ARTICLE ORIGINAL / ORIGINAL ARTICLE

CALCIUM LEVEL IN THE FIRST DECIDUOUS MOLAR CROWN IN RELATION TO FEEDING MODE DURING EARLY CHILDHOOD

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Marlène H. KHOURY FREIHA[1], Jean-Marc MEYER[4], Maha H. DAOU[1], Nada FARHAT MCHAYLEH[2]
Carol M. SUKHN[5], Edgard NEHME[3]

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ABSTRACT • Teeth mineralization is an essential factor in preventing dental decay as calcium is the most important mineral in the hard structure of the teeth. In the first six months after birth, calcium is provided to infant mostly via milk. The first deciduous mandibular molar crown is developed roughly during this suckling period and can be considered as one of many calcium intake indicators.

This study is the first in the world of this kind to measure the calcium levels in the first deciduous molar crown in a group of 9- to 12-year-old children at the time of their natural exfoliation, living in Lebanon over the last decade, attending pediatric dentist clinics in Beirut and who were either breast- or formula-fed. Children with mixed feeding mode were eliminated from the study. Only children fed either feeding mode in the first six months of their lives were included. Calcium levels (g/kg) in molars were determined by ICP/MS following a microwave acid digestion.

The mean calcium level of the first deciduous molar in our study was 358 ± 150 g/kg. Comparing the two groups, it was 370 ± 166 g/kg in the breastfed children and 347 ± 136 g/kg in the formula-fed children. There was no significant statistical difference between the two modes (p = 0.580) in terms of teeth calcium content. No significant difference was found between girls and boys (p = 0.440).

Keywords : calcium, teeth mineralization, first deciduous molar crown, breast-feeding, infant formula-feeding

Khoury Freiha MH, Meyer J.-M. Daou MH, Farhat Mchayleh N, Sukhn CM, Nehme E. Le taux de calcium dans la couronne de la première molaire temporaire en relation avec le mode d'alimentation durant la petite enfance. J Med Liban 2017 ; 65 (2) : 96-100.

RÉSUMÉ • La minéralisation des dents est un facteur essentiel dans la prévention de la carie dentaire, le calcium (Ca) étant le minéral le plus important dans la structure des tissus durs de la dent. Dans les six premiers mois après la naissance, le Ca est fourni à l'enfant la plupart du temps par le lait. La couronne de la 1re molaire temporaire mandibulaire se développe au cours de cette période et peut être considérée comme l'un des nombreux indicateurs d'admission de Ca. Cette étude est la première au monde à mesurer le taux de Ca dans la couronne de la 1re molaire temporaire mandibulaire d'un groupe d'enfants de 9 à 12 ans, au moment de l'exfoliation naturelle, vivant au Liban au cours de la dernière décennie, qui se présentent aux cabinets de dentisterie pédiatrique à Beyrouth et qui ont eu un allaitement exclusif, soit maternel, soit aux laits infantiles dans les six premiers mois de leur vie. Les enfants ayant eu un allaitement mixte ont été éliminés. Les taux de Ca (g/kg) dans les molaires ont été déterminés par ICP/MS après digestion par acide aux micro-ondes.

Le taux moyen de Ca de la 1re molaire temporaire dans notre étude était de 358 ± 150 g/kg ; de 370 ± 166 g/kg chez les enfants qui ont eu un allaitement maternel et de 347 ± 136 g/kg chez ceux nourris aux laits infantiles. Il n'y avait pas de différence statistiquement significative entre les deux modes (p = 0,580) en termes de contenu de Ca dans les dents. Aucune différence significative n'a été trouvée entre les filles et les garçons.

Mots-clés : calcium, minéralisation dentaire, couronne de la première molaire temporaire, allaitement maternel, allaitement aux laits infantiles

INTRODUCTION

The best mode to provide calcium to infants and to establish dietary calcium requirements has always been hotly debated in the international scientific community (WHO). In fact, infant formula- and breast-feeding are the two modes of infants feeding [1]. There is a general consensus stating that 55 to 60% of the calcium from human milk is well absorbed (Abrams *et al*., 1997) [2]. Fomon indicated, in 1993, that calcium absorption efficiency is about 60% with human milk and 40% with infant formulas during the first four months of life [3], other studies show that calcium absorption is highest in doses ≤ 500 mg Ca/dm^3 milk [4]. Based on the work of J.G. Dorea in 1999 who compiled the work of 169 authors, it was established that the calcium content in mothers' milk varied from 84 mg/dm^3 to 462 mg/dm^3 (with an average of 252 mg/dm^3) [5]. Infant formula milk contains twice this average calcium concentration and this is due to the lower bioavailability of calcium from infant formula relatively to or in comparison with mothers' milk [6]. So theoretically, formula-fed infants receive more calcium than breast-fed infants. The calcium content in formula milk ranges from 50 mg/kcal to 140 mg/kcal equivalent to 300 mg/dm^3 and 800 mg/dm^3 as per FAO-WHO and ESPGHAN Committee recommendations [7].

