole non-registered births in 2000 (1). Being recognized as an index of health, growth and development age is of great interest to physicians, dentists and anthropologists as well as forensic experts. In the context of uncertain date of birth, biological ages like skeletal age, morphological age, secondary sex character and dental age have been developed as maturity markers to estimate age (2). Tooth development may be a pragmatic measure of maturity and may serve as a maturity marker for any individual. Especially in growing children, dental-age evaluation is accepted as the most precise, dependable, and rapid technique to estimate age (3). There is maximum degree of association of CA with derived age (DA) than any other biological maturity markers as it is believed to be least affected by malnutrition (4).
Among various dental disciplines DA assessment becomes very essential for orthodontists to devise the treatment modalities of several kinds of malocclusion (2) and paedodontists to know stage of dental maturity of a child to identify the systemic disturbances (5). Age estimation is valuable in bio-archaeology and human anthropology too as it gives substantial information

regarding ancestors. In forensic dentistry, quite often there is a need to determine the age for various medico-legal purposes including personal identification (6-8).

Radiographic assessment among several methodologies of dental age is well-known because of the simplicity, non-invasiveness and reproducibility (9). Fundamentally, this technique noted the mineralization phases of teeth recognized in radiographs and coded them to the corresponding values presented in charts suggested by multiple studies in different populations. The objective of several population based studies on dental age assessment methods is attributed to the wide range of morphological variations among modern human populations with regards to chronology of crown and root formations as well as tooth calcifications (10, 11). Acharya (12) evaluated the age in an Indian population using Demirjian's method and derived a formula which suited an Indian population. The present study was an attempt to determine predictive accuracy of Demirjian's, modified Demirjian's and Acharya's DA assessment method in Odisha (Eastern India) children of age group 3-18 years.

MATERIALS AND METHODS:

A total of 522 (boys=251, girls=271) orthopantomographs along with birth certificates were collected from a cohort of children and adolescents of age group 3-18 years requiring orthopantomograph for treatment planning. Children with developmental anomalies, malnutrition and endocrine disorders, prematurely born children and children with birth defects were excluded from the study group. This cross-sectional study was approved by ethical Committee (Ref No/DMR/IMS-SH/SOA/16026), of Institute of Medical Sciences (IMS) and Sum Hospital, Siksha 'O' Anusandhan Deemed to be University Bhubaneswar (Odisha state, India). Date of birth deducted from date of orthopantomogram yielded CA of the child after which it was converted to the decimal age. For example, patients with ages ranging from 3.00 to 3.99were denoted as the 3-year old group.

While the age group of 7-16 has been selected in modified Demirjian's method, Demirjian had chosen 3-18 years in his study. Therefore the age group in this study has been confined to 3-18 years to obtain a fair comparison. A larger sample size was chosen to minimize the sampling error (Standard error = 4.5). The acceptable margin of error usually falls between 4% to 8% at 95% confidence interval.

Development stages of seven left mandibular permanent teeth were focused in Demirjian's method of age estimation. All teeth were rated on a scale 'A to H' comparing the tooth with Demirjian's calcification chart. The sum of the stages was converted into a maturity score using the tables and graphs provided in the original paper (2) which then was transformed into a DA.

In modified Demirjian's method, the third molar was taken into consideration. The radiograph was compared to tooth development chart and each tooth was assigned any one of the developmental stages from 0 to 9. Corresponding to the selected developmental stage, each tooth was given a numerical score as provided in the original paper. Eight numerical scores were obtained, which were added to attain a total maturity score (S) which was then substituted in Demirjian's formula to derive the DA.

In addition India-specific formula developed by Acharya was also tested on the Odisha sample. The procedure mentioned above for modified Demirjian's method was followed to calculate the total maturity score (S) value and DA was estimated using India-specific formula (12).

The data collected by each method were remodeled to a SPSS Version 21 statistical program to execute the statistical analysis. To observe the normality of distribution of data Kolmogorov-Smirnov test was applied which produced a significant result ($p < 0.05$). Therefore a non-parametric test, Wilcoxon signed rank test was employed. The significance of the difference between CA and DA was evaluated. A P-value of < 0.05 was accepted as statistically significant.

Taking total maturity score obtained by adding individual scores corresponding to developmental stages of tooth in x-axis and CA in y-axis a polynomial regression equation was derived. This formula was again applied in the given sample (n=522) to find out the accuracy of predicting age. Mean absolute error of estimating age by Demirjian's, modified Demirjian's, Acharya's method and the equation derived in this study were found out to compare the predictive accuracy.

Fifty radiographs were again scored according to all three methods by the same observer and another observer. The intra-class correlation coefficient analysis for intra-observer and inter-observer reliability expressed as r value presented in Table 1. This process has minimized non-sampling error.

Table I. Intra-observer and inter-observer correlation coefficient for reliability

Reliability	Methods		
	D	MD	AA
Intra (r value)	0.910-0.924 *	0.920-0.951 *	0.923-0.945 *
Inter (r value)	0.977-0.998 *	0.879-0.885*	0.913-0.959*

*- $p<0.05$

RESULTS

Reliability of the data was established upon the r-value nearing to one in both inter- and intra-class correlation coefficient analysis as presented in table number 1. Comparisons between the mean ages derived by the three studied methods and the mean CA showed that there was an overestimation by Demirjian's and Acharya's formula while the modified Demirjian's method underestimated all values (Table 2).

Pearson's correlation analysis (Table 3) showed a moderately strong correlation between CA and DA by Demirjian's (boys - r value = 0.854, p=0.000; girls - r value = 0.779, p=0.000) and modified Demirjian's methods (boys - r value = 0.860, p=0.000; girls - r value = 0.859, p=0.000). However age derived by Acharya's formula showed a lower correlation (r value = 0.385, p=0.000) with CA in boys and moderately strong correlation in females (r value = 0.718, p=0.000). Line diagram comparing mean differences of CA and DA by three methods in boys (Figure 1) and girls (Figure 2) indicated that in Demirjian's method the difference was very big in age group of 3, 4, 11 and 16-18 years whereas the difference is limited to 1-1.5 years in other age groups. The difference between CA and DA in modified Demirjian's method indicated that the difference of maximum 1.5 years till age group of 15 years after which the difference increases. Age derived by this method closely resembling the CA except in age group 16-18 years. The difference between CA and DA by Acharya's method showed that there was a big difference in the younger age group whereas this difference decreases as age advances. Because none of the three methods could accurately predict dental age in Odisha children of age group 3-18 years, there was a need to derive a polynomial regression equation that suits this population. Taking total maturity score obtained by adding individual scores corresponding to developmental stages of tooth in x-axis and CA in y-axis a polynomial regression equation was derived as follows.

For girls Age = $0.0026\ S^2 - 0.1876\ S + 9.9751$ (S- total maturity score) (correlation coefficient = 0.87, 95% confidence interval)

For boys Age = $0.001\ S^2 + 0.0349\ S + 2.952$ (S- total maturity score) (correlation coefficient = 0.84, 95% confidence interval).

Error of less than one year is regarded as good whereas an error rate of more than 2 years is regarded as inaccurate (12). The results (Table 4) indicated that Demirjian's and modified Demirjian's method having MAE > 1 could predict the DA with fair accuracy in Odisha children of 3-18 age group whereas Acharya's formula having MAE > 2 could predict dental age with lower accuracy in Odisha children of age group 3-18. Odisha specific method could predict age with better accuracy when compared to Demirjian's, modified Demirjian's and Acharya's method. The accuracy of prediction by Odisha specific method is in fact better in girls compared to boys.

Table 2. The mean age of boys (n = 251) and girls (271) calculated by different methods

	Boys (n = 251)			Girls (n=271)		
	Mean (±Std. Deviation)	Minimum	Maximum	Mean (±Std. Deviation)	Minimum	Maximum
CA	12.05 ±4.08	3.00	18.00	13.43 ± 3.48	5.00	18.00
DA-MD	11.50 ±3.67	2.00	16.00	12.32 ±2.96	3.00	16.00
DA-AA	14.73 ±3.12	10.00	19.00	15.22 ±3.41	9.00	20.00
DA-D	12.42 ±3.63	5.00	16.00	13.88 ±2.82	6.00	16.00

Table 3. Pearson correlation coefficient analysis: Correlation of CA age and dental age

		MD	AA	D
Male CA	Pearson correlation N	0.860 (0.000) 251	0.385 (0.000) 251	0.854 (0.000) 251
Female CA	Pearson correlation N	0.859 (0.000) 271	0.718 (0.000) 271	0.789 (0.000) 271

derived by all three methods for boys and girls in the age group 3-18 years
Correlation is significant at the 0.01 level (2-tailed).

Figure 1 – Line diagram showing comparison between mean differences of CA age and age derived by three methods in boys

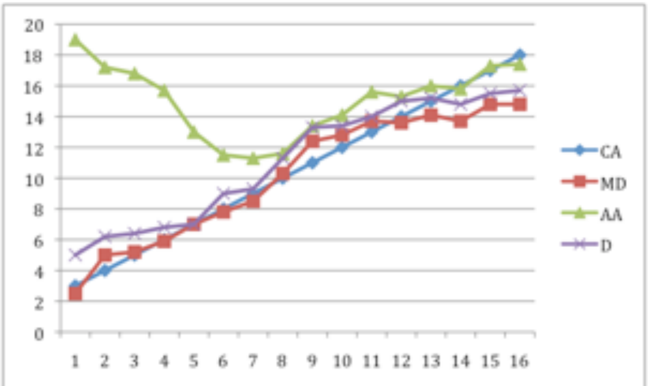

Figure 2 - Line diagram showing comparison between mean differences of CA age and age derived by three methods in girls.

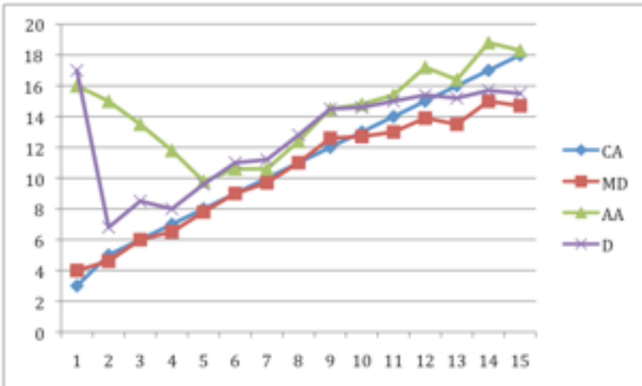

Table 4. Mean absolute error (MAE) of Demirjian's, modified Demirjian's, Acharya's India specific regression formula and Odisha specific age estimation methods.

	Boys (mean±std deviation)	Girls (mean±std deviation)
MD	1.64±1.4	1.7±1.5
AA	3.5±3.5	2.5±2.2
D	1.6±1.4	1.8±1.6
ODISHA	1.2±1.1	0.8±0.9

Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Methodologies for estimating an individual's growth and development are of great importance in fields like forensic odontology and anthropology. In spite of the controversial influence of systemic factors such as hormonal and nutritional status upon tooth development it is believed that this harmonizes well with the CA (6). Therefore, knowledge regarding the stages of tooth development has long been used for estimation of age. From the pre-natal era of tooth development till adulthood, between the ages of 2.5 to 18 years radiological method may be implemented for age estimation (13-15).

