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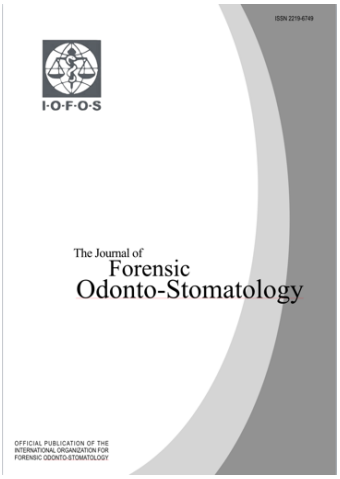

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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CONTENTS

The applicability of the original and revised Demirjian standards to age estimations of 5-15 year old Indian children

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The authors declare that they have no conflict of interest.

KEYWORDS

age estimation, Demirjian's method, seven-tooth method, four-tooth method, Indian children

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ABSTRACT

Background: The Demirjian method has been the most widely tested method for the estimation of the dental age of children and adolescents. However, only three studies have compared Demirjian's original and revised seven-tooth methods, four-tooth method and alternate four-tooth method, none of them conducted on an Indian population.

Aim: The present study aimed to compare the applicability of the original and revised seven-tooth and four-tooth and alternate four-tooth standards for age estimation of 1200 Indian children aged 5-15 years old.

Design: The study was designed as a retrospective cross-sectional study.

Results: Demirjian's original seven-tooth method overestimated age by 0.64 ± 1.44, 0.75 ± 1.50 and 0.69 ± 1.46 years in boys, girls and the total sample, respectively. Demirjian's revised seven-tooth method overestimated age by + 0.24 ± 0.80, + 0.11 ± 0.81 and + 0.19 ± 0.80 years in boys, girls and the total sample, respectively. Demirjian's original four-tooth method overestimated age by 0.79 ± 1.59, 0.59 ± 2.77 and 0.72 ± 2.30 years in boys, girls and the total sample, respectively. Demirjian's alternate four-tooth method overestimated age by 1.31 ± 1.07, 1.20 ± 1.10 and 1.26 ± 1.08 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 gender-based differences were observed only with Demirjian's revised seven-tooth and original four-tooth methods ($p < 0.05$).

Conclusion: The revised seven-tooth standards most accurately predicted the age of the study sample (mean prediction error = 2.28 months), followed by the original seven-tooth, four-tooth and alternate four-tooth standards. The Demirjian original seven-tooth method was significantly more accurate in boys compared to girls, while the reverse was true for the Demirjian revised seven-tooth and original four-tooth methods.

INTRODUCTION

On the back of much early research by several investigators that pointed to tooth formation being a more reliable indicator of dental age than tooth emergence, Demirjian, Goldstein and Tanner[1] developed a method of estimating dental maturity based on relative and not absolute measurements of eight stages which they described as

observable during the development of the seven mandibular teeth of a French-Canadian population. The authors derived self-weighted maturity scores for each stage of each tooth, separate for males and females, and constructed centile charts that allowed the conversion of the numerical maturity score, derived by their method of assessment, to dental age. Following a subsequent study on a larger sample of the same French-Canadian origin, Demirjian and Goldstein[2] updated their original self-weighted scores.

Demirjian's original and revised seven-tooth methods required an orthopantomograph (OPG) for assessment of the dental development. Where OPGs could not be obtained, Demirjian and Goldstein[2] recommended the use of periapical radiographs of four teeth (the molars and premolars), which they considered a separate system and for which they presented separate scores and standards. Where the first molar was missing, the central incisor was assessed in place of the molar, the development of the two teeth being chronologically almost the same. Separate scores and standards for this group of four teeth were also presented.

Since its introduction more than four decades ago, the Demirjian method[2] of age estimation has emerged as the most widely researched and applied technique in dental age estimation of children and adolescents. While a large majority of studies have tested the revised seven-tooth method, globally only three studies have compared Demirjian's original and revised seven-tooth methods, four-tooth method and alternate four-tooth method.[3-5] No such study on any Indian population is yet available in the dental literature. Therefore, the present study aimed to compare the applicability of the original and revised seven-tooth and four-tooth and alternate four-tooth standards for age estimation of 5-15 year old Indian children.

MATERIALS AND METHODS:

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 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 years) for each subject. Panoramic radiographs 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, 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 (22), lack of image clarity (8) or inadequate information regarding the date of birth (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. 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.

Calculation of chronological age: The dates of birth and of panoramic radiography 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 panoramic radiograph was made, to obtain the chronological age (CA) in decimal years.

Calculation of dental age: All digital radiographs meeting the selection criteria were viewed on the same LCD monitor using a magnifying glass for improved visualization. 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 FDI system. For both seven-tooth methods, the 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. In the original seven-tooth method (D7-O), the alpha-numeric stages 0 to H were converted to the original self-weighted gender-specific numerical scores of Demirjian and Goldstein and Tanner.[1] In the revised seven-tooth method (D7-R), the revised self-weighted scores of Demirjian and Goldstein[2] were utilized. In the four-tooth method (D4-O), the left mandibular premolars and first and second molars were assessed and in the alternate four-tooth method (D4-A), the left mandibular central incisor, premolars and second molar were assessed. Scoring for these two methods was done using the separate self-weighted scores described by Demirjian and Goldstein.[2] In all methods, the individual scores were summed to obtain a total maturity score or dental score, which was converted to a dental age (DA) using the Demirjian, Tanner and Goldstein tables.[1]

Reproducibility of measurements: 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 2 months without any knowledge of gender or age or of the stages assigned in the first evaluation. Two well-trained examiners independently evaluated 100 radiographs using Demirjian's method of dental staging, after a period of mutual calibration and without any knowledge of age or gender, in order to allow an analysis of inter-examiner agreement.

Data analysis: All statistical analyses and data management were performed using the Statistical Package for Social Sciences 19.0 (SPSS Inc., Chicago, IL, USA) for Windows and MS-Excel (Microsoft Office 2010). Analyses were made for each gender 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 ≤ 0.05 was considered statistically significant.

The accuracy of each method of age estimation was determined by the mean difference between the dental age and the chronological age (DA–CA) for each gender 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 gender and age group, and the total sample, with whiskers indicating the range (Figures 1-5). 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 gender and age group, for the total sample and between methods. Independent t-test was employed for comparisons of DA-CA between genders. The correlation between DA and CA was analysed using Spearman's rank correlation coefficient for each gender and for the total study sample. Intra- and inter-examiner agreements are expressed as percentages. Cohen's kappa coefficient was used to calculate the degree of reliability of these agreements.

RESULTS

The distribution of radiographs by age and gender is presented in Table 1.

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
12	12.0 - 12.9	55	10.98	91	13.02	146	12.17
13	13.0 - 13.9	57	11.38	82	11.73	139	11.58
14	14.0 - 14.9	59	11.78	58	8.30	117	9.75
15	15.0 - 15.9	21	4.19	29	4.15	50	4.17
Total sample	5.0 - 15.9	501	100	699	100	1200	100

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. Intra- and inter-examiner agreements for Demirjian's dental staging were 93% and 86% respectively, with Kappa values of 0.90 and 0.81 indicating almost perfect agreement.

In the present study, the mean D7-O dental ages for girls and boys were 11.42 ± 0.14 years and 11.44 ± 0.11 years, respectively. The mean DA-CA values for girls, boys and the total sample (+0.75 ± 1.50, +0.64 ± 1.44 and +0.69 ± 1.46 years, respectively) were statistically significant ($p < 0.001$). Significant differences between mean DA and CA were observed in all age groups ($p < 0.001$) except groups 9 and 15 in girls and 9, 12, 13 and 15 in boys ($p \leq 0.05$). In girls, the D7-O method overestimated age by +0.17 to +1.12 years in all age groups. In boys, overestimations ranged from +0.16 to +1.34 years (Table 2).

Table 2: Comparison of chronological and D7-O dental ages by gender and age group

Gender	Age group (years)	N	CA	DA	DA-CA	p value#
			Mean ± SD (years)			
GIRLS	5	24	5.46 ± 0.33	6.51 ± 0.36	1.05 ± 0.77	**0.006**
	6	39	6.57 ± 0.32	7.52 ± 0.18	0.96 ± 1.01	**<0.001**
	7	46	7.52 ± 0.26	7.99 ± 0.19	0.48 ± 1.32	**0.020**
	8	50	8.51 ± 0.31	8.76 ± 0.17	0.26 ± 1.11	0.556
	9	55	9.48 ± 0.30	10.23 ± 0.26	0.75 ± 1.93	**0.010**
	10	55	10.55 ± 0.32	11.37 ± 0.21	0.82 ± 1.62	**<0.001**
	11	40	11.44 ± 0.32	12.56 ± 0.27	1.12 ± 1.64	**<0.001**
	12	55	12.49 ± 0.32	13.15 ± 0.24	0.66 ± 1.86	**0.004**
	13	57	13.46 ± 0.30	14.56 ± 0.17	1.10 ± 1.19	**<0.001**
	14	59	14.48 ± 0.28	15.19 ± 0.18	0.71 ± 1.37	**<0.001**
	15	21	15.48 ± 0.27	15.64 ± 0.15	0.17 ± 0.64	0.192
	Total	501	10.68 ± 2.87	11.42 ± 0.14	0.75 ± 1.50	**<0.001**
BOYS	5	23	5.56 ± 0.29	6.90 ± 0.16	1.34 ± 0.76	**< 0.001**
	6	40	6.52 ± 0.31	7.62 ± 0.23	1.10 ± 1.37	**< 0.001**
	7	58	7.48 ± 0.29	8.11 ± 0.12	0.63 ± 0.88	**< 0.001**
	8	58	8.47 ± 0.29	9.25 ± 0.16	0.79 ± 1.24	**< 0.001**
	9	78	9.46 ± 0.28	9.80 ± 0.20	0.34 ± 1.68	0.156
	10	100	10.45 ± 0.29	11.00 ± 0.15	0.56 ± 1.53	**0.001**
	11	82	11.51 ± 0.30	12.20 ± 0.17	0.70 ± 1.44	**< 0.001**
	12	91	12.44 ± 0.30	12.98 ± 0.17	0.55 ± 1.62	0.012
	13	82	13.41 ± 0.31	13.92 ± 0.20	0.52 ± 1.73	0.005
	14	58	14.47 ± 0.31	15.39 ± 0.12	0.93 ± 0.93	**< 0.001**
	15	29	15.24 ± 0.25	15.41 ± 0.22	0.16 ± 1.17	0.051
	Total	699	10.81 ± 2.60	11.44 ± 0.11	0.64 ± 1.44	**<0.001**
Total sample		120	10.75 ± 2.72	11.44 ± 0.08	0.69 ± 1.46	**<0.001**

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

Table 3: Comparison of chronological and D7-R dental ages by gender and age group

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

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

The mean D7-R dental ages for girls and boys were 10.79 ± 2.86 years and 11.05 ± 2.71 years, respectively. The mean DA-CA values 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 DA and CA were observed in age groups 6, 8, 9, 12, 14 and 15 in girls and 6, 7, 8, 10, 11, 14 and 15 in boys ($p \leq 0.05$). In girls, the D7-R 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 in groups 5 and 12, respectively (Table 3).

The mean D4-O dental ages for girls and boys were 11.27 ± 0.14 years and 11.60 ± 0.11 years, respectively. The mean DA-CA values for girls, boys and the total sample (0.59 ± 2.77, 0.79 ± 1.59 and 0.72 ± 2.30 years, respectively) were statistically significant ($p < 0.05$). Significant differences between mean DA and CA were observed in all age groups except 8, 9, 12 and 15 for girls and age groups 9 and 15 for boys ($p \leq 0.05$). In girls, the method overestimated age by +0.07 to +1.59 years in all age groups. In boys, overestimations ranged from +0.26 to +1.66 years (Table 4).

The mean D4-A dental ages for girls and boys were 9.57 ± 0.11 years and 11.28 ± 0.11 years, respectively. The mean DA-CA values for girls, boys and the total sample (1.20 ± 1.10, 1.31 ± 1.07 and 1.26 ± 1.08 years, respectively) were statistically significant ($p < 0.05$). Significant differences between mean DA and CA were observed in all age groups except 7, 10, 11 and 13 for girls and 9, 10 and 11 for boys ($p \leq 0.05$). In girls, the method overestimated age by +0.60 to +1.69 years in all age groups. In boys, overestimations ranged from +0.70 to +1.67 years (Table 5).

Significant gender-based differences were observed in mean DA-CA with the D7-R and D4-O methods ($p = 0.005$ and < 0.001, respectively), but not with the D7-O and D4-A methods ($p > 0.05$). With both the D7-R and D4-O methods, the mean DA-CA was significantly lower in girls than in boys (Table 6).

Strong linear correlations were observed between CA and DA for girls, boys and the total sample with all methods (Table 7).

An inter-method comparison of mean DA-CA values revealed statistically significant ($p < 0.05$) differences in girls, boys and the total sample (Table 8).

DISCUSSION

The Demirjian method[2] has been the most widely tested method for the estimation of the dental age of children and adolescents. However, only three studies have compared Demirjian's original and revised seven-tooth methods, four-tooth method and alternate four-tooth method,[3-5] none of them conducted on an Indian population. Hence, the present study aimed to compare the applicability of these four methods to age estimation of a sample of 1200 Indian children - 501 female and 699 male - aged 5 to 15 years, selected 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.

Radiographic views of the developing maxillary permanent teeth are often obstructed by bony structures of the maxilla. The teeth of the mandible, on the other hand, are quite clearly visible in an OPG. Hence, only the mandibular teeth were evaluated in the present study, as in some other studies.[1,4,6] Since it has been well-established that a very high degree of symmetry exists between the teeth of the left and right sides,[1,6,7] only the mandibular teeth of the left quadrant were assessed. Third molar germs were excluded from assessment because of the high degree of variability observed in the genesis and development of these teeth.[8,9] Further, studies have reported no improvement in accuracy of age estimation methods when the developmental status of the third molar was included.[10]

Table 4: Comparison of chronological and D4-O dental ages by gender and age group

Gender	Age group (years)	N	CA	DA	DA-CA	p value#
			Mean ± SD (years)			
GIRLS	5	24	5.46 ± 0.33	7.05 ± 0.27	1.59 ± 1.24	**< 0.001**
	6	39	6.57 ± 0.32	7.57 ± 0.13	1.00 ± 0.84	**< 0.001**
	7	46	7.52 ± 0.26	8.02 ± 0.17	0.50 ± 0.96	**0.003**
	8	50	8.51 ± 0.31	8.58 ± 0.15	0.07 ± 0.79	0.660
	9	55	9.48 ± 0.30	9.99 ± 0.26	0.51 ± 1.49	0.154
	10	55	10.55 ± 0.32	11.00 ± 0.22	0.45 ± 1.76	**0.048**
	11	40	11.44 ± 0.32	12.11 ± 0.29	0.67 ± 2.00	**0.019**
	12	55	12.49 ± 0.32	12.87 ± 0.25	0.38 ± 2.35	0.109
	13	57	13.46 ± 0.30	14.38 ± 0.20	0.92 ± 2.95	**< 0.001**
	14	59	14.48 ± 0.28	15.11 ± 0.19	0.63 ± 3.13	**< 0.001**
	15	21	15.48 ± 0.27	15.60 ± 0.16	0.12 ± 2.71	0.313
	Total	501	10.68 ± 2.87	11.27 ± 0.14	0.59 ± 2.77	**< 0.001**
BOYS	5	23	5.56 ± 0.29	7.22 ± 0.12	1.66 ± 0.55	**< 0.001**
	6	40	6.52 ± 0.31	7.78 ± 0.20	1.26 ± 0.93	**< 0.001**
	7	58	7.48 ± 0.29	8.10 ± 0.11	0.62 ± 0.59	**< 0.001**
	8	58	8.47 ± 0.29	9.28 ± 0.18	0.81 ± 1.16	**< 0.001**
	9	78	9.46 ± 0.28	9.72 ± 0.19	0.26 ± 1.58	0.541
	10	100	10.45 ± 0.29	11.02 ± 0.17	0.57 ± 1.68	**< 0.001**
	11	82	11.51 ± 0.30	12.40 ± 0.18	0.89 ± 1.87	**< 0.001**
	12	91	12.44 ± 0.30	13.42 ± 0.18	0.86 ± 1.97	**< 0.001**
	13	82	13.41 ± 0.31	14.24 ± 0.19	0.65 ± 1.85	**< 0.001**
	14	58	14.47 ± 0.31	15.16 ± 0.10	0.69 ± 0.90	**< 0.001**
	15	29	15.24 ± 0.25	15.50 ± 0.26	0.26 ± 1.08	0.051
	Total	699	10.81 ± 2.60	11.60 ± 0.11	0.79 ± 1.59	**< 0.001**
Total sample		1200	10.75 ± 2.72	11.47 ± 0.08	0.72 ± 2.30	**< 0.001**

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

Table 5: Comparison of chronological and D4-A dental ages by gender and age group

Gender	Age group (years)	N	CA	DA	DA-CA	p value#
			Mean ± SD (years)			
GIRLS	5	24	5.46 ± 0.33	6.87 ± 0.25	1.69 ± 1.21	**< 0.001**
	6	39	6.57 ± 0.32	7.54 ± 0.14	1.05 ± 0.68	**< 0.001**
	7	46	7.52 ± 0.26	7.73 ± 0.14	0.77 ± 1.05	0.122
	8	50	8.51 ± 0.31	8.18 ± 0.12	0.75 ± 0.64	**< 0.001**
	9	55	9.48 ± 0.30	8.90 ± 0.20	1.36 ± 1.43	**< 0.001**
	10	55	10.55 ± 0.32	10.04 ± 0.24	1.21 ± 1.18	0.016
	11	40	11.44 ± 0.32	11.26 ± 0.32	1.38 ± 1.27	0.595
	12	55	12.49 ± 0.32	11.52 ± 0.31	1.58 ± 1.18	**0.021**
	13	57	13.46 ± 0.30	11.57 ± 0.38	1.40 ± 0.93	0.002
	14	59	14.48 ± 0.28	10.11 ± 0.40	1.22 ± 0.99	**< 0.001**
	15	21	15.48 ± 0.27	9.02 ± 0.57	0.60 ± 0.37	**< 0.001**
	Total	501	10.68 ± 2.87	9.57 ± 0.11	1.20 ± 1.10	**< 0.001**
BOYS	5	23	5.56 ± 0.29	7.21 ± 0.11	1.67 ± 0.57	**< 0.001**
	6	40	6.52 ± 0.31	7.75 ± 0.16	1.31 ± 1.10	**< 0.001**
	7	58	7.48 ± 0.29	8.01 ± 0.08	0.70 ± 0.73	**< 0.001**
	8	58	8.47 ± 0.29	8.76 ± 0.15	1.14 ± 1.11	**0.343**
	9	78	9.46 ± 0.28	9.23 ± 0.18	1.25 ± 1.18	0.002
	10	100	10.45 ± 0.29	10.40 ± 0.17	1.37 ± 1.13	0.137
	11	82	11.51 ± 0.30	11.84 ± 0.22	1.37 ± 1.22	0.414
	12	91	12.44 ± 0.30	13.04 ± 0.21	1.50 ± 1.17	**0.007**
	13	82	13.41 ± 0.31	14.11 ± 0.21	1.57 ± 1.07	**< 0.001**
	14	58	14.47 ± 0.31	15.61 ± 0.11	1.30 ± 0.57	**< 0.001**
	15	29	15.24 ± 0.25	15.65 ± 0.21	0.96 ± 0.87	**0.002**
	Total	699	10.81 ± 2.60	11.28 ± 0.11	1.31 ± 1.07	**< 0.001**
Total sample		1200	10.75 ± 2.72	10.56 ± 0.09	1.26 ± 1.08	0.322

