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ARTICLE ORIGINAL/ORIGINAL ARTICLE

IMPACT of BREAST vs. FORMULA FEEDING MODE on PRIMARY TEETH LEAD LEVEL

Retrospective Clinical Study and Literature Review

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Khoury Freiha MH, Daou MH, El Osta N, Sukhn CM, Nehme E. Impact of breast vs. formula feeding mode on primary teeth lead level. Retrospective clinical study and literature review. J Med Liban 2014 ; 62 (1) : 14-21.

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ABSTRACT : Lead exposure is a global environmental problem inducing lifelong adverse health effects. Children's exposure to lead, from birth to 6 months, is typically dominated by dietary sources. The first primary mandibular molar could provide an integrated record of lead exposure. The aim of this study is to detect the level of lead in the first primary molar of a group of Lebanese children born between 1990 and 2000, living in Beirut and suburbs, and to compare the amount of lead according to gender and feeding mode.

MATERIALS AND METHODS : A group of 53 first prima- ry molars belonging to 53 Lebanese children (31 girls and 22 boys), and aged 9 to 12 years old, were included in the study. The primary outcome variable of the study was the amount of lead level (mg/kg) in the first prima- ry mandibular molars taken at the time of the natural exfoliation and measured by ICP/MS (Induc-tively Coupled Plasma Mass Spectrometry). Children includ- ed in the study were fed exclusively with formula feed- ing or maternal feeding for six months.

RESULTS : The mean lead level of the first primary molar was 0.678 mg/kg ± 0.717. The mean lead was significantly higher among children fed with instant formula milk (0.876 ± 0.802) than breastfed children (0.455 ± 0.540), (*p* = 0.044). No significant difference was found between girls (0.595 ± 0.548) and boys (0.794 ± 0.905), (*p* = 0.440).

CONCLUSION : Within the limitations of this study, we found a statistically significant difference in the teeth lead level between breastfeeding and formula feeding, in favor of breastfeeding. This difference proves once more the importance of breastfeeding ; on the other hand, the Lebanese children accumulated lead in their teeth to levels below the international levels.

Keywords : lead level, bioindicator, ICP/MS, first primary molar, breastfeeding, formula feeding.

RÉSUMÉ : L'exposition au plomb est un problème environnemental global, induisant des effets indésirables sur la santé. De 0 à 6 mois, l'exposition des enfants au plomb est généralement due à leur alimentation. La 1re molaire mandibulaire lactéale pourrait être un indicateur de l'exposition au plomb. L'objectif de cette étude est de déterminer le taux de plomb dans la 1re molaire mandibulaire lactéale d'un groupe d'enfants libanais nés entre 1990 et 2000, vivant à Beyrouth et dans les banlieues, et de le comparer selon le sexe et le mode d'allaitement.

MATÉRIEL ET MÉTHODE : Cinquante-trois 1res molaires lactéales appartenant à 53 enfants libanais, 31 filles et 22 garçons âgés de 9 à 12 ans, ont été inclues dans l'étude. La variable principale de l'étude était la teneur en plomb (mg/kg) dans les 1res molaires mandibulaires lactéales récupérées au moment de l'exfoliation natu- relle et mesurée par ICP/MS, (Spectrométrie de masse à plasma couplé par induction). Les enfants inclus dans l'étude ont été nourris exclusivement avec du lait infantile ou par allaitement maternel pendant 6 mois.

RÉSULTATS : La moyenne du taux de plomb dans la 1re molaire lactéale est de 0,678 mg/kg ± 0,717. Elle est significativement plus élevée chez les enfants nourris au lait infantile (0,876 ± 0,802) que chez ceux nourris au lait maternel (0,455 ± 0,540), (*p* = 0,044). Aucune différence significative n'a été trouvée entre les garçons (0,794 ± 0,905) et les filles (0,595 ± 0,548) (*p* = 0,440).

CONCLUSION : Dans les limites de notre étude, une dif- férence statistiquement significative du taux de plomb dans les dents a été trouvée entre l'allaitement maternel et l'allaitement au lait infantile en faveur de l'allaitement maternel, prouvant une fois de plus son importance; par contre il s'est avéré que les enfants libanais accumulent du plomb dans les 1res molaires lactéales à un niveau infé- rieur aux niveaux internationaux.