Conventionally, Demirjian popularized an age estimation method among individuals of all age groups (2) based on stages of development of all seven teeth in the left side mandible. Although this technique encouraged understanding the divergence of the dental maturity for an individual, it was not found to be accurate in every population (16). The modification of Demirjian's technique incorporated the third molar for applicability among a wider age-group and derived regression formulae for assessing age in French children. This method too in populations apart from the French demonstrated patterns of comparatively delayed or advanced dental development (7, 10, 17, 18). This motivated several authors to check the applicability of Demirjian's method and to advocate population specific standards. Meantime polynomial functions were introduced in forensic science by Chaillet and Demirjian in 2004 (16) which were found to be highly reliable. In fact application of standards formulated for European population in Indian population may lead to misrepresentations of the result. Therefore, with an objective of suitability of above two methods in Indian population Acharya (12) studied radiographs of predominantly south-western Indian population and derived an Indian regression formula. This Indian specific regression formula has been found to estimate the age variably in few parts of India (19, 20). A thorough literature analysis along with the result of this study indicates that the there is a need for population specific formulae to predict DA because of the considerable variation between and within populations (21). A thorough literature analysis demonstrated discrepancies in dental development within and among populations (22, 23).With the objective of checking suitability of above mentioned three DA estimation methods in Odisha (Eastern India) population we have conducted this study and found out that none of the three methods of age estimation can be applied suitably to Odisha population between age group of 3-18 years.

An overestimation of CA by 1.16 years and 1.37 years in boys and girls respectively by Demirjian's method as found in this study is in accordance with most of the studies conducted on various populations like Chinese (17), Iranian (24, 25), Turkish (22), Malayan (6, 26) and Tunisian (27). Even in India, study among various states has produced similar results (28-31). Overestimation of age ranged from 0.14 years (29) to 3.04 years (28) in boys and 0.04 years (29) to 2.82 years (28) in girls among Indians. Contrary to this study Demirjian's method underestimated CA in Kuwaiti (32), Turkish (33), North Chinese (34) and appeared to be accurate in Norwegian children (35). This method was also found to be accurate in school going children of few states of India (37-39). Modified Demirjian's method was found to underestimate the CA by 0.55 (±2.09) years and 1.08(±1.82) years for boys and girls respectively. With a handful of studies applying this method overestimation of CA was observed in population of Sydney (36), Hungary (41) and Tibet (37) whereas underestimation of CA by modified Demirjian's method was the conclusion in a few Indian populations (38-40).

In the present study, Acharya's India-specific regression formula over estimated dental age by 2.73 (+4.08) years in boys and 1.85(+2.62) years in girls. Overestimation of age by AA method was also found in studies conducted in Chennai (20) south Indian, (38, 39, 41) and Haryana, north India (19) population. Overestimation ranged from 0.21 years (39)to 1.72 years (38) in boys and 0.47 years (41) to 1.91 years (38) in girls. However AA method was found to underestimate the age in Gujarati population though the lower value of mean absolute error allowed its applicability (42). Of importance is the finding that as age increases the accuracy of prediction by Acharya's method increases. This finding was in concordance with that of Khorate et al (40) who conducted a study in Goan population and reported that for age group from 10.01 to 19 years the mean absolute error was less than 1.5 year. For individuals under seven years Acharya's age estimation methods over estimated with mean absolute errors panning from 10.9 to 17.62. Acharya in his study (12) in a south-western Indian population has applied the India specific regression formula to the wider age group of 7-25 years whereas Acharya's method has been applied to an age group of 3-18 years in this study. Therefore this Odisha specific regression formula may be considered as a modification of Acharya's formula for the age group 3-18 years of Indian population. However this can only be confirmed with further application of this formula in other Indian populations aged 3-18 years.

Efficacy of age prediction is represented by the mean absolute error (MAE) (43, 44) which is calculated by subtracting the CA from the derived dental age. An error of less than one year is accepted as good whereas an error of more than two years is regarded as inaccurate (12). Because Demirjian's and modified Demirjian's methods resulted in mean absolute error <2 years, it may be concluded that these two methods may predict the dental age with fair accuracy While Acharya's method having an error rate of >2 may not predict the age accurately the formula derived in this study with MAE 1.2 in boys and 0.8 in girls may predict age more accurately especially in children younger than seven years. A close comparison between the age derived by modified Demirjian and this study in both genders may indicate a close comparison of maturity standard of French and Odisha children with slight advanced dental development in

Odisha children. Most of the researchers have in fact supported the delayed growth in French children when compared to other populations (45-47).

The possible reasons which can explain this variability in predicting dental age could be attributed to variations in ethnicity, dietary habits, environmental factors, socio-economic status, that differ in various population groups and most importantly reasonable time difference between studies on the development of tooth among these children. Consequence of malnutrition on tooth development remains controversial with inconsistent conclusions suggesting a significant impact (48, 49) as well as no impact (4, 50). Supporting the earlier studies (51,52), the present study is demonstrating that girls are ahead of boys in tooth development in Demirjian's and Modified Demirjian's method but not in Acharya's study owing to sexual dimorphism during mineralization of teeth. Sexual dimorphism is defined as the differences among both genders in regards to biology. In humans, the sexual dimorphism is minimally appreciable before the onset of puberty. At puberty because of surge of hormones modifications occur in the skeletal system and beginning of secondary sexual characteristics, such as the widening of the pelvis in females. However our study has shown early development of tooth in girls compared to boys for the 3-18 years age group.

CONCLUSION

Digital orthopantomographs offer the advantage of accurate estimation of developmental stages of teeth precision accuracy of which are dependent upon very few variables like age distribution, sample size and statistical approach. To conclude, for the dental age estimation, stage of calcification may be a better indicator and population specific polynomial regression formulae should be derived through population specific standards of tooth development. There may be incorporation of all population specific standards in a common database which may be used for determining DA in forensic as well as anthropological requirements. Having a statistically significant correlation with chronologic age Odisha specific DA estimation formula derived in this study may appropriately be used to estimate DA in Odisha population aged 3-18 years. This study also offers new avenue for further research with an objective to test this method in other Indian populations aged 3-18 years.

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The applicability of the Demirjian, Willems and Chaillet standards to age estimation of 5-15 year old Indian children

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KEYWORDS

Age estimation, Demirjian's method, Willems' method, Chaillet's method, Indian children

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ABSTRACT

Background: Demirjian's method of age estimation has been reported to overestimate age and Willems' method to give consistently more accurate results. Not enough, however, is known about the applicability of Chaillet's standards.
Aim: The present study aimed to compare the accuracy of Demirjian's, Willems' and Chaillet's standards in age estimation of 5 to 15 year-old Indian children.
Design: In this cross-sectional observational study, three methods were compared for accuracy in estimating the age of 1200 Indian children aged 5-15 years.
Results: Demirjian's method overestimated age by +0.24 ± 0.80 years, +0.11 ± 0.81years and +0.19 ± 0.80 years in boys, girls and the total sample, respectively. With Willems' method, overestimations of +0.09 ± 0.80 years, +0.08 ± 0.80 years and +0.09 ± 0.80 years were obtained in boys, girls and the total sample, respectively. Chaillet's method underestimated age by -0.12 ± 0.69 years, -0.45 ± 0.88 years and -0.25 ± 0.83 years in boys, girls and the total sample, respectively. Statistically significant differences were observed between dental and chronological ages with all methods ($p < 0.001$). Significant sex-based differences were observed only with Demirjian's and Chaillet's methods ($p < 0.05$).
Conclusion: Willems' method was the most accurate in age estimation, followed by Demirjian's and Chaillet's methods. While Demirjian's method was more accurate than Chaillet's in females, Chaillet's method better predicted the age of males.

INTRODUCTION

First proposed more than four decades ago, the Demirjian method[1] has emerged as the most widely researched and applied technique in dental age (DA) estimation of children and adolescents. This is owing largely to the simplicity of the method, as well as the radiographic and schematic illustrations of tooth development and accompanying description, which the original[1] and subsequent works[2] provided. However, Demirjian's French-Canadian standards have been reported to consistently overestimate age in most populations, including a Belgian-Caucasian population studied by Willems and co-workers,[3] which highlighted the need for population-specific databases. In order to avoid this overestimation, these latter researchers adapted Demirjian's maturity scores using a weighted ANOVA and constructed new tables for boys and girls, from which a maturity score could be directly expressed in years. The Willems method is increasingly recognized as a more accurate predictor of age in recent years. Chaillet and co-workers,[4] adapted Demirjian's scores for the same Belgian population using polynomial functions and constructed another set of tables which they observed to be more accurate for this population. The dental literature abounds with reports[4-18] on the applicability of the Demirjian method and its variations to non-Indian and Indian populations. Extensive research[7,8,11,14,16-20] has been carried out using the Willems method as well. However, previous research on Indian populations is limited by inadequate sample sizes, age structures, grouping and approaches to statistical analysis.[16-19] Tests of the application of the Chaillet standards to non-Indian populations are few,[4,5,21-23] and while one study[24] did test these standards in an Indian population, the sample size was relatively small, the age range was 9 to 20 years and the Demirjian 8-teeth method was used for dental staging. Hence, the present study proposed to evaluate and compare the validity of the Demirjian, Willems and Chaillet standards in determination of chronological age (CA) of a reasonably large sample of 1200 Indian children aged 5 to 15 years.

METHODOLOGY:

This study was designed as a cross-sectional observational study. Ethical clearance was obtained from the Ethical Committee, Pacific Dental College and Hospital, Udaipur, India. Parents/ guardians had signed an agreement with the dental institution that dental records and radiographs could be used only for research and educational purposes without the possibility of personal identification.

Sampling method: A convenience sampling method was employed, all radiographs being digital and made during the period from January 2012 to September 2015 of children aged between 5.0 and 15.9 years who had sought treatment at the Department of Paediatric Dentistry, Pacific Dental College and Hospital, Udaipur, Rajasthan, India, and required an orthopantomograph (OPG) as part of the investigation protocol.

Selection criteria: Both parents of all children were of Indian origin and nationality. Only patients with a documented date of birth and date of radiography in the oral health record were included to facilitate verification of the chronological age (in completed decimal years) for each subject. Orthopantomographs with image distortion due to improper position or movement of the patient during exposure, and incomplete image or lack of clarity resulting from an improper exposure technique were excluded. Also, radiographs were excluded from the study if the patient had any history of surgical/medical treatment or systemic illness with the potential to cause significantly delayed or early development, significant numbers of teeth other than third molars missing either congenitally or due to disease and trauma, or malformation of teeth or obvious dental pathology that could affect tooth development.

Final sample: Of the 1303 radiographs collected, 103 did not meet the selection criteria owing to either congenital absence of several teeth (n = 22), lack of image clarity (n = 08) or inadequate information regarding the date of birth (n = 73). Thus, a final sample of 1200 OPGS of 699 male and 501 female Indian children aged 5 to 15 years was selected for the study. The distribution of radiographs by age and sex is presented in Table 1. Radiographs of patients aged 5.0 to 5.9 years were included in age group 5, of those aged 6.0 to 6.9 years in age group 6 and so on. Thus, age group 15 consisted of children aged 15.0 to 15.9 years.

Table 1: Distribution of the study sample by age and gender

Chronological age (years)		Females		Males		Total	
Age group	Age range	N	%	N	%	N	%
5	5.0 - 5.9	24	4.79	23	3.29	47	3.92
6	6.0 - 6.9	39	7.78	40	5.72	79	6.58
7	7.0 - 7.9	46	9.18	58	8.30	104	8.67
8	8.0 - 8.9	50	9.98	58	8.30	108	9.00
9	9.0 - 9.9	55	10.98	78	11.16	133	11.08
10	10.0 - 10.9	55	10.98	100	14.31	155	12.92
11	11.0 - 11.9	40	7.98	82	11.73	122	10.17

Calculation of chronological age: The dates of birth and of making the OPG were obtained from the hospital records. A function of Microsoft Excel was used to calculate the difference between the recorded date of birth and the date on which the OPG was made, to obtain the chronological age (CA) in decimal years.