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

Table 6: Intra-method comparison between genders of mean DA-CA

Gender	N	D7-O		D7-R		D4-O		D4-A	
		Mean DA-CA ± SD (years)	p value	Mean DA-CA ± SD (years)	p value	Mean DA-CA ± SD (years)	p value	Mean DA-CA ± SD (years)	p value
Girls	501	0.75 ± 1.50	0.200	+ 0.11 ± 0.81	**0.005**	0.59 ± 2.77	**<0.001**	1.20 ± 1.10	0.083
Boys	699	0.64 ± 1.44		+ 0.24 ± 0.80		0.79 ± 1.59		1.31 ± 1.07	

Independent t-test; p≤ 0.05 = significant

Table 7: Correlation between chronological and dental ages by method used

Method	r / p values	Females	Males	Total sample
D7-O	r value	0.965	0.950	0.959
	p value	**< 0.001**	**< 0.001**	**< 0.001**
D7-R	r value	0.957	0.962	0.961
	p value	**< 0.001**	**< 0.001**	**< 0.001**
D4-O	r value	0.960	0.954	0.958
	p value	**< 0.001**	**< 0.001**	**< 0.001**
D4-A	r value	0.959	0.947	0.953
	p value	**< 0.001**	**< 0.001**	**< 0.001**

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

While assessing dental age, it is important to consider not only 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 within and between examiners for Demirjian's method of dental staging were obtained in percentages and measured by Cohen's kappa coefficient, which is a more robust measure than simple percentage agreement calculation, taking into account the agreement occurring by chance.[11] Intra- and inter-examiner agreements for Demirjian's dental staging were observed to be 90% and 91%, respectively, with a kappa coefficient of 0.83. The difference in mean DA-CA was not significant between two assessments by one examiner or between two examiners. Other studies have reported kappa values ranging from 0.67 to 0.96 for intra-examiner agreements[12,13] and from 0.68 to 0.92 for inter-examiner agreements.[14,15]

In the present study, overall as well as by age group, a significant difference was observed between the mean DA and CA with all four methods in both genders, a finding which is in agreement with the observations of Flood et al.[4] in a South Australian population. However, in an earlier study by Flood et al.[5] on a Western Australian population, no significant differences between the mean DA and CA were observed overall in males, with all but the Demirjian's original seven-tooth method. In females, overall significant differences were observed with all except the four-tooth method.

Table 8: Inter-method comparison of the accuracy of age estimation

	Method with mean DA-CA		Difference in mean DA-CA	95% CI of DA-CA	Absolute difference	p value#
	(years ± SD)			Years		
Girls (N = 501)	D7-O	D7-R	-0.64 ± 0.84	0.049 to 0.197	0.51	0.004
		D4-O	-0.16 ± 0.84	-0.417 to -0.269	0.68	**< 0.001**
	D7-R	D4-O	0.48 ± 1.10	-0.315 to -0.123	0.86	**< 0.001**
		D4-A	0.96 ± 0.97	-0.045 to 0.125	0.69	0.983
	D4-O	D4-A	0.61 ± 0.66	0.202 to 0.317	0.50	**< 0.001**
	D4-A	D7-O	-0.45 ± 0.65	0.026 to 0.140	0.48	0.001
Boys (N = 699)	D7-O	D7-R	0.40 ± 0.87	0.000 to 0.286	0.47	0.077
		D4-O	0.15 ± 0.90	-0.162 to 0.043	0.72	0.277
	D7-R	D4-O	0.55 ± 1.02	-0.393 to -0.158	0.81	**< 0.001**
		D4-A	1.07 ± 1.01	-0.155 to 0.075	0.74	0.359
	D4-O	D4-A	0.52 ± 0.83	0.126 to 0.249	0.65	**< 0.001**
	D4-A	D7-O	-0.67 ± 0.83	-0.149 to 0.020	0.61	0.042
Total (N = 1200)	D7-O	D7-R	-0.50 ± 0.86	-0.035 to 0.0.62	0.48	0.773
		D4-O	0.03 ± 0.88	-0.287 to -0.187	0.70	**< 0.001**
	D7-R	D4-O	0.53 ± 1.05	-0.283 to -0.164	0.83	**< 0.001**
		D4-A	1.07 ± 0.99	-0.062 to -0.050	0.72	0.615
	D4-O	D4-A	0.54 ± 0.76	-0.174 to 0.261	0.59	**< 0.001**
	D4-A	D7-O	-0.57 ± 0.76	-0.023 to 0.063	0.55	0.506

Wilcoxon Signed-rank test; $p \leq 0.05$ = significant

Phillips reported an overestimation of age by an average of 0.89 years using the original seven-tooth method on Tygerberg dental patients; a random mixture of Caucasoid and Khoisanoid children. He also observed similar overestimations in samples of Indian and Negroid children from Kwa-Zulu Natal. He derived correction factors and found them to improve the accuracy of method significantly.[16,17] The Demirjian's revised seven-tooth method has been reported to consistently overestimate age in various populations by up to +1.23 years in males and +1.20 years in females.[18] A study by Flood et al. reported overestimations of +0.61 years (m) and +0.75 years (f), +0.49 years (m) and +0.47 years (f), +0.31 years (m) and +0.62 years (f) and +0.49 years (m) and +0.70 years (f), using the original seven-tooth, revised seven-tooth, four-tooth and alternate four-tooth standards, respectively, in a South Australian population.[4] Another study by

Flood et al. reported overestimations of +0.51 years (m) and +0.63 years (f), +0.19 years (m) and +0.41 years (f), +0.04 years (m) and +0.25 years (f) and -0.20 years (m) and +0.37 years (f), using the original seven-tooth, revised seven-tooth, four-tooth and alternate four-tooth standards, respectively, in a Western Australian population.[5] A similar study by Akkaya et al. reported overestimations of +0.53 years (m) and +0.66 years (f), +0.33 years (m) and +0.62 years (f), +0.21 years (m) and +0.57 years (f) and +0.08 years (m) and +0.61 years (f), using the original seven-tooth, revised seven-tooth, four-tooth and alternate four-tooth standards, respectively, in a Turkish population.[3] In the present study overestimations were obtained, of +0.64 years (m) and +0.75 years (f), +0.24 years (m) and +0.11 years (f), +0.79 years (m) and +0.59 years (f) and +1.31 years (m) and +1.20 years (f), using the original seven-tooth, revised seven-tooth, four-tooth and alternate four-tooth standards, respectively.

Significant gender-based differences were observed with the D7-R and D4-O methods in the present study dental age of girls being more advanced than that of boys. This gender difference has been attributed to the faster biological and dental maturation in girls, which leads to a higher DA compared to CA.[19] However, some other studies[13,20] have reported a higher DA compared to CA in boys than in girls.

Several factors can affect the accuracy or precision of an age estimating method, such as the quality of the reference material (sample), reliability of the method and biological variability in dental development.[21,22] Hence, no age estimation method can be expected to predict the exact age of every individual. Differences between chronological and estimated ages of up to 12 months can be considered to be within normal standards,[23] although smaller intervals are desirable.[24] In the present study, mean prediction errors ranged from 1.32 to 15.72 months.

Figure 1: Box-plot of mean DA-CA males aged 5-10 years

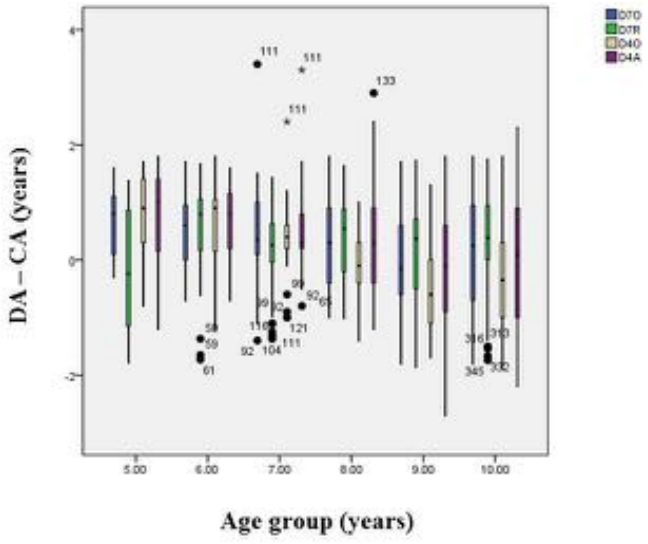


Figure 2: Box-plot of mean DA-CA males aged 11-15 years

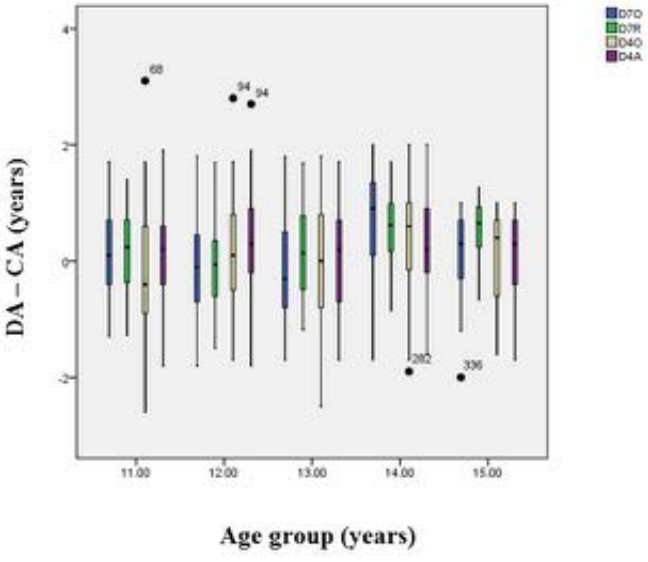


Figure 3: Box-plot of mean DA-CA females aged 5-10 years

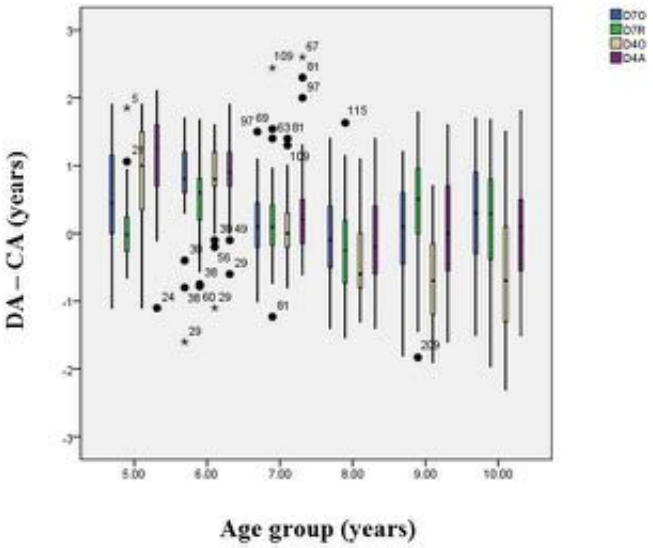


Figure 4: Box-plot of mean DA-CA females aged 11-15 years

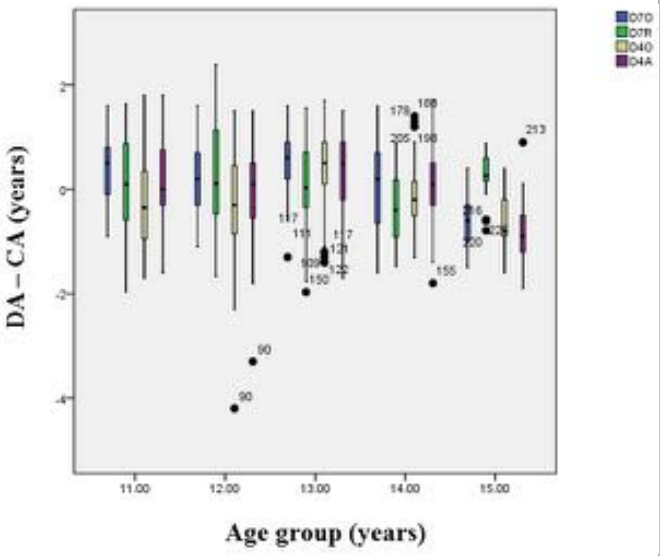
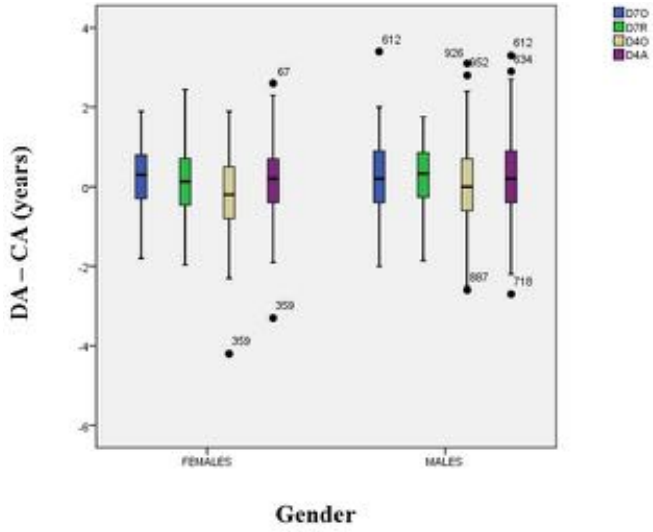


Figure 5: Box-plot of mean DA-CA by gender for the total sample



CONCLUSION

From the results of this study, it was concluded that all the four Demirjian methods overestimated age. The revised seven-tooth standards most accurately predicted the age of the study sample (mean prediction error = 2.28 months), followed by the original seven-tooth, four-tooth and alternate four-tooth standards. The Demirjian original seven-tooth method was significantly more accurate in boys compared to girls, while the reverse was true for the Demirjian revised seven-tooth and Demirjian original four-tooth methods. The Demirjian alternate four-tooth method was almost equally accurate in both genders. Significant differences between dental age and chronological age were observed with all the methods.

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CT and MR imaging used in age estimation: a systematic review

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KEYWORDS

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ABSTRACT

Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) may be useful tools in assessment of age of an individual. This article presents a review of published studies using CT or MRI in dental age estimation. They were published between July 2004 and September 2017 investigating different types of teeth, methods and formulae for age estimation. Twenty-seven articles were included. The different studies show good results, and it seems that a combination of different types of teeth, methods (depending on the degree of root formation) and cooperation between different disciplines in the same study gives a higher accuracy.

INTRODUCTION

An English dentist named Edwin Saunders is said to be the first to apply tooth development in age estimation when he published a pamphlet to the English Parliament in 1837.[1] Since then several articles have been written regarding dental age estimation and many methods have been described. In 1896, one year after Conrad Röntgen discovered X-rays, radiology was first used in forensic science. Since 1955 radiology has been an important tool in age estimation.[2] Teeth are appropriate to use in age assessment because they are very resistant to time, mechanical, chemical and physical influences. They are also very little influenced by environment, nutrition and living conditions.[3]

Age estimation is important in forensic medicine both for unidentified human remains and living individuals in single cases as well as in mass disasters. It is also one of a number of methods to determine if a person is an adult or a child in asylum applications, in youth sports, immigration and adoption, and in criminal as well as civil procedures. It is also important in orthodontic and paediatric dentistry and in anthropology. In living individuals, the recommendation is that, the age assessment is made by evaluation of growth and development on radiographs of the teeth, a physical examination and a radiograph of the hand and/or radiographs or CT of the clavicles.[4] The highest reliability for dental age estimation occurs when several teeth are in development (until the age of 12-14 years), and the most common methods in use have been tested by several researchers both theoretically and in practice.[5] As a person grows the age estimations are relatively more imprecise because of the high variability of physiological age indicators.[5] The third molars are the only teeth still developing after the age of 16 years and

up to late teens early twenties. These teeth are therefore used in age estimation of adolescents and young adults, but they are also the most variable tooth regarding time of formation and development.

Many methods have been applied to assess a person's age. The most common methods have been presented by Demirjian et al. in 1973, Moorrees, Fanning and Hunt (MFH) in 1963, London Atlas of Human Tooth Development and Eruption (QMUL) in 2010, Mincer et al in 1993, Olze et al. in 2005 and different formulae to determine the ratio between pulp and tooth volume like Kvaal et al. in 1995 and Cameriere in 2004.[6-11] The methods grade or measure dental development into mineralization and eruption stages, secondary dentine deposition and the degree of root formation.

In 2009-2010 the radiographic visibility of the periodontal ligament and of the root pulp of the fully mineralised mandibular third molars was assessed by Olze et al. [12,13]

Periapical radiographs and OPG are conventional radiographic methods that show two-dimensional images. Since the late 1990s several articles have been written about three-dimensional imaging: Computed Tomography (CT) came in 1972, Micro Computed Tomography (Micro-CT) in early 1980s, Cone-Bean Computed Tomography (CBCT) in 1996 and x-ray free imaging like Magnetic Resonance (MR) came in 1985.

CT scans are non-invasive, practical, cause no magnification errors due to geometric distortion, and present three-dimensional (3D) images of the third molar whether it is angulated or superimposed on adjacent structures.[14] Micro-CT is even more accurate, but is best in small areas and on extracted teeth.[15] Vaandevoort et al. (2004) were the first who investigated the potential of using Micro-CT in age estimation.[16] CBCT gives more detailed information, less artefacts, is cheaper and has a lower radiation dose than CT, but is not used as a routine in forensic medicine.[17] Multi-detector computed tomography (MDCT) is often used in forensic medicine since it has become more common to perform a post-mortem CT before autopsy.

The disadvantages with CT scans is the high radiation dose, high costs and artefacts caused by metal materials in for example fillings and implants (especially in the molar region). In countries were radiation is not recommended or allowed, some research with x-ray free imaging (MRI) has been tested for age estimations based on molar development. Both CT and MR imaging are dependent on good scanning protocols that provide the desired spatial resolution to distinguish what we are looking for within a clinically acceptable acquisition of time.[18]

The aim of this article is to give a review of the articles that have been written about estimating age from CT and MR imaging. The articles written on this topic have different limitations, selection of teeth and methods employed. This present article will try to sample the literature and find the gaps, problems and advantages which can lead us to further research.