Mots-clés: plomb, bioindicateur, ICP/MS, première molaire lactéale, allaitement maternel, lait infantile.

INTRODUCTION

Lead is a heavy metal that exists in relatively small quantities in the earth's crust with no biological value [1]. In the last few decades, human activities and extensive use of lead in industry have resulted in its redistri-bution in the environment leading to a contamination of air, water, and food which eventually led to a significant rise in lead concentration in human body organs [2]. Thereafter, lead exposure became a global environmental problem inducing lifelong adverse health effects damaging the nervous, hematopoietic, renal, endocrine,

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reproductive and skeletal systems [3]. The environmental lead exposure is associated with an increased prevalence of dental caries [4-5]. Children are more susceptible to lead exposure than adults because they have greater gastrointestinal absorption and less effective renal excretion in addition to other different behaviors. Children, with elevated levels of lead in their blood, are at risk of learning and behavioral problems, reduced intelligence and other serious health effects even at concentrations below 10 µg/dl, the limit globally recommended for children (EPA, 1997) [6-7].

In Lebanon, research over the last two decades has detected lead in air, water and food [8-10]. Outdoor lead pollution stemmed from the use of leaded gasoline and from years of war where fire ammunition containing high amount of lead was introduced. Before the phase-out of lead in gasoline, it was believed that inhalation was the predominant route of exposure to the Lebanese general public [10]. Indoor pollution was mainly caused by lead household paints which were phased out after 1980. In houses built before, paint crumbles can mix with house dust and soil, where they may be unwittingly ingested or inhaled by young children [11].

Besides the indoor lead pollution, the infant's exposure to lead, from birth to six months, is typically dominated by dietary sources [12]. The two main dietary sources of milk for infant are either a formula or breast milk. In the past, lead intake in children was thought to be influenced by storage of infant formulas in lead-soldered cans [13]. New regulations were set in mid 80s to correct this situation [14]. As for breast milk, there are indications of higher bioavailability of lead from human milk than from infant formula, where similar blood levels were found in breastfed infants as in bottle-fed, although the dietary intake of lead was much lower in breastfed infants [15]. As for the lead intake from water, it cannot be completely overruled since the water network system in Beirut and suburbs is at least sixty years old and has not undergone any real maintenance during the war. It is suspected that lead has been leaching from the water network system due to the presence of lead pipes in old homes [16].

Most studies among children have used blood-lead (BPb) levels as a marker of exposure; but lead in the blood has a short half-life of 30 days and reflects recent exposure. Consequently, other matrices such as hair, teeth and bones which are known to store metals for a long time should be considered to evaluate the exposure in children and to estimate their total body lead burden.

Around 95 percent of the lead is found in bone tissues; it can be stored for more than 30 years and subsequently be released to other tissues or organs [17-18].

Nevertheless, teeth accumulate lead over a longer period of time and provide an integrated record of lead exposure from intrauterine life until the teeth are exfoliated [19]. The development of the first mandibular primary molar usually occurs between the fifth intrauterine month and six months of extra-uterine life [20]. The crowns of the other teeth complete their development before that date [21]. Moreover, unlike in bone, there is no turnover of apatite in teeth [22]. For that reason, the dental hard tissues are relatively stable and metals deposited in teeth during development are retained [18]. It has been also noted that the lead burden is more pronounced in children than in adults and higher lead levels have been reported in primary teeth than in permanent teeth [23]. Consequently, the accumulation of lead during the tooth development could serve as bioindicator to identify the long-term lead exposure of the child [24-25].

To our knowledge, no publication concerning the lead level in the teeth of Lebanese children population has been conducted. It's thus vital to detect the lead level in order to help the Lebanese health authorities update the health regulations pertaining to children protection, to assess and classify the level of the uptake of Lebanese children, relatively to children in other countries, for health bill projections.

The objectives of this study were to detect the lead level in the first primary molar of a group of Lebanese children living in Beirut and suburbs, to compare the mean amount of lead according to gender and according to feeding mode. In order to compare the results of our study with the results from other countries, an extensive literature review has been conducted and lead levels in teeth reported from around the world from 1989 to 2012 have been summarized in a table for comparison.

