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Tooth Calcification in Low-Weight Human Fetuses

Calcificación de Dientes en Fetos Humanos de Bajo Peso

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ABSTRACT: Tooth calcification begins during the intrauterine life and is related to the nutritional status of fetus. The purpose of this study was to evaluate the dental calcification status of 15 low-weight human fetuses aged from the 14th to 28th weeks of intrauterine life through radiographic analyses and von Kossa histochemical technique, which is specific to the detection of calcium and other mineral salts in tissues. After dissection of jaws, the mandibles were submitted to lateral and occlusal radiographies. Three mm thick slide sections of jaws samples were stained by conventional and von Kossa methods. As results, radiographic analysis did not exhibit any suggestive image of tooth mineralization, although rudiments of mandibular ossification could be noted. Hematoxilin and eosin sections revealed tooth germs in advanced phases of odontogenesis, but relative delay in tooth development was observed. The von Kossa staining did not show any positivity to the deposition of mineral salts in tooth germs from maxilla and mandible, supporting our findings related to the delay of dental development that was found in studied sample. Hence, we suggest that there is relation between the fetal low-weight and the calcification and maturation status of teeth.

KEY WORDS: odontogenesis, calcification, nutritional imbalances, low-weight, von Kossa.

INTRODUCTION

Odontogenesis is a result of a complex cellular and molecular reciprocal induction between ectomesenchyme, oral mesenchyme and oral epithelium beginning from the 27th day of embryonic development. It may be divided in stages (bud, cap and bell) that in its conclusion results in the dental tissues, such as the dental enamel and dentin (Nanci, 2001; Galassi *et al*., 1997; Arana-Chavez & Arana-Chavez, 1997; Tsujigiwa *et al*., 2013; Yoshizaki & Yamada, 2013).

Dental enamel is the only mineralized tissue arising from epithelium and becomes acelular after its complete maturation. It is the hardest tissue of the human body and its composition is represented by calcium phosphate crystals in the shape of hydroxyapatite that is permeated to 1% of organic matter full of protein, with small percentiles of carbon hydrates, lipids and 2% of water. The responsible cells for enamel formation are the ameloblasts (enameloblasts), which pass by several functional stages during amelogenesis (Nanci; Tsujigiwa *et al*.).

Dentin has 70% of its weight constituted by mineral content also in the shape of hydroxyapatite, besides 18% of organic matter and 12% of water. It is the most important component of the tooth, which borrows its shape. Dentin has a connective nature; it is covered by enamel in the crown, and by cementum in the root. Subjacent and involved by the dentin the pulp is located. Together both structures are called dentin-pulp complex since they interact with each other from early development to complete maturation of their structures (Nanci; Galassi *et al*.; Arana-Chavez & Arana-Chavez; Tsujigiwa *et al*.; Yoshizaki & Yamada).

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Mineral deposition during odontogenesis occurs immediately after organic matrix secretion by ameloblasts and odontoblasts, which are initially deposited at the beginning of the bell stage (Tsujigiwa *et al*.). It involves a process depending on performance of local and systemic factors as, for example, the nutritional balance, once severe nutritional privations during a critical time of the odontogenesis may result in several defects on the structure of the hard dental tissues, such as the enamel (Nanci; Bello Perez *et al*., 1997).

How the teeth formation begins at the intrauterine life and it seems to be related to the nutritional status of the fetal environment (Bello Perez *et al*., 1997), in this study, the calcification during the odontogenesis was investigated in low-weight human fetuses, through radiographies and von Kossa histochemical technique, which is based on the affinity of the silver ions of binding to carbonates and phosphates that in tissues are invariably associated to calcium ions.

MATERIAL AND METHOD

After approval by the Ethical Research Committee from the University of Pernambuco (CEP/UPE), 15 fetuses with proven low-weight, which suffered normal abortion, were collected from Hospitals located at Recife, Pernambuco, Brazil, and fixed in 10% buffered-formalin solution.