Data collection: All digital radiographs meeting the selection criteria were saved as jpeg images and viewed on the same LCD monitor using Windows Photo Viewer and a magnifying glass for improved visualization. The examiners were allowed to use resources of image improvement such as brightness, contrast and zoom. Each OPG was coded with a numerical ID to avoid examiner bias. Age and sex of the subjects were thus unknown to the examiner. Nomenclature for teeth assessed was assigned according to the Fédération Dentaire Internationale (FDI) system. Seven mandibular teeth of the left side (excluding the third molar) were evaluated by the Demirjian's dental staging method.[1] Once the stage that most accurately described the stage of development of the tooth in question was identified, the corresponding alpha-numeric rating (0 to H) was assigned to that tooth. The alpha-numeric stages 0 to H were converted to the revised self-weighted gender-specific numerical scores of Demirjian and Goldstein.[2] The individual scores for were summed to obtain a total maturity score or dental score (DS), which was converted to a dental age (DA) using the Demirjian, Tanner and Goldstein tables.[1]

In Chaillet's method, the maturity scores provided by Chaillet et al.[4] were summed to obtain the total maturity score, which was converted to a dental age using the tables constructed by the same authors, while in the Willems method, the maturity scores provided by Willems et al.[3] for each tooth were summed to directly provide the dental age in years.

Reproducibility of measurements: Two well-trained examiners independently evaluated 100 radiographs using Demirjian's method of dental staging, after a period of mutual calibration without any knowledge of age or sex, in order to allow an analysis of inter-examiner agreement. Ultimately, a single examiner assessed all radiographs. Intra-examiner agreement was assessed by having one examiner re-evaluate the same 100 radiographs after a period of two months without any knowledge of sex or age or of the stages assigned in the first evaluation.

Data analysis: All statistical analyses and data management were performed using Statistical Package of Social Sciences 19.0 (SPSS Inc., Chicago, IL, USA) for Windows and MS-Excel (Microsoft Office 2010). Analyses were made for each sex and age group, and for the total sample. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test the normality of the data. As the sample showed a non-normal distribution, non-parametric tests were applied. For all tests, a p value $\leq$ 0.05 was considered statistically significant.

The accuracy of each method of age estimation was determined by mean difference between estimated dental age and the chronological age (DA–CA) for each sex and age group, and the total sample. A positive result indicated an overestimation, and a negative result indicated an underestimation of age. Box-plot graphs are used to present the mean DA-CA of each sex and age group, and the total sample, with whiskers indicating the range. Absolute accuracy was determined by means of the absolute differences between DA and CA of girls and boys and the total sample for each method. The Wilcoxon signed rank test was applied to assess the significance of DA-CA for both methods for each sex and age group, for the total sample and between methods. Independent t-test was employed for comparisons of DA-CA between sexes. The correlation between DA and CA was analysed using Spearman's rank correlation coefficient for each sex and for the total study sample. Inter- and intra-examiner agreements are expressed as percentages. Cohen's kappa coefficient was used to calculate the degree of reliability of these agreements. Regression analyses were performed and gender-specific equations were derived for all three methods.

RESULTS

The mean age (± SD) of the entire sample was 10.75 ± 2.72 years, those of girls and boys being 10.68 ± 2.87 and 10.81 ± 2.60, respectively. Inter- and intra-examiner agreements were 86% and 93% respectively, with Kappa values of 0.81 and 0.90 indicating almost perfect agreement.

In the present study, the mean chronological and Demirjian dental ages for girls were 10.68 ± 2.87 and 10.79 ± 2.86 years, respectively, while for boys the values were 10.81 ± 2.60 and 11.05 ± 2.71 years, respectively. The mean differences between dental and chronological ages for boys, girls and the total sample (+0.24 ± 0.80, +0.11 ± 0.81 and

+0.19 ± 0.80 years, respectively) were statistically significant ($p < 0.01$). Significant differences between mean dental and chronological ages were observed in age groups 6, 8, 9, 12, 14 and 15 for girls and 6, 7, 8, 10, 11, 14 and 15 for boys ($p \leq 0.05$). In girls, Demirjian's method overestimated age by +0.06 to +0.54 years in all age groups, with the exception of groups 8 and 14, for which underestimations of -0.21 and -0.37 years, respectively, were obtained. In boys, overestimations ranged from +0.15 to + 0.59 years in most age groups, with underestimations of -0.13 and -0.02 years for groups 5 and 12, respectively (Table 2).

Table 2: Comparison of chronological age (CA) and Demirjian dental age (DA) by gender and age group

Gender	Age group (years)	N	CA	DA	DA-CA	p value*	p value#
			Mean ± SD (years)				
GIRLS	5	24	5.46 ± 0.33	5.53 ± 0.58	+ 0.07 ± 0.43	0.543	0.846
	6	39	6.57 ± 0.32	7.11 ± 0.58	+ 0.54 ± 0.38	**< 0.001**	**< 0.001**
	7	46	7.52 ± 0.26	7.65 ± 0.67	+ 0.13 ± 0.47	0.097	0.167
	8	50	8.51 ± 0.31	8.30 ± 0.73	- 0.21 ± 0.44	**0.049**	**0.048**
	9	55	9.48 ± 0.30	9.84 ± 0.77	+ 0.36 ± 0.43	**0.001**	**0.001**
	10	55	10.55 ± 0.32	10.72 ± 0.85	+ 0.17 ± 0.52	0.173	0.101
	11	40	11.44 ± 0.32	11.58 ± 0.84	+ 0.14 ± 0.54	0.352	0.357
	12	55	12.49 ± 0.32	12.75 ± 0.87	+ 0.26 ± 0.53	**0.048**	**0.049**
	13	57	13.46 ± 0.30	13.52 ± 0.83	+ 0.06 ± 0.50	0.607	0.502
	14	59	14.48 ± 0.28	14.11 ± 0.62	- 0.37 ± 0.39	**< 0.001**	**< 0.001**
	15	21	15.48 ± 0.27	15.73 ± 0.51	+ 0.25 ± 0.26	**0.024**	**0.023**
	Total	501	10.68 ± 2.87	10.79 ± 2.86	+ 0.11 ± 0.81	**0.003**	**0.002**
BOYS	5	23	5.56 ± 0.29	5.43 ± 0.98	- 0.13 ± 0.42	0.551	0.503
	6	40	6.52 ± 0.31	7.05 ± 0.78	+ 0.53 ± 0.44	**< 0.001**	**< 0.001**
	7	58	7.48 ± 0.29	7.72 ± 0.67	+ 0.24 ± 0.46	**0.015**	**0.004**
	8	58	8.47 ± 0.29	8.89 ± 0.61	+ 0.42 ± 0.97	**< 0.001**	**< 0.001**
	9	78	9.46 ± 0.28	9.61 ± 0.82	+ 0.15 ± 0.45	0.111	0.069
	10	100	10.45 ± 0.29	10.76 ± 0.84	+ 0.31 ± 0.47	**< 0.001**	**< 0.000**
	11	82	11.51 ± 0.30	11.66 ± 0.70	+ 0.15 ± 0.37	**0.035**	**< 0.001**
	12	91	12.44 ± 0.30	12.42 ± 0.74	- 0.02 ± 0.45	0.705	0.541
	13	82	13.41 ± 0.31	13.56 ± 0.78	+ 0.15 ± 0.36	0.088	0.106
	14	58	14.47 ± 0.31	15.06 ± 0.58	+ 0.59 ± 0.46	**< 0.001**	**< 0.001**
	15	29	15.24 ± 0.25	15.80 ± 0.40	+ 0.56 ± 0.35	**< 0.001**	**< 0.001**
	Total	699	10.81 ± 2.60	11.05 ± 2.71	+ 0.24 ± 0.80	**< 0.001**	**< 0.001**
Total sample		1200	10.75 ± 2.72	10.94 ± 2.78	+ 0.19 ± 0.80	**< 0.001**	**< 0.001**

*Paired t test, #Wilcoxon Signed Rank test: $p \leq 0.05$ = significant

The mean chronological and Willems' dental ages for girls were 10.68 ± 2.87 and 10.76 ± 3.04 years, respectively, while for boys the values were 10.81 ± 2.60 and 10.90 ± 2.66 years, respectively. The mean differences between dental and chronological ages for boys, girls and the total sample (+0.09 ± 0.80, +0.08 ± 0.80 and +0.09 ± 0.80 years, respectively) were statistically significant ($p < 0.05$). Significant differences between mean dental and chronological ages were observed in age groups 6, 11, 13 and 14 for girls and 8, 10, and 15 for boys ($p \leq 0.05$). In girls, Willems' method overestimated age by +0.01 to +0.61 years in most age groups, while underestimating age by -0.02 to -0.25 years, in groups 7, 8, 10 and 13. In boys, overestimations ranged from +0.01 to +0.35 years in all age groups, with the exception of age group 12 for which an underestimation of -0.10 years was obtained (Table 3).

Table 3: Comparison of chronological age (CA) and Willems' dental age (DA) by gender and age group

Gender	Age group	N	Mean age ± SD (years)		Mean DA-CA (years)	p value#
			CA	DA		
GIRLS	5	24	5.46 ± 0.33	5.47 ± 0.86	+ 0.01 ± 0.84	0.964
	6	39	6.57 ± 0.32	6.81 ± 0.70	+ 0.24 ± 0.64	**0.033**
	7	46	7.52 ± 0.26	7.39 ± 0.78	- 0.13 ± 0.75	0.191
	8	50	8.51 ± 0.31	8.46 ± 0.73	- 0.05 ± 0.63	0.765
	9	55	9.48 ± 0.30	9.52 ± 0.78	+ 0.04 ± 0.79	0.811
	10	55	10.55 ± 0.32	10.53 ± 0.81	- 0.02 ± 0.85	0.694
	11	40	11.44 ± 0.32	11.73 ± 0.89	+ 0.29 ± 0.84	**0.032**
	12	55	12.49 ± 0.32	12.53 ± 0.83	+ 0.04 ± 0.88	0.744
	13	57	13.46 ± 0.30	13.21 ± 0.71	- 0.25 ± 0.70	**0.018**
	14	59	14.48 ± 0.28	15.09 ± 0.93	+ 0.61 ± 0.93	**< 0.001**
	15	21	15.48 ± 0.27	15.63 ± 0.37	+ 0.15 ± 0.42	0.126
	Total	501	10.68 ± 2.87	10.76 ± 3.04	+ 0.08 ± 0.80	**0.049**
BOYS	5	23	5.56 ± 0.29	5.74 ± 0.73	+ 0.18 ± 0.73	0.301
	6	40	6.52 ± 0.31	6.62 ± 0.77	+ 0.10 ± 0.84	0.183
	7	58	7.48 ± 0.29	7.62 ± 0.78	+ 0.14 ± 0.73	0.153
	8	58	8.47 ± 0.29	8.69 ± 0.69	+ 0.22 ± 0.76	**0.043**
	9	78	9.46 ± 0.28	9.56 ± 0.88	+ 0.10 ± 0.90	0.410
	10	100	10.45 ± 0.29	10.65 ±0.82	+ 0.20 ± 0.80	**0.015**
	11	82	11.51 ± 0.30	11.53 ± 0.75	+ 0.02 ± 0.75	0.705
	12	91	12.44 ± 0.30	12.34 ± 0.70	- 0.10 ± 0.67	0.145
	13	82	13.41 ± 0.31	13.42 ± 0.76	+ 0.01 ± 0.81	0.891
	14	58	14.47 ± 0.31	14.54 ±0.68	+ 0.07 ± 0.75	0.553
	15	29	15.24 ± 0.25	15.59 ±0.76	+ 0.35 ± 0.82	**0.048**
	Total	699	10.81 ± 2.60	10.90 ± 2.66	+ 0.09 ± 0.80	**0.002**
Total sample		120	10.75 ± 2.72	10.84 ± 2.83	+ 0.09 ± 0.80	**< 0.001**

#Wilcoxon Signed Rank test: $p \leq 0.05$ = significant

The mean chronological and Chaillet's dental ages for girls were 10.68 ± 2.87 and 10.23 ± 2.83 years, respectively, while for boys the values were 10.81 ± 2.60 and 10.69 ± 2.68 years, respectively. The mean differences between dental and chronological ages for boys, girls and the total sample (-0.45 ± 0.88, -0.12 ± 0.69 and -0.25 ± 0.83 years, respectively) were statistically significant ($p < 0.001$). Significant differences between mean dental and chronological ages were observed in age groups 8, 10, 11 and 12 for girls and 5, 6, 8 and 14 for boys ($p < 0.05$). In girls, the Chaillet method underestimated age by -0.04 to -0.93 years in all age groups, except group 5 for which an overestimation of +0.20 years was obtained. In boys, underestimations ranged from -0.01 to -0.36 years in all age groups, while overestimating age by +0.06 to +0.29 years in group 5, 8 and 14 (Table 4).