MATERIALS AND METHODS

Study population

During the period 2000 till 2008, Lebanese children within the age group of 9 to12 years living in Beirut and suburbs, and presenting one healthy first primary mandibular molar nearing exfoliation were asked to participate in the study.

Participants were recruited from the Department of Pediatric Dentistry at the Faculty of Dentistry at Saint-Joseph University of Beirut and four private pediatric dental clinics in Beirut city and suburbs.

Data about feeding modes was also collected from the parents since the crown mineralization of the first primary molar occurs in part during the period of suckling (breastfeeding or formula feeding) from birth to six months of age. The socioeconomic level of infants and children was wide-ranging, from low to high.

The protocol of this study was submitted to the ethical research committee at Saint-Joseph University of Beirut, Lebanon. Written informed consent was obtained from the parents.

METHODS

Fifty-three children and 53 first primary molar were included in the study. The teeth were divided in two groups depending on the mode of feeding: exclusive breastfeeding or exclusive formula feeding.

TABLE I
MEAN LEVEL LEAD* in the FIRST PRIMARY MOLAR in BOYS & GIRLS ACCORDING to BREAST FEEDING or FORMULA MILK

	Groups	N	Mean	SD
Boys	Breast milk	9	0.543	0.675
	Instant formula milk	13	0.969	1.025
	Total	**22**	**0.794**	**0.905**
Girls	Breast milk	16	0.406	0.466
	Instant formula milk	15	0.797	0.571
	Total	**31**	**0.595**	**0.548**
Children	Breast milk	25	0.455	0.540
	Instant formula milk	28	0.876	0.802
	Total	**53**	**0.678**	**0.717**

*In mg/kg **SD:** standard deviation

Four qualified pediatric dentists performed the extraction or collection of the first primary mandibular molar. The anesthetic product used was Mepivacaine 2% special with adrenaline 1/100000 (Scandicaine special Septodont®). All instruments used during extraction were stainless steel (Hu-Friedy®). After exfoliation or extraction, the tooth was rinsed with 0.1% nitric acid HNO_3 followed by a rinse with deionized water to avoid contamination of the sample by extrinsic lead. The extracted tooth was placed in a zipped bag, labeled with the number of the sample and type of the tooth. Samples were sent directly for analysis to the Environment Core Laboratory (ISO 17025 accredited) at the American University of Beirut. A microwave digestion was undertaken on the whole tooth after external cleaning with deionized water. The tooth 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 program of the ETHOS microwave was set as follows: 5 minutes to reach 200 ºC, 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 Inductively Coupled Plasma Mass Spectrometry (ICP/MS) [Agilent 7500 ce] using a stringent quality control consisting of a blank of digestion, certified reference material with an average recovery of 87% and a spiked blank. The sample was then processed on the Ion Coupled Plasma Mass Spectrometry (ICP/MS) [Agilent ce 7500], 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. No duplicates or matrix spikes were undertaken due to the nature of the sample. Detection limit of the procedure was 0.05 mg/kg and data was reported as mg of Pb/kg of tooth.

Inclusion criteria

Children fed exclusively with breast milk or instant formula for six months.

Exclusion criteria

Were excluded from this study children with health problem.

STATISTICAL ANALYSIS

The primary outcome variable of the study was the amount of lead level in mg/kg in the first primary mandibular molars. Variable was tested for normal distribution using Kolmogorov-Smirnov test. Two-way analyses of variance, followed by univariate analyses or multiple comparisons, were conducted to explore significant difference in mean lead level according to gender and feeding modes. The alpha error was set at 0.05. The statistical analyses were performed using a software program (SPSS for Windows, version 17.0, Chicago, IL, USA).

RESULTS

Fifty-three Lebanese children (31 girls and 22 boys) were included in the study and 53 first primary molars were studied. The mean lead level of the first primary molar was 0.678 mg/kg ± 0.717. The mean lead was significantly higher among children feeding with instant formula milk (*p* value: 0.044). No significant difference was found between girls and boys (*p* value: 0.440) (Table I, Figure 1).

