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HUMAN TEETH APATITE MICROANALYSIS: DETERMINATION OF INDIVIDUALS' AGE AND SEX FOR FORENSIC APPLICATIONS

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***Abstract* -** Teeth, as well as other hardened body structures, are extremely useful in forensic analysis and identification of human remains. Due to their compositional characteristics, they are preserved over time and are resistant to high temperatures, making them a fundamental in contexts where individuals cannot be identified visually or by other means. Current studies of the crystal structure properties of human dental apatite (hydroxyapatite) have been conducted and highlight its decrease in function of age, but fail in establishing a mathematical relationship between these parameters. This study performed X-ray diffractometry (XRD) and electronic microprobe analyses (EPMA) in 29 teeth aiming at determining an age and sex criteria of identification based on the crystalline reticulum's parameters results, crystallite size and chemical composition of dental apatite. Through these methods it was possible to recognize that decreasing contents of calcium correlate to the formation of mineral phases that alter the original dental composition, causing a decrease in crystallinity with advancing age, and most importantly, it was possible to verify, as a result of a theoretical model, here proposed, that crystallinities values are not absolute, but a spectrum for a certain age: where permanent non-third molar vary between 20 and 45 µm, with a range of $\pm$2µm for the same age; deciduous teeth between 50 and 57 µm, with a range of $\pm$1µm for the same age and third-molar teeth, above 60 µm.

***Keywords**: Dental Apatite; Forensic Mineralogy; Microanalysis*

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1. INTRODUÇÃO

Teeth are formed by minerals from the apatite's isomorphic group, mostly hydroxyapatite, $Ca_5(PO_4)_3OH$, with carbonate concentrations ranging from 4 to 6%. This ion is the main responsible for structural changes that can occur due to multiple substitutions in crystallographic sites (Leventouri et al. 2009). Current studies have focused on microstructural and crystallographic analyzes of hydroxyapatite, aiming at a better understanding of the formation of caries and abrasion processes, (Siddiqui et al. 2014), as well as the synthesis of single crystals of this mineral (Zhuang et al. 2013; Okada et al. 2015), primarily highlighting the decrease in the overall average crystallite size in relation to aging. However, there are few studies available in literature regarding the relationship between structural and crystallographic parameters of dental apatite and sex/age of individuals, especially as a possible support tool for Forensic Sciences, Wilson (2009).

Teeth are classified into incisors, canines, premolars and molars; they are anatomically constituted of crown and root and, histologically constituted, by enamel, dentin, cementum and pulp (Fig. 1). The pulp has a nourishing, protective and repairing function, is not mineralized and corresponds to the vascularized part of the teeth. The cementum is a calcified tissue that covers the root surface, and has a percentage of inorganic material ranging between 40 and 60%. The dentin protects the pulp and supports the enamel; and it is formed by inorganic and organic constituents in a proportion of 70% for the mineral part, 20% for the organic matrix and 10% of water, approximately; it contains the so-called dentinal tubules, hollow canaliculi that, with advancing age, may be obstructed by amorphous mineralized materials (Yoshiyama et al.1996). The enamel is highly resistant and mineralized, largely, by oriented hydroxyapatite crystals (97%), the other 3% are composed of water and organic materials distributed among the crystals, Nanci (2013).

In this article, data obtained by X-ray diffractometry (XRD) and electronic microprobe analyses (EPMA) of human teeth, from consenting donors, will be presented aiming to establish an age and sex criteria of identification based on the crystalline reticulum's parameters results, crystallite size and chemical composition of dental apatite.

2. MATERIALS AND METHODS

The initial sample universe consisted of 40 teeth, from consenting donors, subdivided based on the donor's gender, tooth typology (<u>deciduous</u>, <u>permanent</u> and <u>third molars</u>) and their mouth position. Bases on a preliminary visual analysis, which took into account the state of conservation and the amount of material for analysis, 29 samples were selected for further evaluation.

The samples were identified by five alphanumeric digits, the first three letters correspond to the initials of the donor's name and the following two numbers correspond to the position of the tooth in relation to the dental arch, the first referring to the quadrant and the second, to the tooth's type, differentiated between deciduous and permanent teeth, according to the International Dental Federation (FDI).

Information on all teeth and donors is shown in Table 1, where data were organized by the ascending order of donor's age and grouped according to sex; the table also shows the type of tooth analyzed, as well as its main visual characteristics.

After the teeth identification, the samples were: i) sawn in half, ii) cleaned, iii) dried, iv) powdered, v) embedded in tablets, vi) polished, vii) received a metal coating, viii) were analyzed by XRD, ix) were analyzed by EPMA.

The samples were sawn in half, vertically, in the direction of the longest length, so that the same tooth could be used in more than one analysis, in case of destructive procedures. The cleaning was conducted in two steps:

i) removal of blood, tartar, caries and fillings, with the aid of a file and microdrill and

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ii) removal of surface impurities with a solution of 1L of ultrapure water, with 20 mL of detergent, during 15 minutes, with subsequent brushing and rinsing, also, with ultrapure water.

To aid in the powdering process, the teeth were placed in ovens in a temperature of 70°C for 24 hours. (Sudarsanan et al. 1968).