Table 4: Comparison of chronological age (CA) and Chaillet dental age (DA) by gender and age grou

Gender	Age group (years)	N	CA	DA	DA-CA	p value#
			Mean ± SD (years)			
GIRLS	5	24	5.46 ± 0.33	5.66 ± 0.84	0.20 ± 0.64	0.308
	6	39	6.57 ± 0.32	6.53 ± 0.67	- 0.04 ± 0.30	0.613
	7	46	7.52 ± 0.26	7.12 ± 0.94	- 0.40 ± 0.67	0.062
	8	50	8.51 ± 0.31	8.00 ± 1.04	- 0.51 ± 0.60	**0.005**
	9	55	9.48 ± 0.30	9.17 ± 0.66	- 0.31 ± 0.53	0.384
	10	55	10.55 ± 0.32	10.11 ± 0.73	- 0.44 ± 0.63	**0.002**
	11	40	11.44 ± 0.32	10.88 ± 0.63	- 0.56 ± 0.73	**0.004**
	12	55	12.49 ± 0.32	11.56 ± 0.69	- 0.93 ± 0.91	**0.001**
	13	57	13.46 ± 0.30	12.64 ± 0.96	- 0.82 ± 1.17	0.893
	14	59	14.48 ± 0.28	14.35 ± 0.99	- 0.13 ± 1.06	0.150
	15	21	15.48 ± 0.27	14.69 ± 0.70	- 0.79 ± 0.90	0.476
	Total	501	10.68 ± 2.87	10.23 ± 2.83	- 0.45 ± 0.34	**0.005**
BOYS	5	23	5.56 ± 0.29	5.85 ± 0.62	0.29 ± 0.71	**0.001**
	6	40	6.52 ± 0.31	6.48 ± 0.88	- 0.04 ± 0.59	0.097
	7	58	7.48 ± 0.29	7.47 ± 0.86	- 0.01 ± 0.25	0.614
	8	58	8.47 ± 0.29	8.53 ± 0.80	0.06 ± 0.37	**0.008**
	9	78	9.46 ± 0.28	9.32 ± 0.95	- 0.14 ± 0.58	0.801
	10	100	10.45 ± 0.29	10.22 ± 0.89	- 0.23 ± 0.62	0.182
	11	82	11.51 ± 0.30	11.38 ± 0.79	- 0.13 ± 0.65	0.504
	12	91	12.44 ± 0.30	12.17 ± 0.84	- 0.27 ± 0.77	0.832
	13	82	13.41 ± 0.31	13.05 ± 0.91	- 0.36 ± 0.86	0.825
	14	58	14.47 ± 0.31	14.71 ± 0.92	0.24 ± 0.92	**0.007**
	15	29	15.24 ± 0.25	15.18 ± 0.69	- 0.06 ± 0.91	0.795
	Total	699	10.81 ± 2.60	10.69 ± 2.68	- 0.12 ± 0.20	**< 0.001**
Total sample		1200	10.75 ± 2.72	10.50 ± 2.75	- 0.25 ± 0.32	**< 0.001**

#Wilcoxon Signed Rank test: $p \leq 0.05$ = significant

The lowest mean DA-CA was obtained with the Willems method, followed by the Demirjian and Chaillet method. Significant sex-based differences were observed in mean DA-CA with the Demirjian and the Chaillet methods ($p = 0.005$ and $p < 0.0001$, respectively) but not the Willems method ($p > 0.05$). With the Demirjian method the mean DA-CA was significantly lower in girls than in boys, whereas, the reverse was true with the Chaillet's method (Table 5).

When the Spearman correlation coefficient test was performed for girls, boys and the total sample, strong linear correlations were observed between CA and DA for all methods, r values ranging from 0.875 to 0.966 and p values < 0.001 (Table 6). An inter-method comparison of mean DA-CA values revealed statistically significant ($p < 0.05$) differences in girls, boys and the total sample (Table 7).

Table 5: Intra-method comparison between genders of mean dental and chronological ages (DA-CA)

Gender	N	Demirjian		Chaillet		Willems	
		Mean DA-CA ± SD (years)	p value	Mean DA-CA ± SD (years)	p value	Mean DA-CA ± SD (years)	p value
Girls	501	+ 0.11 ± 0.81	**0.005**	- 0.45 ± 0.34	**<0.001**	0.08 ± 0.80	0.830
Boys	699	+ 0.24 ± 0.80		- 0.12 ± 0.20		0.09 ± 0.80	

Independent t-test; p≤ 0.05 = significant

Table 6: Correlation between chronological and dental ages by method

Method	r / p values	Females	Males	Total sample
Demirjian	r value	0.957	0.962	0.961
	p value	**< 0.001**	**< 0.001**	**< 0.001**
Chaillet	r value	0.837	0.877	0.841
	p value	**< 0.001**	**< 0.001**	**< 0.001**
Willems	r value	0.966	0.959	0.962
	p value	**< 0.001**	**< 0.001**	**< 0.001**

Spearman's rank correlation coefficient: r = Spearman's rho, p = significant

Table 7: Inter-method comparison of mean dental and chronological ages (DA-CA) by gender and age group

	Method with mean DA-CA		Difference in mean DA-CA	95% CI of DA-CA	Absolute difference	p value#
	(years ± SD)			Years		
Females (N = 501)	Demirjian	Willems	-0.03 ± 0.80	0.05 to 0.16	0.61	**< 0.001**
		Chaillet	-0.56 ± 0.85	0.48 to 0.62	0.62	**< 0.001**
	Willems	Chaillet	0.53 ± 0.84	-0.53 to -0.40	0.65	**< 0.001**
Males (N = 699)	Demirjian	Willems	-0.15 ± 0.80	0.09 to 0.16	0.56	**< 0.001**
		Chaillet	-0.36 ± 0.75	0.05 to 0.14	0.58	**0.012**
	Willems	Chaillet	0.21 ± 0.75	-0.16 to 0.44	0.56	**< 0.001**
Total (N = 1200)	Demirjian	Willems	-0.10 ± 0.80	0.03 to 0.26	0.58	**< 0.001**
		Chaillet	-0.44 ± 0.83	-0.20 to 0.54	0.60	**< 0.001**
	Willems	Chaillet	-0.34 ± 0.82	-0.34 to -0.19	0.62	**< 0.001**

#Wilcoxon Signed Rank test: p ≤ 0.05 = significant

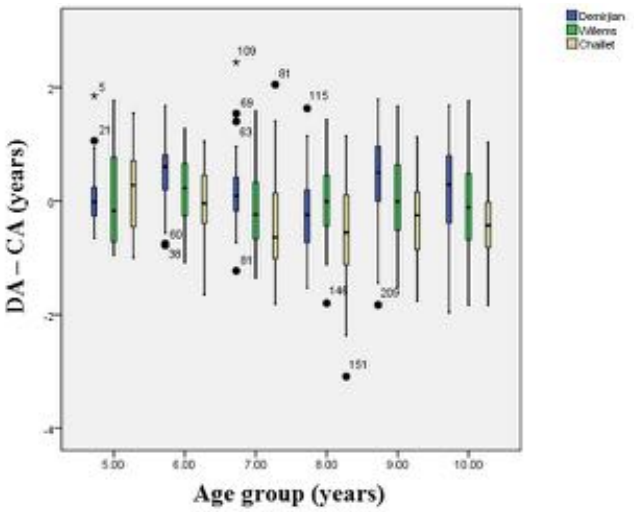
Box-plot graphs 1-5 present the mean DA-CA of each sex and age group, and the total sample,with whiskers indicating the range.Regression analyses were performed and the following equations were derived:

For the Demirjian method:
Males: CA = 0.914 + 0.883 × DA
Females: CA = 0.316 + 0.961 × DA

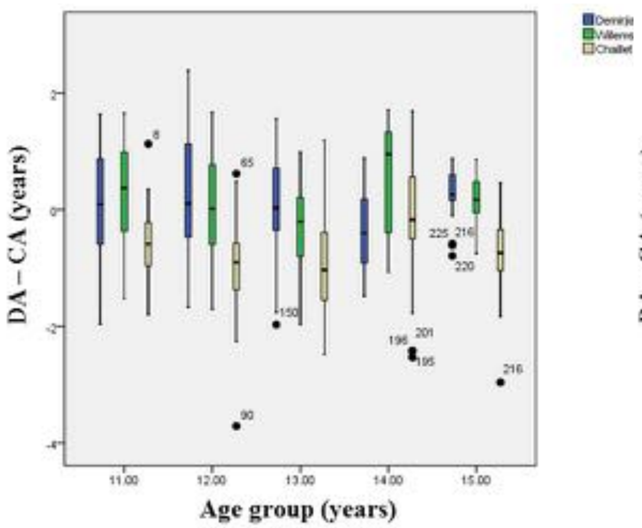
For the Willems method:
Males: CA = 0.622 + 0.934 × DA
Females: CA = 0.894 + 0.909 × DA

For the Chaillet method:
Males: CA = 2.104 + 0.791 × DA
Females: CA = 0.768 + 0.969 × DA

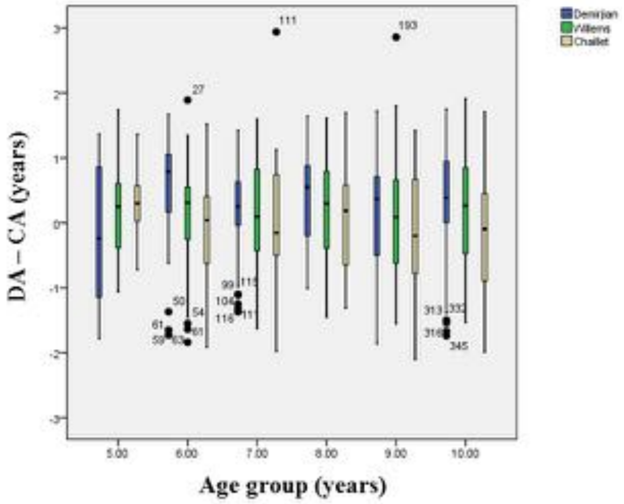
Graph 1: Box plot of mean DA-CA of females aged 5-10 years



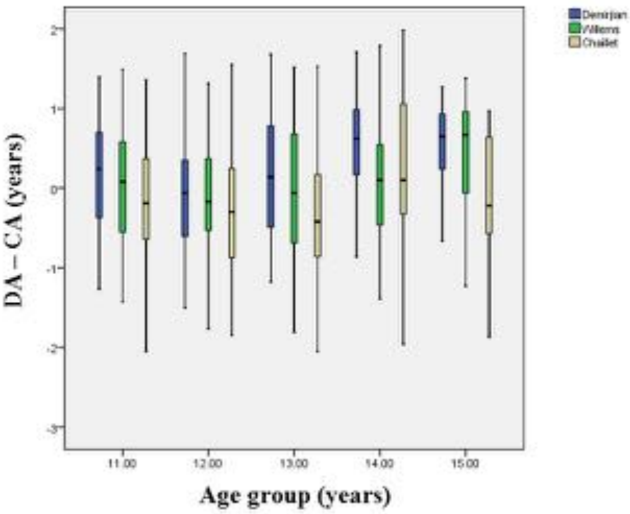
Graph 2: Box plot of mean DA-CA of females aged 11-15 years