Concerning the developmental stage of the fetuses, the length of femur was the parameter utilized to estimate the gestational age of specimens. Thus, dissection of fetal femurs was necessary to perform better measurement of these bones and hence to estimate the gestational age of studied fetuses (Hadlock *et al*., 1984; Callen, 1996).

The fetal weight was obtained in grams with a digital balance (B-6000, Micronal, Brazil) and this procedure was necessary to fill bioethics requirements, once, according to Brazilian civil law, the fetuses could not weigh more than 500 g. Furthermore, fetal weight permitted to make a comparison with gestational ages by mean of commended values (12) and verify whether fetal development was really normal or not.

The weight, length of femur and gestational ages of 15 studied fetuses may be checked in Table I. The estimated gestational age was between 14th (±1.0) e 28th (±2.0) weeks of fetal development. In Table II the pronounced difference between the fetal weights and the expected values for a normal fetal development can be observed. The percentile average for this difference was 42.5%.

The lateral and occlusal radiographic incidences of surgically removed fetal mandibles were performed by a x-ray apparatus for dental use (Pró-70, Brazil) through protocol technique previously calibrated for these analyses (70Kvp, 8mA, exposure time of 0.3 seconds). Due to technical difficulty, the radiographic study of the fetal maxillas was not carried out.

All specimens were paraffin-embedded and serial 3 mm thick sections were performed and stained by hematoxilin and eosin (H-E) with the purpose of identifying the tooth germ development stage, and stained by von Kossa histochemical technique that is specific to mineral salts detection. For the von Kossa technique, the bone tissue adjacent to the tooth germs was used as positive internal control. These histological sections were visualized under light microscopy and the results were noted and descriptively and statistically analyzed by mean of the SPSS 10.0 software (SPSS, EUA), with the p value established at 0.05.

Table I. Distribution of the fetuses weight, femoral size and gestational age. Percentile of 50%.

Fetus	Weight (g)	Femur size (cm)	Gestational age (weeks)
1	14.3	1.6	14
2	17.7	1.7	15
3	29.6	1.9	15
4	34.7	2.0	16
5	47.4	2.2	16
6	34.8	2.3	17
7	32.9	2.8	18
8	153.5	3.7	21
9	227.4	4.0	23
10	195.7	4.0	23
11	302.2	4.5	24
12	318.0	4.6	25
13	295.0	4.8	26
14	321.4	4.8	26
15	378.1	5.4	28

Variability estimates (±2 DP); 12–18 weeks= ±1.0 week; 18–24 weeks= ±1.8 week; 24–30 weeks= ±2.0 week.

Table II. Relation between weight, gestational age and the fetuses development.

Gestational age (weeks)	Fetal weight (g)	
	*Expected weight**	*Weight of studied fetuses*
14	45	14.3
16	110	47.4
18	200	32.9
24	630	302.2
26	820	295
28	1000	378.1

*Reference values to specimens fixed in 10% buffered formalin during more or less two weeks.

RESULTS

Figure 1 shows no suggestive radiographic image of tooth germ, although only some general characteristics of mandibular ossification could be noted such as: 1) independently of the performed radiographic incidence, radiographic density was more elevated in the central regions of the mandibular body, and the same progressively increased with the fetal gestational ages including the regions close to the mandibular symphysis and ascendent ramus; 2) early formation of the mandibular canal corticals in those fetuses above 15th week of development; and 3) no specimen showed median-fusion of the mandibular symphysis.