When comparing the level of lead in this group of Lebanese children to the international level, we found that Lebanon is in the lower rank (Figure 2). The international community lead levels varied from 0.3 mg/kg to 10.2 mg/kg. More detailed information is provided in Table II.

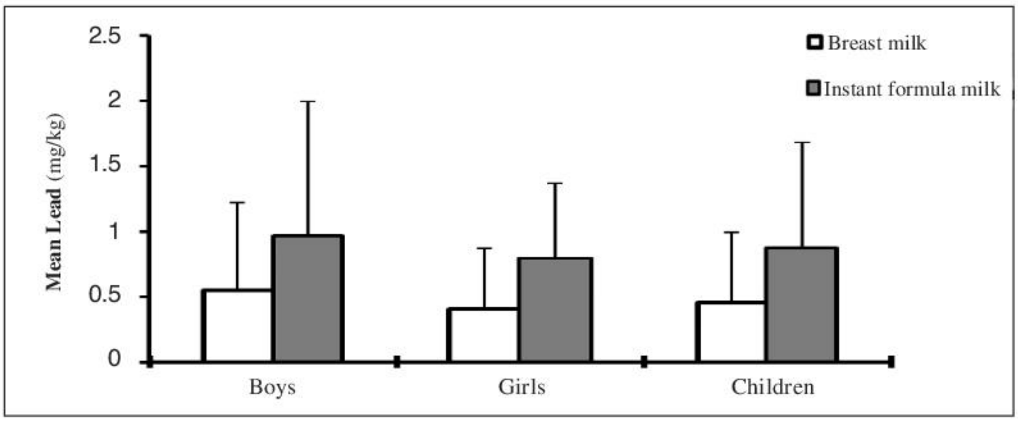

Figure 1

Mean lead and standard deviation among different groups (boys and girls) fed with instant formula or breast milk.

