DECIDUOUS TEETH AND BLOOD LEAD LEVELS IN CHILDREN OF BASRAH CITY

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ABSTRACT
To determine lead exposure among children of Basrah a total of 100 deciduous whole teeth were collected from 100 children. Teeth and blood were analyzed for lead concentrations using atomic absorption spectrophotometry and ALAD activity by colorimeter. Children were between 5 and 15 years old (56 males and 44 females). The study period extended from January 2005 till the end of May 2006. Laboratory investigations included measuring teeth and blood lead levels and ALAD activity, A questionnaire was used to gather information on each person, such as age, sex, place where the person lives, the mean blood lead level (BLL), deciduous teeth lead level (DLL) and aminolevulinic acid dehydratase (ALAD) were 11.42±2.7µg /dl, 7.6±3.93 μg/g and 202±19.74 U/ml RBC respectively. BLL, DLL ALAD activity was significantly different according to the age, sex and urban areas. There was a significant correlation (P<0.01) between all parameters (age, BLL and DLL and ALAD) which is included in this study. In conclusion, the levels of lead in deciduous teeth and blood serves as a dosimeter for lead exposure which originates from different sources, also there were high correlation between BLL and DLL and ALAD.

INTRODUCTION
Lead, which represents a non-essential trace element, presents in living organism in limited amounts. It is one of the most important and widely distributed pollutants in the environment. It has serious problems in poisoning due to excessive use of lead in industry and automobile fuel. Environmental lead exposure is a public health problem on global level. Lead gets into the body through food, water and air. Environmental pollution, agricultural technology and food processing are the main sources of exposure. Absorption and retention in the body depends on age, chemical environment of the gastro-intestinal tract and nutritional status of the individual. The mean biological life of lead in blood is about 30 days, after which majority (70-95%) of it is accumulated within the long-lived compartments of bones, with the elimination half-life of 5-10 years. Measurement of blood lead levels is the most accurate method of determining the actual current exposure in individuals and populations. Any blood lead level above the designated threshold is recognized as a cause of concern. For children, the threshold is 10 µg /dl. For adults, the threshold considered is 25 µg /dl because many researchers believe that adverse effects begin to occur in adults at least at this level (If not lower levels). Lead levels in deciduous, permanent and dental caries have given great attention to investigate recent and past exposure to lead. Levels reported ranged from less than 1 to 60 µg pb/g tooth for different communities in the world with lower levels in deciduous teeth and it is increased steadily with age. Lead easily crosses the placental barrier and accumulate in calcified tissue such as teeth. Formation of the enamel of deciduous incisors starts three month after conception and ends about one month after birth. Blood flow through the enamel is terminated after its formation, so deciduous tooth enamel considers to be a suitable medium for the assessments of lead exposure. Lead is powerful inhibitor of enzymes containing sulphohydryl (SH) group and a number of these enzymes are concern with heme synthesis. Aminolevulinic acid dehydratase (ALAD) is the second enzymes in the heme biosynthesis pathway and is one of the most sensitive indicators of lead accumulation due to exposure, that is responsible for the conversion of ALA into porphobilinogen. The immediate effect of the inhibition of (ALAD) due to lead exposure is an increased (ALA) in plasma and blood, which then lead to increased urinary of (ALA). The objective of this work was to study deciduous tooth, blood lead levels and ALAD activity in Basrah children and to relate lead levels to sociodemography and environmental factors.

MATERIALS AND METHODS
The present study was conducted during the period from January 2005 till the end of May 2006 in which one hundred children deciduous tooth were collected from Basrah. They were
attending a dentist clinic for treatments. Their ages ranged between 4 to 15 years. There were 56 males and 44 females. A questionnaire form was set for each individual, including age, sex, address, social class, educational level, exposure to lead from gasoline automobile, and residency (urban or rural).

**Sampling and analysis:**
Blood sample (~4 ml) was collected from each subject into disposable plastic tubes containing EDTA and delivered to the laboratory for immediate acid digestion. Lead estimation was carried out by the method of Dale et al, 1976,[17] with the use of Atomic Absorption Spectrophotometry, (Pye Unicam model SP 9). Erythrocytes ALAD activity was determined using a modified aerobic method.[18] The assay of ALAD in whole blood depends on direct colorimetric estimation of the amount of porphobilinogen produced from added ALAD after incubation for one hour. The results were corrected to constant haematocrit values as the enzyme only occurs in the erythrocytes. Also, each tooth was initially socked in a dilute nitric acid and cleaned with distilled water to remove any adhering tissues, then dried and weight. The digestion method was done by using acid mixture of HNO₃: HClO₄: H₂O in a ratio of 1:2:1 in which teeth digestion for 24 hour at 37°C following the procedure by Ahmed[7] for deciduous teeth. The dense clear solution was used for aspiration into a Pye Unicam SP9 atomic absorption spectrophotometer.

**Statistical analysis**
All results were expressed as mean±SD. The data were analyzed statistically by one-way analysis of variance (ANOVA) while the correlation between the data was tested statistically by simple linear regression test by using computer SPSS program. The criterion for significance was (P<0.05).