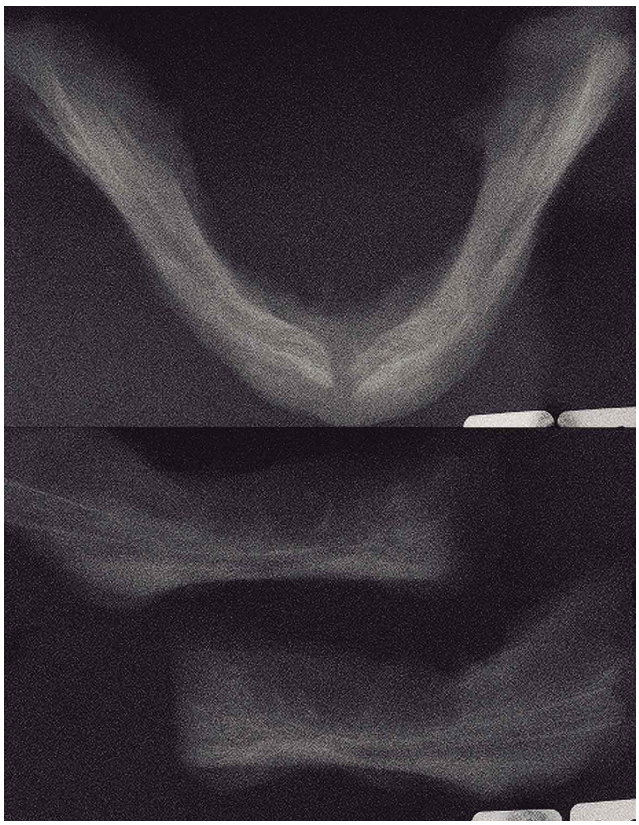

Fig. 1. Radiographs of the 26-weeks fetus mandible. Figure above – Occlusal incidence - Note the inner and outer corticals of the mandibular canal and the no-fusion of symphysis in the median line. Figure below – Lateral incidence - See the superior and inferior corticals of mandibular canal and the higher intensity of bone mineralization in the anterior region of the inferior margin of mandible.

H&E sections exhibited dental germs from final bud stage (14th week) to the bell stage (24th week), what, considering the parameters listed by Figún & Garino (1994) about normal teeth development, a relative delay on the dental development of the studied fetuses could be seen. Moreover, in those dental germs of the fetuses above 24th week of development we observed the presence of the enamel and dentin matrixes (Fig. 2). The slides stained by von Kossa

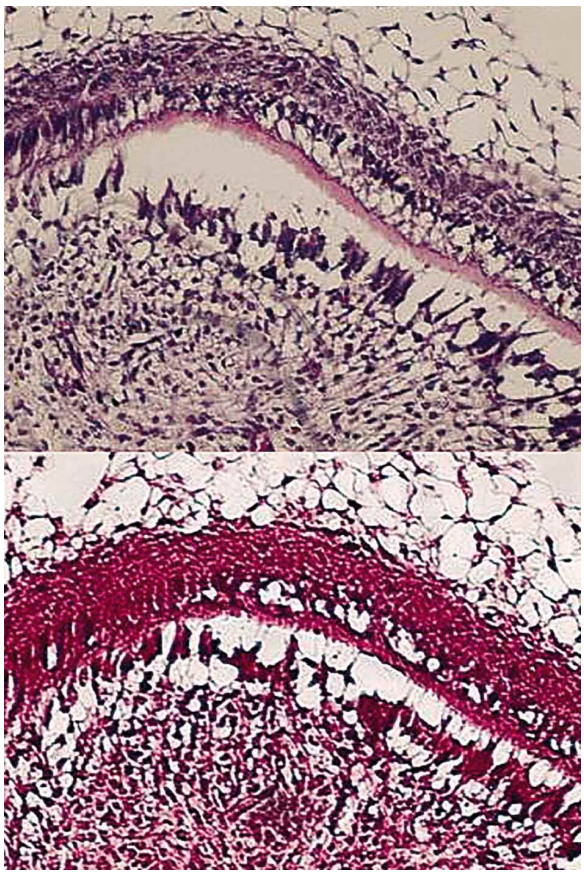

Fig. 2. Comparison among histochemical techniques in maxilla of a 28-weeks fetus. Figure above – H&E staining showing dentin deposition between ameloblasts and odontoblasts. Figure below – Von Kossa staining showing no positive black deposits in the dentin deposition area (H&E, Von Kossa, 400X).

showed no positive-stain within the tooth germs (Fig. 2). However, inside the adjacent and maturing bone tissue it was possible to visualize positive-black-stain compatible with Von Kossa staining (Fig. 3).