MATERIALS AND METHODS:

Criteria for the selection were restricted to original articles of three-dimensional (3D) imaging in human dentition published in English after July 2004 and up to September 2017. Two-dimensional (2D) imaging was not included. The chosen articles were analysed and the characteristics of each article were systemized to give a short overview of the main points. A search was made in the following data-bases: Pubmed, Springer Link, Directory of open journals (DOAJ), Science Direct Journals (Elsevier), Oria, Annex Publishers, DentoMaxilloFacial Radiology (DMFR), RSNA, Wikipedia, Wiley Online Library, Digital Medicine, Journal of Oral Medicine and Pain (JOMP), Cochrane Library, Ovid and Google. Search words were: CT, MRI, age estimation, forensic dentistry.

RESULTS

The initial search resulted in 155 articles and after excluding 2D imaging there were 27 articles which included dental age estimation and 3D imaging for the review. These are presented in Table 1. The table shows that there are several methods used in age estimation with 3D imaging (Demirjian et al., MFH, multi-factorial with bones and teeth, Mincer et al., Olze et al., and calculations of the pulp/tooth volume). 23 articles with CT imaging include both single- and multi-rooted teeth while in MR imaging (4 articles) studies only multi-rooted teeth (molars) are included.[19] Studies with a large sample and an even age distribution for both males and females had the highest correlation coefficient. The coefficient varied in single- or multi-rooted teeth and in which calendar year the research was carried out.

DISCUSSION

To see if CT and MR imaging are useful tools in age estimation it is best to focus on the research with a large number of teeth and an even distribution of age in the test population, the highest correlation coefficient and comparison with conventional radiographs, so that we can see if the method can be an alternative to two-dimensional imaging.

In all articles the selection criteria were also good health, normal growth and dental conditions, and in the case of third molars no head injuries that affected the visualization of the tooth.[14] Teeth with metal restorations were excluded on CT because of the artefacts.[15]

The year the research was carried out is important because the imaging quality is better when modern machines are used and the scanning protocols have improved with experience. The various CBCT systems available differ with certain technical characteristics like the spatial resolution, the field of exploration and the contrast.[20] With large sample sizes and the newest CBCT operating modes and optimized software the research will be even more reliable.[20] The latest multi-detector scanners are able to provide thinner slice thickness which is optimal for assessing dental development.[5] All this can influence the accuracy.

As several studies have shown that there are differences in development between girls and boys, both genders ought to be represented.[21-24] Aboshi et al. used mandibular premolars as they are less prone to damage when compared to canines and incisors and they have simpler and more stable root morphology when compared to molar teeth.[25] There might be differences between upper and lower jaw as well as regional variations, but there is no difference between right and left side of the jaw.[14,23,24]

The stage of tooth development determines the method of age estimation. Pulp cavity/tooth volume is used when the roots are completed, while methods with different eruption and mineralisation stages and charts are used in age estimation in children and adolescents when the teeth are still developing.

The Demirjian et al. stages were used by Bassed et al. for the mandibular third molar when comparing CT with OPG.[5,26] This study reported some disagreements so Cantekin et al. decided to use CBCT images. Statistical analysis showed strong correlation between age and third molar development for both genders.[14] Cameriere et al. compared measurement of open apices of third molars and Demirjan stages to test chronological age over 18 years old in living subjects with 2D imaging.[27] This could also be used with 3D imaging.

The MFH stages of tooth development has also been tested on CT images with two people estimating, one experienced and one inexperienced.[28] Ages were underestimated by 10% using MFH method. Still compared to chronological ages, the accuracy rates of estimated ages based on CT was high (0,8-0,9 where the ratio of 1,0 indicated that the estimate was identical to the chronological age of each case).

With the individual variations of the root development of a third molar[5] cooperation between disciplines (forensic odontology, medical imaging, human growth and development and anatomy) might be required to maximise the accuracy of age estimates. In 2011 Bassed et al. carried out research using CT imaging and a multi-factorial method using the third molar tooth, the medial clavicular epiphysis and the spheno-occipital synchrondrosis.[26] Using only the third molar and the clavicle gave a good result. It seems like the uncertainty is higher the older the individual is, and this method may only be used when selected dental and skeletal sites are not fully developed.

The dimensions of the dental pulp show negative correlation with age. Kvaal et al. in 1995[29] described a non-destructive method for age estimation by linear measurements on radiographs. Two-dimensional images accumulate only horizontal and parallel aspects of the tooth, not the complete three-dimensional morphological changes of the pulp. Because of the fast-developing technology of CT machines, only research written after 2009 and research with large samples are relevant today. Ge et al. in 2015 used first molars and concluded that the pulp chamber is a useful index for age estimation, but gender and tooth position plays an important role.[30] Therefore, it is recommended to use gender and tooth specific equations.

Agematsu et al. and Sakuma et al. found a significant difference between gender and tooth position. A higher correlation between reduction in pulp volume and aging was observed in females than in males and a higher correlation between aging and decrease in pulp size was observed in the mandibular central incisors than in the mandibular second premolars.[15,31] There was also a difference

between Micro-CT and MDCT. The age-related formation of the secondary dentine is directly related to the decrease of the pulp cavity volume while the volume of an entire tooth was mainly affected by the attrition of the enamel. Thus, a pulp cavity/tooth volume ratio may not reflect the real change from secondary dentine deposition, they found that the pulp chamber volume was more accurate than the volume calculation of the whole tooth because of high image contrast between dentine and pulp chamber. Micro-CT is more accurate, but the average difference in this study was less than in earlier studies. The explanation may be modern machines. It will always be more difficult to define the junction between enamel, dentine and pulp in CT images than in OPG, but the contrast is getting better with new technology. From table 1 it is evident that studies with small samples give low correlation coefficient.[16,32-34] The correlation coefficient is also higher when using Micro-CT which give more accurate measurements because of more spatial resolution than CBCT and CT.[22,25,31] Though the latest research after 2013 shows that it is possible to achieve high correlation coefficient with large samples and modern machines. The research should simplify dental volume measurements through geometric approximation of the different parts of the tooth. It is important to remember that it easily can be inaccurate measurements because of the difficulty to find exactly the junction between tooth structures like the pulp-dentine junction. In addition, some teeth can have irregular volumes. The time may differ between methods and may vary from less than 15 minutes up to 3 hours, but shorter exposure times may require higher doses and incur larger costs.[25,32,34]

Ge et al. (2015) investigated pulp chamber volume of first molars in relation to age on CBCT images in a large sample with focus on multi-rooted teeth.[30] Because of the complex root system of first molars and to simplify the segmentation procedure, it was decided to calculate the volume of the coronal pulp chamber with age. The difference in volume between genders and the difference between the volumes of maxillary molars and mandibular molars was significant.

Another study by Ge et al. had only an average difference of 4% between the pulp volumes in multi-rooted molars and 6% in single-rooted premolars obtained from Micro-CT and CBCT.[35] The study tried to find out which type of tooth in the same dentition had the best relationship between age and pulp cavity volume in 13 types of teeth. The teeth were divided into single- and multi-rooted teeth excluding third molars. The total volume of tooth pulp cavity was calculated while for multi-rooted molars the pulp chamber floor was set as the "cut plane" because of the complex root system. The maxillary second molar gave the highest correlation coefficient with age (both in male, female and pooled gender samples). The maxillary canines showed the lowest correlation coefficient between volume and age. The explanation may be the difference in function of the teeth and location in the arch. The canines are located at the turning point of the dental arch and after reconstruction, the 3D images at a turning point will be less clear and accurate than those obtained at a planar field where the molars are located. The pulp chamber of the molars is also bigger so it is easier to see the junction between pulp and dentine.

Tardivo, et al. presented the highest correlation coefficient when using canines.[33] He chose canines because of their high level of survival, they undergo less wear, they have the largest pulp volume compared to other single-rooted teeth and the mandibular canines present the most important sexual dimorphism. This research also had a reasonably large sample with an even age distribution for both males and females. Maybe the results would have been even better if he had also used the maxillary second molar as GE et al. did, or maybe Tardivo et al. had a better protocol that minimized the importance of the location at the dental arch.[33,35]

Penaloza et al. used the Kvaal method on volumetric data from CBCT and concluded that it was less accurate because in some cases the border between the pulp chamber and the dentine, or tooth and bone was more blurred than in dental radiographs. There was also a difference in resolution between the different machines and settings. It was also more time consuming than using 2D images because of the need to properly align the teeth in the sagittal and coronal plane.[36] Volume calculations depend also on a good software.[37,38]

In most countries it is not ethically acceptable to use radiation without a diagnostic indication in living individuals. Therefore, research with MR imaging as a tool in age estimation has been performed. The research is quite new, presented in 2015 and has used the Demirjian's stages of tooth development and Olze's stages on eruption on

molars. Baumann et al. did a study with two separate and independent dentists who evaluated the different stages of mineralization and eruption of the molars in all four quadrants - one using OPG and the other MRI.[24] The different stages could be identified equally well both for OPG and MRI. It was easier to evaluate the mineralization stages on MRI than OPG because the roots on OPG are often superimposed (OPG is a projection method). It was the opposite with the evaluation of the eruption stages because the dental crown is prone to metallic artefacts in MRI (the roots are not influenced by artefacts). The results showed that MRI has a tendency to give lower stages compared to OPG. The mineralization stages can also be affected by the imaging procedures. In the dental follicle mineralised dental tissue is surrounded by watery content creating sufficient contrast, so early stages of tooth development are clearly displayed on MRI. Later stages are more challenging, as the most significant characteristics are the remnant of the dental follicle and closing of the apex.[39] In these stages the lack of contrast between dental tissue and bone can make the measurement difficult and inaccurate. Therefore, in MRI imaging it is very important to use a protocol which optimises the contrast. Baumann et al. also observed method-dependent differences with regard to the mineralization stages in some of the examined molars.[24] MRI is radiation free, but at present takes more time and is much more expensive than OPG. Another factor which must be considered is gender differences in tooth development.[21]

It has been shown that the image quality in OPG was lower in older age groups and higher in women than in men.[40] This is explained by forensic investigations as age-related changes of the dental pulp and sex differences of the skull geometry and this must also be considered in three-dimensional imaging.

De Tobel, et al.'s study showed that images taken in the sagittal plane gave 100% assessable mandibular third molars, compared to 58.8% in the axial plan.[18] The same study tried to develop the best protocol (to visualise all third molars) suitable for forensic age estimation, but a control study is needed to see if this protocol can be used.[18] The research concluded that if a method for age estimation with MRI is used, a sequence with an almost isotropic voxel size is necessary. The difference between the in-plane resolution and the slice thickness should be limited. Stern et al. has developed a method like this for left hand MRI.[41] It is also important to study further whether MRI in all planes is necessary, or if it is sufficient with only one plane. Reference samples should be scanned with suitable protocols, all stages of the third molars and include individuals from 8-24 years.[42] De Tobel et al. found the appearance of third molars on MRI different from the dental radiographs and created an appropriate staging technique.[43] The study confirmed that the mandibular molars were in the same or more advanced stages than the maxillary ones, but no difference was observed between left or right side in the same jaw. In up to 2.2% of the control measurements a two-stage difference occurred between the observers.

There are no recommendations on how to present research in this field, and the various statistical analyses have different limitations. Bias can occur in the planning, data collection, analysis and publication phases of research. The size of the test sample (individuals of known age) in a study is important to get reliable results. It is also important to include an equal number in each age group and an age range which covers the variation in the test population to avoid age mimicry. For example, an age group of 20-80 years needs more than hundred because this will only give 1.67 each year. Both genders must be equally represented and intra- and inter-observer accuracy and repeatability of the measurements must be tested. It is important that the technical error of measurements is as low as possible and the coefficient of reliability is as high as possible. Gelbrich et al. said that only reference data with uniform age distribution or adequate correction for potential bias should be cited for forensic age estimation.[40] Reference data with low bias and small values of mean absolute difference are recommended to estimate age and point estimates and age intervals should be accompanied by a measure of variation.[44]. In the assessed studies some of the criteria mentioned above is not fulfilled: too small samples, the sexes are pooled and the sample do not have an even age distribution. On the other hand, some of the studies are results of cooperation between different researchers.

The most common statistical method in age estimation is regression analysis.[45] The Demirjian method frequently overestimates young individuals and underestimates elderly individuals when using this analysis.[6,45] The most appropriate

statistical method is maybe a model that allows for multiple related age predictors to be integrated for age estimation e.g. the Bayesian model.[43]

It seems that when different age-related changes are used together it gives higher confidence than when one is used on its own.[26] This needs more research and can be the ultimate method for age estimation when collaborating conventional radiographs with CT or MRI of the hand/wrist and other skeletal changes. Maret et al. had an hypothesis that quantitative measurements of the alveolar bone and surrounding cortical bone could be combined with other variables like the volume of each component of the teeth to estimate the age of a living subject.[20] Swasty et al. evaluated the cortical thickness, height and the width with CBCT and determined the relationships between these parameters and age.[46] The variations of these mandibular variables indicated changes in overall shape that were dependent on age and probably the forces generated during function.[20] Other factors that could influence the cortical thickness and anatomy were face type and gender.

Table 1. Characteristics of the chosen articles

Author	Year	Country	Total amount	Age	Sex	Teeth	Method	Imaging	correlation results
Vandevoort, Cleynenbreugel, Bielen, Lambrechts, Weves, Peirs and Willems[16]	2004	Belgium	43	24-66	N/A	Single-rooted teeth	Pulp/tooth volume	Micro-CT SkyScan byba	r=0,31
Yang, Jacobs and Willems[32]	2006	Belgium	19	23-70	11 f 8 m	Incisors, canines and premolars	Pulp/tooth volume ratio	Cone-beam CT- 3D Accuitomo	r=0,29
Someda, Saka, Matsunaga, Nakahara, Hirata and Hashimoto[22]	2009	Japan	155	12-79	79 f 76 m	Mandibular central incisors	Volumes of enamel, dentine and pulp cavity	Micro-CT-HMX225 ACTIS4	r=0,67 m r=0,76 f volume ratio pulp cavity/ tooth volume excluding enamel
Graham, O`Donnel, Craig, Walker, Hill, Cirillo, Clark, Gledhill and Schneider-Kolsky[28]	2009	Australia	96	<15	N/A	Incisors Deciduous Permanent	MFH (Moorrees, Fanning and Hunt) Experience-based estimation	CT-Toshiba	r=0,9
Bassed and Hill[43]	2010	Australia	2	0,5-3,5 year	N/A	N/A	Demirjian	CT-Toshiba Aquilion 16	Useful tool in identification
Aboshi, Takahashi and Komuro[25]	2010	Japan	50	20-78	23 f 27 m	Mandibular first and second premolars	Pulp/tooth volume	Micro CT-SMX-130CT-SV	r=0,625 mandibular first r=0,698 mandibular second

Agematsu, Someda, Hashimoto, Matsunaga, Abe, Kim, Koyama, Naito, Ishida and Ide[31]	2010	Japan	N/A	20-79	75 f 73 m 54 f 56 m	Mandibular central incisor Mandibular second premolar	Pulp/tooth volume	Micro-CT HMX225 ACTIS4	r=0,75 r=0,67 r=0,58 r=0,56
Tardivo, Sastre, Ruquet, Thollon, Adalian, Leonetti and Foti[33]	2011	France	58	14-74	32 f 26 m	Canines	Pulp/ tooth volume	CT	r=0,32 f r=0,47 m
Bassed, Briggs and Drummer[5]	2011	Australia	667	15-25	216 f 451 m	Mandibular third molar	Demirjian	CT-Toshiba Aquilion 16	100 % females and 96 % males with completed roots were over 18 years old
Bassed, Briggs and Drummer[26]	2011	Australia	605	15-25	184 f 421 m	Mandibular third molar, clavicle and an intact spheno-occipital synchondrosis	Demirjian Schulz Powell and Brodie	CT-Toshiba Aquilion 16	These three methods used together gives 95 % confidence than when each marker is used alone
Star, Thevissen, Jacobs, Fieuws, Solheim and Willems[34]	2011	Belgium	64 32 15	10-65	N/A	Central Incisor Lateral Incisor Canine First premolar Second premolar	Pulp/tooth volume	CBCT Scanora 3 D	r=0,41 r=0,07 r=0,23
Jagannathan, Neelakantan, Thiruvengadam, Ramani, Premkumar, Natesan, Herald and Luder[48]	2011	India	140	10-70	N/A	Mandibular canines	Pulp/tooth volume	CBCT 3D Accuimoto	r=0,397
Sakuma, Saitoh, Suzuki, Makino, Inokuchi, Hayakawa, Yajima and Iwase[15]	2012	Japan	136	14-79	31 f 105 m	Mandibular first premolars	Pulp cavity/ tooth volume	CT-NewTom 3G	r=0,76 and 95% confidence
Cantekin, Sekerci and Buyuk[14]	2013	Turkey	649	9-25	319 f 330 m	Mandibular third molar	Demirjian	Cone-beam CT-NewTom 3G	r=0,78 f r=0,80 m

Tardivo, Sastre, Catherine, Leonetti, Adalian and Foti[33]	2014	France	840	15-85	N/A	Canines	Pulp/tooth volume	CT-Siemens sensation	r=0,915-0,964
Porto, Neto, Pontual and Catunda[49]	2015	Brazil	118	22-70	60 f 58 m	Maxillary central incisors	Pulp cavity / tooth volume ratio	Cone-beam CT i-Cat Next Generation	r=0,21 r=0,15 m r=0,297 f
Ge, Ma, Li, Zhang and Ma[30]	2015	China	373 maxillary 372 mandibular	12-69	N/A	First molar	Pulp/tooth volume	Cone-beam CT NewTom VG Micro-CT	Significant differences in sex and tooth position r=0,66 maxillary r=0,604 mandibular More accuracy than CBCT
Pinchi, Pradella, Buti, Baldinotti, Focardi and Norelli[50]	2015	Italy	148	10-80	91 f 57 m	Maxillary left central incisors	Pulp/tooth volume	CBCT Scanora 3D	The age cohorts between 30 and 59 years showed the highest accuracy r=0,58
De Angelis, Gaudio, Guercini, Cipriani, Gibelli, Caputi and Cattaneo[51]	2015	Italy	91	17-80	49 f 42 m	Maxillary right third molar	Pulp/tooth volume	CBCT-i-cat Next Generation	r=0,485 r=0,263 Total r=0,389
Baumann, Widek, Merkens, Boldt, Petrivic, Urshler, Kirnbauer, Jakse and Scheurer[24]	2015	Austria	27	13,6-23,1	19 f 8 m	Molars (262, mineralization, 274 eruption)	Demirjian and Mincer (mineralization) Olze (eruption)	MR MagnetomTrio,a Tim System,Siemens AG	Good correlation between MRI and OPG MRI tended to give slightly lower stages
Guo, Olze, Ottow, Scmidt, Schulz, Heindel, Pfeiffer, Vieth and Scmeling[21]	2015	Germany	517	12-24	248 f 269 m	Mandibular third molars	Demirjian	MR Philips 3,0 Achieva	MRI is an X-ray free alternative to OPG in assessing mineralization
Ge, Yang, Li, Zhang and Ma[35]	2016	China	240	16-63	115 f 125 m	13 types of teeth	Pulp/tooth volume	CBCT-New Tom VG	Maxillary second molar has the best result r=0,64

Penaloza, Karkhanis, Kvaal, Nurul, Kanagasinga, Franklin, Vasudavan, Kruger and Tennant[36]	2016	Australia	101	15-75	55f 46m	Maxillary central and lateral incisors and The second premolar Mandibular lateral incisor, canine and first premolar	Kvaal	CBCT both Kodac and i-Cat.	The accuracy was outside an acceptable range and more time consuming than dental radiographs.
De Tobel, Hillewig and Verstraete[39]	2016	Belgium	52	14-26	N/A	Third molars	Demirjian and Köhler	MR Magnetom, a Tim system, Siemens AG	3T MRI has advantages compared to OPG, but further research is necessary.
Màrquez-Ruiz, Trevino-Tijerina, Gonzàlez-Herrera, Sànches, Gonzàles-Ramirre and Valenzuela[37]	2017	Spain	135	14-23	62f 73m	Maxillary and mandibular third molar	Demirjian Pulp/tooth volume	Multi-slice helical high resolutionLi gt-Speed VCT CT system	Virtual CT imaging is an alternative to OPG for the assessment of third molar mineralization.
Penaloza, Karkhanis, Kvaal, Vasudavan, Castelblanco, Kruger and Tennant[38]	2017	Australia	21	15-63	12f 9m	Maxillary canines	Pulp/tooth volume	CBCT 9000 3D Extraoral Imaging System	Not showing better results than methods based on dental radiographs.
De Tobel, Phlypo, Fieuws, Politis, Verstraete and Thevissen[43]	2017	Belgium	309	14-26	146 f 163 m	Third molars	To develop an MRI specific staging technique for the development of third molars	Magnetom Trio Tim, Siemens	The new staging technique showed comparable reproducibility and performance.