Of the samples selected for XRD, half were pulverized and gritted on agate gravel for homogenization. The powder obtained was placed in pressed tablets and taken for analysis in a Panalytical diffractometer, Empyrian model, with X-Celerador detector and CuKα 0.154060 nm emission line, according to the θ vs θ geometry; the analyzes were carried out at the Minerals and Rocks Analysis Laboratory (LAMIR), at the Federal University of Paraná (UFPR); diffractograms parameter measurements were refined according to the Scherrer equation, using the Origin 9.0 software. Miller indices were determined by the structural model's numerical simulation proposed by Holly Springs for hydroxyapatite (Fernández-Escudero et al. 2020) using Vesta software. The determination of the line widths (FWHM - Full Width at Half Maximum), used in the Scherrer equation for the determination of the average crystallite size per diffraction plane, was obtained by Gaussian adjustment of the peaks. For peaks between 29º and 34º, the Gaussian fit was obtained by the deconvolution of three chosen peaks within this range (Ivanova et al. 2001; Wilson et al. 1999).

On the half of the teeth kept intact, EPMA analyzes were conducted at the Laboratory of Microanalysis of the Institute of Geosciences (IG) of the Federal University of Pará (UFPA), employing equipment from the Jeol Brand, model JXA-8230, with dosage of P_2O_5, CaO, Na_2O, K_2O, MgO, SiO_2, SO_3, SrO, Al_2O_3 and the elements F and Cl. The selected samples were embedded in a heated epoxy resin, gritted with 200nm, 600nm and 1000nm abrasive and 1,500nm sandpaper until the embedded tooth outcropped. After this process, a chemical-mechanical polishing was carried out with a diamond paste of 6nm, 3nm, 1nm, 0.25nm and colloidal silica to smooth the surface; metallization was performed with C, in 5 pulses of 2 seconds, in a Quorum metallized, model Q150T-ES.

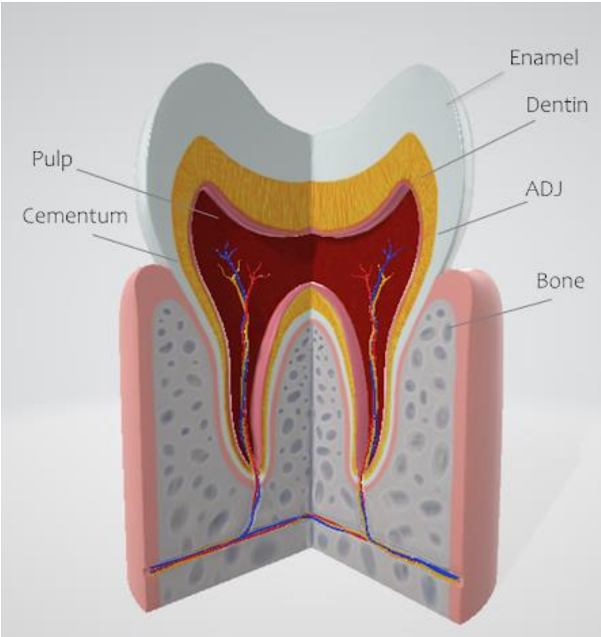

Figure 1. Tooth's histology, highlighting the division between enamel, dentin, pulp and cementum, as well as its supporting structure. The acronym ADJ corresponds to the structure called Amelodentinary Junction, which is the boundary between the enamel and the underlying dentin that forms the solid architecture of a tooth. Modified from Nanci (2013).

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Table 1. List of donors divided by sex and age, as well as the main characteristics of the analyzed teeth. For more details on sample identification, see text.

DECIDUOUS TEETH				
Sample	Sex	Age (year)	Teeth type	Visual Description
DAS-83	F	6	Lower jaw right Canine	Milky white, was a long time outside the mouth
DAS-81	F	7	Lower jaw central-right Incisive	Milky white, was a long time outside the mouth
MAN-61	F	8	Upper jaw central - left Incisive	Milky white, was a long time outside the mouth
THE-85	F	9	Lower jaw right Second Molar	Milky white, was a long time outside the mouth
DAS-73	F	10	Lower jaw left Canine	Milky white, was a long time outside the mouth
THE-73	F	10	Lower jaw left Canine	Milky white, was a long time outside the mouth
THE-65	F	11	Upper jaw left Second Molar	Milky white, was a long time outside the mouth
MAN-65	F	11	Upper jaw left Second Molar	Milky white, was a long time outside the mouth
MAN-85	F	12	Lower jaw right Second Molar	Milky white, was a long time outside the mouth
MAU-81	M	7	Lower jaw central - right Incisive	Milky white, was a long time outside the mouth
NIS-73	M	8	Lower jaw left Canine	White, translucent
GAL-61	M	8	Upper jaw central - left Incisive	Milky white, was a long time outside the mouth
GAL-83	M	9	Lower jaw right Canine	Milky white, was a long time outside the mouth
LUS-65	M	9	Upper jaw left Second Molar	Milky white, was a long time outside the mouth
GAL-65	M	11	Upper jaw left Second Molar	Milky white, was a long time outside the mouth
MAU-65	M	11	Upper jaw left Second Molar	Milky white, was a long time outside the mouth
MAU-73	M	12	Lower jaw left Canine	Milky white, was a long time outside the mouth
MAU-85	M	12	Lower jaw right Second Molar	Milky white, was a long time outside the mouth

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Table 1. Continuation.