As all studied cases did not show the presence of calcification of the dental germs, no statistical tests could be performed and, for this reason, the found results were only descriptively analyzed and presented.

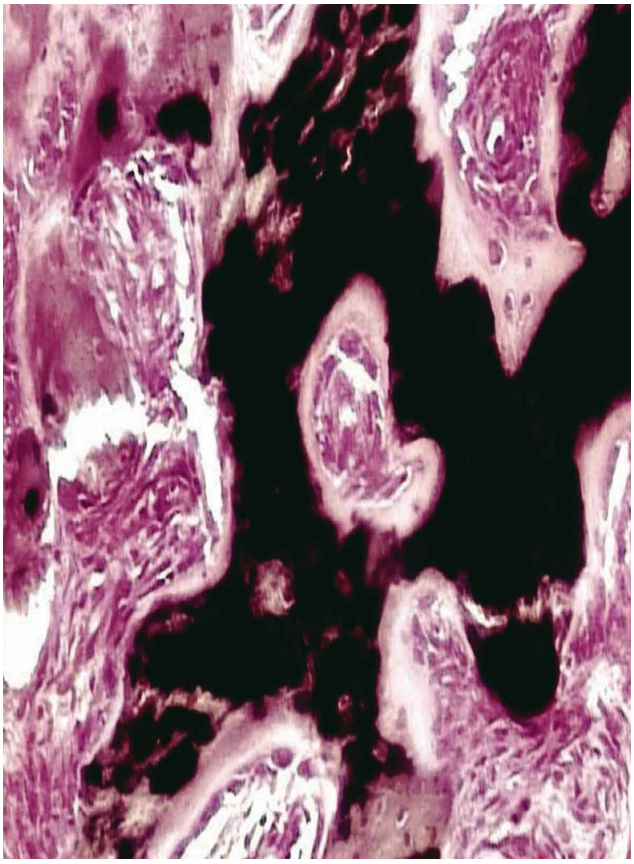

Fig. 3. Bone tissue adjacent to the dental germs of a 28-weeks fetus showing consistent and positive von Kossa black deposits (Von Kossa, 400X).

DISCUSSION

At first, Rezende (1991) and Cunningham *et al*. (2000) affirm that fetal weight is a parameter intimately dependent on environmental, social and economical factors. For this reason, it was necessary to consider that the studied fetuses were from public Brazilian maternities, with larger attendance of low-income mothers, with nutritional deficiencies that, unfortunately, could not be better investigated, but certainly is responsible for the pronounced low-weight of the fetuses used in this work.

How teeth begin their development and maturation during the intrauterine life, the nutritional status of fetus seems to be very important to the normal maturation of dental tissues. According to Menoli *et al*. (2003) the main nutrients involved in odontogenesis are calcium, phosphate, vitamins A, C, D and the protein/energy balance. Vitamin A is responsible for the sustenance of the integrity of ectodermic tissues. Vitamin D facilitates calcium and phosphorus homeostasis, interfering in dental calcification. Vitamin C is essential to the maintenance and functioning of intercellular substances and collagen. Phosphorus adds solidity to the dental structure and lipids and carbohydrates ensure the energy needed in odontogenesis.