Departments of [1]Pediatric Dentistry,[2]Pediatric Dentistry and Community, [3]Diagnosis and Oral Medicine, Faculty of Dental Medicine, Saint-Joseph University, Beirut, Lebanon; [4]School of Dentistry, University of Geneva, Switzerland; [5]Environment Core Laboratory, Faculty of Medicine, American University of Beirut, Lebanon.
*Corresponding author: *Marlène Khoury Freiha, DDS*. e-mail: marlene.khouryfreiha@usj.edu.lb

Despite this fact, in the breast milk, both calcium and phosphorus amounts increase during lactation, and neither element is affected by the calcium and phosphorus levels of the mother's serum [5]. On the other hand, the oligosaccharides and PTHRP (parathyroid-hormone-related protein) breast milk contains increase the efficiency of calcium absorption and calcium metabolism in children [8,9].

While the average calcium concentration values in permanent teeth are known to be around 37.5% [10], the literature lacks studies about calcium levels in deciduous molar crowns in children fed either mode.

When comparing the dental enamel microstructure and mineral composition of the permanent and deciduous teeth, the primary enamel structure showed a thinner density and lower calcium and phosphorus levels [11].

For the deciduous teeth, studies in the literature concentrated on measuring calcium in different components of hard structure of the crown (enamel or dentin) [12-14]; however, none has been done to measure the calcium level in the crown itself (enamel, dentin and pulp) and attempt to link this level to the feeding mode during early childhood. Previous studies have shown that malnutrition affects tooth mineralization leading an increased rate of dental caries later in life [15]. However, little is known about the effects of children dietary intake during the suckling period on the structure of deciduous molar crowns. Hypothetically, children's higher calcium intake during the suckling period might influence tooth mineralization, enhance enamel resistance to acidic environment and reduce the risk of development of decay and dental erosion, an international problem that affects children's oral health [16,17].

This applies particularly to Lebanon where the decay level is the highest in the whole Middle East [18-21]. Greater knowledge is needed to reduce the incidence of dental caries, greater knowledge is needed not only of the deleterious effects of malnutrition [22-24], but also of the beneficial effects on dental health of appropriate dietary intake during dental development .

Consequently, we conducted this study to examine the influence of breast-feeding or infant formula-feeding on the dental mineralization in the first six months of the infant's life. The aim of this study was to compare the amount of calcium in the first deciduous molar crown according to the feeding mode in a group of Lebanese children.

MATERIAL AND METHODS:

Study population

Fifty-four children 9- to 12-year-old living in Lebanon over the last decade and attending pediatric dental clinics were enrolled in this study. Participants, recruited in Beirut and its suburbs, came from all over Lebanon. Data about feeding modes from birth to 6 months of age was collected from parents. Written informed consent was obtained from the parents. The protocol of this study was submitted to the ethical research committee at Saint-Joseph University in Beirut, Lebanon, and was approved by this committee.

The recruitment conditions were: 1) children fed exclusively with either breast milk or infant formula milk for six months, with healthy mothers free of any health problems during the lactation period, and 2) having a sound exfoliated tooth. Children and mothers with chronic health problems and/or with mixed feeding mode were excluded.

ANALYSIS

Group study

Fifty-four children with first deciduous molars were divided in two groups depending on the mode of feeding in infancy: exclusive breast-feeding (25 samples) and exclusive formula-feeding (29 samples).

Qualified pediatric dentists performed the collection of the first deciduous mandibular molar crown; the tooth was rinsed with 0.1% nitric acid (HNO_3) to remove any external calcium that could have been inadvertently introduced.

Chemical analysis

The crown was placed in a zipped bag, labeled with the number of the sample and the type of tooth. Samples were sent for analysis to the Environment Core Laboratory (ISO 17025 accredited) at the American University of Beirut. A microwave digestion was undertaken on the crown after external cleaning with deionized water. The crown was dried, weighed, put in a Teflon vessel with 3 ml of nitric acid (ICP/MS grade purchased from Fisher) and 2 ml of hydrogen peroxide (Fisher). The crown of the first primary mandibular molar was analyzed for calcium level by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

The program of the ETHOS microwave was set as follows: 5 minutes to reach 200ºC, then this temperature was sustained for another 5 minutes. Once digested, the sample was then diluted to 50 ml with deionized water and run on ICP/MS [Agilent 7500 ce] equipped with a cell dynamic range. The instrument was tuned on the day of use and data were calculated using external standards from two different suppliers and one internal standard (Purchased from Agilent and Absolute Standards) and a repetition of the curve every ten samples.