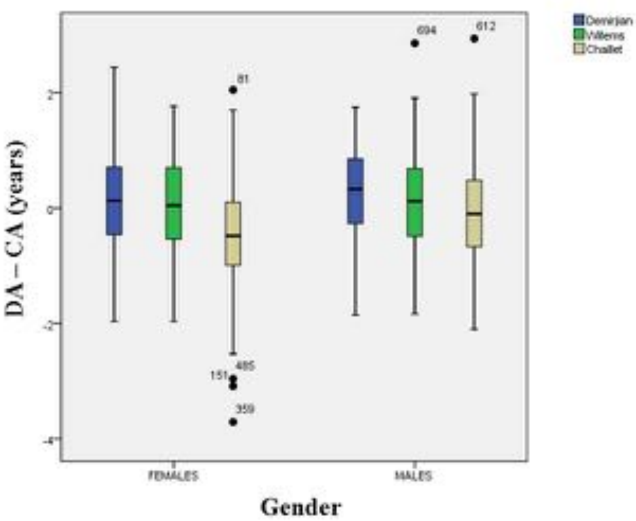
Graph 3: Box plot of mean DA-CA of males aged 5-10 years



Graph 4: Box plot of mean DA-CA of males aged 11-15 years



Graph 5: Box plot of mean DA-CA by gender for the total sample



DISCUSSION

Owing to the consistent overestimations of age reported with Demirjian's French-Canadian standards in most populations, Willems and his co-workers[3] and Chaillet et al.,[4] developed new dental maturity standards using weighted ANOVA and polynomial functions, respectively, for the same Belgian population. While the Demirjian and Willems methods have been widely tested, the Chaillet standards have not, especially on Indian populations. Therefore, the present study compared the Demirjian, Willems and Chaillet methods on a sample of orthopantomographs of Indian children aged 5 to 15 years, obtained by a convenience sampling method. This method is preferred by most researchers because it is fast, inexpensive and easy and the subjects are conveniently accessible.

Only the mandibular teeth were evaluated in the present study because, unlike the developing maxillary permanent teeth whose radiographic views are often obstructed by bony structures of the maxilla, the teeth of the mandible are quite clearly visible in an OPG. Further, a very high degree of symmetry is known to exist between the teeth of the left and right sides, only the seven mandibular teeth of the left quadrant were assessed. Third molar germs were excluded from assessment because of the high degree of variability observed in third molar genesis and development.

While assessing dental age, it is important not only to consider the proximity of the estimated age to the actual or chronological age, but also the reproducibility of the age estimation method. In the present study, agreements between and within examiners for Demirjian's method of dental staging were obtained in percentages and measured by Cohen's kappa coefficient. This coefficient is a more robust measure than simple percent agreement calculation, taking into account the agreement occurring by chance. Inter- and intra-examiner agreements for Demirjian's dental staging were observed to be 91% and 90%, respectively, with a kappa coefficient of 0.83. The difference in mean DA-CA was not significant between examiners or between two assessments by the first examiner. Other studies have reported kappa values ranging from 0.68 to 0.92 for inter-examiner agreements[9,10] and from 0.67 to 0.96 for intra-examiner agreements.[11,12]

The Demirjian method has been found to consistently overestimate age in various populations, with a majority of studies reporting overestimations of up to +1.23 years in males and +1.20 years in females.[13] The Willems method has, in recent years, been accepted as a more accurate method of age estimation with reports of overestimations of up to +0.55 in males and +0.53 years in females.[14] Reports of underestimations of age with both these methods, although available, are few. In the present study, overestimations of age by +0.24 and +0.09 years for males and by +0.11 and +0.08 years for females, were obtained using the Demirjian and the Willems methods respectively. Studies testing the Chaillet's multi-ethnic international maturity standards[21] have reported overestimations of +0.28 and +0.37 years in males and +0.09 and +0.21 years in females of Bosnian-Herzegovinian[22] and Spanish Caucasian populations,[23] respectively. Underestimations have been reported of -0.48 and -0.18 years in males and -0.61 and -0.59 years in females of Venezuelan[23] and French[8] populations, respectively. In the present study, underestimations were obtained of -0.12 years in males and -0.45 years in females, with Chaillet's original standards for Belgian children.

Significant sex-based differences were observed with the Demirjian and Chaillet methods in the present study but not with the Willems method. This sex difference has been attributed to the faster biological and dental maturation in girls, which leads to a higher dental compared to chronological age.[15] However, some other studies[12,16] have reported a higher dental age compared to chronological age in boys than in girls.

The accuracy or precision of an age estimating method may be affected by the quality of the reference material (sample), reliability of the method and biological variability in dental development.[25,26] Hence, it is important to accept that no age estimation method can predict the exact age of every individual. In the present study, significant correlations were observed between the chronological and estimated dental ages with all the three methods tested. The Willems method was the most accurate in age estimation, followed by the Demirjian and the Chaillet methods. While the Demirjian method was more accurate than the Chaillet method in females, the Chaillet method better predicted the age of males compared to the Demirjian method.

Most studies on non-Indian[7,27] and Indian[16,17] populations have found the Willems method to be more accurate than the Demirjian method, while one Indian study[18] reports the reverse.
The mean prediction errors obtained with all three methods for the sample ranged from 1.08 to 3.0 months. Although smaller intervals are desirable, differences between chronological and estimated ages of up to 12 months can be considered to be within normal standards.[28] While the low mean prediction errors suggest that the published standards of the age estimation methods tested could be suitable, gender-specific formulae were derived in the present study, which could increase the accuracy of these methods when applied to Indian populations.
The main limitation of this study would be the use of a convenience sample, which may be subject to sampling bias and may not be representative of the entire population.[29] However, obtaining a random sample was not practical. Further, exposing children to X-radiation for obtaining orthopantomographs when not indicated for diagnosis or treatment may raise ethical concerns. With the use of convenience sampling, we could prevent unnecessary exposure to radiation. By including a large sample of varied age and of both sexes, we have attempted to reduce the sampling bias of a convenience sampling.

CONCLUSIONS

From the results of this study, it was concluded that the Willems and Demirjian methods overestimated age, while the Chaillet method underestimated age. The Willems method most accurately predicted the age (mean prediction error = 1.08 months for both sexes) of the study sample, followed by the Demirjian and Chaillet methods. The Demirjian method was significantly more accurate in girls compared to boys while the reverse was true for the Chaillet method. The Willems method was equally accurate in both sexes. Significant differences between estimated dental age and chronological age were observed with each of the methods. The gender-specific formulae derived in the present study could increase the accuracy of these methods when applied to Indian populations.

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New insights into odontological exploration of drowning using rat model - A pilot study.

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ABSTRACT

Dental forensics for the resolution of unnatural death remains an underdeveloped field. Accordingly, an experimental study was conducted with six to seven months old Wistar rats that were drowned in order to identify key postmortem features and pattern of dental decomposition. The visual, structural and elemental changes were assessed periodically. Based on mode of death, they were designated as SB (euthanized and soil buried), FWD (fresh water drowned) and SWD (sea water drowned). Postmortem features as well as the structural and elemental patterns of decomposition of teeth were analyzed with Field Emission Scanning Electron Microscopy (FE-SEM) and Energy Dispersive Spectroscopy (EDAX) periodically for two months. The periodic observation of elemental changes in the teeth of SB, FWD and SWD rats allowed us to derive an equation using linear regression analysis to relate the degree of dental decomposition with the time since death. The difference in pattern of surface deterioration was also observed. The present findings could provide a better knowledge in resolving unnatural deaths and supporting evidence for legal prosecution.

INTRODUCTION

Drowning is one of the most common forms of asphyxial death. It is broadly classified as typical and atypical based on the extent of fluid accumulation within lung's air passages.[1] In drowning, it is always difficult to confirm the cause and time since death because of decomposition and because the bodies are frequently mutilated by aquatic animals.[1] Also, putrefaction inevitably leads to gas formation in soft tissues, which adds further uncertainties in the determination of time of death.[2] The teeth, by contrast , are comparatively preserved due to their hard and calciferous properties. The manifestation of drowning occurs throughout the body with pink tooth being one of the most prominent features. The reason behind this appearance is not clear, but such a phenotype is predominant in asphyxial death.[3, 19]

Thomas Bell (1829) was the first one to note the pink tooth.[4] The Christie murder in 1953 was one of the landmark cases where pink tooth became an important Forensic biomarker since. The exhumed body expressed pink coloration of both tooth and skin. The accused confessed that the victim was strangulated and thus was subjected to carbon monoxide poisoning. The evidence of pink tooth reconfirmed the

strangulation.[4] Beeley and Harvey (1973), Whittaker et.al (1976), Clark and Law (1984), Brondum and Simonsen (1987) and Van Wyk (1987) did major work on pink tooth.[5, 6]
In animal models, the appearance of pink tooth was demonstrated in cat, golden hamsters, female dog and rats.[5, 6, 20] These studies were only qualitative in nature wherein the appearance of pink tooth under various conditions and modes of death were highlighted.
In the present work, we made an attempt to find out postmortem changes apart from pink tooth appearance in rats that were subjected to fresh and sea water drowning (FWD and SWD rat) experimentally. The SB rat was euthanized and buried to simulate natural death and decomposition was used as a reference for comparisons to FWD and SWD rats. The pathophysiology of death in fresh and sea water drowning were different[1] which was the reason for the present work with FWD and SWD rats. The visual, elemental, and structural features exhibited in rat teeth (SB, FWD and SWD) were studied over a period of two months.
Currently, there are two methods that allow to determine the time of death: Rate method and concurrence method, with the former used in the present work.[7]

MATERIALS AND METHODS:

Study design

This study was approved by the Institutional Animal Ethics Committee (IAEC) of Saveetha Medical College and the approval number is SU/CLAR/RD/027/2017. The animals in the lab were maintained according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, India). Three Wistar rats of six to seven months old were used in the experiment and based on their mode of death induced they were designated as SB (euthanized and soil buried), FWD (fresh water drowned) and SWD (sea water drowned). The dental formula of Wistar rat is I 1/1 C 0/0 M 3/3 and hence a rat contains 16 teeth.[8] Overall, 48 teeth (including SB, FWD and SWD rats) were observed to record the postmortem changes in this study. This includes 12 incisors and 36 molars. Fresh water was collected from a Lake, sea water from Bay of Bengal and soil collected from the college campus was categorized as red soil. The other materials utilized for research were isoflurane (Raman and Weil, Mumbai), surgical blade no 22, BP handle no 4, toothed forceps, desiccator, Mackintosh sheet, diamond disc, micro motor and 10% formalin (SB chems, Tamilnadu).