Abbreviations:

Total amount: number of participants	f: female	m: male
N/A: not applicable	Age= years	OPG: Orthopantomography
MR: Magnetic Resonance	MRI: Magnetic Resonance Imaging	CT: Computer Tomography
CBCT: Cone-beam CT		

CONCLUSION

Both CT and MR imaging may be useful tools in age estimation, but more research is needed before these tools can be used for this purpose. Since CBCT is more commonly used in dental clinics, more image samples for retrospective studies may be available in the future. Further and extensive research may also lead to new methods in which mandibular third molars are used to determine if a person is younger or older than eighteen years of age. It would also be interesting to do more research in cooperation with x-ray and/or CT of the hand/wrist region and clavicles to get as accurate a result as possible. The quality of the machines and scanning protocols are improving and will give better standards of the images. This will give us possibilities for research with larger numbers of test subjects and therefore even more reliable results and more specific conclusions.

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Age and gender related changes of salivary total protein levels for forensic application

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ABSTRACT

Saliva is one of the most commonly encountered biological fluids found at the crime scene. Forensic science including forensic odontology is focused on the positive identification of individuals. The salivary protein profiling can help in personalization by the changes associated with age throughout life and gender. These changes also seem to vary with the dietary habits, environmental factors and geographical areas. Thus, the aim of present study is to estimate these changes in salivary total protein concentration and profiling in individuals of Gujarat, India. The association of total protein concentration and protein content with the age, gender, tooth eruption, functions of the protein and its physiological significance are also intended for study in this population. One hundred unstimulated whole saliva samples from study subjects of Gujarat population were collected and grouped based on age and gender. Total protein concentration was determined by Bradford assay; also protein was separated and analyzed using Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS PAGE). T Test and ANOVA were used for statistical analysis. The concentration of Total Protein was found to be between 2-4 mg/ml. It showed a positive correlation with age and gender. It can be concluded more protein bands were prominently present in the adolescents group followed by children and lastly in the adults groups.More high (more than 80 kDa) and low (less than 30 kDa) molecular weight proteins are seen in children and adolescents than adults. SDS PAGE allowed identification and comparison of group variabilities in protein profiles. The total salivary protein showed an association between the parameters under this study which will aid in the individual identification in the field of forensics.

INTRODUCTION

Forensic science including forensic odontology deals with the dental evidences and its examination that is used in recognition of the crime and positive identification of an individual. Teeth, physical injuries, bitemarks, lip prints and oro-facial structures are the normal evidences used in forensic odontology investigations. Over the last decade or so there has been a growing interest in saliva and its use as a diagnostic tool as an alternative to blood or urine.[1] In recent times, the importance of saliva has been brought into focus for individualization and victim identification in mass disasters. Saliva is composed of about 99% water, 1% solid; 0.5% inorganic substances

(chloride, sodium, phosphorus, calcium, potassium, nitrogen, oxygen, bicarbonate, dissolved carbon dioxide and trace elements) and 0.5% organic substances (protein, vitamin, lipid, hormone-like substance, free amino acid, urea, microbial, shed cells and antimicrobial substances).[2] Components of saliva may serve as biomarkers because the composition of oral fluid is responsive to behavioral, mechanical, genetic or ontogenetic stimuli.[3] Saliva is often detected in scenes of crime along with bite marks or lip prints where the oral cavity may have been involved.[1] Saliva can act as an aid in cases where the direct examination of bitemarks or lip prints cannot lead to a positive conclusion due to elasticity of the skin or substrate, malocclusion, partial impressions, multiple or distorted impressions, avulsion bites leading to tear of the tissue and unavailability of records . Saliva also leads to identification of individuals associated with crime scenes like homicide, assault, child or elder abuse, poisoning, drug or alcohol abuse and other criminal cases. Saliva is a body fluid usually deposited with oral evidences when oral tissues come in contact with skin, food, clothes, cutlery, glass and cigarette. Forensic investigation and analysis of saliva is preferred due to ease of availability at a crime scene, effective collection methods and huge variation in the biological composition throughout an individual's life. The added advantage of its noninvasive method of collection even by individuals with limited training and avoidance of intrusion of private functions while collection under direct supervision, makes saliva a popular fluid for forensic analysis. Serological and cellular analysis of obtained saliva is of immense use in identification of the accused.[1]

Proteomics and genomics are gaining popularity as remarkable tools in forensics. Proteins are found in different forms based on their molecular weight, size, structure and functions. Specific array of proteins have distinctive character and function which helps to gain information of a particular individual. Molecular changes in proteins are reflected in the salivary composition and serve as genetic markers. Salivary biomarkers may be used in detecting and diagnosing systemic diseases based on the relativity between a specific disease and salivary protein changes. It also provides vital information in identifying a person involved in crime.[4] Total salivary protein concentration and protein profiling demarcates age and gender dependent differences. Thus it is essential to understand the standard baseline of the variations found in the protein profiles of a population. The major salivary proteins comprise amylases, acidic, basic, and glycosylated proline-rich proteins (PRPs), statherins, histatins, peroxidases, cystatins, and mucins constitutes the proteome. Concentration of protein in whole saliva is ~2000-4000 µg/mL.[5] The main functions of salivary proteins are protection of teeth, pH maintenance, anti-microbial and anti-fungal properties, decrease in demineralization by pellicle formation, remineralization of enamel, caries prevention, inhibition of microbial growth and defence to maintain oral health. Functions of salivary proteins may depend on the molecule's location or site of action.[6]

The identification of individuals by salivary protein profiling can aid in personalization by the changes observed associated with age throughout life and gender. The aim of present study is to estimate these changes in one hundred individuals of Gujarat population using salivary total protein. The present study quantitatively estimates the protein concentration of saliva in different age groups and gender with the help of Bradford Assay. It also focuses on the qualitative approach to determine salivary proteome of individual and pooled samples using SDS PAGE.

MATERIALS AND METHODS:

Study design and saliva sampling

This study was done to examine and compare the salivary proteome in individuals during the course of dental eruption in children and throughout the adult life. Samples were analyzed individually for inter-individual variations as well as pooled to assess inter-group differences. For this study unstimulated whole saliva specimens were collected from 100 healthy individuals of Gujarati population. Written consent was taken from each participant before the collection. Whole unstimulated saliva was collected in the morning between 8 a.m. to 10 a.m. No edentulous individuals were included for the subject selection. Healthy individuals with no acute or chronic disease between the age group of 3 to 60 years were included. Any consumption of water, food or drugs one hour prior to sample collection was excluded. The subjects were equally divided into children group 1 (3 to 6 years) - children with primary dentition: 8 subjects, group 2 (7 to 12 years) – children with mixed dentition: 25

subjects, adolescents (13 – 25 years) - permanent dentition until third molar eruption: 34 subject sand adults (26-60 years) - post dental eruption: 33 subjects. Subjects were also divided on basis of gender: 53 males and 47 females. Table 1 shows age and gender distribution of the study subjects. Nearly 5 ml of saliva sample was collected in sterile eppendorf within 5 minutes time window, fellowed by addition of 0.5 M EDTA for preservation. Immediately after collection saliva samples were centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatants were gently aspirated with a pipette without disturbing the pellet. The supernatants were frozen and preserved at −20°C. Prior to analysis, aliquots were thawed and used for analysis.

Table 1. Table shows distribution of subjects for this study according to (A) age and (B) gender.

A	
Groups according to Age	**No. of subjects**
Children 3 to 6 years (Primary dentition)	8
Children 7 to 12 years (Mixed dentition)	25
Adolescents 13 to 25 years (Permanent dentition until third molar eruption)	34
Adults 26 to 60 years (Post dental eruption)	33
Total	100

B	
Groups according to Gender	**No. of subjects**
Female	47
Male	53
Total	100

Quantification of salivary proteins

The standard Bradford assay[7] suitable for measuring between 10 and 100 µg protein was used in the present study to determine the concentration of salivary proteins. The approximate protein concentration in the sample was unknown so a range of dilutions (1, 1/5, 1/10, 1/20, 1/100) were assayed randomly in 4 samples to optimize the dilution. The samples and protein standards were measured at A595 for absorbance in Vis-UV Spectrophotometer between 5 and 60 minutes. The total protein concentration was calculated using the BSA standard calibration curve. Statistical analysis ANOVA test was done to compare the estimated age and chronological age of different groups and T- test was done for gender differentiation.

Qualitative analysis by SDS-Page gel electrophoresis

Proteins from saliva were extracted by TCA - Acetone - DTT method as described earlier[8]. The pellet obtained at the end of the process was used for SDS-PAGE gel electrophoresis for separation and analysis of salivary proteins. SDS-PAGE[9] was performed in gel caster and power bank assembly (SE260 from Hoefer Inc., USA) using 15% polyacrylamide resolving gel and 5% stacking gel. 1x sample buffer was added to 5 µL of the prepared samples. Electrophoresis was carried out at constant voltage of 180 V for three hours. MAGSPIN-34 MAG Universal prestained protein ladder was used as a reference. Coomassie brilliant blue colorimetric staining protocol was used for staining the gels. Gel Documentation System Photo scanner was used for gel image analysis.

RESULTS

The proteins present in the saliva were quantified by the Bradford assay. In the present study the total protein content was calculated in all saliva samples with 20 as the optimum dilution factor. The protein concentration obtained from Bradford assay was found to be 1.75 ± 0.75 mg/ml in children having primary dentition (3-6 years). It increased with age in children having mixed dentition to 2.31 ± 0.81 mg/m. It slightly decreased to 2.25 ± 0.74 mg/ml in adolescents having permanent dentition up to the eruption of third molar (13-25 years). Protein content was found to be 2.05 ± 0.91 mg/ml in adults (26-60 years) corresponding to decrease than adolescents group as shown in Table 2.

Table 2. Table shows the total protein concentration in saliva samples obtained by Bradford Assay of various age groups. The average protein concentration was found to be increasing form adolescent group to children up to adults. Whereas in pooled samples concentration decreased from children to adolescent to adult group. The association of protein concentration with the eruption of teeth can also be assessed.

Group	Average Total Protein Concentration (mg/ml)	Total Protein Concentration in pooled samples (mg/ml)
Children 3 to 6 years (Primary dentition)	1.75 ± 0.75	2.60
Children 7 to 12 years (Mixed dentition)	2.31 ± 0.81	3.68
Adolescents 13 to 25 years (Permanent dentition until third molar eruption)	2.25 ± 0.74	3.25
Adults 26 to 60 years (Post dental eruption)	2.05 ± 0.91	2.71

ANOVA statistical analysis was done for the age groups. The P value was found to be 0.60(P > 0.05) showing no significant difference between the age groups. Total Protein content in pooled saliva samples was done for each group. It showed saliva protein concentration obtained from children (3- 6 years) was 2.60 mg/ml, increased in children (7-12 years) at 3.68 mg/ml and decreased to 3.35 mg/ml in adolescents (13-25 years). Further protein concentration was found to decrease at 2.71 mg/ml in adults (26-60 years).

Our results showed higher salivary total protein in female than males as shown in Table 3.

Table 3. Table shows the total protein concentration in saliva samples obtained by Bradford Assay of gender groups. The average protein concentration and total protein concentration in pooled samples was found to be increased in female group than male group.

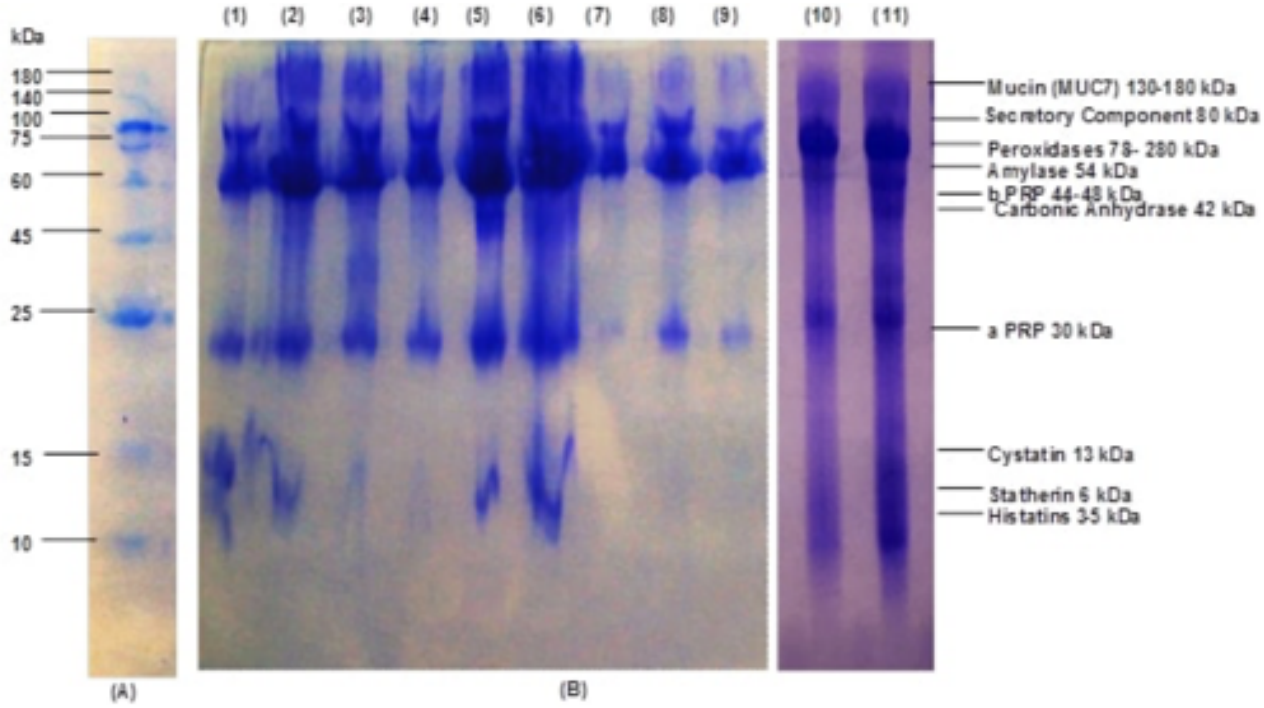
Group	No. of subjects	Average Total Protein Concentration (mg/ml)	Total Protein Concentration in pooled samples (mg/ml)
Female	47	2.25 ± 0.85	3.06
Male	53	2.06 ± 0.80	2.60

The total protein concentration in females was found to be 2.25 ± 0.85 mg/ml and in males was found to be 2.06 ± 0.80 mg/ml. T test statistical analysis was done for the gender groups. P value was found to be 0.28 (P > 0.05) showing no significant difference between females and males. Total protein content in pooled saliva samples was found to be 3.06 mg/ml and 2.6 mg/ml in females and males respectively.

The total protein concentration in pooled samples was greater than the average total protein concentration in all the age as well as in gender groups.

The SDS PAGE of each age group and pooled samples from each group shows the protein bands that are identified according to their molecular weight[10] as shown in Figure 1.

Figure 1: SDS-PAGE gel of salivary protein (A) shows the Prestained Protein Ladder gel (B) shows saliva samples of different groups (1) Age 3 (2) Age 7 (3) Age 12 (4) Age 13 (5) Age 18 (6) Age 25 (7) Age 27 (8) Age 46 (9) Age 60 (10) Pooled female sample (11) Pooled male sample



Our results showed that more protein bands were prominently present in the adolescents group followed by children with mixed dentition of age group 7-12 years followed by children with primary dentition of age group 3-6 years and lastly in the adults groups. High molecular weight protein band like Mucin (MUC7- 130 to 180 kDa) was present in both groups of children and adolescents but absent in adults. Secretory component of IgA (80 kDa), peroxidases (78 kDa), amylase (54 kDa) protein bands were seen in all the age groups. aPRP- proline rich protein band (30 kDa) and bPRP- proline rich protein band (44-48 kDa) were also seen in all the age groups. We observed the presence of carbonic anhydrase (42 kDa) only in the adolescent group and children with mixed dentition of age group 7-12 years saliva samples but its absence in adults. Low molecular weight protein bands like cystin (13 kDa), statherin (6 kDa) and histatin (3-5 kDa) bands are not very clearly seen in the adults but seen in both groups of children and adolescents. The SDS-PAGE gels of the pooled samples of all the age groups showed similar protein bands as compared to the individual sample. All protein bands in female pooled samples as well as in male pooled samples which are similar to the bands seen in individual samples.