Sample	Sex	Age (year)	Teeth type	Visual Description
PERMANENT TEETH (EXCEPT THID MOLARS)				
DAC-11	F	40	Upper jaw central - right Incisive	Dark brown colored
DAC-22	F	40	Upper jaw lateral - left Incisive	Dark brown colored
DAC-23	F	40	Upper jaw left Canine	Dark brown colored
ROS-12	F	58	Upper jaw lateral - right Incisive	Dark yellow colored
MAA-13	F	62	Upper jaw right Canine	Decayed
MAA-27	F	62	Upper jaw left Second Molar	Amalgam filling removed, normal color
JOO-27	M	41	Upper jaw left Second Molar	Amalgam filling removed
LEF-11	M	57	Upper jaw central - right Incisive	Yellow colored, smoker
LEF-22	M	57	Upper jaw lateral - left Incisive	Yellow colored, smoker, periodontal disease
JOB-11	M	68	Upper jaw central- right Incisive	Yellow colored, intense tartar
JOB-12	M	68	Upper jaw lateral - right Incisive	Yellow colored, intense tartar
JOB-13	M	68	Upper jaw right Canine	Yellow colored, intense tartar
TAD-43	M	60	Lower jaw right Canine	Advanced surface abrasion
JUA-23	M	63	Upper jaw left Canine	Blackened root, yellowish tooth
ROM-21	M	104	Upper jaw central - left Incisive	Yellow, fractured, with little time out of the mouth
ROM-11	M	104	Upper jaw central - right Incisive	Yellow, very fractured, small amount of material, little time out of mouth
ROM-47	M	104	Lower jaw right Second Molar	Yellow, fractured, decayed, with little time out of the mouth
ROM-25	M	104	Upper jaw left Second pre-molar	Yellow, fractured, decayed, with little time out of the mouth
THIRD MOLARS				
ISI-18	F	22	Upper jaw right Third Molar	Included, milky white
PAM-48	F	23	Lower jaw right Third Molar	Semi-included, milky white
MAL-28	F	60	Upper jaw left Third Molar	Cancer patient, in chemotherapy
LUI-18	M	27	Upper jaw right Third Molar	Semi-included, milky white

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3. RESULTS AND DISCUSSION

3.1. Dental apatite's crystallinity

Structural data on dental apatite were obtained from the analysis of the diffractograms'peaks (002), (211), (300), (112) and (310), which are included in the ICDD hydroxyapatite sheet 09-432. To calculate the average crystallite size, the parameters used were the width at half height (FWHM) of the peak in the diffractogram, as well as the corresponding 2θ Bragg angles to each peak. The average crystallinity was obtained based on the Scherrer equation (Ivanova et al. 2001; Wilson et al. 1999). Table 2, which was organized based on typology (deciduous, permanent non-third molars and third molars), position (quadrant in the mouth), sex and age of individuals, and average tooth crystallinity, shows the results of these calculations for the 29 samples analyzed.

Regarding the deciduous teeth, the average crystallites'size for female teeth was 53,869 µm and for male, 54,113 µm. It is important to note, however, that the DAS-73 and THE-73 samples, although equivalent to the same type of tooth, in individuals of the same sex and age, show a slight variation of ±1µm in the crystallinity value.

In all deciduous teeth analyzed, the largest crystallinity size was related to the samples of younger donors (Fig. 2). There was little noise; peak widths at half height were very similar to each other and showed constant intensity, with approximately 600 counts.

The average crystallinity's calculation for permanent non-third molar teeth became hindered due to the difficulty in obtaining teeth of the same type and age in opposite sexes. Thus, the only possible comparison, in this case, was between crystallinity and age, which was observed to be an inverse correlation, noticed in all analyzed teeth (Table 2). Teeth aged between 40 and 41 years showed crystallinities around 40 µm, ranging between 40.175 and 44.215 µm. Those aged between 57 and 58 years had an average crystal size between 38.176 and 38.512 µm, with little variation; those aged between 62 and 63 years ranged from 34,555 to 37.771 µm. Teeth aged 68 years had an average crystal size between 31.606 and 33.206 µm, with the lowest crystallinity observed in the canine (tooth 13). It is noteworthy that the teeth of the 104-year-old patient displayed the lowest crystallinity, ranging between 20.51 and 26.31 µm.

The XRD analysis of permanent non-third molar teeth (Fig. 3A) exhibited the diffractograms' peak intensity of approximately 500 counts, with widening of the Bragg peaks of and greater noise. The peaks with the best differentiation were those from younger samples (40 and 41 years), with no evident separation regarding sex.

The average crystallinity of third molar teeth varied between 64.601 and 67.683 µm (Table 2), which highlighted these teeth' anomalous behavior, indicating values higher than those of deciduous teeth and quite deviant from those found for other permanent teeth. In Fig. 3B, it is possible to notice the comparison between third molar teeth, where the diffractograms presented higher counts (600 cps), lower noise, as well as good differentiation between the peaks. The average values of crystallinity (Table 2) border on the characteristics of deciduous teeth. This peculiarity is probably due to the fact that these teeth have a late hatching, or are extracted before their complete hatching, thus spending less time in the mouth, and therefore being less worn.