Procedures

SB rat were subjected to overdose of anaesthesia and euthanized, while FWD and SWD rats were subjected to fresh and sea water drowning respectively after mild anaesthesia in the laboratory at an optimum room temperature of 26°C. Video recordings of both fresh and sea water drowning of rats were collected (data not shown). Immediately upon death confirmation, SB rat was buried in soil while FWD and SWD rats were kept in a tub containing fresh and sea water respectively for 24 hours. After 24 hours, the maxilla and mandible of rats were resected, buried in soil in the case of SB rat, immersed in fresh and sea water filled containers for FWD and SWD rats correspondingly. The containers were kept open and maintained at an optimum temperature ranging from 25 to 28 °C. To prevent complications from contagious disease such as bubonic or pneumonic plague, the maxilla and mandible of the rats were resected from dead animals and monitored for two months. The FWD and SWD rat lungs were removed by dissection and preserved in 10% formalin for histopathological examination. Visual observation was done to record the changes taking place in the skeleton (maxilla and mandible) and teeth of the rats. Both fresh and sea water utilized for experimental drowning were analyzed by ICP-MS inductively coupled plasma mass spectrometry to understand their chemical composition.[9] The ICP-MS equipment used for water analysis was the Agilent 7700X instrument (model#-G3281A) with an octopole based collision/reaction cell. After every ten days, one incisor tooth from each rat was taken and subjected to FE-SEM with EDAX to monitor the changes happening elementally. The model of the instrument used for FE-SEM with EDAX was CARL ZEISS SUPRA 55 FE-SEM which has resolution capacity of 0·8nm (magnification ranging from 100X to 1,000kx). Before analyzing the sample in FE-SEM with EDAX, sample preparation was done that includes sectioning (cross section) of the tooth with diamond disc. The standard size of the sample for observing in FE-SEM with EDAX is less than 1×1cm, so specifically from the cervical to apex region was sectioned excluding apical end. This sectioned part was observed under FE-SEM at three different but fixed points for consistency. The cross sectioned rat teeth were subjected to gold coating with sputtering instrument and analyzed by FE-SEM with EDAX.[10]

In this study, only the circumpulpal dentin and pulpal region of the incisor tooth were examined by FESEM with EDAX and elemental quantification determined. In total, analysis with FE-SEM with EDAX was done four times with each session having an interval of ten days. Hematoxylin and eosin staining were performed for lung tissue sections. Light microscope was used to observe the slides and optical images were captured.

Statistical analysis

The elemental changes of the tooth for two months were observed periodically by EDAX. Standard two-tailed unequal variance T-test was used for statistical analysis of the results. The elemental changes, taking place in the dentine and pulpal region of four incisor teeth over time were assessed by linear regression analysis using Sigmaplot 13 (Systat software, USA)

RESULTS

The elemental quantification of fresh and sea water were given in the appendix in table 1. On observation of the drowned rats, copious white froth with bloody tinge was noted on the nasal and oral cavity of SWD rat alone. However these signs were not seen in FWD rat. The nasal and oral cavity images of SB, FWD and SWD rats are given in Fig 1 - A, B, C. The isolated maxilla and mandible of the three rats were observed regularly to study the decomposition changes. On checking the mobility of tooth with probes, it was found that the mobility of tooth started earlier in SWD rat compared to others. Within a time period of 72 hrs after drowning, most of the teeth of SWD rat got mobilized. In SB and FWD rats, the teeth were firm in the socket. However, the maxilla and mandible of all three rats showed observable color differences. In particular, the maxilla and mandible of SB, FWD and SWD rats appeared black, white and pale white in color respectively. The images of the maxilla and mandible of the three rats after 72 hrs post death are shown in Fig 2 - A-C.

Fig. 1: Images showing the oral and nasal cavity of dead rats after euthanasia or SB (A), fresh water drowning or FWD (B) and sea water drowning or SWD (C). All rats shown were subjected to anaesthesia before death. The copious froth is evident in the oral and nasal regions of SWD rat alone.

Fig. 2: The appearance of maxilla and mandible regions of SB(A), FWD (B) and SWD (C) rats 72 hrs after death

The onset of pink tooth was evident 96 hrs after death in both FWD and SWD rats, while in SB rat there was no such signs. The pink tooth evident in FWD and SWD rats are displayed in Fig 3 (A, B).

The intensity of color and extent of pink tooth in FWD and SWD rats were compared and found to be quite phenotypically different. Within 2 to 3 days, the pink tooth slowly faded off in the FWD rat while in SWD rat it was present until 14 days postmortem. After 14 days, the pinkish coloration slowly started to fade from the SWD samples as well. Mainly, pink tooth was evident in the mandibular incisor teeth of the FWD rat while in SWD rat it was found in all the four incisors which included both the maxillary and the mandibular archs. The remaining maxillary and mandibular molars and maxillary incisors tooth of FWD rat and all molars of SWD rat didn't manifest pink tooth. In SB rat, none of the tooth expressed pink tooth phenomena. The quantification of pink teeth among three rats are given in the table 1.

Fig. 3: Evidence of pink tooth in the incisors of FWD (A) and SWD (B) rats, 96 hrs after death.

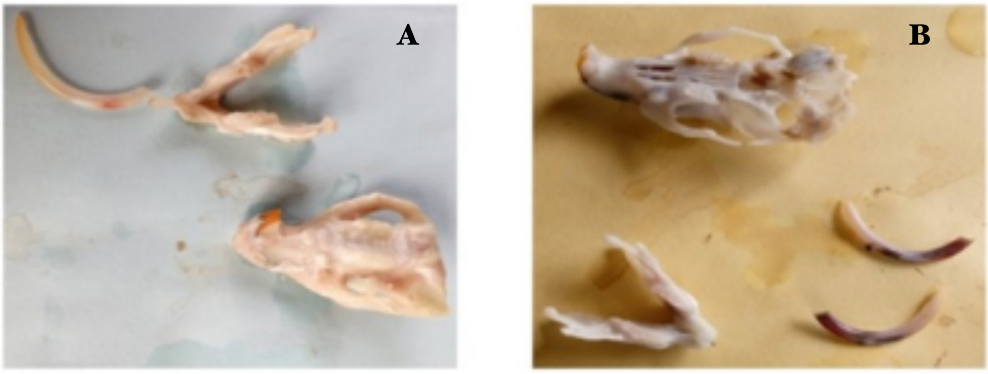


Table 1: the evidence of pink tooth among three rats were given below.

Evidence of pink teeth among three groups	
Number of rats	3
Number of teeth(overall)	48
Number of pink teeth evident in SB rat	0
Number of pink teeth evident in FWD rat	2
Number of pink teeth evident in SWD rat	4
Number of pink teeth evident in anterior region of FWD rat (incisors)	2
Number of pink teeth evident in posterior region of FWD rat (molars)	0
Number of pink teeth evident in anterior region Of SWD rat (incisors)	4
Number of pink teeth evident in posterior region Of SWD rat (molars)	0

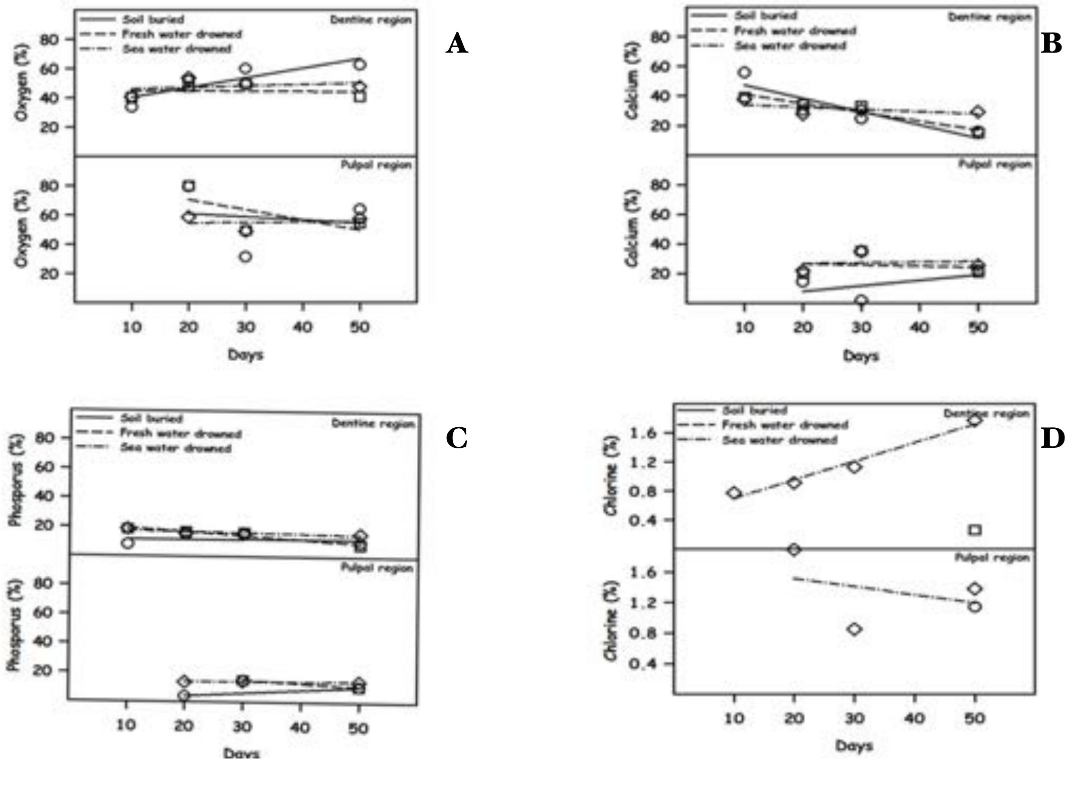
SB-euthanized and soil buried rat: FWD- fresh water drowned rat: SWD sea water drowned rat.

Collapse or atelectatic changes of the alveoli is common in both fresh and sea water drowning but is more prominent in the latter.

Alveoli appeared empty and without the lining pneumocytes in FWD and SWD rat lungs. Capillaries were dilated and more congested in SWD compared to FWD (Appendix Fig.1 - A, B). Edema which is more pronounced grossly can also be detected microscopically in both drowning interventions, but are more predominant in SWD cases. Inflammatory infiltrates composed of macrophages and neutrophils are seen as a reaction to the pulmonary edema due to hemodynamic disturbances (Appendix Fig 1C). Endothelial lining is injured and denuded in SWD, but not in the FWD (Appendix Fig 1D, E).

Through EDAX data, the elements such as oxygen, calcium and phosphorous were consistently detected in the dentine region of the incisor teeth (SB, FWD and SWD rats) in all the four sessions. When observing the dentine from SB rat, potassium and aluminium were found in addition to oxygen, calcium and phosphorous. However in the dentine of FWD rat incisors, sodium and magnesium were found other than the three elements of oxygen, calcium and phosphorous. In the dentine of SWD rat incisors, the sodium, magnesium and chlorine are evident in addition to the three common elements. Other elements such as zinc, carbon and Sulphur were detected in the dentine of incisors from SB rat. Copper was evident in the fourth session analysis of dentine for both SB and FWD rat incisors. A linear regression analysis was used for analyzing all the four sessions EDAX data (Fig.4 A-D).

Fig 4 A. The oxygen element weight % in the dentine and pulpal region of the incisor teeth of Wistar rats after a time period of asphyxial (drowning) death with reference control. Continuous line (SB rat (n=4 incisors) – simulating natural death and buried in soil); medium line (FWD rat (n=4 incisors) - fresh water drowned); dash dot (SWD rat (n=4 incisors) - Sea water drowned). B. The calcium element weight % in the dentine and pulpal region of the incisor teeth of Wistar rats after a time period of asphyxial (drowning) death with reference control. Continuous line (SB rat (n=4 incisors) - simulating natural death and buried in soil); medium line (FWD rat (n=4 incisors) – fresh water drowned); dash dot (SWD rat (n=4 incisors) - Sea water drowned). C. The phosphorus element weight % in the dentine and pulpal region of incisor teeth of Wistar rats after a time period of asphyxial (drowning) death with reference control. Continuous line (SB rat (n=4 incisors) - simulating natural death and buried in soil); medium line (FWD rat (n=4 incisors) - fresh water drowned); Dash dot (SWD rat (n=4 incisors) – Sea water drowned). D. The chlorine element weight % in the dentine and pulpal region of incisor teeth of Wistar rats after a time period of asphyxial (drowning) death with reference control. Continuous line (SB rat (n=4 incisors) - simulating natural death and buried in soil); medium line (FWD rat (n=4 incisors) - fresh water drowned); dash dot (SWD rat (n=4 incisors) - Sea water drowned).