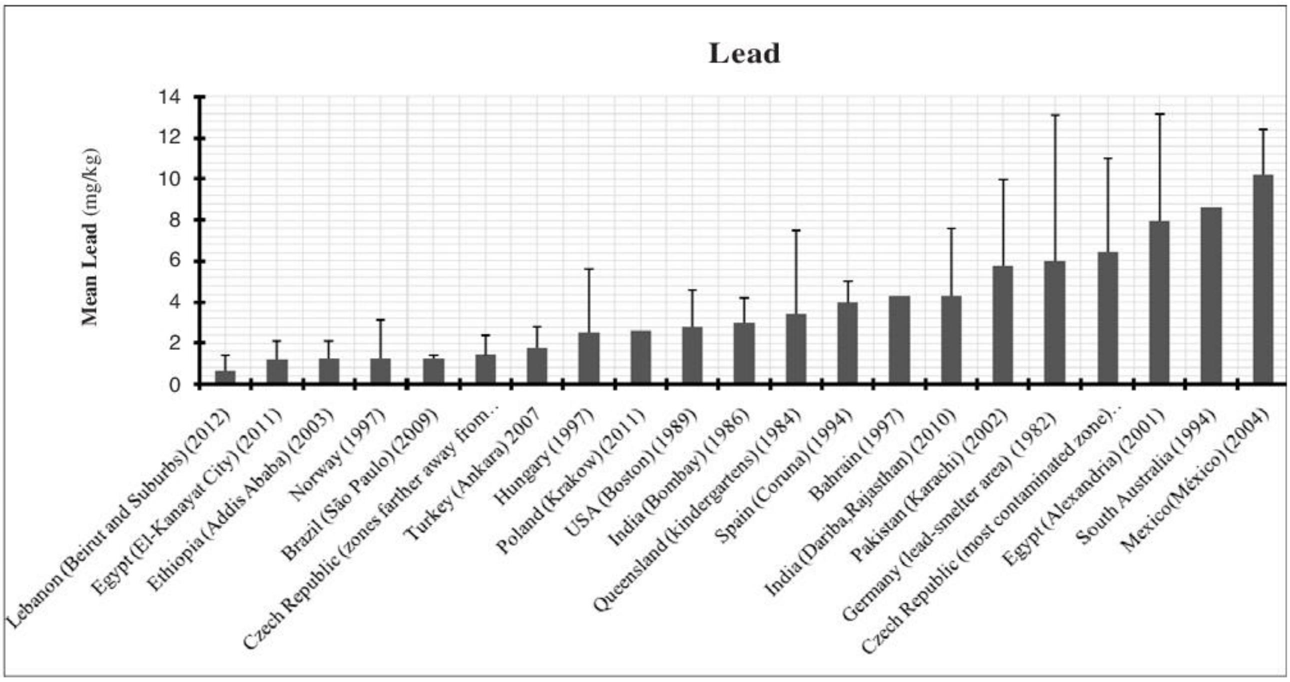

FIGURE 2. Mean lead level in teeth of the group of Lebanese children compared to lead levels in populations of other countries ranging from 0.3 mg/kg to 10.2 mg/kg

DISCUSSION

Our study found a statistically significant difference in the teeth lead level between breastfeeding and formula feeding, in favor of breastfeeding. This difference proves once more the importance of breastfeeding. It is imperative to consider the mode of feeding during the formation of the crown of the first mandibular molar, because its development usually occurs between the fifth intra-uterine month and the sixth of extra-uterine life [21] and the children would be fed with breast milk or instant formula milk prepared with bottled water. Moreover, no studies have been done in Lebanon about lead level in breast milk. In previous studies, a correlation between mother milk lead level and children teeth lead level has been detected [35]; however, in this study we could not have measured lead level in the mother breast milk because this study is considered a retrospective study. A study by Gulson [45], which studied lead concentrations in breast milk, showed that in the United Arab Emirates (UAE) breast milk has the highest lead level (70 ± 17 parts per billion). This is believed to be due to the fact that over 94% of the UAE women used kohl (lead-bearing) cosmetics, a habit which is not practiced by Lebanese women [45].

A quick glance at the results of the present study proved that there is no difference between boys and girls because gender is not possibly an influencing factor [29-30, 32]

The use of the first primary mandibular molar as a bioindicator has been reemphasized in this study. Even though the lead levels in this group of Lebanese children were lower than the rest of the international community, they were nevertheless detectable. It was observed that the highest levels of lead were measured in industrialized countries.

Lead levels in all environmental media (except soil) have since declined, and currently, the predominant route of lead intake for the general adult population is oral from food and drinking water [46-48]. This decline of air pollution does not apply however to all geographical locations around the planet especially if these studies were undertaken in relatively clean environment and not in underdeveloped countries.

In Lebanon, there are conceivably many factors which could have contributed to this low level. One of the possible reasons is that Lebanon is not classified as an industrial country. Moreover, the war which was believed to be a major source of lead was already over at the time the children were born (around 2000). Although the lead level in outdoor atmosphere in Lebanon is fourfold the international recommended level [10], this has not affected much the children uptake because in the first six months (inclusion criteria), children are more protected and spend most of their time indoors. It is also possible that since children were born after the phase out of lead from paint, the indoor pollution was also either at its lowest at the time of metal uptake or because children between 0 and 6 months do not play and interact with paint crumbles and do not mix with house dust and hence minimum intake from inhalation is considered.

Other possible sources which could have contributed to the lead uptake such as water can be probably ruled out for two reasons: Lebanese have been consuming in

TABLEAU II **COMPARISON OF TEETH LEAD LEVEL BETWEEN LEBANON AND OTHER COUNTRIES WITH THE FOLLOWING COMPONENTS:**
Country, location, age of children, type of teeth, number of children, number of samples, mean in mg/kg ± range or SD (when available), reference to the method used (when available), authors, year & ref. # in this article.