The graph in Fig. 4A illustrates the variation between crystallinity and age of the individuals. The distribution over three distinct fields is clearly seen for each of the types of teeth analyzed; the greatest dispersion occurs in the set of permanent non-third molar teeth due to the greater age gap of donors.

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Table 2. List of donors divided by sex and age, as well as the main characteristics of the analyzed teeth. For more details on sample identification, see text.

Sample	Sex	Age (years)	Medium Crystallinity (µm)
DECIDUOUS TEETH			
DAS-83	F	6	56,356
DAS-81	F	7	54,812
DAS-73	F	10	51,882
THE-73	F	10	52,246
THE-65	F	11	53,265
MAN-65	F	11	54,046
MAN-85	F	12	51,276
NIS-73	M	8	54,36
GAL-83	M	9	53,974
LUS-65	M	9	56,417
GAL-65	M	11	53,162
MAU-85	M	11	52,238
MAU-73	M	12	50,603
PERMANENTS TEEH EXCEPT MOLAR THIRD			
DAC-11	F	40	44,216
DAC-23	F	40	40,06
DAC-22	F	40	43,397
ROS-12	F	58	38,316
MAA-27	F	62	37,771
JOO-27	M	41	40,175
LEF-22	M	57	38,177
JUA-23	M	63	34,955
JOB-12	M	68	33,207
JOB-11	M	68	31,606
ROM-21	M	104	20,509
ROM-11	M	104	22,797
ROM-47	M	104	26,315
ROM-25	M	104	22,372
THIRD MOLAR			
ISI-18	F	22	64,602
LUI-18	M	27	67,683

For deciduous teeth, also subdivided according to sex, there is an evident negative correlation regarding age (Fig. 4B), with no significant variations, however, regarding the sex of the donor. The same trend is observed for the population of permanent non-third molar teeth, with a slight increase in crystallinity in female teeth (Fig.4C).

From the data shown in Table 2, excluding the third-molar permanent teeth, a graph was formulated (Fig. 5) correlating the parameters of age (years) and crystallinity (µm). The correlation is clearly negative and has a linear trend, which made it possible to delineate a theoretical line equation $y= 56.376 - 0.3275x$, on which y represents the crystallinity (µm) and x the age (years). The coefficient of determination of this theoretical trend line was $R2>0.9788$, that is, the theoretical model - represented by the trend line - explains about 98% of the variability of the response data, denoting its high reliability in relation to the data samples.

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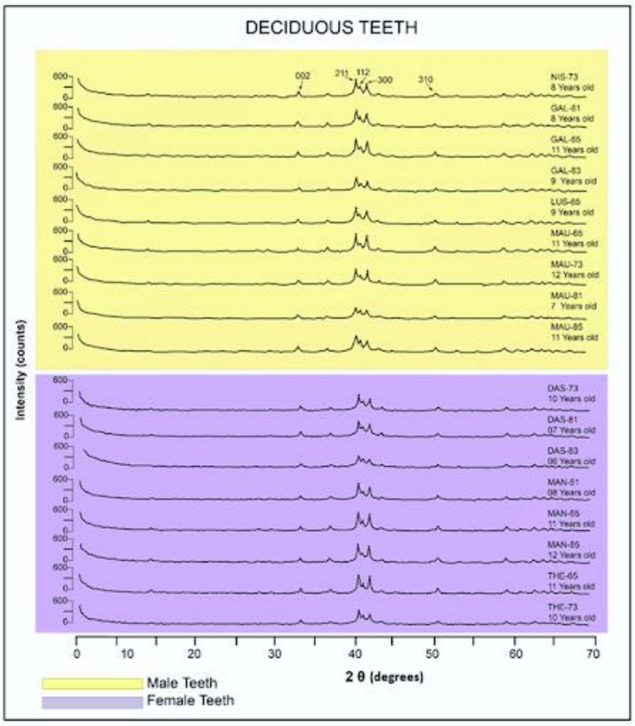

Figure 2. Diffractograms for sets of male and female deciduous teeth, indicating the peaks (002), (211), (112), (300) and (310), which were used to calculate average crystallinity. See Table 2.

To calculate the absolute error, relative to the theoretical model provided by the trend line (Fig. 5), the true ages of the donors, in Table 2, were subtracted from the theoretical ages obtained by the line equation (Fig. 6). About 70% of the analyzed teeth showed an absolute error of 5 years. For another 15%, the associated error was between 5 and 7 years. Thus, the theoretical model proposed here allows the estimation of the age of a tooth with an error of ± 5 years, based on the average crystallinity values of the apatite, with a 70% probability of correctness. Considering an error of 7 years, the probability of obtaining the correct age of an individual, based solely on the average crystallinity value of apatite, increases to 85%.