While analyzing the oxygen element content of four sessions of the dentine region in SB, FWD and SWD rats, the r^2 value was significant in the dentine region of SB rat and the probability of prediction was 87 %. The oxygen element percentage shows a positive correlation with days in the dentine region of SB rat incisor tooth. An equation was derived for SB rat(y=33·84+0·6629x) to estimate the time since death approximately with the help of percentage of oxygen element present in dentine region. For FWD and SWD rats, the r^2 value of oxygen analysis was not significant both in dentine and pulpal region. For the calcium EDAX data analysis of dentine and pulpal region, the r^2 value was significant in the dentine region of SB and FWD rats. The percentage of calcium negatively correlated with days in the dentine region of SB and FWD rats. Based on that, an equation was derived for estimating time since death for SB rat (y=56·10 -0·89x) and FWD rat (y= 46·95-0·599x) approximately with the help of calcium composition in the dentine region. The probabilities of estimating time since death with the above equations are 88% and 96% respectively. For SWD rat, the r^2 value obtained for calcium percentage was not significant both in the dentine and pulpal region. While analyzing the phosphorous EDAX data, the r^2 value was significant for FWD and SWD rats in the dentine region. The percentage of the phosphorous element in the dentine region of FWD and SWD rats were negatively correlated. Based on the above data, the derived equation for FWD and SWD rats was(y=22·10-0·257x) and (y=18·27-0·062x) respectively. The probability of prediction of time since death with the above equations derived from phosphorous percentages in the dentin are 94% and 72% correspondingly. While observing the dentine region of SWD rat incisor tooth for four sessions, the level of chlorine consistently increased on every succeeding session and hence positively correlated. By assessing the quantity of chlorine in the dentine region for the four sessions, an equation was derived for SWD rat (y=0·443+0·0255x) with probability of prediction as 98%.The rest of the elements are not consistently evident in the all four sessions of analysis, so they are not statistically assessed. Table 2 shows the equation derived from the elements. Correlation of various elements (%) with days post death and the differential detection of the elemental percentages in the dentine and pulpal region in SB, FWD and SWD rats were given in appendix table 2 and 3.

Table 2: Derived Equations for estimating time since death from elemental degradation with the help of linear regression.

Element	Region	Equations		
		SB	**FWD**	**SWD**
Oxygen	Dentine	y=33·84+0·6629x	-	-
	Pulpar	-	-	-
Calcium	Dentine	y= 56·10-0·89x	y=46·95-0·599x	-
	Pulpar	-	-	-
Phosphorus	Dentine	-	y= 22·10-0·257x	y= 18·27-0·062x
	Pulpar	-	-	
Chlorine	Dentine	-	-	y = 0·443+0·0255x
	Pulpar	-	-	-

The FE-SEM images of dentine and pulpal region of incisor teeth of SB, FWD and SWD rats were studied and interpreted results are given below.

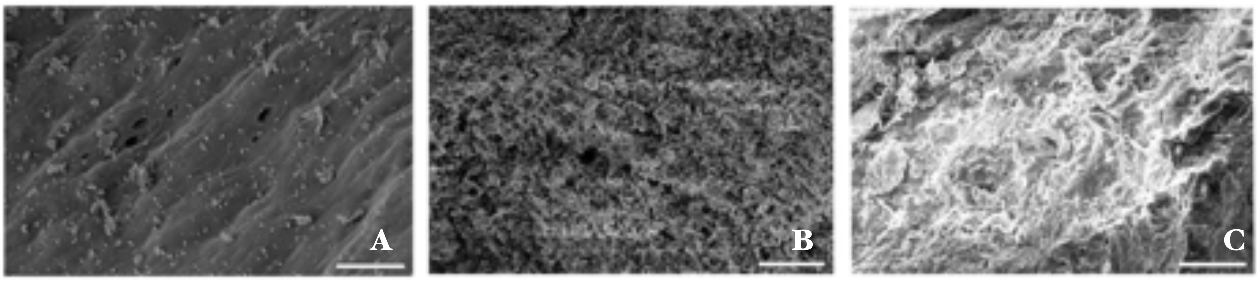
I. Dentine region

The results in surface morphology were not consistent, with select areas of the sample in each of the groups suffering from severe degradation even by day 10, while some areas appear relatively intact after day 50. Thus, morphology of such regions cannot be reliably used for sample differentiation and dating.

II. Pulpal region

The damage to the pulpal surface of teeth in SB, FWD and SWD rats are more mild and much more differentiated compared to the dentine region. In particular, the pulpal surface of SB rat teeth (Fig 5A) exhibited minimal damage even after 50 days. On the contrary, the damage to the pulpal surface is progressively worse for FWD and SWD rat's incisor teeth. FWD rat incisor teeth shows deep pores in the pulpal surface (Fig 5B) but the surface roughness of the latter is highest among the three (SB, FWD and SWD rats) and is most pronounced at day 50 without noticeable pores (Fig 5C). Calcification of the pulpal surface seems to be diminished going from SB, FWD and SWD rats teeth, although the difference has not been determined to be significant.

Fig 5 FE-SEM image of incisor tooth of SB (A), FWD (B) and SWD(C) rats captured on 50th day after death showing pulpal surface manifests the variations in surface structural decomposition features.



DISCUSSION

The occurrence of pink tooth in asphyxial death is a constant feature in forensic medicine but literature on this topic are not sufficient in scientific databases.[11]Consequently, studies carried out on pink tooth (a key manifestation of asphyxia death) in humans and laboratory animals are very scarce. Any investigations were restricted to qualitative studies. Hence, the present study has been focused on quantitatively assesing some unexplored features to the pink tooth phenomenon from drowning cases. The features include visual, elemental and structural changes observed over a period of 2 months after death in these animals.

After death due to diagenetic changes, the enamel and dentine allow entry of water which leads to mineral deposition in teeth that consequently changes the color, composition and surface texture of the teeth.[12] Quantitative elemental analysis of fresh and sea water was carried out in this study to establish the effect of elements on rats teeth of FWD and SWD.

Atelactic changes in alveoli, capillaries dilatations, oedema and macrophage infiltration proves the ante mortem drowning in the Wistar rats.[13] The oedema, the capillary dilatation and congestion are predominant in sea water drowning indicating higher level of destruction of lungs than in the fresh water.[14]

At 96 hours after death, the entire maxilla and mandible regions of SB rat appeared black indicating loss of vascularity. This shows the vascular tissue decomposition rate is faster in soil buried as compared to the maxilla and mandible kept in fresh and sea water. The cause for early decomposition of vascular tissues could be the effect of microorganisms in soil that enters through the vascular bundles and cellular region consequently leading to decomposition. This rapid decomposition of vascularity in the skeletal part would have caused the early necrosis in the skeletal and dental part of SB rat as compared to FWD and SWD rats.[15] Interestingly, the teeth of SB rat were rigidly fixed and could not be extracted easily from the maxilla and mandible of SB rat at 96 hours which indicates the outer surface of the skeletal structures (maxilla and mandible) are intact. The comparatively mobility of teeth, 72 hours after death in SWD

rat indicates that decomposition in bone tend to follow the peripheral region rather than the vascular bundles as reported for inhumed cases. The early mobility of teeth in SWD rat could be possibly due to faster rate of decomposition around the neck of the tooth.[15]
The time taken for pink tooth occurrence in fresh and sea water drowning were around 96 hrs. The reason for time delay in the occurrence of pink teeth appearance in humans was explained as follows: The diameter of erythrocytes (7.5µm) are larger than the diameter of dentinal tubules (3µm). Thus, there should be the resistance to entry of erythrocytes by the dentinal tubules for several hours after death.[6] When autolysis of erythrocytes is accomplished in teeth, it leads to seepage of blood components into dentinal tubules which results in pink tooth. Obviously, a similar kind of mechanism would have occurred in rat teeth which delayed the pink tooth. The pink tooth in FWD rat faded off at an earlier time period as compared to the SWD rat, an observation which can be explained by the FE-SEM results. The pores are deep in the pulpal surface of FWD rat teeth due to deterioration. These deep pores encouraged easy seepage of erythrocytes which consequently led to earlier fading of pink tooth in FWD rat. FE-SEM image of the pulpal surface of SWD rat incisor tooth show that the pores are not very evident which may be the reason for late disappearance of pink tooth. The extent and color variation of pink tooth visualized in fresh and sea water drowning may be due to the variations in mineral content, carbon dioxide and salinity level. The intense color of pink tooth in SWD rat could be attributed to the increased salinity of sea water, [5] which is reflected in the present work (Fig 3b).
There are more likely, species variations in the appearance and distribution of pink tooth. For instance, pink tooth was evident only in anterior teeth in FWD and SWD rats while in posterior region, it was not observable. The distribution of pink tooth given by Campobasso et al (2006) in ship wreckage case shows that pink tooth was evident in both anterior and posterior region indicating the difference in occurrence between humans and animals.[16] The reason for predominance of pink tooth in anterior region as compared to posteriors in rats could be due to the difference in the root morphology and dimensions which plays the role in pooling of blood in the cervical region of the tooth. The anterior tooth of rats have single root and tooth are tubular in structure which may allow pink tooth occurrence predominantly in anterior than posterior teeth.
The elements present in the Wistar rat dentin are Ca, P, F, Mg, Na and Zn. Based on the different areas of dentin like mantle and circumpulpal, the percentage of the element varies.[17]
In SB rat, the level of oxygen increased in the dentine region of incisor tooth for every succeeding analysis whereas the calcium levels were found to be decreased. The work of Pipenbrick et.al (1987) on bones explains about the calcium carbonate formation in the Volkmann's canals of carcasses. Their findings revealed that calcium from hydroxy apatite crystals reacted with carbonate in soil to form calcium carbonate which leads to further decomposition of bone.[18] The present EDAX data of incisor teeth from four sessions of SB rat shows similar kind of results such as calcium decrease and oxygen increase on every succeeding sessions which correlates with Pipenbrick's findings of decomposition mechanism in the inhumed bone. The FE-SEM interpretation also reconfirms the EDAX data showing maximum calcium decreases in the dentine region of the incisor tooth of SB rat compared to others. While analyzing phosphorous content in the dentine of SB rat, it appeared the levels kept fluctuating during the sessions assessed. In contrast, Pipenbrick et al (1987) mentioned that phosphorous was reduced very prominently in inhumed as compared to the original bone.[18] Therefore, a long term observation of inhumed teeth might give results comparable with Pipenbrick's findings. The degree of pulpal elemental changes in SB rat is not substantial to provide any clues for the study. The elements such as oxygen, calcium and phosphorous consistently decreased in the dentine region of FWD rat teeth with succeeding sessions. In the dentine region of SWD rat teeth, oxygen and chlorine increased while calcium and phosphorus decreased. The cause for such elemental changes in the dentine region of FWD and SWD rat's incisor teeth needs further exploration.

CONCLUSION

The subtle differences in pink tooth features observed between fresh and sea water drowning allows one to discriminate between the two and is focus of the present study. Even though pink tooth occurrence is believed to be due to internal mechanism like increased intrapulpal pressure, the extent and duration of pink tooth as well as color intensity could be influenced by the external environment. The tracking of elemental and structural changes during decomposition of teeth over time helps to determine the approximate time since death. Although the present study is limited by the small sample size of studied animals, the results were consolidated with reasonable number of tooth samples from each group for both morphological evaluation and element analysis. More robust and discrete conclusions on elemental and structural alterations of teeth are likely to be generated, if the number of rats is increased in a future study. Undoubtedly, this work can create a base for many future works on asphyxial deaths and post-mortem pink tooth evaluation.