DISCUSSION

The concentration of total protein in whole saliva obtained in the study using Bradford Assay was found to be in the normal range between 2.0-4.0 mg/ml.[5] The dilution factor 20 was found to be most appropriate in our study for the concentration of salivary proteins according to the absorbance range as it showed absorbance in the optimum range (<1). Each individual sample from all age groups contained a specific and different protein concentration which may help in differentiating each person. The age and total protein concentration showed a positive correlation. Our study shows difference in protein concentration of young, adolescents and adults. This is in accordance with results shown by Katie P. Wu et al.[11] Our results show linear increase in protein concentration with age until development of permanent teeth. This is in accordance with the studies by earlier reports by Nagler and Hershkovich,[12] Deshpande et al.[13,14] and Vibhakar et al.[6] Our results are also in

accordance with results by Kalipatnapu et al.[15] showing that protein content increase until middle age and remains constant in adults, further it decreases with advancing age. Our study also shows that children aged 7-12 years with mixed dentition show more protein concentration than children aged 3-6 years with primary dentition because of differences in salivary gland development. Katie P. Wu et al. also concluded that age 12-14 years show more protein concentration than 3-11 years.[11] Our study also shows the same results until the age of 14 years. Statistical analysis shows no significant difference between the age groups. These results were in accordance with study results by Shiv kumar et al.[3] Higher protein concentration was found in females than in males. This was also shown in studies by Dodds et al.[16] Statistical analysis showed no significant difference between males and females similar to results by Shivkumar et al.[3] and Vibhakar et al.[6] but in contrast with results by Dodds et al. which stated that significant sex differences in salivary protein concentrations exist.[16]

SDS PAGE showed high individual variability of the saliva protein band patterns in our study. The present study showed that the protein profiles differ with the age groups, which can be correlated to the teeth eruption in children (Primary and mixed dentition), adolescents (Permanent dentition until third molar eruption) and adults (Post dental eruption). This is in accordance with the study by M. Morzel et al.[17] More high and low molecular weight proteins are seen in both groups of children and adolescents than adults. Adolescent and children saliva were seen to contain both higher (more than 80 kDa) and lower molecular weight protein (less than 30 kDa). However, lower molecular weight protein lesser than 25 kDa was not observed in adults and was found mostly in children and adolescent saliva samples. These results were also reported by Shivkumar et al.[3] as they stated that children contained high molecular weight proteins and low molecular weight proteins of >90 kDa and <30 kDa respectively. Deshpande et al.[13] also showed the number of peaks of high molecular weight proteins (>70 kDa) observed in primary, mixed and permanent dentition did not show any statistically significant difference, though the average number of peaks in permanent dentition was higher than primary and mixed dentition age groups.

We have also tried to correlate the results of our study with the functions of the protein[18,19] and the physiological significance of the same. Mucin provides protection against bacterial protease activity, provides viscoelasticity and lubrication. The mucin protein band absent in adults may suggest that the adults have decreased viscoelasticity and are more prone to bacterial colonization. The secretory component of IgA is known to play a role in immune functions. Peroxidases are bactericidal and fungicidal agent and help prevent decalcification of enamel. Amylase is known to have function of hydrolysation (digestion) of starch and protection by selective binding to microorganisms. Proline Rich Proteins help in remineralization and caries protection. All these protein families are found in all the groups in our study and so these functions can be correlated to physiological functions in all ages but the concentration may differ with age. The protein band of carbonic anhydrase was found in adolescents and children with mixed dentition of age group 7-12 years. The function of carbonic anhydrase is known to be protective in nature involved in salivary pH regulation. The low carbonic anhydrase in adults can be associated with an increase in prevalence of caries. Statherins inhibit crystal growth of calcium phosphate salts and inhibit its precipitation. It also binds to bacteria and hydroxyapatite. Histatins also bind hydroxyapatite, complex with metal ions, inhibit crystal growth of calcium phosphate salts and stimulate wound-closure. Similarly, cystatins also weakly bind to hydroxyapatite. These protein bands are seen only in both children groups and adolescents but absent in adults. This can be correlated to the eruption and maturation of teeth in children and adolescents up to the eruption of the third molar at the age of around 25 years. Histatin bands are also seen in children and adolescents but absent in adults. Histatins are anti-fungal (potent C. albicans growth inhibitors) and anti-bacterial (inhibits activity of *P. gingivalis* associated with forms of periodontal disease). Histatin 1 protects tooth enamel and pellicle formation whereas Histain 5 is the most potent candidacidal. The absence of histatin band in the adults may be associated with the increased susceptibility of

adults to periodontal and fungal (candidial) diseases. This aspect has to be further studied for more accurate differentiation and individualization. Presence or absence of certain protein family bands in children, adolescents or adult saliva samples needs to be further investigated for its putative physiological significance.

CONCLUSION

The age and total protein concentration showed a positive correlation with age and gender. Protein content increases from children with primary dentition to children with permanent dentition until adolescence, further remains constant in adults and decreases with advancing age. It is concluded that there was no significant difference between the age groups as well as between females and males. Inter- and intra- group variability of the protein profiles by SDS-PAGE allowed the quantification and comparison of protein profiles from all individual and group samples successfully. It can be concluded more protein bands were prominently present in the adolescents group followed by children with mixed dentition of age group 7-12 years followed by children with primary dentition of age group 3-6 years and lastly in the adults groups. More high (more than 80 kDa) and low (less than 30 kDa) molecular weight proteins are seen in both groups of children and adolescents than adults. It was thus concluded in our study that there is correlation of the total protein concentration and protein content with the age, gender, tooth eruption, salivary gland development, functions of the protein and its physiological significance.

Saliva has a great potential in this field which has to be further explored. The importance of the protein component in saliva was highlighted in the present study. It also shows that it may prove to be of great importance in investigations where DNA may not be obtained. Since whole saliva can be collected quickly and non-invasively from many crime scenes, they may prove to be useful protein profiling media for age estimation or gender determination and criminal investigation by the forensic expert. This study establishes age related changes in human salivary total protein levels and also constructs a catalogue for age estimation gender determination using saliva which will aid in individual identification in forensic cases.

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DISCLAIMERS

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the University.

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Palate shape and size and palatal rugae morphology of children with anterior open bite and normal vertical overbite

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KEYWORDS

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ABSTRACT

Purpose: To determine differences between open bite and normal vertical overbite regarding distribution, size and clinical appearance of palatal rugae, depth and length of the palate, intercanine and intermolar widths and arch perimeter.

Methods: A cross-sectional study was performed in 264 superior models were studied with a 3D analysis system. A total of 132 individuals with AOB and 132 individuals with normal vertical overbite were evaluated, chosen from public schools with ages between 8 and 16 years. Palatal anthropometric features were evaluated. Qualitative analysis of palatal rugae was performed, exploring the shape, direction, unification and sensitivity of the palate. The Mann Whitney and Chi Square tests were used for statistical analyses.

Results: The average age was 11.37 +/- 2.27 years for normal overbite and 11.87 for anterior open bite, with 54.9% of women. No significant differences were found between subjects with AOB and subjects with normal vertical overbite regarding intermolar or intercanine width. The maxillary length and depth and the height and width of palatal rugae were lower in the AOB group. The most common rugae shapes were curved and wavy, predominating in the horizontal direction with a parallel distribution.

Conclusion: Qualitative evaluation demonstrated asymmetry in the shape, direction and unification of rugae in both groups. Most arch measurements were greater in individuals with AOB.

INTRODUCTION

An anterior open bite (AOB) is present when no vertical overlap between the incisors occurs and an interincisal separation can be measured.[1] Subjects with this class of malocclusion can show other impairments associated with open bite such the presence of a narrow palate, prominent palatal rugae, atypical deglutition and a forward position of the tongue at rest.[2-4]

The widening of the palate occurs primarily during the first 5 years of life, at the level of the intermaxillary and interpalatine sutures. In later stages of development, any increase in the width is the result of bone apposition on the outer surfaces of the maxillary and buccal eruption of permanent teeth, generating an increase of up to 2.2 mm in intermolar width.[5] Width increases are correlated with the vertical growth of the alveolar process, whose direction in the upper arch is divergent, forming the palatal walls.[6]

A narrow or triangular palate is consistent with an abnormal lingual position at rest and during swallowing because the tongue does not remain in the palatal rugae but on the floor of mouth, exerting force on the teeth. A low position of the tongue can generate expansion of the lower dental arch and promote collapse of the upper arch.[2]

The palatal rugae are crests located in the anterior part of the palatal mucosa on each side of the palatine raphe and behind the incisive papilla. They appear by the third month of intrauterine life (weeks 12–14) with connective tissue covering the palatine process of the maxillary bone. The palatal rugae growth and development are controlled by epithelial-mesenchymal interactions through the extracellular matrix.[5] With an increasing size of the front of the palate in the early years of life, the length of the rugae and the distance between them increases; from this moment, the model of rugae orientation becomes clearer and remains unchanged throughout life.[7-10] The number of palatal rugae and their arrangement, shape and length are particular to each individual, similar to fingerprints.[11] Physiologically, the palatal rugae are involved in the oral stage of swallowing and help to improve the relationship between food and the taste receptors on the dorsal surface of the tongue. Additionally, the rugae participate in speech and sucking.[12-14]

Palatal rugae are considered as stable references for the quantitative analysis of models due to their shape, design and features and because they are not altered by tooth eruption or loss[15]. Although palatal rugae show little bilateral symmetry in their distribution pattern, the number of rugae on each side varies between three and five; they are not extended posteriorly beyond the anterior half of the hard palate and never cross the midline. The anterior palatal rugae are generally more prominent than the posterior ones.[14] A relationship has been observed between the clinical appearance and size of the palatal rugae and the presence of open bite,[4] for which an increased size has been reported.

Moreover, individuals with AOB can show kinaesthetic and proprioceptive limitations generated by alterations in reciprocal contact between the physiological contacts of the lingual tip and palatal rugae and by the difficulty in recognizing the tongue location within the oral cavity.[16] It has been stated that this alteration in rugae proprioception is influenced by the size of the palatal rugae. Finally, it has been found that a slight roughness, corresponding to the pressure exerted by the tongue at a resting position, and pronounced or hypertrophic palatine folds may occur due to a lack of stimulation of the tongue at rest or during swallowing; however, this criterion is not the result of studies with sufficient evidence.[17]

Being able to determine the differences in palatal features and size between individuals with AOB and individuals with normal vertical overbite (NVO) is critical to establish other aetiological factors involved, which helps to establish a treatment scheme according to the interventions required by the patient. There are a few studies available in the literature that compare the shape and size of the maxilla and palatal rugae [8,9,18] between individuals with AOB and NVO, but none of them compares the shape and size of the palatal rugae. It should be normal to find that AOB patients have a narrower palate and thicker palatal rugae. Therefore, the aim of the present study is to determine if there are differences between patients with AOB and with NVO regarding the distribution, size and clinical appearance of the palatal rugae, depth, palatal length, intercanine and intermolar widths and arch perimeter.

MATERIALS AND METHODS:

A cross-sectional study was performed in which a total of 264 cast models were obtained from schoolchildren from 5 public schools in the municipality of Envigado. A total of 132 dental models were obtained from children with AOB, and 132 dental models were obtained from children with NVO. The sample size was calculated based on 2% AOB prevalence with a 95% confidence interval and a sampling error of 7%, based on a population of 22.955 inhabitants. The 264 students selected met the following inclusion criteria: schoolchildren aged 8–16 years with AOB and NVO from public schools from the municipality of Envigado, with four upper and lower incisors fully erupted and without posterior crossbite. Children whose parents did not sign the consent form, individuals who had a mental syndrome and facial and/or skeletal malformations, children who received or were in interceptive and/or corrective treatment and children with finger and lower lip sucking habits

were excluded. AOB definition considered for the study was the following: anterior teeth that do not reach the line of occlusion and do not contact the antagonists by at least 1 mm, as measured from the incisal edges of upper incisors to the incisal edges of lower incisors. While NVO definition was: incisal edges of the mandibular incisors in contact with the palatal surfaces of the maxillary incisors, with approximately one-third of the crowns of the lower incisors covered.

This research was approved by the bioethics committee of the Cooperative University of Colombia. The informed consent and assent forms were signed before beginning the study.

Impressions were obtained in alginate, the powder and liquid were mixed in a ratio according to the manufacturer's instructions, and the mixture was poured into type III plaster (the ratio of water to powder was obtained by dividing the water volume by the powder weight). Two consistencies were prepared: one consistency was more fluid to copy perfectly palatal rugae and teeth surfaces, while the second consistency was denser and was poured over the first one to finish the filling of the impression.

The superior models of AOB and NVO were digitised by the company i3D with an optical 3D scanner (The ATOS Core Kinematics) using a lens with a distance of 440 mm, a volume of 300 x 230 x 230 mm and a scanner with a precision of 15 microns. The measurements were performed on the 3D digital dental models using GOM's inspection software; palatal rugae patterns and measurements and palatal measurements: intercanine width, intermolar width, arch perimeter, arch length, anterior arch length, palatal depth, anterior arch width and rugae size (Table 1) were performed by a single examiner after calibration.

A qualitative evaluation of the rugae was performed to determine their clinical appearance and distribution (figure 1). Each ruga was classified as straight, wavy, curvy or circular based on the classification of Kapali et al[19]. The direction and unification were recorded according to the classification of Thomas and Kotze[20] (Table 1); similarly, unification was described according to the ruga origin and course.

The clinical assessment of palatal sensitivity was performed by a speech therapist. Subjects were seated and a mild sensory stimulus was applied in the anterior-posterior direction in a linear and circular way on the median raphe and on the rugae edges (figure 2). The reaction shown by the subject was classified as normal sensitivity, hypersensitivity or hyposensitivity.[17]

A total of 10 3D digital dental models were chosen for the calibration by the examiner in the models measurements. All measurements were performed at two time points by two independent examiners. Inter- and intra-observer reproducibility for all variables were tested by repeated landmark identification. Two sets of measurements were quantified with two weeks between them. For each variable, Dahlbergs's error was calculated and values between 0.05 to 0.6 mm were obtained. A less than 10% of the maximum value was obtained for each variable.

Statistical Analysis

Quantitative variables were assessed by a non-parametric comparison (Mann-Whitney U test). A Chi square test of independence was performed for qualitative variables, and the association among the variables was verified. Values of $p \leq 0.05$ were considered significant differences using SPSS v 19.

RESULTS

A total of 264 dental models of schoolchildren from five schools of the municipality of Envigado were considered for this study. They were divided into two groups based on the type of bite: 132 with NVO and 132 with AOB. The average age in the NVO group was 11.37 ± 2.27 years, and the average age in the AOB group was 11.87 ± 2.84 years, which demonstrates that the groups were comparable based on the age ($p = 0.117$).

The general data showed that 54.9% of the students were female, both groups had a higher proportion of females with the highest percentage in the AOB group at 59.1%. ($p = 0.174$).

Significant differences ($p = 0.003$) between the groups were found in the mean arch length when evaluating the dental arch features, and the group with the greatest length was the AOB group at 26.99 mm ± 2.67 (Table 2). No significant differences between the groups were found for the intercanine or intermolar widths.

Similarly, significant differences ($p = 0.000$) were found when comparing the posterior and anterior palatal depths between the groups. Both depths were greater in the AOB group, with values of 18.59 ± 2.76 and 16.04 ± 2.41, respectively (Table 2).

Table 1. Measurements for the anthropometric features of the palate

Measurement	Definition	
Intercanine width	Straight line between cusp tips of right and left canines or the middle of the facet resulting from attrition. The measurement was not performed when one or both of the canines were absent (30).	
Intermolar width	Straight line measured between the centre point of the mesial fossa of the right molar and the mesial fossa of the left molar. The measurement was not performed when one or both of the molars were absent (30).	
Arch perimeter	Sum of four segments: from distal surface of primary second molars or mesial surface of first permanent molar on one side (passing over the contact points) to mesial deciduous or permanent canine on both sides. The other segments were measured from mesial deciduous or permanent canine to a point between two central points on both sides (30).	
Arch length	Straight distance from interdental papilla tip between upper central to a tangent through mesial surfaces of the second molars (30).	
Anterior arch length	Corresponds to the perpendicular distance from the interincisive papilla to a tangent line formed by the interpapillary line of premolars or deciduous molars.	
Palatal depth	Distance from the occlusal plane to the posterior and anterior palatal depth. To take these measurements, first a plane of three points was established using the mesio-palatal cusps and one disto-palatal cusp from the first upper molars. Then, a tangent was made through the midline of the model to section it sagitally and to be able to measure posterior and anterior depth.	**Posterior palatal depth:** with the cutaway model, the distance from the occlusal plane to the depth of the palate, having as a reference the mesiobuccal cusp of the first upper molar, is measured.
		Anterior palatal depth: with the cutaway model, the distance from the occlusal plane to the depth of the palate at the level of the interpremolar or deciduous intermolar is measured.
Anterior arch width	Distance form interpremolar or deciduous intermolar papilla to the contralateral side.	
Rugae size	The first 3 and/or 4 rugae pairs were measured. The rugae size was determined according to the height and width. First, the rugae are outlined with software, and a tangent is drawn on the more elevated area that cuts the ruga cross-sectionally, forming the ruga curve (Figure 3). Then, the ruga width is measured at the level of the curve base, taking both edges as reference, to later measure the distance between them and proceed with the measurement of the ruga height, drawing a perpendicular line from the highest edge of the curve to the base (Figure 1). Both right and left rugae were measured for 3 or 4 pairs in each model. The right rugae were identified with the letter A, and according to their number, they were ordered as A1 (the first ruga of the right side), A2 (the second ruga of the right side), and so on until the fourth ruga. The left side rugae were identified with the letter B, and according to the number they were ordered in a similar manner (Figure 4).	