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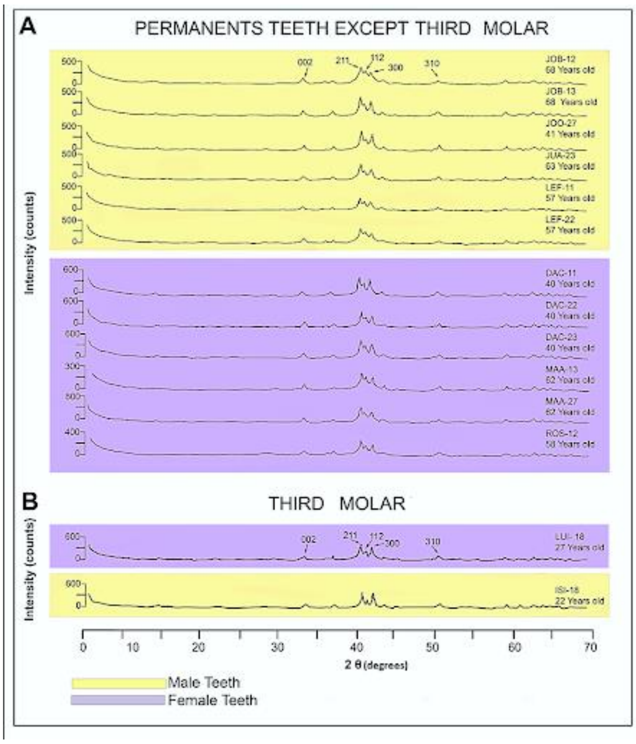

Figure 3. (A) Diffractogram of male and female for non-third molar permanent teeth, indicating the peaks used to calculate the crystallinity of apatite. Younger donors' samples show better differentiation between these peaks. (B) Diffractograms for third molar teeth, with higher average total count than permanent non-third molar teeth, less noise and peaks, in general, more differentiated.

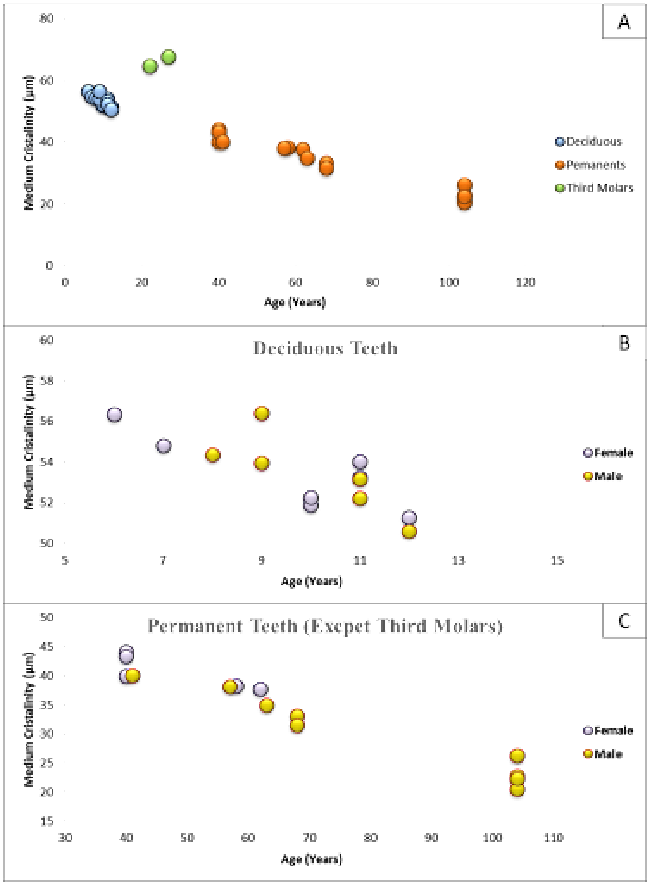

Figure 4. Distribution of the degree of crystallinity of dental apatite versus age of individuals for deciduous teeth, permanent non-third molars and third molars. (B) Distribution of the degree of crystallinity of dental apatite versus age of individuals for primary teeth according to sex. There is a clear negative correlation between age and degree of crystallinity, but the relationship with sex is not evident. (C) Distribution of the degree of crystallinity of dental apatite versus age of individuals for permanent non-third molar teeth according to sex, with the same observation made for the correlation in (B).

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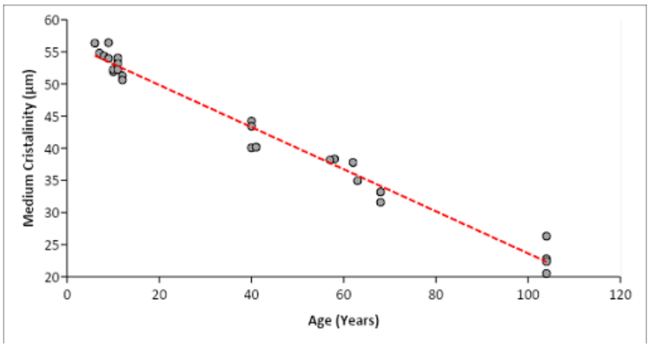

Figure 5. Relation between age (years) and crystallinity (µm) parameters; trend line relative to the data and coefficient of determination of the linear model relative to the analyzed data set. For details, see text.