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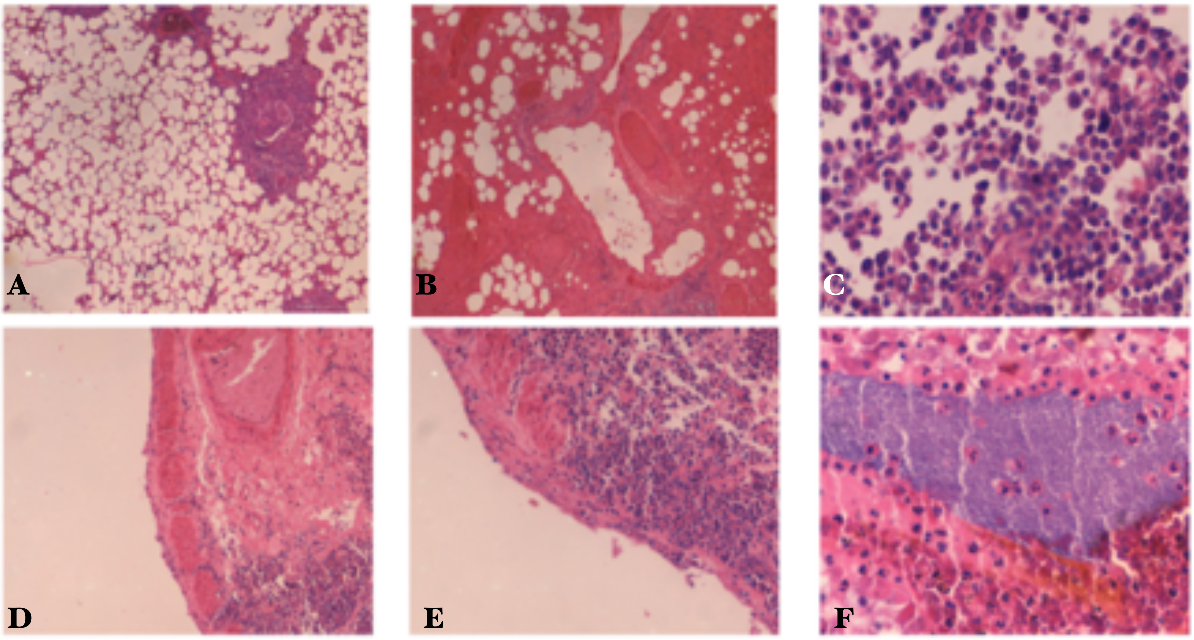
SUPPLEMENTAL APPENDIX

Appendix Figure 1: Dilatation of the alveoli and the capillaries in fresh water drowning 40X(A), Dilatation and edema of the alveoli and more congested capillaries in sea water drowning 40X (B), Inflammation composed of macrophages and neutrophils in sea water drowning 400X (C), Endothelial injury and denudation of the lining cells in the sea water drowning 100X (D), Higher power view of the denuded endothelium in sea water drowning 200X (E), Bacterial colonies as evident of sea water drowning as against fresh water 400X (F).



Appendix Table 1: Elemental analysis of fresh and sea water by ICP-MS.

Elements	Concentration	
	Fresh water	Sea water
Na23	50mg/dl	6450mg/dl
Mg24	84mg/dl	849mg/dl
S34	643mg/dl	1587mg/l
Cl35	2·210mg/	79·665mg/l
K39	2·412mg/l	2·412mg/l
Ca43	0·16421mg/l	2·206mg/l
Fe56	0·00621mg/l	0·003704mg/l
Zn66	0·007718mg/l	0·004831mg/l

Appendix Table 2: Correlation of various elements (%) with days post death in the dentine and pulpal region in SB, FWD and SWD rats.

Element	Region	SB rat	FWD rat	SWD rat
Oxygen	Dentine	r^2=**0·7544**	r^2=0·00924	r^2=0·094
		β_0=33·64	β_0=45·27	β_0=45·22
		β_1=0·6229	β_1= -0·026	β_1=0·096
	Pulpal	r^2=0·018	r^2=0·425	r^2=0·0072
		β_0=65·16	β_0=85·25	β_0=53·93
		β_1= -0·213	β_1= -0·69	β_1=0·029
Calcium	Dentine	r^2=**0·792**	r^2=**0·929**	r^2=0·339
		β_0=56·10	β_0=46·95	β_0=35·44
		β_1= -0·89	β_1= -0·599	β_1= -0·151
	Pulpal	r^2=0·299	r^2=0·021	r^2=0·011
		β_0=0·459	β_0=28·25	β_0=25·94
		β_1=0·371	β_1= -0·081	β_1=0·046
Phosphorous	Dentine	r^2=0·0035	r^2=**0·891**	r^2=**0·521**
		β_0=11·54	β_0=22·10	β_0=18·27
		β_1=0·012	β_1= -0·257	β_1= -0·062
	Pulpal	r^2=1	r^2=1	r^2=0·165
		β_0= -	β_0= -	β_0=13·88
		β_1= -	β_1= -	β_1=7·21
Chlorine	Dentine	r^2= -	r^2= -	r^2=**0·973**
		β_0= -	β_0= -	β_0=0·443
		β_1= -	β_1= -	β_1=0·0255
	Pulpal	r^2= -	r^2= -	r^2= 0.100
		β_0= -	β_0= -	β_0=1.733
		β_1= -	β_1= -	β_1= -0.011

The statistical result of the elements in the dentine and pulpal region of SB, FWD and SWD rats were given above. *the bolded figures indicate the significant values. Y (element %) = $\beta_0 + \beta_1$ x(days)

Appendix Table 3: The differential detection of the elemental percentages among the SB, FWD and SWD rats

Elements	Dentine region			Pulpar region		
	SB	**FWD**	**SWD**	**SB**	**FWD**	**SWD**
Oxygen	+++	-	-	-	+	-
Calcium	+++	+++	+	-	-	-
Phosporous	-	+++	++	-	-	-
Chlorine	-	-	+++	-	-	-

The + for elemental percentage was given based on the r value. r>0·3= +, r>0·5=++ and r>0·7= +++.

The utilization of small amounts of residual endodontic material for dental identification

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The authors declare that they have no conflict of interest.

KEYWORDS

Forensic identification, Edentulous, Extruded endodontic material

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ABSTRACT

Dental information is one of the three scientific methods of identifying a deceased person. However, when an investigator is faced with dental ante-mortem information that indicates the deceased has had all his teeth extracted, it may be assumed that the dental information will not be useful, especially if no retained roots are visible in the post-mortem triage. The following case report highlights that careful examination including radiography, may reveal specific detailed information which was useful for identification to be established. Two small radiopaque objects were located in the apical area where the upper left canine root apex would have been. The radiopacities size, location, positioning to both each other and to the left maxillary sinus corresponded to ante-mortem radiographs. This case reveals an unusual use of extruded root canal material being of evidential value even though the tooth was extracted.

INTRODUCTION

Following the death of any person, identification is required for legal and ethical reasons.[1] For non-visual cases the three scientific methods of identification accepted by INTERPOL are fingerprinting, DNA and dental comparison.[2] DNA testing has proven to be very successful in matching biological material. However, DNA testing may be costly, delayed (depending upon the resources available for the local authorities) compromised by contamination and degraded by heat.[3] In regards to fingerprinting, the deceased may also not be on the registry to provide a match. Dental identification based on comparative analysis of post-mortem dental information to ante-mortem information is recognized to be reliable and time efficient.[4,5] Comparisons to anatomical landmarks and morphological structures such as sinus patterns, stylus abnormalities and medical prostheses, dependent upon ante-mortem information being available, may sometimes be utilised when the dental information is limited, either ante-mortem or post-mortem.[4-8]

Occasionally when the investigator receives dental ante-mortem information that indicates that the deceased is edentulous in maxillary and/or mandibular arches with or without full upper and/or lower dentures, too often it is assumed that the dental information has little or no forensic value for identification. Also there could be insufficient dental records, no ante-mortem radiographs available or the recovered dentures are unlabelled. Previous literature has discussed the utilisation of root canal treatment comparison for identification where the roots are still present [9-12] but there is no literature available on the use of retained extruded material for identification purposes. The following case demonstrates that dental radiographic examination of edentulous areas could provide valuable forensic evidence for identification.

THE CASE

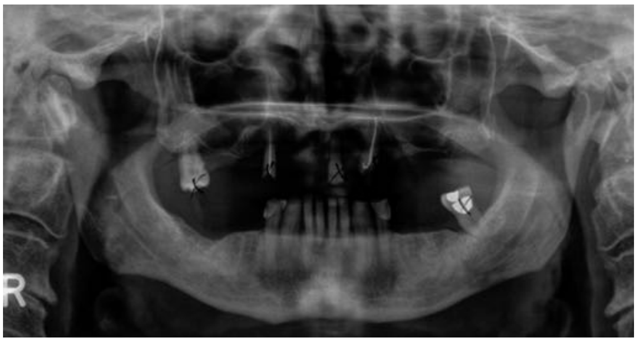
A deceased adult male in an advanced stage of decomposition was presented for odontological identification. Upon visual dental examination, it was noted that the maxillary arch was edentulous and the lower arch consisted of eight teeth with severe attrition, abrasion and erosion. There were only minor adhesive restorations present.

Upon detailed dental periapical radiography, two small radiopaque objects (one oblong in shape and the other more circular) were located in the apical area where the upper left canine root apex would have been (Fig. 1).

Fig. 1: Post-mortem radiographic image showing the two radiopaque root filling material objects positioned high and adjacent to the left maxillary sinus

The radiopacities size, location, positioning to each other and to the left maxillary sinus corresponded to the endodontic materials present on an ante-mortem orthopantomograph (OPG). The ante-mortem OPG was taken when the deceased still retained his upper left canine tooth with the overfilled root canal obturation at its apical area (Fig. 2).

Fig. 2: The ante-mortem orthopantomograph showing the pre-extraction positioning of the numbered 23 tooth together with the extruded root filling material compared with the maxillary sinus



Magnification of this OPG can be seen in Fig. 3. Further evidence was observed on a more recent ante-mortem OPG which was substandard in quality but contained sufficient detail to confirm that these radiopacities were still present post-extraction (Fig. 4). As the types of post-mortem and ante-mortem radiographs are of different styles and affected by technical issues such as dissimilar x-ray angulations and orientation of the patient's head, this situation does not allow accurate metric analysis, however comparative analysis is possible. This radiographic evidence, coupled with the information from the lower dentition, was sufficient to establish the identity of the deceased.

Fig. 3: Detail of Fig.2 showing the two radiopaque root filling material objects at the apex of the root

Fig. 4: A more recent (but blurred) ante-mortem orthopantomograph showing the upper loss of the numbered 23 tooth but the retention of the extruded root filling material

DISCUSSION

An initial triage assessment of a deceased person requiring non-visual identification with both or either full upper and lower dentures might suggest that dental examination would prove difficult[13], suggesting other methods are required. In most cases this would be correct, however if other methods were not successful or in the case of a multiple victim disaster, a dental examination should be conducted as there is a possibility that detailed information might be discovered. As well as root filling material a dental radiographic examination could highlight visible anatomical features including retained roots, maxillary sinuses and trabeculae patterns[14,15]. Artefacts could also be highlighted, including amalgam tattoos which may not correspond to the exact position of an ante-mortem radiographic record due to movement if embedded within soft tissue only[16], implants, medical stabilization devices (which consist of small plates and screws) and foreign bodies which can be compared with ante-mortem radiographs. Nonetheless, physiological or pathological changes affecting the dental structures should not be discounted when comparing the features between the post-mortem and ante-mortem radiographs.

Although this case is unusual, it provides evidence for the necessity of thorough dental radiographic examination of edentulous areas even though initial investigations might suggest that these areas are of little evidential value.

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