Table 2. Anthropometric features based on study group

MEASUREMENT	NVO n = 132 $\dot{X}$ (SD)	AOB n = 132 $\dot{X}$ (SD)	P Value
Intermolar	46.90 ± 2.96	47.31 ± 3.18	0.252
Intercanine	34.21 ± 2.67	33.65 ± 2.77	0.065
Perimeter	74.12 ± 4.67	73.28 ± 5.45	0.069
Total length	26.01 ± 2.03	26.99 ± 2.67	0.003*
Anterior width	35.47 ± 2.72	35.57 ± 3.25	0.562
Anterior length	18.77 ± 2.44	19.02 ± 2.15	0.219
Posterior depth	16.74 ± 2.36	18.59 ± 2.76	0.000*
Anterior depth	14.48 ± 2.26	16.04 ± 2.41	0.000*
Height of first right ruga	0.74 ± 0.23	0.84 ± 0.25	0.020*
Height of first left ruga	0.81 ± 0.27	0.94 ± 0.30	0.000*
Height of second right ruga	0.61 ± 0.19	0.71 ± 0.18	0.000*
Height of second left ruga	0.66 ± 0.24	0.78 ± 0.27	0.000*
Height of third right ruga	0.58 ± 0.20	0.65 ± 0.23	0.008*
Height of third left ruga	0.59 ± 0.21	0.71 ± 0.24	0.000*
Height of fourth right ruga	0.46 ± 0.20	0.49 ± 0.22	0.352
Height of fourth left ruga	0.47 ± 0.16	0.56 ± 0.22	0.003*
Width of first right ruga	2.8 ± 0.62	3.08 ± 0.65	0.033*
Width of first left ruga	2.97 ± 0.71	3.2 ± 0.73	0.01*
Width of second right ruga	2.40 ± 0.46	2.51 ± 0.49	0.136
Width of second left ruga	2.43 ± 0.49	2.55 ± 0.55	0.70
Width of third right ruga	2.28 ± 0.49	2.27 ± 0.42	0.985
Width of third left ruga	2.24 ± 0.50	2.30 ± 0.51	0.280
Width of fourth right ruga	2.10 ± 0.69	2.13 ± 0.57	0.600
Width of fourth left ruga	2.03 ± 0.47	2 ± 0.54	0.179

*Significance $p \leq 0.05$ Mann-Whitney U test

In the quantitative evaluation of the rugae, significant differences were found when comparing the height of the first three rugae on both the right and left sides between the groups.

The rugae with the greatest heights were found in the AOB group (Table 2). Significant differences between the NVO and AOB groups were found in the width of the first ruga, with a greater width for rugae from AOB patients (3.08 ± 0.65; p = 0.033) (Table 2).

The qualitative analysis revealed that the rugae shape was asymmetric. The first rugae on the right side had a straight shape in individuals with NVO and a wavy shape in individuals with AOB. A higher proportion of the curvy shape was found on the left side for both groups. The wavy shape was predominant in the posterior rugae of the NVO group for both the right and left sides. In contrast, straight and wavy rugae were found in the same proportion in the AOB group (Table 3).

General asymmetry was found when assessing the direction. When evaluating the first right ruga, a higher proportion of horizontal rugae were found in both the NVO and AOB groups. On the left side, most rugae had a forward direction in both types of bite. For the posterior rugae, the horizontal direction predominated, and only the third and fourth rugae of the NVO group showed a higher proportion of the backward direction (Table 3 and 4).

Table 3. Classification of rugae based on direction

RUGA	RIGHT			LEFT		
	NVO	AOB	p value	NVO	AOB	p value
1	Horizontal 67.4%	Horizontal 68.2%	0.631	Forward 52.3%	Forward 58.3%	0.416
2	Horizontal 52.3%	Horizontal 49.2%	0.622	Horizontal 49.2%	Horizontal 47%	0.493
3	Backward 44%	Horizontal 53.8%	0.064	Horizontal 45.5%	Horizontal 56.1%	0.219
4	Backward 31.1%	Horizontal 39.4%	0.645	Horizontal 34.1%	Horizontal 45.5%	0.542

*Significance p ≤ 0.05 - Chi square test

Table 4. Classification of rugae based on unification. For palatal rugae unification, parallel rugae predominated in both study groups without significant differences.

RUGA	RIGHT			LEFT		
	NVO	AOB	p value	NVO	AOB	p value
1	Parallels 69.7%	Parallels 68.9%	0.791	Parallels 68.9%	Parallels 81.1%	0.065
2	Parallels 73.5%	Parallels 71.2%	0.755	Parallels 78%	Parallels 78%	1
3	Parallels 87.9%	Parallels 92.4%	0.547	Parallels 90.9%	Parallels 88.6%	0.362
4	Parallels 69.7%	Parallels 81.7%	0.645	Parallels 65.2%	Parallels 78.8%	0.564

*Significance p≤0.05 - Chi square test

Figure 1: Features and distribution of palatal rugae shape

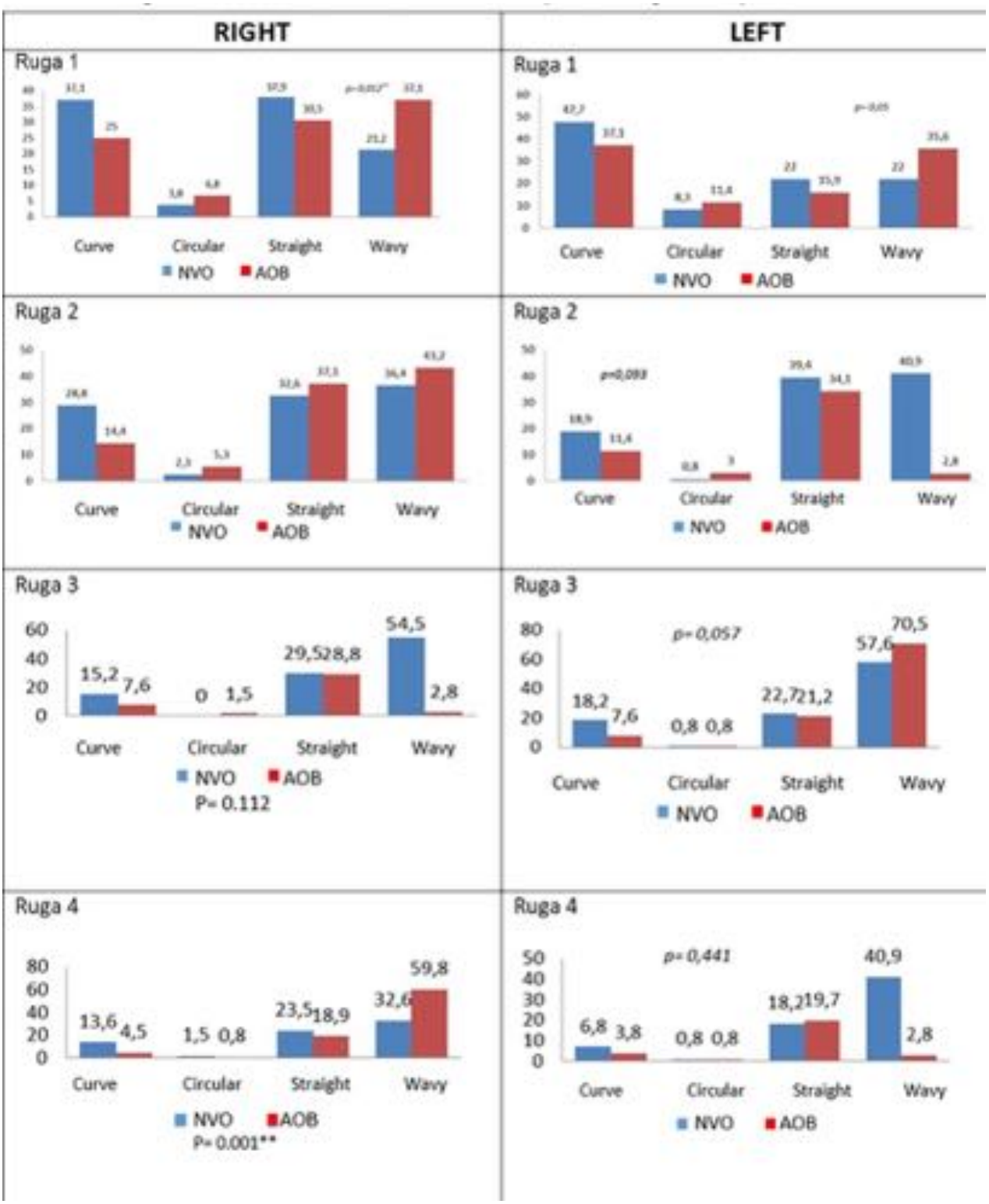


Figure 2: Evaluation of rugae sensitivity

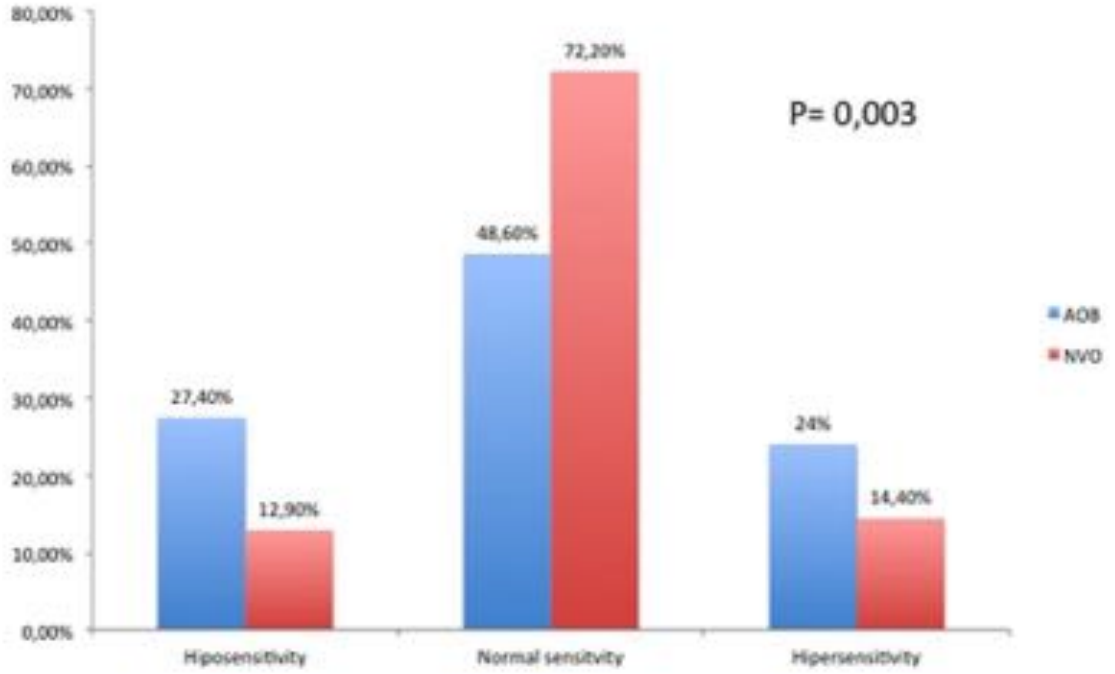


Figure 3: Outlined rugae and denomination

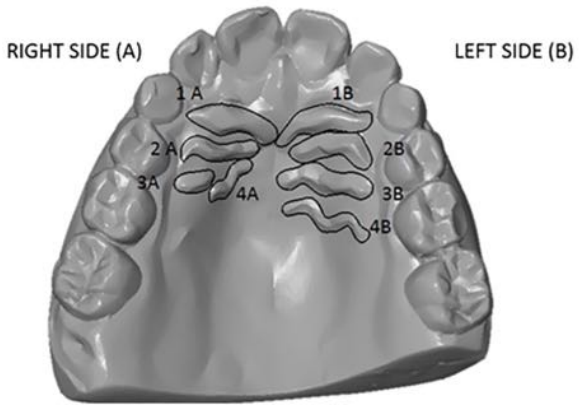
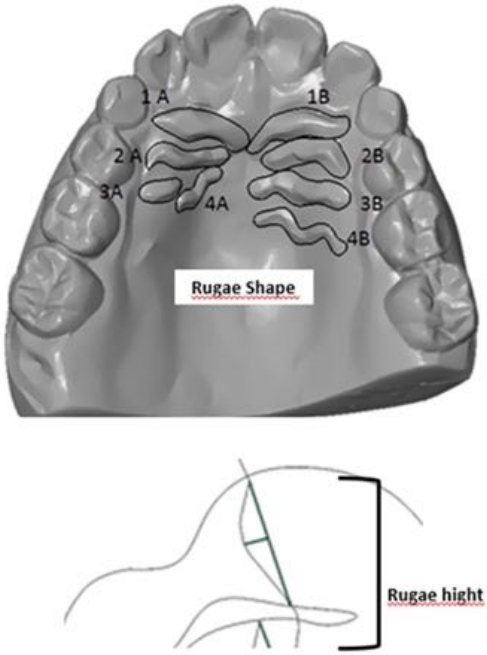


Figure 4: Rugae shape and size measurement method



When evaluating rugae sensitivity for the total sample, 59.2% of students showed normal sensitivity. A higher percentage (p= 0.003, chi square test) of normal sensitivity was found in the NVO group (72.7%) than in the AOB group (46.6%).

DISCUSSION

In the present study subjects with AOB were found to have larger arch length and depth, and larger rugae height and width than NVO. Function has been proven also to be different in AOB subjects, like deglutition and phonation during clinical or radiographic studies,[2,21] but occlusal characteristics sometimes are difficult to be objectively evaluated during clinical examination.

Currently, 3D scanner technology and reconstruction with virtual models are being widely used in dentistry for various applications and are becoming accurate and reliable techniques.[22] The use of 3D reconstruction allows the measurement of specific distances that would be difficult

to obtain with sufficient accuracy with conventional methods, especially topographic measurements.[23]

Even though intermolar and intercanine widths in our study did not show significant differences between individuals from the AOB and NVO groups, this result contrasts with that of various studies showing that individuals with AOB have a narrow maxilla with posterior crossbite.[19,24] The difference in the results can be explained because these studies use only individuals with deciduous or permanent dentition (with an average age of 14 years), whereas in our study, most individuals had mixed dentition with an average age of 11 years. Individuals with mixed dentition have a shorter period of muscle imbalance. Additionally, the way the diagnosis of open bite is performed differs. Hsu[24] uses a method based on model analysis, while in the present study the assessment was performed clinically. Moreover, some studies included individuals with mouth breathing, low position of the tongue and non-nutritive sucking, which may influence the arch dimensions.[25-27] Additionally, this difference can be attributed to the absence of uniformity in the sample size, as some authors such as Machado [26] and Sousa[25] used larger sample sizes (5,522 and 864, respectively) than the present study.

Significant differences between groups were found for arch length, which was greater for the AOB group, in agreement with the findings reported by Melsen et al [28] who showed how the vestibular inclination of the teeth of individuals with AOB is responsible for this difference.[26]

The present study showed that posterior and anterior palatal depth was higher in the AOB group, supporting the cephalometric results on the posterior dentoalveolar height of AOB patients.[27] Furthermore, the vertical dimension of these patients has not been studied thoroughly with models; our study is in contrast with the Hsu findings, in which models were sectioned distally from the upper first molar to the central fossa, outlining a tangent to the fossa of the contralateral molar and then measuring the palatal depth from this tangent. No significant differences were reported between the AOB group and the group with normal occlusion.[24] The difference in results is likely due to the design of the current study in which the measurements of depth were performed using a virtual 3D modelling program, which allowed measurement from the deepest part of the palate to the occlusal plane at the level of the mesio-buccal cusp of the upper first molar.

In the present study, 53.4% of individuals with AOB showed an alteration in normal sensitivity, which can be associated with tongue thrust and the presence of rugae with greater height and width than measurements in the NVO group according with other studies.[29] Similar to the findings reported by Premkumar et al,[29] when evaluating oral sensory perception in patients with AOB with tongue thrust and comparing them with the normal occlusion control group without habits, individuals with AOB had less ability to perceive shapes and texture, which was influenced by the palatal size, shape and surface.[29] The palatal rugae are involved in the motor-sensory feedback mechanism required for the proper maturation of orofacial functions because of the presence of proprioceptive receptors.[30] Maturation is linked to local sensory experience and the development of brain function.

Normal sensitivity in this area is essential for the installation of appropriate language patterns at rest to clarify the articulatory point of different parts of the tongue against the palate during the deglutition function and speech.[17] Furthermore, in animal studies, the palatal rugae are low when they are under continuous pressure of the tongue and are prominent in animals that have altered tongue position. [30] According to some authors, the tongue may lose the ability to recognize its spatial location in the oral cavity during rest and function in a proprioceptive or kinaesthetic alteration. However, analytical and experimental studies in humans are needed to determine the degree of association.

The prevailing rugae shape in the NVO group was curvy and straight, while that of the AOB group was curvy and wavy; these results are consistent with those of other studies that compared the patterns of the palatal rugae in different studies. They found that the most common forms in the African group were wavy and curvy, while the straight shape was more common in Caucasians (20). Furthermore, in Sudanese, Indian and Egyptian populations,[30] the wavy and curvy shapes are the most common and represent more than 55% of the ridges for both sexes (30). The orientation of the palatal rugae was asymmetrical in the present study, similar to the result reported for other populations.[30] This asymmetry contrasts with the assumption that the development of rugae is a coordinated process that occurs along the palate, which indicates that there are differential growth rates between the two sides. With regard to unification, more parallel rugae were found in both groups, meaning that convergent or divergent unification was scarce, which is in agreement with Danish results showing convergent or divergent unification at a rate of only 5%.[30]

CONCLUSION

Individuals with AOB show different shapes and sizes of the palatal rugae compared with individuals with NVO, even though palatal size was different, it was non-statistical significant. It would be ideal to perform an analytical study that allows the determination of the type of association between thick and prominent palatal rugae and the presence of open bite, and also to determine if the differences between AOB and NVO are associated with lingual protrusion.

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AVAILABILITY OF SUPPORTING DATA:

The database is available in:
https://mynotebook.labarchives.com/
The data is in anonymized form that complies with data protection/privacy laws.

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Dental age estimation in children with chromosomal syndromes

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KEYWORDS

age estimation, London Atlas, Down syndrome, Williams syndrome, dental maturation, forensic age assessment

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ABSTRACT

When the age of an individual is unknown, age assessment refers to the procedures through which authorities try to establish the chronological age of an individual. Dental evidence demonstrated to be very effective in estimating age and dental mineralization is largely deemed a process scarcely influenced by major diseases and nutritional or environmental factors which can affect child growth. This research aims to understand the possible influence of genetic syndromes on dental maturation of affected individuals.

The sample is composed of a test sample of 159 chromosomal affected children, 69 males and 90 females, and a control sample of 157 healthy children, 77 males and 80 females aged between 4,49 and 19,8years. London Atlas was applied to estimate dental age on OPGs (orthopantompographies).

No statistical significant difference has been found in dental estimates between syndromic and healthy individuals. Moreover no statistical significant difference emerged between sexes and age cohorts. Children affected by Down or Williams syndromes nor mean error neither the mean accuracy per cohort of age show differences compared to non-affected subjects.

The London Atlas can be validly applied to age estimation of individuals with multiple agenesis as in Down and Williams syndromes, even if it a slight overestimation of age occurs systematically in syndromic as well as in healthy samples. The current findings suggest that dental maturation is a very stable biological process scarcely affected by even serious illnesses as genetic syndromes.

INTRODUCTION

Age is one of the main characteristics of the biological profile reconstruction of an individual and age estimation is necessary to determine if the subject is accountable for his actions in criminal law, shall undergo specific obligations (educational, for instance) or should receive specific aides or other providences from the state administration or for other important administrative and civil issues (health care, immigration, adoption, driving license, passport release, marriage - to cite only the most common fields of application).