Real age (years)	Teoretical age (years)	Error (Years)
6	0	6
7	5	2
10	14	4
10	13	3
11	9	2
11	7	4
12	16	4
8	6	2
9	7	2
9	0	9
11	10	1
11	13	2
12	18	6
40	37	3
40	50	10
40	40	0
58	55	3
62	57	5
41	49	8
57	56	1
63	65	2
68	71	3
68	76	8
104	110	6
104	103	1
104	92	12
104	104	0

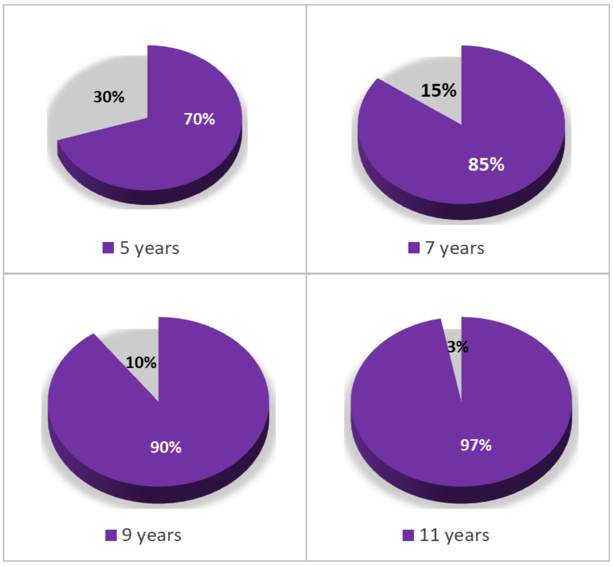

Figure 6. Absolute error of the proposed model in relation to the real data shown in Table 2. About 70% of the analyzed teeth had an absolute error of ± 5 years; in 15% of the analyzed teeth, the associated error is between 5 and 7 years.

3.2. Dental apatite microanalysis

Electron microprobe analyzes measured P_2O_5, CaO, MgO, Na_2O, K_2O, SiO_2, Cl, SO_3 and SrO in the enamel and dentin of three teeth: THE-85 (deciduous, female, 9 years old), TAD-43 (permanent not third- molar, male, 60 years old) and MAL-28 (third molar, female, 60 years old). The characteristics of these teeth are shown on Table 1; the analytical values on Table 3.

Fig. 7 shows the distribution of these elements along the enamel and dentin based on a chemical profile performed on samples from the three teeth analyzed. For the 9-year-old female deciduous tooth (THE-85) there was a slight decrease in P_2O_5 from the enamel towards the dentin (Fig. 7A); Na_2O exhibited decreasing concentrations close to the ADJ (dentinal-enamel junction) and stabilized in the dentin; Cl had a slow and gradual content decrease in the same direction (enamel - dentin); CaO was stable in the enamel, and decreased in levels closer to the ADJ, with a slight increase in the dentin; K_2O had a stable concentration in the enamel, with a slight increase closer to the ADJ, maintaining it in the dentin contents; SO_3 had low stable contents in the enamel, which grew from the ADJ, with an abrupt decrease in the dentin, until the concentrations were stabilized; MgO contents rose from enamel and slightly fluctuated in the dentin after an abrupt increase close to ADJ; there was no significant variation of SiO_2 in the enamel and dentin,

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where concentrations fluctuated along the profile; SrO showed the highest percentages close to ADJ, with an abrupt decrease in the dentin.

For the non-third-molar permanent tooth (TAD-43), male aged 60 years (Fig. 7B) there were higher concentrations of P_2O_5 in the enamel, with an abrupt drop towards the ADJ and, finally, its contents were stabilized in the dentin, similarly to the deciduous tooth's behavior; Na_2O increased towards the ADJ, then slightly decreased, with a new substantial increase and subsequent stabilization in the dentin, contrary to what was observed in the dentin of the deciduous tooth (Fig. 7A); Cl displayed a slow and gradual decrease similarly to what was observed in the deciduous tooth; CaO was stable in the enamel, exhibited a decrease close to the ADJ and stabilized in the dentin; K_2O showed a similar behavior, but it decreased markedly in enamel up to the ADJ, with a slight increase in the dentin, contrary to what was observed for deciduous teeth. MgO and SO_3 had a similar behavior: concentrations that increased from the enamel towards the dentin, with stabilization from the ADJ. MgO behaved similarly to deciduous teeth; in the latter, SO_3 showed an abrupt drop in concentrations before stabilization. SiO_2 had oscillating contents, but with a decrease from the enamel towards the dentin; SrO exhibited slightly higher values in the enamel, with a drop close to ADJ and oscillations in the dentin.

The third molar tooth MAL-28, from a 60-year-old female donor (Fig. 7C), showed a slight decrease in P_2O_5 concentrations, similarly to what is observed in the other teeth analyzed; Na_2O increased in the enamel, decreased towards the ADJ, with a slight decrease in the dentin contents; Cl had a behavior similar to the one mentioned above, that is, an abrupt decrease in the enamel towards the dentin; CaO behaved similarly to previous teeth: stable concentrations in the enamel and slight decrease from the ADJ towards the dentin. K_2O behaved differently from those previously shown: there was a gradual increase in the enamel, a decrease close to the ADJ, with subsequent stabilization in the dentin; SO_3 has a similar behavior to the teeth described above, but in the analyzed third molar it showed a sharp drop in the dentin. Stable concentrations of MgO occurred in the enamel, with growth close to the ADJ and decrease in the dentin; the highest concentrations of SiO_2 were found in the dentin, where abrupt growth was verified close to the ADJ, with a brusque drop in the dentin contents. SrO had sudden fluctuations in contents, both in the enamel and dentin.