Country	City/Area/Location	Age groups (years)	Type of teeth	Children N	Samples N	Mean mg/kg ± range or SD	Authors year
Lebanon	Beirut & Suburbs	9-12	First primary mandibular molar	53	53	0.678 ± 0.717[a]	Present study (2013)
Poland	Krakow	5-7	All incisors types, whole teeth*	285	424	2.6[e] (1.2-5.9)	Barton HJ (2011) [26]
Egypt	El-Kanayat City	5-12	All types	64	NM	1.2 ± 0.89[a] (0.34-4.01)	Amr MA (2011) [27]
Ethiopia	Wonji Shoa Sugar Estate (Gr 1), Rift Valley (Gr 2) & Addis Ababa (Gr 3 traffic)	Primary schools NM	Primary incisors	Gr 1: 49 Gr 2: 51 Gr 3: 46	146	Gr 1: 0.59 ± 0.38[c] Gr 2: 0.39 ± 0.38 Gr 3: 1.27 ± 0.85	Tvinnereim HM *et al.* (2011) [28]
Brazil	Contaminated area in São Paulo City	5-10	All types whole teeth	74	74	1.28 ± 0.11[a]	Arruda-Neto JD *et al.* (2009) [24]
Turkey	Ankara (Gr 1: heavy traffic and high air pollution) & Balikesir (Gr 2: less traffic)	4-15	All types, whole teeth	263	297	Gr 1: Ankara-Centre[b] 1.30 ± 0.59 Gr 2: Balikesir-Centre 1.77 ± 1.03	Karahalil B *et al.* (2007) [29]
México	México City Metropolitan Zone	5-13	All types, whole teeth	79	79	10.2 ± 2.2[c]	Baez A *et al.* (2004) [30]
Pakistan	Nine primary schools in Karachi	Mean age: 7.6	All types, whole teeth	9 schools	309	5.78 ± 4.2[b] (0.42-39.75) Incisors: 6.42 ± 4.19 Canines: 4.91 ± 5.12 Molars: 4.50 ± 2.67	Rahman A *et al.* (2002) [31]
Egypt	Alexandria City (Gr 1 urban) & Kafr El-Sheikh Province (Gr 2 rural)	6-12	All types, whole teeth	60: Gr 1: 30 Gr 2: 30	60	Gr 1: 7.96 ± 5.20[b] Gr 2: 4.97 ± 3.77[b]	Omar M (2001) [32]
Kuwait	Industrial & suburban areas of Kuwait	Kuwaiti residents: 3-74	Whole incisor	216	NM	Boys[b] - Industrial: 2.89 (1.98-3.41) Suburban: 2.84 (2.56-3.10) Girls - Industrial: 2.69 (2.31-3.21) Suburban: 2.42 (1.65-2.92)	Bu-Olayan AH *et al.* (1999) [33]
Norway	19 counties	NM	Whole teeth	1373	2746	1.27 ± 1.87[a]	Tvinnereim HM *et al.* (1997) [34]
Bahrain	Bahrain	5-15	All types, whole teeth	269	280	4.3[b] (0.1-60.8) 35 % > 4 (concentration toxique)	Al-Mahroos F *et al.* (1997) [35]
Czech Republic	Area around the Pribram lead smelter	Elementary school children NM	All types, whole teeth	162	162	1.43[c] in zones farther away from the point source Confidence limit (1.11-1.84) 6.44 in the most contaminated zone Confidence limit (3.95-10.50)	Cikrt M *et al.* (1997) [36]
Hungary	Children's Dental Polyclinic of Miskolc: North-East Hungary, urban-industrial air pollution	4-16	Primary molars, permanent molars	312	335 deciduous, 65 permanent teeth	2.52 ± 3.1[b]	Selypes A *et al.* (1997) [37]
Germany	Rural & urban areas of western Germany (Gr 1) & eastern Germany (Gr 2)	6	Incisors, whole teeth, upper jaw only	790	790	(Gr 1) Western: 1.61 ± 1.57[b] (Gr 2) Eastern: 1.98 ± 1.72	Begerow J *et al.* (1994) [38]
Spain	Coruna	10	Incisors, molars, whole teeth	43	43	3.96 ± 1.07[b]	Gil F *et al.* (1994) [39]
Australia	Lead smelter town of Port Pirie, South Australia	7	Central upper incisor	262	262	8.6[e]	McMichael AJ *et al.* (1994) [40]
USA	Boston	6-8	Primary incisors	85	85	2.8 ± 1.8[e] / Range 0.3 to 12.8	Rabinowitz MB (1989) [41]
India	Bombay residents	1-14	Incisive, canines & molars	179	179	3.01 ± 1.22[d] / Range 0.22 to 14.4	Khandekar RN *et al.* (1986) [42]
Queensland	Children attending kindergartens	4-9	Deciduous teeth	292	292	3.4[e] 0 to 29 ± 4.1	Clegg DE *et al.* (1984) [43]
Germany	302 children living in a lead-smelter area (Gr 1) 85 children living in a non-polluted rural area (Gr 2)	6	Deciduous teeth (incisors)	387	NM	Gr 1: 6.0 ± 7.1[c] / Range: 1.49-38.5 Gr 2: 3.9 ± 4.2 / Range: 1.6-9.4	Ewers U (1982) [44]