When the age of an individual is unknown, age assessment refers to the procedures through which authorities try to establish the chronological age of an individual, utilizing any attempts including documentary evidence, psychological

assessment, medical examination. The latter procedures try to estimate the age of an individual by converting age-related biological markers to chronological age. The term "estimation" (other than age "determination") defines more precisely the real limits inherent to this sort of expertise.

Biological age is measured through recognition of growth and maturational milestones achieved in different biological systems as the skeleton, the dentition or some soft tissues. Generally speaking, the estimation is much easier till the age threshold of full maturity of the main used indicators (which is around 16 years), because after that age problems rise due to the completion of the maturation of the main parameters for the estimation, i.e. ossification of the wrist and the second molar roots apex. As soon as the child reaches maturity, and therefore the examined markers become mature, the same markers are no longer informative; the only information they provide is the likely age, or better, range of ages, when the individual reached the adult state, again based on population norms, and this serves only as a lower limit for their likely chronological age.

Methods based on the permanent teeth calcification provide reliable and accurate tools for estimating the age of children. They are largely adopted for auxological reasons, when just an evaluation of the overall developmental stage of the individual is requested and therefore just an approximate result is needed, a practice very far from the requested accuracy of an examination performed for forensic purposes.

When estimating age in medico-legal and forensic practice, dental mineralization is largely deemed a process scarcely influenced by major diseases and nutritional or environmental factors which can affect child growth, unlike the maturation of, for example, the skeleton, probably because the skeletal age is more sensitive than dental age to whatsoever insults.

According to the latter affirmation, the aim of this research is to understand the possible influence of genetic syndromes on dental maturation of affected individuals.

Moreover, since individuals affected by Down syndrome often undergo adoption, and sometimes are undocumented [1,2], aim of the present research is also the analysis of the accuracy of the dental methods of age estimation applied on individuals affected by genetic syndromes and the understanding if the dental methods are reliable tools to estimate age in the syndromic individuals[3-6].

MATERIALS AND METHODS:

This study was conducted with the prior approval of the local ethical committee.

The sample is composed of a total of 316 Ortopantomographies (OPGs) of subjects aged between 4,49 and 19,98 years, 146 male and 170 female Italian subjects who underwent dental check-up or treatments provided by the Meyer Children University Hospital in Florence. The total sample is divided in a test sample of 159 chromosomal affected children, 69 males and 90 females, and a control sample of 157 healthy children: 77 males and 80 females. Since no data about the family origins were available for the study we therefore assumed as "Italian" all the children with an Italian surname.

The syndromes included in the sample are: Down (DS), Turner , Williams (WS), Klinefelter, De George and Wolf-Hirschorn (table 1)

Table 1: Composition of the sample

	Females	Males	Tot OPGs
DOWN	46	37	124
WILLIAMS	5	5	21
TURNER	5	0	7
DE GEORGE	0	1	1
KLINEFELTER	0	2	5
WOLF-HIRSCHORN	0	1	1
OPG of Affected children	69	90	159
OPG of Non-affected children	80	77	157

These syndromes were chosen for the research because they have similar genetic and/or chromosomal influence on the overall somatic development [7-18]. Age and other clinical information of the patients, except sex, were not disclosed to the operator for both samples. The control sample was chosen with a quite similar

distribution of age and gender as the test sample, and subjects had unremarkable medical history.
The London Atlas of tooth development and eruption (LA)[19] was adopted as the method of choice to estimate the age for both samples, and the procedure has been performed by a forensic odontologist expert in age estimation of children. This method was chosen because it enables the age estimation even in the case of multiple agenesis, an anomaly very often present in these syndromes [20]
The Demirijan's and Cameriere's methods, commonly applied in age estimation procedures [21-25], cannot be comfortably applied in our sample of syndromic patients: very often multiple agenesis are present and very often is found an agenesis of the premolars[26], teeth necessary even for the four teeth Demirijan method[27]. Moreover, in such syndromic children some teeth can appear distorted in the OPG due to lack of cooperation during the radiography execution [26]. In these cases, also the Cameriere method cannot be used. Such type of difficulties are confirmed also by Van der Linden [28] who pointed out the lack of collaboration of the DS children and especially of the youngest. He also reported difficulties due to the different shape of the roots (shorter and blunter), a morphological characteristic which is present in DS and in other syndromes.

Data were collected in Excel 2003® and analyzed with Windows MiniTab®. After the test for normal distribution, an One-way Anova was carried out in the male and female samples.
The results obtained from the sample affected by chromosomal syndromes were then compared with the estimations obtained from the non-affected children control sample in order to evaluate the possible influence of chromosomal syndromes on the dental maturation process.
Three months after the first evaluation, the concordance correlation coefficient and the intra-rater agreement were calculated on 30 OPGs randomly chosen and submitted to a second qualified forensic odontologist.

RESULTS

The normality test showed a normal distribution in all groups and the One-way ANOVA adopted in the males and females samples revealed no statistical differences. (fig. 1)
The intra- and inter-rater agreement resulted to be 93% and 90% respectively.
The results are reported in table 2, which shows the difference between the chronological age (CA) and the estimated age (EA) in both genders and reveals that the trend is similar with no significant difference in affected children (AFF C) compared with non-affected population (NAFF C.) The mean, minimum and maximum errors are quite similar in AFF C and NAFF C.

Figure 1: One-way ANOVA between male and female. No statistical differences. P< 0,05.

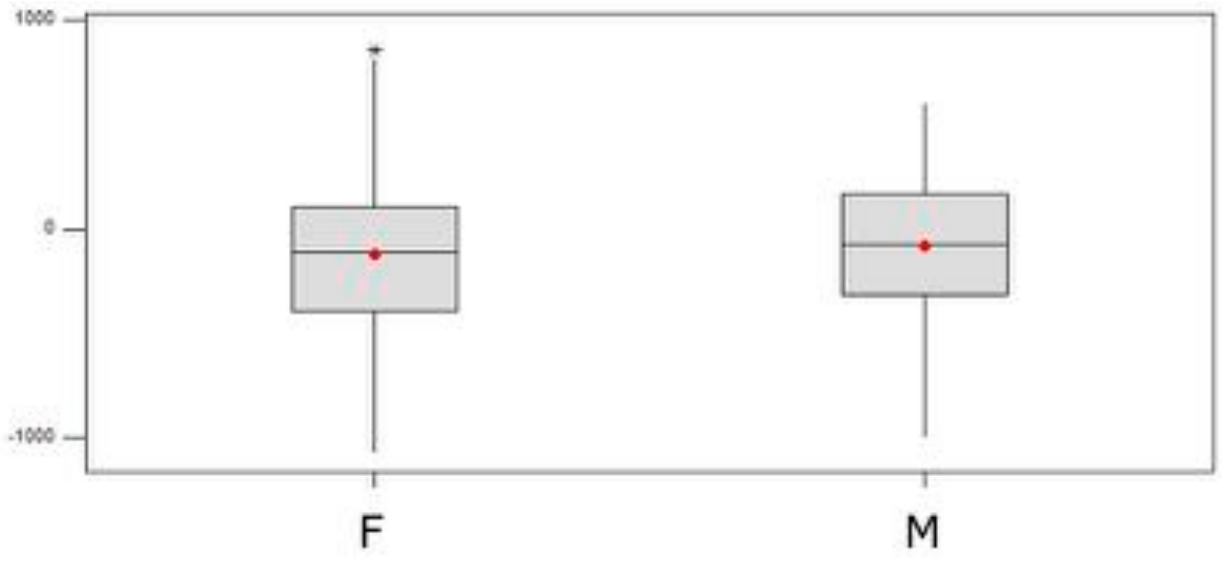


Table 2: Differences between CA and EA(CA-EA) in Chromosomal affected children and non-affected children. Minimum, maximum and mean values are reported.

		CA-EA MIN days	CA-EA MAX days	CA-EA Mean days
AFF C	Male	-8	-745	-37,8
	Female	2	-889	-165
NAFF C	Male	-7	-1004	-141
	Female	0	-1072	-188

The range of difference between the chronological age and the estimated age of the affected children is not different from the non-affected children and from the error range very commonly found in dental age estimation procedures performed with the usually adopted methods in healthy individuals. Errors of estimated age are slightly lower for males both for NAFF C and AFF C.

Table 3 shows the mean estimated age compared to mean chronological age per cohort of ages in affected and non affected children. A trend to overestimate age emerged for both samples and all cohorts of ages. The last cohorts for AFF C should be evaluated with caution since several children presented the agenesis of the third molars. This condition implied a constant underestimation when the complete mineralization of the second molar was reached. The operator continues to assign the maximum age that London atlas provides for the second molar complete formation (16.5 years) since no useful information is available from the third molar.

Table 3: Comparison of mean CA and mean EA for age cohort in both populations (Affected and non-Affected)[1] Mean CA=Mean Chronological age, Mean EA = Mean Estimated age , [2]Diff= Mean CA – Mean EA

	AFF C			NAFF C		
Age cohort	Nr	Mean CA/ MeanEA [1]	Diff [2]	Nr	Mean CA/ MeanEA[1]	Diff[2]
4	6	4,73/5	-0,27	3	4,77/5,16	-0,39
5	8	5,63/5,62	0,01	7	5,62/5,78	-0,16
6	14	6,46/6,71	-0,25	10	6,47/6,5	-0,03
7	9	7,34/7,38	-0,05	12	7,46/7,91	-0,46
8	7	8,35/9,07	-0,72	19	8,46/8,39	0,07
9	11	9,47/10,04	-0,58	10	9,41/9,5	-0,09
10	14	10,53/11,21	-0,69	16	10,43/10,75	-0,32
11	16	11,51/12,43	-0,93	18	11,42/11,36	0,06
12	14	12,64/13,64	-1	17	12,47/13,02	-0,55
13	10	13,57/14,4	-0,83	11	13,43/13,68	-0,25
14	12	14,42/14,5	-0,08	9	14,34/14,61	-0,27
15	9	15,45/16,05	-0,61	8	15,31/15,87	-0,57
16	12	16,41/16,91	-0,5	8	16,48/15,75	-0,73
17	6	17,57/17,33	0,24	4	17,71/18	-0,29
18	5	18,45/18,1	0,35	2	18,52/18,5	0,02
19	4	19,50/19,25	0,25	5	19,59/19,9	-0,31

Figure 2: Means of CA-EA in Affected children and Non affected children per cohort of age; whole sample

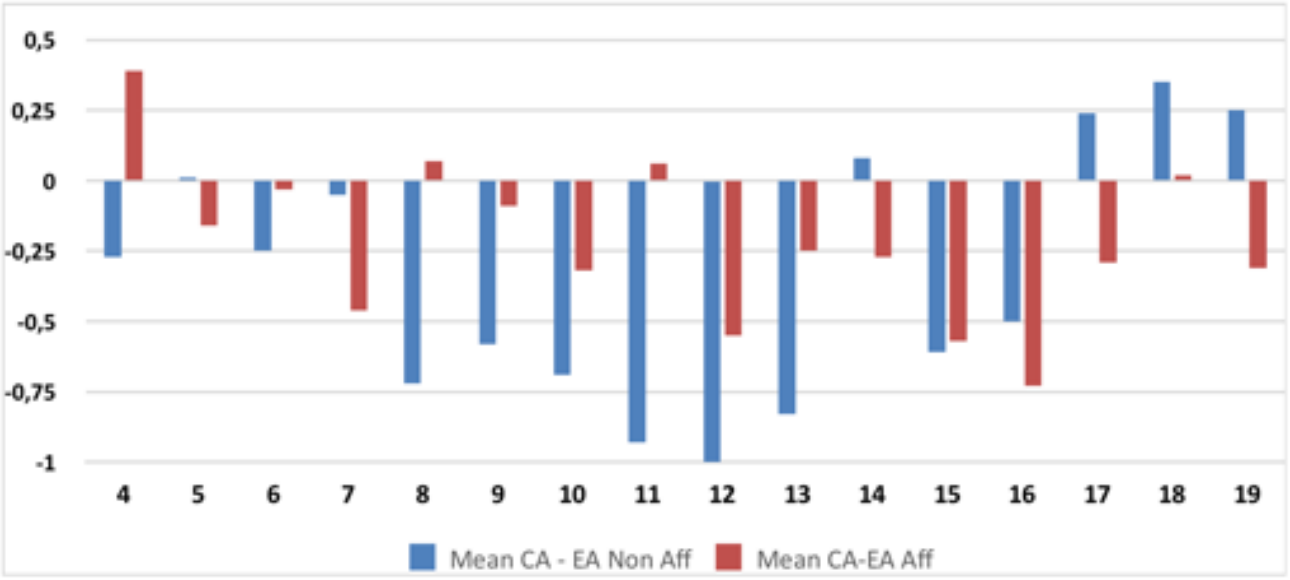


Fig. 2 reports the comparison of the mean differences of CA – EA for the affected and non-affected samples per cohort of age. The London Atlas shows an evident tendency to overestimate age for both populations and differences between affected and non-affected samples are not significant.

Given the large prevalence of children affected by Down and Williams syndromes, a specific analisys of the results was provided for these two syndromes. The test sample (non affected children) was recalibrated for number of subjects, gender and age to be quite similar to the affected samples separately considered for Down or Williams syndrome.

For these samples the mean error, but also accuracy, intended as |CA-EA| was calculated. Since the absolute value of the errors has been considered, there is no mutual compensation from the mean and it is therefore possible to determine the deviation of the predicticted value (estimated age) from the real one (chronological age).

Table 4 and figures 3,4 show the value of mean errors and mean accuracy attributable to non affected samples compared to DS or WS individuals.

Figure 3 shows the means of CA-EA and accuracy assumed as the absolute value of the differences between chronological age and estimated age for different cohorts both for NAFF C and children AFF by DS. Both samples consisted of 124 OPGs and NAFF sample was chosen from the general sample considered above (table 1) in order to have a very similar composition to AFF C (age and gender distribution) .

Figure 4 reports the mean CA-EA and accuracy for different cohorts both for NAFF C and children affected by WS. The compared sample (NAFF C and AFF C) were composed of 21 children.

The London Atlas resulted to overestimate age in both populations and this result is quite relevant especially in the control sample of unaffected individuals. Even if the analyzed sample is too small to draw definitive conclusions, the present results suggest to apply caution when London Atlas is used to Italian individuals, exactly because of its tendency to overestimate age in all cohorts and in both genders.

Table 4: Comparison of non affected children with children affected by Down and Williams syndrome. Mean accuracy and mean error per cohorts of age

	DOWN SYNDROME 124 OPGS NAFF C 124 OPGS				WILLIAMS SYNDROME 21 OPGS NAFF C 21 OPGS			
Age cohorts	Mean Accuracy AFF C	Mean Accuracy NAFF C	Mean CA-EA AFF C	Mean CA-EA NAFF C	Mean accuracy AFF C	Mean accuracy NAFF C	Mean CA-EA AFF C.	Mean CA-EA NAFF C
4					0,42	0,69	0,42	-0,69
5	0,43	0,7	-0,23	-0,15			-0,43	-0,61
6	0,73	0,41	-0,19	0,24	0,43	0,61		
7	0,79	0,38	-0,28	-0,05				
8	0,63	1,11	0,14	-0,72	0,22	0,78	-0,16	-0,78
9	0,41	0,71	-0,01	-0,58	0,8	1,13	-0,11	-0,4
10	0,9	1,17	-0,15	-0,69	0,27	0,4	0,02	-0,4
11	0,87	1,41	0,17	-0,93	1,02	0,78	-0,05	-0,78
12	0,99	0,45	0,45	-1	1,21	0,59	-1,21	-0,59
13	0,95	0,88	-0,08	-0,83	0,78	0,76	-0,78	-0,76
14	1,23	1,06	-0,19	-0,51	1	1,01	-1	-1,01
15	0,71	0,7	0,44	-0,5	0,07	0,34	-0,03	0,11
16	0,74	0,77	0,73	-0,56				
17	1,04	0,75	-0,18	0,03	0,74	1,37	-0,74	1,37
18	0,52	0,46	-0,52	0,24				
19	0,66	0,64	-0,43	-0,16				

Figure 3: Mean error and accuracy for non affected children compared to children affected by Down syndrome. Accuracy = Mean |CA-EA|; AFF C : affected children, NAFF C: non affected children