These results are compatible with those presented in the literature, which show a decrease in the calcium content in dentin as a function of age and, consequently, the formation of mineral phases that alter the original dental composition, causing a decrease in crystallinity with advancing age, Leventouri et al., (2009). It can be seen in Fig. 7B and C and in Table 2, a general trend towards a decrease in the concentration of CaO in dentin and a decrease in the average size of dental apatite crystals as a function of the age of the individuals.

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Table 3. EPMA analytical results (%wt) in female deciduous tooth (9 years old); male permanent non-third male (60 years old) and female third molar (60 years old). Points 1 to 3 refer to enamel analyses; 4 to 6, in dentin.

THE-85 (Deciduous - Female- 9 years old)											
Nº	F	Na2O	MgO	Al2O3	SiO2	CaO	K2O	P2O5	Cl	SO3	SrO	Total
1	0,080	0,606	0,403	0,000	0,031	50,182	0,032	40,093	0,531	0,006	0,009	91,819
2	0,000	0,873	0,397	0,001	0,032	49,967	0,031	39,684	0,354	0,011	0,000	91,270
3	0,017	1,054	0,423	0,000	0,019	48,980	0,026	39,178	0,220	0,013	0,019	89,892
4	0,080	0,829	1,529	0,000	0,032	42,811	0,036	35,731	0,112	0,370	0,036	81,507
5	0,014	0,831	1,554	0,002	0,025	37,287	0,037	31,260	0,056	0,213	0,001	71,261
6	0,000	0,866	1,694	0,000	0,035	40,357	0,041	33,862	0,057	0,206	0,000	77,105
Minimum	0,000	0,606	0,397	0,000	0,019	37,287	0,026	31,260	0,056	0,006	0,000	71,261
Maximum	0,080	1,054	1,694	0,002	0,035	50,182	0,041	40,093	0,531	0,370	0,036	91,819
Average	0,032	0,843	1,000	0,001	0,029	44,931	0,034	36,635	0,222	0,137	0,011	83,809

TAD – 43 (Permanent- Male - 60 years old)												
Nº	F	Na2O	MgO	Al2O3	SiO2	CaO	K2O	P2O5	Cl	SO3	SrO	Total
1	0,025	0,797	0,478	0,000	0,038	50,274	0,049	41,149	0,418	0,008	0,054	93,185
2	0,001	0,991	0,613	0,005	0,019	48,748	0,050	39,676	0,209	0,000	0,074	90,339
3	0,000	1,096	0,655	0,000	0,023	48,400	0,042	39,531	0,128	0,022	0,074	89,942
4	0,103	0,610	1,364	0,000	0,008	34,092	0,021	29,114	0,097	0,206	0,056	65,606
5	0,000	0,766	1,463	0,000	0,011	36,701	0,030	32,061	0,069	0,226	0,072	71,383
6	0,000	0,742	1,474	0,000	0,008	36,239	0,033	31,450	0,066	0,228	0,052	70,277
Minimum	0,000	0,610	0,478	0,000	0,008	34,092	0,021	29,114	0,066	0,000	0,052	65,606
Maximum	0,103	1,096	1,474	0,005	0,038	50,274	0,050	41,149	0,418	0,228	0,074	93,185
Average	0,022	0,834	1,008	0,001	0,018	42,409	0,038	35,497	0,165	0,115	0,064	80,122

MAL – 28 (Third Molar - Female - 60 years old)												
Nº	F	Na2O	MgO	Al2O3	SiO2	CaO	K2O	P2O5	Cl	SO3	SrO	Total
1	0,000	0,757	0,436	0,000	0,013	50,314	0,029	39,910	0,412	0,002	0,011	91,791
2	0,000	0,692	0,430	0,000	0,020	48,677	0,029	39,841	0,272	0,011	0,004	89,915
3	0,054	1,219	0,507	0,002	0,014	48,703	0,048	39,183	0,141	0,002	0,001	89,819
4	0,004	0,860	1,488	0,000	0,024	40,260	0,027	34,116	0,095	0,265	0,009	77,125
5	0,000	0,805	1,463	0,000	0,034	36,074	0,027	32,658	0,076	0,237	0,000	71,357
6	0,038	0,746	1,240	0,000	0,024	35,960	0,031	29,780	0,067	0,180	0,017	68,052
Minimum	0,000	0,692	0,430	0,000	0,013	35,960	0,027	29,780	0,067	0,002	0,000	68,052
Maximum	0,054	1,219	1,488	0,002	0,034	50,314	0,048	39,910	0,412	0,265	0,017	91,791
Average	0,016	0,847	0,927	0,000	0,022	43,331	0,032	35,915	0,177	0,116	0,007	81,343