The detection limit of the procedure was 0.05 mg/kg and data was reported as mg of Ca/kg of tooth.

Statistical analysis

The statistical analysis was performed using the Statistical Program for Social Sciences (SPSS for Windows, version 20, Chicago, IL, USA). Results were considered statistically significant for a p-value < 0.05. The primary outcome variable of the study was the amount of calcium level in (g/kg) in the first deciduous mandibular molars after natural exfoliation. The variable was tested for normal distribution using Kolmogorov-Smirnov test. One-way analysis of variance followed by univariate comparisons were conducted to explore significant differences in mean calcium level according to feeding modes.

RESULTS

The descriptive statistics of this study are presented in Table I. The mean calcium level of the first deciduous molar crown was 357 ± 149 g/kg. The breast-fed children crown calcium mean level was 370 ± 165 g/kg and 346 ± 136 g/kg for the formula-fed children. No significant difference was found between the two feeding modes ($p = 0.580$). No significant difference was found between girls and boys ($p = 0.440$) (Figure 1).

DISCUSSION

To the best of our knowledge, this study is the first to evaluate the calcium level in the deciduous molar crown of children in Lebanon or elsewhere in the world, taking into consideration the feeding mode and the whole hard structure of the crown. We have chosen this particular part of the tooth at this particular age because the mineralization of the first primary molar crown occurs roughly during the period of suckling (breast-feeding or formula-feeding), from birth to six months of age, while the root mineralization takes place later and the exfoliation happens in the 9 to 12 years old [25,26].

There is a conception that calcium is higher in the teeth of breast-fed children, but our study does not back up this concept. Since the aim of this retrospective study is to compare the calcium levels in the first deciduous molar crown in this age group [9 to 12 years] according to breast- and formula-feeding modes, and since no statistically significant difference was found in the crown calcium levels between the two groups, it can be deduced that although both feeding modes obviously affect the teeth mineralization in general, they do not affect the calcium levels.

Many plausible reasons may explain the lack of difference between the two modes in terms of calcium even though literature abounds with articles more in favor of breast-feeding and claims that calcium absorption is definitely higher with breast milk. These reasons can be divided as follows: calcium/phosphorus (Ca/P) ratio, vitamin D, and environmental and food pollution.

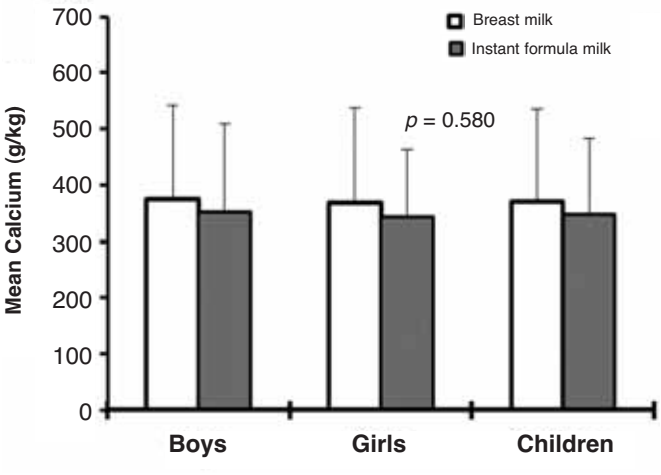

FIGURE 1

Mean calcium level and standard deviation among different groups. *The difference in calcium levels in the deciduous molar crown between breast- and formula-feeding groups, supposedly in favor of breast-feeding, is not statistically significant, p considered significant < 0.05. No significant difference either between boys and girls.*

TABLE I

MEAN AND STANDARD DEVIATION OF CALCIUM (g/kg) IN DIFFERENT GROUPS*

Gender	Groups	Number per group	Calcium Mean ± SD
Boys	Breast milk	9	373 ± 169
	Instant formula milk	14	350 ± 158
	Total	23	360 ± 159
Girls	Breast milk	16	368 ± 169
	Instant formula milk	15	343 ± 120
	Total	31	356 ± 145
Children	Breast milk	25	370 ± 165
	Instant formula milk	29	346 ± 136
	Total	54	357 ± 149

*A difference in the teeth calcium level between breast- and formula-feeding was found in favor of breast-feeding but not statistically significant. No significant difference between boys and girls ($p = 0.440$).

Calcium-phosphorus ratio

The most important factor influencing calcium absorption is the calcium/phosphorus ratio in food [7].

