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 primary 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 primary mandibular molars taken at the time of the natural exfoliation and measured by ICP/MS (Inductively Coupled Plasma Mass Spectrometry). Children included in the study were fed exclusively with formula feeding 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.

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 redistribution 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,
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 to 12 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.
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 HNO₃ 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](image_url)

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

![Figure 2](image-url)
<table>
<thead>
<tr>
<th>Country</th>
<th>City/Area/Location</th>
<th>Age groups (years)</th>
<th>Type of teeth</th>
<th>Children N</th>
<th>Samples N</th>
<th>Mean mg/kg ± range or SD</th>
<th>Authors/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lebanon</td>
<td>Beirut &amp; Suburbs</td>
<td>9-12</td>
<td>First primary mandibular molar</td>
<td>53</td>
<td>53</td>
<td>0.678 ± 0.717</td>
<td>Present study (2013)</td>
</tr>
<tr>
<td>Poland</td>
<td>Krakow</td>
<td>5-7</td>
<td>All incisors types, whole teeth*</td>
<td>285</td>
<td>424</td>
<td>2.6 ± (1.2-5.9)</td>
<td>Barton HJ (2011) [26]</td>
</tr>
<tr>
<td>Egypt</td>
<td>El-Hanayyat City</td>
<td>5-12</td>
<td>All types</td>
<td>64</td>
<td>NM</td>
<td>1.2 ± 0.86* (0.34-4.01)</td>
<td>Amr MA (2011) [27]</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>Wonji Shoa Sugar Estate (Gr 1), Rift Valley (Gr 2) &amp; Addis Ababa (Gr 3 traffic)</td>
<td>Primary schools NM</td>
<td>Primary incisors</td>
<td>Gr 1: 49</td>
<td>146</td>
<td>Gr 1: 0.59 ± 0.38*</td>
<td>Túrvinereim HM et al. (2011) [28]</td>
</tr>
<tr>
<td>Brazil</td>
<td>Contaminated area in São Paulo City</td>
<td>5-10</td>
<td>All types whole teeth</td>
<td>74</td>
<td>74</td>
<td>1.26 ± 0.11*</td>
<td>Arruda-Neto JD et al. (2009) [24]</td>
</tr>
<tr>
<td>Turkey</td>
<td>Ankara (Gr 1: heavy traffic and high air pollution) &amp; Balikesir (Gr 2: less traffic)</td>
<td>4-15</td>
<td>All types whole teeth</td>
<td>263</td>
<td>297</td>
<td>1.36 ± 0.59</td>
<td>Karahalil B et al. (2007) [29]</td>
</tr>
<tr>
<td>México</td>
<td>Mexico City Metropolitan Zone</td>
<td>5-13</td>
<td>All types, whole teeth</td>
<td>79</td>
<td>79</td>
<td>10.2 ± 2.2*</td>
<td>Baez A et al. (2004) [30]</td>
</tr>
<tr>
<td>Pakistan</td>
<td>Nine primary schools in Karachi</td>
<td>Mean age: 7.6</td>
<td>All types, whole teeth</td>
<td>9 schools</td>
<td>309</td>
<td>5.78 ± 4.2* (0.42-39.75)</td>
<td>Rahman A et al. (2002) [31]</td>
</tr>
<tr>
<td>Egypt</td>
<td>Alexandria City (Gr 1 urban) &amp; Kafr El-Sheikh Province (Gr 2 rural)</td>
<td>6-12</td>
<td>All types, whole teeth</td>
<td>80; Gr 1: 30</td>
<td>60</td>
<td>Gr 1: 7.96 ± 5.20*</td>
<td>Omar M (2001) [32]</td>
</tr>
<tr>
<td>Kuwait</td>
<td>Industrial &amp; suburban areas of Kuwait</td>
<td>Kuwait residents: 3-74</td>
<td>Whole incisor</td>
<td>216</td>
<td>NM</td>
<td>Boys*: Industrial: 2.86 (1.98-3.41)</td>
<td>Bu-Olayan AH et al. (1999) [33]</td>
</tr>
<tr>
<td>Norway</td>
<td>19 counties</td>
<td>NM</td>
<td>Whole teeth</td>
<td>1373</td>
<td>2765</td>
<td>1.27 ± 1.87*</td>
<td>Túrvinereim HM et al. (1997) [34]</td>
</tr>
<tr>
<td>Bahrain</td>
<td>Bahrain</td>
<td>5-15</td>
<td>All types, whole teeth</td>
<td>269</td>
<td>280</td>
<td>4.3* (0.1-60.8)</td>
<td>Al-Mahroos F et al. (1997) [35]</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>Around the Pribram lead smelter</td>
<td>Elementary school children, NM</td>
<td>All types, whole teeth</td>
<td>162</td>
<td>162</td>
<td>1.43* in zones farther away from the point source</td>
<td>Cikrt M et al. (1997) [36]</td>
</tr>
<tr>
<td>Hungary</td>
<td>Children’s Dental Polyclinic of Miskolc: North-East Hungary, urban-industrial air pollution</td>
<td>4-18</td>
<td>Primary molars, permanent molars</td>
<td>312</td>
<td>335</td>
<td>2.52 ± 3.1*</td>
<td>Selupers A et al. (1997) [37]</td>
</tr>
<tr>
<td>Germany</td>
<td>Rural &amp; urban areas of western Germany (Gr 1) &amp; eastern Germany (Gr 2)</td>
<td>6</td>
<td>Incisors, whole teeth, upper jaw only</td>
<td>790</td>
<td>790</td>
<td>(Gr 1) Western: 1.61 ± 1.57*</td>
<td>Begerow J et al. (1994) [38]</td>
</tr>
<tr>
<td>Spain</td>
<td>Coruna</td>
<td>10</td>
<td>Incisors, molars</td>
<td>43</td>
<td>43</td>
<td>3.96 ± 1.07*</td>
<td>Gil F et al. (1994) [39]</td>
</tr>
<tr>
<td>Austria</td>
<td>Lead smelter town of Por: Pirie, South Australia</td>
<td>7</td>
<td>Central upper incisor</td>
<td>262</td>
<td>262</td>
<td>8.6*</td>
<td>McMichael AJ et al. (1994) [40]</td>
</tr>
<tr>
<td>USA</td>
<td>Boston</td>
<td>6-8</td>
<td>Primary incisors</td>
<td>85</td>
<td>85</td>
<td>2.8 ± 1.8* / Range 0.3 to 12.8</td>
<td>Rabinowitz MB (1989) [41]</td>
</tr>
<tr>
<td>India</td>
<td>Bombay residents</td>
<td>1-14</td>
<td>Incisive, canines &amp; molars</td>
<td>179</td>
<td>179</td>
<td>3.01 ± 1.22* / Range 2.24 to 14.4</td>
<td>Khandaker RN et al. (1986) [42]</td>
</tr>
<tr>
<td>Queensland</td>
<td>Children attending kindergartens</td>
<td>4-9</td>
<td>Deciduous teeth</td>
<td>292</td>
<td>292</td>
<td>3.4*</td>
<td>Clegg DE et al. (1994) [43]</td>
</tr>
<tr>
<td>Germany</td>
<td>302 children living in a lead-smelter area (Gr 1)</td>
<td>85 children living in a non-polluted rural area (Gr 2)</td>
<td>Deciduous teeth (incisors)</td>
<td>387</td>
<td>NM</td>
<td>Gr 1: 6.0 ± 7.1*, Range: 1.49-38.5</td>
<td>Ewers U (1982) [44]</td>
</tr>
</tbody>
</table>

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 has been 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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