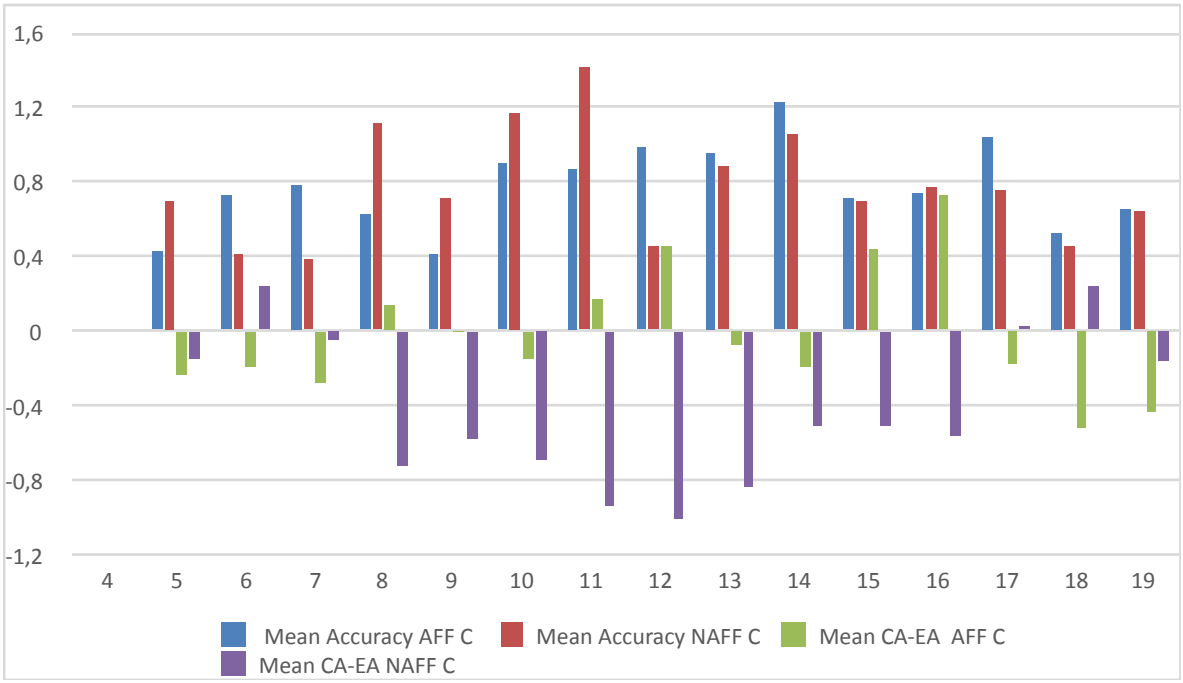
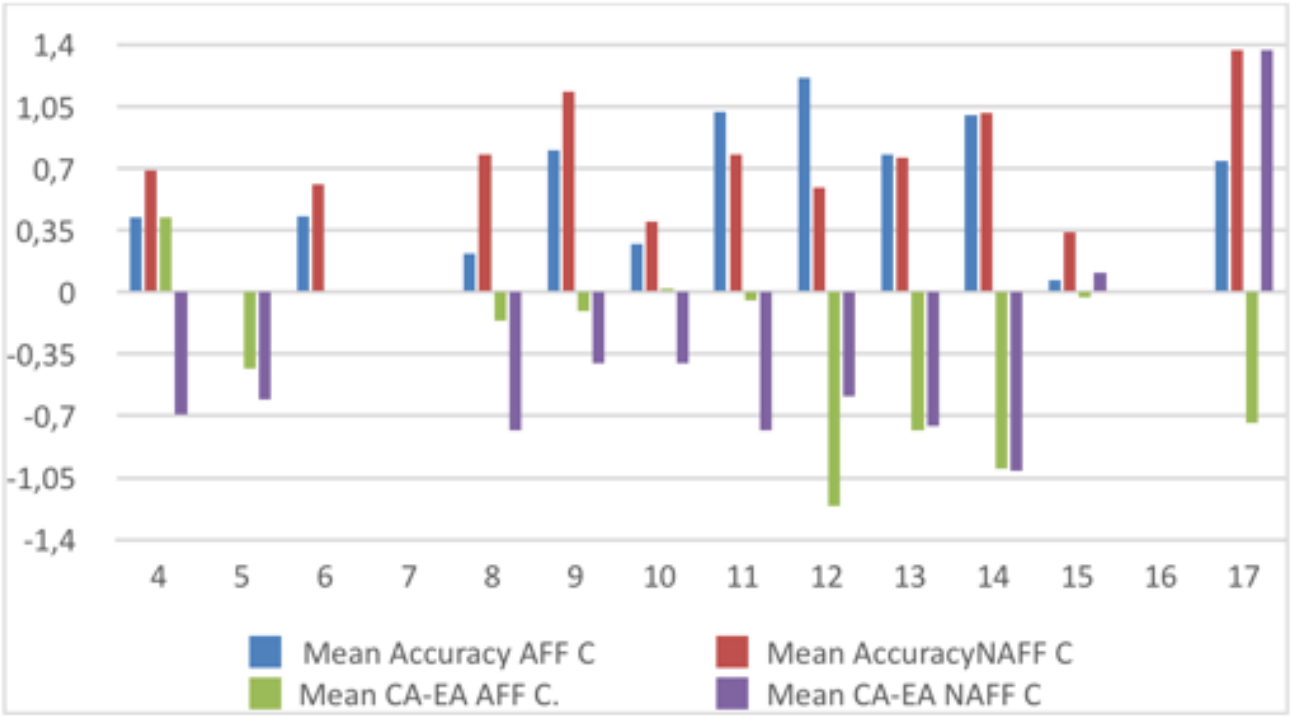


Figure 4: Mean error and accuracy for non-affected children (NAFF C) compared to children affected by Williams syndrome. Accuracy = |CA-EA|



The separate evaluation of the results from the Down syndrome (DS) and Williams syndrome (WS) affected individuals was then performed to learn if special differences between the two syndromes could be detected. As seen in table 4 and figures 3-4, no statistically significant differences emerged between estimated age and chronological age between the DS and WS individuals compared to the NAFF children.
Just as an observational and not definitively or statistically significant data, considering the small number of cases examined in our sample, we observed that WS females showed a slightly faster dental maturation than WS males.

DISCUSSION

Chromosomal disorders and syndromes, which arise from numerical and structural defects of the chromosomes, often include manifestations affecting the craniofacial region. Many of these chromosomal and multifactorial disorders present characteristic oral manifestations[3], such as, for instance, multiple agenesis and delayed teeth eruption in deciduous and permanent dentitions. These dental features are not unique to people with DS even if they occur more frequently in people with DS [9,26].

Very few studies are present in the Literature, however, about the issue of the influence of these syndromic affections on dental maturation in syndromic individuals.
In the study of Leila Abou Hala, the accuracy of dental age and skeletal age methods was evaluated, in order to estimate chronological age in individuals with Down syndrome. In the conclusions she stated that "*more caution is required for age estimation for DS individuals, since they present much more variation than non-ds individuals*"[4].
Other Authors, on the other hand [1,5,6,28], reported that no difference is detectable between healthy and syndromic individuals.
With these opposite thesis in mind we therefore performed the age estimation of syndromic cases with a dental method, the London Atlas.
The analysis of the results we obtained and the comparison between the chronological and the estimated age with dental methods allow us to say definitely that there are no significant differences between the samples, and therefore that there is no difference in dental maturation between the syndromic individuals sample and the control one. We can therefore affirm, in full accordance with Diz et al.[1], Mari Ellis Leonelli de Moraes [5, 6], M. S. van der Linden et

al. [28], that no slowing or acceleration of the dental maturation can be seen in Down syndrome affected individuals.
The analysis of the results coming from the DS individuals shows that the CA/EA difference is negative, as shown exactly by the results from all the other samples, but the ranges are even lower than those coming from the healthy children.
The analysis of the results coming from the Williams syndrome affected individuals shows the same trend of the DS and healthy individuals. The sample, however, is made of just 21 cases; the conclusions which can be drawn in these cases are therefore just indicative and preliminary.
With these premises, we can say that the dental methods, as the LA is, can be anyway considered valid tools for the age estimation of syndromic individuals.

CONCLUSION

Despite any influence that the genetic and chromosomal alterations could have on the development of the oral system, no statistical significant difference has been found in dental age estimations between syndromic and healthy individuals in our samples. No statistical significant difference has been found between sexes and age cohorts. No difference has been found between syndromic and healthy individuals dental maturation.
From the data drawn from our research we can suggest that dental maturation is a very stable biological process scarcely affected by even serious illnesses as genetic syndromes are. In these cases the evidence taken from the dental system is the most reliable in age estimation procedures since dental maturation results much less affected by environmental, nutritional and pathological factors than the skeletal development.
The London Atlas, can be considered a valid tool for age estimation of individuals with multiple agenesis, a very frequent characteristic found in such syndromic cases, and especially in Down and Williams syndromes, even if slight overestimation of age occurs systematically in syndromic as well in healthy Italian individuals.

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Quality assurance in forensic odontology

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KEYWORDS

forensic odontology, quality assurance, IOFOS recommendations, Interpol's guide

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ABSTRACT

Quality assurance or quality control is a term and concept coming from the industry. Here it is most important. All products must have a minimum quality and variation in size, for example, must be kept within certain strict limits. There must be a system to control this. May be not every single product is controlled, but spot tests must be taken. Measures must be taken to improve the quality if it is not good enough.
This concept has been transferred to medicine, odontology, and consequently also to forensic odontology. These areas have in common with industry the production of that certain products. However, they are usually handmade and not produced in an industrial process. In addition, dentistry is a great deal of art and judgement and quality control of these factors may be difficult. In this paper, I will focus on forensic odontology. What are the problems? What can we do and cannot do? In addition, how can we assure the quality of the work, the assessment and conclusion, and the report? I have some personal opinions on that and I will give some suggestions.
Quality assurance on an international level is difficult. Conditions and juridical systems are different in different countries. Especially forensic odontologists are different and have different opinions. This presentation will be relevant to the ongoing discussion and attempts at revising the IOFOS' guidelines for quality assurance.

QUALITY CONTROL IN INDUSTRY

Quality assurance is an idea coming from the industry. There they call it quality control. In the production of certain products, there is a need to keep the variation of each sample within certain dimensional limits. Only a minimum variation is acceptable. In addition, there is a need to make sure that the quality is good enough so that the product will not stop functioning after a short time. Therefore, certain descriptions defining the quality are set up. Often the quality controllers have a special task to control that the quality is satisfactory and that products that do not satisfy the defined quality should be withdrawn from the market.

NEED FOR QUALITY CONTROL IN MEDICINE AND DENTISTRY

Obviously, in both medicine and dentistry patients may be dissatisfied with the quality of the service or treatment offered because of a poor technical quality or poor patient treatment.

This may sometimes lead to claims for compensation. It is also bad for the reputation of doctors and dentists. Therefore, the idea that some kind of quality control could improve the situation came up both on a state level, from the health authorities, and from the professional organizations.
It is possible to produce a description of what is intended as quality. However, the professional organizations and the professionals have had difficulties in accepting quality controllers, because there is no tradition for looking into the work of a dentist or a doctor. Especially, dentists feel uncomfortable in accepting that a control of their works is performed by other persons. Therefore, they invented a new system to make sure the quality was good enough. That was quality assurance. They wanted to implement systems whereby the dentist could control the quality of his own work.

QUALITY ASSURANCE

Quality assurance by definition implies that all measures are taken to assure that the quality of the given service is satisfactory both for the patient and for the dentist. A textbook describing the final product and the procedure to follow to obtain a satisfactory result is part of the quality assurance. Naturally, all teaching and demonstrations contribute to quality assurance. Scientific research in the subject can also help to improve quality. Diagnosis and treatment should as far as possible be based on scientific evidence.
Professional organizations have been mostly involved in the definition of the steps in the procedure, implying that following the procedure properly the result will be satisfactory. In dentistry may be difficult to define an acceptable final result and also it may be difficult to control that. The starting point in a restoration may be very variable and very often improvisation may be required. This is almost impossible to judge and understand afterwards. The recommendation is that you should not be too eager to criticize the work of other dentists.
Most state authorities and professional organizations have therefore focused their activity to obtain acceptable quality and on the steps to follow, and how to perform these steps. This has resulted in a point for point description of the procedures to be followed. The state health authorities may thus require that the dentists write a procedure handbook available in the practice. Updates of such a book must be done from time to time, and the updates dated. This may also simplify the record keeping. Important steps in a procedure, which you should normally note, can be omitted by just referring to the procedures. However, any deviations from the procedures and why they actually occurred become important and should be recorded.
The understanding that quality is important is not new neither in medicine nor in dentistry. People have tried to set up systems to improve the quality of the service and work. Long before the definition of the concept of quality assurance, the preparation of forms to use was a way to make sure that the surgeon did not forget important steps. If you followed the form, you should therefore have an optimal chance of having performed a quality treatment.

MINIMUM QUALITY/OPTIMUM QUALITY

It is always a question if the quality obtained is an optimal quality or a minimum quality. In industry, the quality defined will always be a minimum quality. However, in dentistry you never see a minimum quality defined: it will always be an optimal quality for the case. Given that only few dentists may be able to deliver optimal quality treatments, there will always be the possibility of a quality improvement. This is a part of quality assurance called quality development.

QUALITY ASSURANCE IN FORENSIC ODONTOLOGY

As a part of dentistry, also forensic odontology should have some kind of quality assurance. Anyone who had the opportunity to read reports from forensic odontologists must admit that sometimes the quality of the reports could have been better. Forensic odontology is a special subject but only in a few countries, it has an acknowledged specialty in the field. In other countries, any dentist can call himself a forensic odontologist. Thus, there is also no special education in the field. Naturally, the knowledge of forensic odontology may be variable. In addition to university-affiliated dentists, many private practitioners become interested in forensic odontology. They often consider forensic odontology a practical area with just a little need of a theoretical background. Accordingly, they are not so interested in reading scientific articles in that field.

Since the time of Amoëdo at the turn of the 18th century, many textbooks have been published. These books have often put the emphasis on histories and cases rather than the more boring technical notes. As examples of this, we may consider the identification. It has been used as an expression without any more discussion of what it really means. Obviously, it may mean different things to different persons. Authors seem to be able to identify without doubt most dead persons. There is no mention that you may never be 100% sure of an identification. Moreover, it is the task of the police, ID-commission or coroner to make the final identification. The forensic odontologist should only make a comparison report, not an identification report. The forensic odontologist is thus only responsible to assess how much the dental evidence may make for the final identification. The forensic odontologist is not responsible for the final identification.

Another technique for quality assurance, especially in identification procedures, is the use of a form. In a form, there are specific spaces to fill in for each important observation. This is excellent, but in my experience, a number of dentists have still not understood that they should fill each field of the form, leaving none of them empty. You should state this explicitly even if there is no information. Otherwise it is not possible to take full advantage of a form.

With the introduction of quality assurance in medicine and odontology, a more formal step by step procedure was suggested. It became mandatory to have a handbook describing the procedures in practice step by step. This also affects the forensic odontologist practice, even if few of us have such a handbook. Many professional organizations, however, introduced procedural steps that the dentists could use. The last edition of American Manual of Forensic Odontology included also description of the procedural steps to follow in bite-mark cases[1]. There is the danger that such procedural steps could tend to cement a procedure and may be an obstacle to improvements. The other problem is that forensic odontologists tend to have their own opinion, which is difficult to change.

The agreement upon the number of steps, and how to perform them, may be extremely difficult. Within one country, as it in the US for instance, it is possible to force the practicing forensic odontologists to follow the accepted procedures. This might not be valid on an international level. This became obvious in an IOFOS meeting at Lillehammer in 2003. Several forensic odontologists from different countries met to draft recommendations for quality assurance for IOFOS. It was practically impossible to agree upon anything. Some people wanted to include procedures which others thought were unnecessary. It became soon clear that on an international level forensic odontologists will never agree upon the exact procedures. It was therefore decided only to define the steps and not to describe how to do them [2].

AMERICAN MANUAL OF FORENSIC ODONTOLOGY AND OTHER BOOKS

This book have been rewritten many times and the 6th edition is now under preparation. The manual, which covers most of the fields in forensic odontology, is actually a textbook, with different chapters written by different authors, authorized by the American Academy of Forensic Odontology (AAFS)[1]. It is, however, unknown which control the AAFS have on each author and the exact text. It also covers the history of forensic odontology and it has a chapter on forensic medicine and jurisprudence and the expert witness testimony, both useful for a thorough understanding of the background of forensic odontology. The book contains also a lot of good advice for practical casework. The chapter on bite-marks also includes the American Board of Forensic Odontology (ABFO) standards for the investigation and the final report as a step by step approach to the work. It also contains a recommendation for a second expert reviewing of the work and conclusions to ensure the quality and reliability.

Another recent textbook from England is Forensic Odontology[3]. It is an easy introductory text, which considers most fields of forensic odontology included the role of forensic odontologists in the protection of vulnerable people. The field of dental injuries in connection with crime or prime target of a crime has however been forgotten. However, this book can be considered an excellent introduction to forensic odontology for dentists who want to go into this field.

IOFOS RECOMMENDATIONS FOR QUALITY ASSURANCE

In 2003, the IOFOS executive took an initiative to call a working meeting to draft recommendations for forensic odontology work. The meeting took place in a mountain cabin close to Lillehammer, Norway. The participants came from many countries of Europe, Asia and Africa. Some suggestions had been sent out to the participants beforehand and the participants were divided in groups to discuss the different fields of forensic odontology. The subjects were age estimation, identification and identification after large disasters, dental injuries, tooth-marks and the forensic odontological report. It was clear that an agreement detailed requirements on technique to execute each step would be extremely difficult to obtain. Therefore, it was decided to agree upon the different steps in the procedure to follow during a case without discussing how to perform the steps. Detailed recommendation on the technique to use should be set up by to the national associations. However, it turned out that there were great differences also in the views of which steps the participants considered necessary . Thus, the steps on which the participants agreed on were written in black, while steps that some would include while others thought they were unnecessary were written in blue [1].

The 2014 Interpol Guide does not contain so much about forensic odontology and seems more a guide for the administrative police work [4]. It is however important for the forensic odontologist to have some knowledge and understanding of it. Only little is included about forensic odontology.

Under the PM examination the guide says however that two or three odontologists should work together. It divides the roles of the forensic odontologists in examiner, recorder and radiography assistant. The Interpol Guide also recommends a double recording of the findings with one forensic dentist as the examiner and another forensic dentist who repeats the data to ensure a correct data entry. In my opinion, this is an unnecessary doubling of the time for the examination, and also the comparison of the data by two persons is time consuming. If there is something the dentist knows, it is recording of teeth and fillings. The procedure, as it is recommended by the Guide, is a distrust of dentists, as such a procedure has not been recommended for any other specialist groups. It is important to use the accepted Interpol nomenclature and forms for the registrations especially in the identification procedure of a foreign individual when the body and report are being sent to another country.

Much of the text is devoted to the excision of the jaws, which may be important under certain conditions. It is important that the excision of the jaws may be done only after a proper authorization from the legal controlling authority and only when deemed necessary. However, an absolute requirement that the jaws should be always kept with the body at all times show that the authors do not know why it is sometimes a good practice to take out the jaws and remove them for later supplementary examination. However, it is imperative to return the removed jaws to the body before it is sent to the relatives for burial, especially if on an international context.

The practicing dentist should keep a copy of the AM records for himself for documentation and control if asked about special details. This problem is of course, avoided if a computer program for dental recording is used as most dentists do today. It is also stressed the necessity to register the name, address, e-mail address and telephone number of the dentist. The original records and radiographs should never be given to relatives after a disaster.

Under the methods of identification, following conclusions are recommended 1. Identification, 2. Identification probable. 3. Identification possible. 4. Identity excluded in case it is necessary to write a report and 5.Insufficient evidenced in case there is no AM or PM material. It is seldom actual to write a report in such cases.

MY RECOMMENDATIONS

It is difficult to orient yourself towards the best practice if you get a forensic odontology case. After my long experience in many years, I may give some recommendations. I know that forensic odontologists want to make professional examinations and reports with a minimum time used. First, I will recommend to follow the practice defined by Interpol as far as it is a good guide, and to use the forms and nomenclature. Any form you use, all fields should be filled in with information. Further, I would recommend to read the IOFOS recommendations for quality assurance and follow them. These recommendations are valid for all the fields of

forensic odontology and under all circumstances. They should be universal. It would be a sign of quality if the forensic odontologist at the end of his report states that the examination and the work have been carried out according to the IOFOS recommendations and that the report written according to the same recommendations. Perform each step of the procedures according to the best knowledge or to the recommendations from the national societies of forensic odontology. To be able to choose the best practice, it is necessary to read both textbooks and scientific articles.

The fact that there is no systematic education in forensic odontology does not excuse the odontologist if he does a lousy job or even ends up with wrong conclusions. We work among professionals in forensic medicine and law. They know their job. They also know how to produce a professional report. They will easily see if a forensic odontologist does not perform following a good standard. Lawyers have often impressed me by their knowledge of dentistry. Do not try to trick them!

The quality assurance systems do not cover all the possible circumstances. Even if recommendations are issued, it is important to use one's own brain. It happens that sometimes it might be necessary to improvise in some aspects of the procedures.

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