In view of possible inconvenient effects of unbalanced ratios between calcium and phosphorus contents in milk, the Ca/P ratio (weight/weight) should be between 1:1 and 2:1. And the 1.7:1 ratio allows for the highest absolute retention of both minerals [27]. In the literature, the average Ca/P ratio in breast milk is 1.7 [5], and currently most infant formulas ensure this ratio [27].

Vitamine D

Another factor influencing calcium absorption is vitamin D. The daily recommended intake of vitamin D during lactation was arbitrarily set at 400 IU/d (10 μg/d) [28-30].

Daily supplementation with 800 IU of vitamin D reduces the prevalence of vitamin D deficiency without showing any improvement in bone mineralization [31]. In fact a maternal intake of 4000 IU/d could achieve substantial progress toward improving both maternal and neonatal nutritional vitamin D status [32].

In Lebanon, studies show that Lebanese population present hypovitaminosis D [33]. Fuleihan *et al*. (2001) suggest that even in a sunny country, Lebanon, hypovitaminosis D is common in schoolchildren, especially girls, in those with a lower socioeconomic status and those who follow the dress code of covered head, arms, and legs [34].

Furthermore, in an international investigation among women with osteoporosis (aged 41 to 96), Lips *et al*. found in 2006 that the mean serum 25-hydroxyvitamin D, 25(OH)D level was the lowest in the Middle East (Lebanon and Turkey) [35].

The preceding findings explain why the expected higher Ca level in breast-fed infants was not found when compared to the level in formula-fed infants. It is suspected that the mothers' milk had low vitamin D level reflecting the low level in breast-feeding women's blood. Infant formula milk is fortified with vitamin D, hence formula-fed babies were able to absorb similar levels as breast-fed babies. In Lebanon, the vitamine D_3 average level in infant formulas is between 8.5 $\mu g/dm^3$ and 13 $\mu g/dm^3$ (340 IU/dm^3 and 520 IU/dm^3), the average is 10 $\mu g/dm^3$ equivalent to 400 IU /dm^3 [personal communication]; the recommended range is 6.5-16.25 $\mu g/dm^3$ [7].

Environmental and food pollution

Despite its unequivocal advantages, studies suggest that extended breast-feeding may increase the risk of mineralization defects in healthy children, possibly because environmental contaminants contained in breast milk interfere with tooth development [36]. Through breast-feeding, large amounts of polychlorinated biphenyls (PCBs), dioxins (polychlorinated dibenzo p-dioxins and dibenzofurans) and cadmium are transferred from the mother to the child [37].

We have observed a decline in the background of dioxin and PCB levels in mothers' milk in the last 20 years [38]; however, cadmium – a component of ammunitions used in wars – is still present as Lebanon has been a war zone for the last forty years. [39, 40]. Cadmium competes with calcium and might replace it because it is heavier or more competing [41,42].

Formulas are free of these substances because formula milk is manufactured according to stringent specifications as highlighted by the current ISO 22000 international standards [43].

The World Health Organization (WHO) recommends that infants should be exclusively breast-fed for 4 to 6 months; exclusive breast-feeding for around 6 months is a desirable goal [44,45] but partial breast-feeding as well as breast-feeding for shorter periods of time are also valuable, as mothers' milk might contain more defensive elements for the baby which may be lacking in a formula. However, regarding calcium absorption, with the limitations of this study, we can state that both feeding modes are no different as far as the dental mineralization is concerned. In cases where breast-feeding is unmanageable, this result may reassure the mothers for the quality of teeth mineralization of their future babies.

This is a pilot study and further studies on a larger sample will be of benefit to confirm our results about the importance of the breast-feeding mode on teeth calcium levels.

CONCLUSIONS

To the best of our knowledge, this study is the first to evaluate the calcium levels in the crown teeth of children, taking into account the feeding mode during early childhood. The lack of significant differences between the two feeding modes is better explained by a balanced formula milk which can compete in terms of calcium content present in breast milk. This is achieved because the infant formula milk is respecting Ca/P ratios, is fortified with vitamins, especially vitamin D, and is almost free of any metals such as cadmium and lead which usually compete with calcium. Therefore, either feeding mode is not believed to affect the teeth mineralization in terms of teeth calcium content. However, due to the high rate of vitamin D deficiency during lactation, we should consider monitoring lactating mothers vitamin D status [30]. It is important to establish screening and supplementation guidelines based on observed regional trends, habits and risk factors, in addition to considerations based on global recommendations [46]. Breast-fed infants should receive daily vitamin D supplementation unrelatedly of maternal vitamin D status [47].

Further research must be undertaken to find if correlations exist between a dental mineralization or dental caries and hypovitaminosis D and between suckling (feeding mode) and MIH (molar incisive hypomineralisation), a dental problem on the rise worldwide .

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