*Whole teeth: enamel, dentine, crown and root. Analytical methods: a: ICP/MS (ICP/MS: Inductively Coupled Plasma Mass Spectrometry) b: AAS (Atomic Absorption Spectrophotometry with Electro Thermal Atomization) c: FGAAS (Graphite Furnace Atomic Absorption Spectrometry) d: DPASV (Differential Pulse Anodic Stripping Voltammetry) e: NM (Not Mentioned) the last two decades bottled water and people relying on ground water as a source could not have been subjected to any levels of lead since the lead level in groundwater sources used in Lebanon was not detectable [9]. Nevertheless, it is not adequate to rule out the lead coming from tap water since no data is available to this date (personal communications).

If water and air were to be ruled out as possible source, the food remains a major suspect which can explain the presence of lead in teeth of Lebanese children in this study. Although the lead level in the Lebanese food baskets is low [8], the mode of feeding or the intrauterine transfer cannot be overlooked. It has been established that the lead could have been taken up by the fetus while still intrauterine; in fact the umbilical cord carries not on-ly the building blocks of life, but also a steady stream of industrial chemicals, pollutants and pesticides that cross the placenta as readily as residues from cigarettes and alcohol. This is possibly the main route of entry [49].

The paucity of infant and children research in Lebanon has certainly limited our ability to correlate the sources of the lead to the teeth accumulation in Lebanese children. The fact that no formula milk or breast milk were tested between 1990 and 2000 was really an obstacle. This study could be expanded into a national database which should include more children and be truly representative.

The number of teeth in this study was limited due to the difficulties in recruitment of appropriate cases. Initially 100 teeth were considered, but given the difficulties of enlisting, this number was reduced due to the loss of patients along the way or due to the time of exfoliation: the child must be seen at the time of the natural exfoliation of the tooth; patients are often seen either before or after the tooth is exfoliated. The number was also limited due to the mode of feeding: mainly the difficulty to find children exclusively breastfed for six months.

This study supports and encourages the initiation and sustaining of exclusive breastfeeding based on the American Academy of Pediatrics guidelines endorsed by WHO/UNICEF, as breastfeeding and human milk are the normative standards for infant feeding and medical nutrition and because contraindications of breastfeeding are rare [50-51].

This study prompts the authorities and policymakers to use the data to start developing guidelines and strategies in environmental public health related to children. An effective implementation of governmental policies can help prevent lead accumulation in children and to identify patients at risk for preventive and curative dental care. For instance, to predict if the current lead level has affected the number of caries, studies around the world attempted to link the lead to caries incidence [4-5]. Of course, in Lebanon this study probably cannot be used to correlate the high amounts of decay with the lead level detected in children [52-53].

The number of Lebanese children considered as high caries risk patients and the relation with lead level and caries support the breastfeeding till 12 months or longer to reduce caries risk. Moreover, it will be interesting to find a correlation between lead level and HMI (hypomineralization molar incisor) in a future retrospective research.

CONCLUSIONS

Frist primary molar can be used as bioindicator of lead uptake and accumulation.

Lebanese population accumulated lead in the first primary molar.

The mean lead level in teeth of this group of Lebanese children is 0.678 mg/kg ± 0.717 (SD) which is well below internationally reported levels.

This study is the first to evaluate the lead level in teeth of children in Lebanon taking into account the mode of feeding. The mean lead level was significantly higher among children feeding on instant formula milk (p value: 0.044). No significant difference was found between girls and boys (p value: 0.440).

A national study and lead screening must be undertaken to cover all the country as well as more dental prevention must be considered for high-risk children in lead intoxication. A standard protocol to identify heavy metals in teeth is required [54].

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