The correlation between factors as nutritional deficiencies, low-weight of fetuses and newborns have been discussed, and the increase of diseases and alterations in dental development, such as enamel hypoplasia, increase of caries susceptibility and delay in the tooth eruption (Infante & Gillespie, 1974; Alvarez, 1995; Li *et al*., 1995; Lai *et al*., 1997; Rugg-gunn *et al*., 1998; Agarwal *et al*., 2003; Machado & Ribeiro, 2004). However, some works show no association between nutritional status and the presence of diseases or other problems related to the development, maturation and physiology of teeth (Hanser-Ducatti *et al*., 2004; Melo *et al*., 2002).

The present work is one study, among few that show the relationship between fetal nutritional status and the dental development, once in the studied low-weight fetuses there was no evidence or any sign of dental mineralization nor for the x-rays incidences, or for the von Kossa staining, and a relative delay in the dental formation was noted, in view of the fact that it was expected to find germs of deciduous teeth with one third of crown already formed (Figún & Garino, 1994).

Tooth calcification could not be observed on the radiographic incidences of fetal mandibles, despite the microscopic evidence of mineral deposition within the crown of the dental deciduous germs. The general characteristics of mandibular ossification were as expected, since it begins from a single point of the membranous ossification related to the principal regional nerve and the Merckel cartilage. With respect to the absent fusion of the mandibular symphysis, it is reported that it happens only after one year of birth (Tsujigiwa *et al*.; Yoshizaki *et al*.).

Evaluation of the histochemical results showed that the suggestive areas of mineralized dental tissue observed in the H&E stained sections did not react with the silver employed in the von Kossa technique. This fact may be justified because the dental germs were in the initial stage of early crown formation (bell stage), where, according to Arana-Chavez & Arana-Chavez, the dentin and enamel matrixes are minimally mineralized (about 15%) and, thus, they were not able to capture sufficient amounts of silver in the teeth germs necessary to be visualized on light-microscopy.

Sunderland *et al*. (1987) also performed a histological study of the initial mineralization in human fetuses. In this study, serial sections of the jaws of 121 fetuses ranging from 10 to 26 weeks post-menstrual age first showed mineralized dentine in the deciduous central incisor at 15-19 weeks, in the lateral incisor at 16-21 weeks, in the canine at 19-22 weeks, in the deciduous first molar at 16-19 weeks and in the second molar at 20-22 weeks. Similarly, these authors found that the age ranges differ from those for initial mineralization common in textbooks, particularly for the molars. However, our specimens were low-weight fetuses.

Despite this paper supporting the importance of maintenance of a satisfactory nutritional status during fetal development to prevent trouble in the dental formation, further research is necessary about this subject, and it would also be interesting to research the relationship between nutritional imbalances and other factors that may interfere in dental development such as genetic disorders and infectious diseases.

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RESUMEN: La calcificación del diente comienza durante la vida intrauterina y se relaciona con el estado nutricional del feto. El propósito de este estudio fue evaluar el estado de calcificación dental de 15 fetos humanos de bajo peso entre las 14 y 28 semanas de vida intrauterina mediante análisis radiográficos y técnica histoquímica von Kossa, específica para la detección de calcio y otras sales minerales en los tejidos. Después de su disección, las mandíbulas fueron sometidas a radiografías laterales y oclusales. Secciones de 3 mm de espesor de las mandibulas fueron teñidas con la técnica Von Kossa. El análisis radiográfico no mostró ninguna imagen sugerente de mineralización dentaria, aunque rudimentos de la osificación mandibular podrían tenerse en cuenta. Las secciones de H-E revelaron la presencia de los gérmenes dentarios en las fases avanzadas de odontogénesis, pero con un retraso relativo en el desarrollo normal. La tinción de von Kossa no mostró positividad a la deposición de sales minerales en gérmenes dentarios, lo que apoya nuestros hallazgos relacionados con el retraso de desarrollo dental que se encontró en la muestra estudiada. Se sugiere que existe relación entre el bajo peso del feto y el estado de calcificación y maduración de los dientes.

PALABRAS CLAVE: odontogénesis, calcificación, desequilibrios nutricionales y de bajo peso, von Kossa.

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