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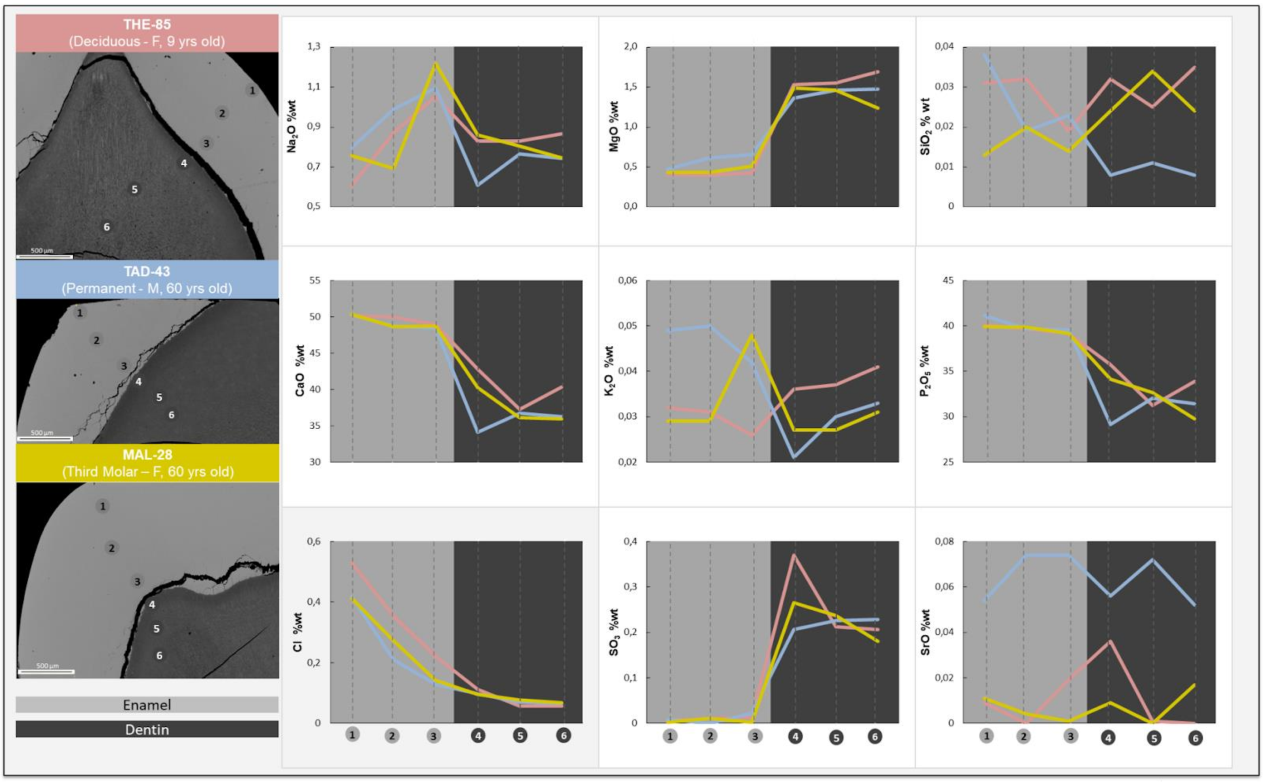

Figure 7 - Analysis by EPMA (%wt), showing the profiles of variations of the elements P_2O_5, CaO, MgO, Na_2O, K_2O, SiO_2, Cl, SO_3 and SrO in enamel and dentin of a deciduous tooth (THE-85), female with 9 years (A); permanent non-third molar (TAD-43), male aged 60 years (B) and permanent third molar (MAL-28), female aged 64 years (C).

4. CONCLUSIONS

This research demonstrates that analysis by type of tooth, as well as its position in the mouth, is fundamental for a more realistic interpretation of this kind of data. Further categorizations into subgroups (female and male) proved to be essential for the analyses. XRD reaffirmed its capability as an accurate technique in separating teeth from adults and children, as well as determining the crystallinity of dental apatite in relation to age - where permanent non-third molar teeth have a crystallinity between 20 and 45 µm; deciduous teeth between 50 and 57 µm and third-molar teeth, above 60 µm. However, even under conditions of sample equality (same sex, same age and same type of tooth) there was a variation in the average size of the crystals, indicating that the crystallinity value is not absolute, but a spectrum that corresponds to a certain age.

For deciduous teeth (Fig. 4 - Table 2), the range that best defined the crystal size for a given age was x ± 1µm, where x is the value of the crystallinity obtained; that is, for a 10-year-old sample, for example, with a crystal size of 51µm, another sample belonging to the same sex and the same type of tooth and it that is also 10 years old will have the value of 51 ± 1µm. For non-third molar permanent teeth, the range of crystallinity for the same age, same-sex tooth type was ± 2 µm. Third molar teeth, on the other hand, are not indicated for establishing a relationship between the degree of crystallinity versus age, as they have a late hatching and showed contradictory results to those expected. The results of XRD have not revealed, so far, good indications for determining the sex of individuals.

The results presented here, especially in regards to the theoretical model that correlates the age of an individual to the crystallinity of the teeth, are of paramount importance for several areas of Forensic Sciences, since despite sophisticated analyses, such as DNA and other identification methods, certain types of disasters -

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especially those that occurred en masse, such as air catastrophes - and/or crimes, where bodies are carbonized, require alternative methods for determining the age, sex and biological profile of individuals.

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