**RESULTS**
Table-1 shows all the biochemical characteristics which were investigated in this study for participant. The mean and SD of age, blood lead level (BLL), deciduous tooth lead level (DLL) and ALAD were 8.96±2.8, 11.42±2.7 μg/dl, 7.6±3.93 μg/g and 202±19.74 U/ml RBC respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>100</td>
<td>4.00</td>
<td>15.00</td>
<td>8.96 ± 2.8</td>
</tr>
<tr>
<td>BLL μg/dl</td>
<td>100</td>
<td>5.00</td>
<td>19.00</td>
<td>11.42 ± 2.7</td>
</tr>
<tr>
<td>DLL μg/g</td>
<td>100</td>
<td>2.00</td>
<td>21</td>
<td>7.6 ± 3.93</td>
</tr>
<tr>
<td>ALAD U/ml RBC</td>
<td>100</td>
<td>160</td>
<td>240</td>
<td>202 ± 19.74</td>
</tr>
</tbody>
</table>

Values were expressed as mean ±SD.

Table-2 shows that BLL and DLL levels for (≤10 years) age group was higher than values in (>10 years) group. This elevation was statistically significant (P<0.001) while ALAD activity was significantly lower (P<0.001).

**Table 2. The mean BLL µg /dl DLL μg/g and ALAD U/ml RBC for participants in relation to age.**

<table>
<thead>
<tr>
<th>Age years</th>
<th>No.</th>
<th>BLL μg/dl</th>
<th>DLL μg/g</th>
<th>ALAD U/ml RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10</td>
<td>29</td>
<td>10.24±1.91</td>
<td>6.61±2.68</td>
<td>209.3±17.3</td>
</tr>
<tr>
<td>≤ 10</td>
<td>71</td>
<td>14.31±2.10***</td>
<td>10.00±5.31***</td>
<td>184.1±12.8***</td>
</tr>
</tbody>
</table>

Values were expressed as mean ±SD.

***Significant difference as compared between two groups (P<0.001).

Increased BLL and DLL of male individuals were statistically significant as compared with that of female (P<0.001) (Table-3). The same table shows that there was a significant decrease in the activity of ALAD males as compared with females (P< 0.001).

**Table 3. The mean BLL µg/g and ALAD for participants in relation to sex.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sex</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>No.</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>BLL μg/dl</td>
<td>13.36±2.88</td>
<td>9.48±2.51</td>
</tr>
<tr>
<td>DLL μg/g</td>
<td>8.92±4.54</td>
<td>6.25±3.15</td>
</tr>
<tr>
<td>ALAD</td>
<td>186.3±20.4</td>
<td>217±18.1</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD.
The same result was obtained concerning the mean BLL, DLL and ALAD activity among participants in relation to residency (urban and rural regions) (Table-4). The mean BLL and DLL were significantly lower for those living in rural region compared to urban area (P<0.0001), while ALAD activity was significantly higher (P<0.0001).

**Table 4. Blood lead levels (µg/dl) DPB µg/g and ALAD by residence.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Residence</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban</td>
<td>Rural</td>
</tr>
<tr>
<td>No.</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>BLL µg/dl</td>
<td>12.64±2.56</td>
<td>9.93±1.91</td>
</tr>
<tr>
<td>DLL µg/g</td>
<td>8.72±4.38</td>
<td>6.22±2.77</td>
</tr>
<tr>
<td>ALAD</td>
<td>195.5±19.1</td>
<td>209±17.7</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD.

Table-5 describes correlation between all biochemical parameters in participation of the study. There was a significant negative correlation as compared age, BLL and DLL with ALAD (P<0.01) whereas comparison between, BLL and DLL with age revealed significant positive correlation (P<0.01). In addition, comparison BLL with DLL shows significant positive correlation.

**Table 5. Correlation coefficient (r) between all biochemical parameters for participants.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age</th>
<th>BLL</th>
<th>DLL</th>
<th>ALAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>1.00</td>
<td>0.690**</td>
<td>0.446**</td>
</tr>
<tr>
<td></td>
<td>BLL</td>
<td>0.690**</td>
<td>1.00</td>
<td>0.523**</td>
</tr>
<tr>
<td></td>
<td>DLL</td>
<td>0.446</td>
<td>0.523**</td>
<td>1.00</td>
</tr>
<tr>
<td>ALAD</td>
<td>-0.654**</td>
<td>-0.716**</td>
<td>-0.491**</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values expressed as correlation coefficient (r).
**Correlation is significant at 0.01 level respectively.

**DISCUSSION**

Blood lead concentration is currently regarded as the most reliable index of lead exposure. Proteins in the ALAD fraction of erythrocytes supernatant have the highest affinity to lead among erythrocytes constituents. The factor most influencing BLL is environmental exposure. Absorbed lead due to exposure will inhibit ALAD activity. ALAD activity may provide better assessment than BLL of the amount of accumulated lead at the site of its effects in the body because it better predicts the amount of chelatable lead excreted in urine, which originates from peripheral blood and soft tissues. At the same level of BLL, the simultaneous ALAD activity is relatively less depressed in subjects with short-term lead exposure or recently increased exposure level. Furthermore, the exposure of lead can decrease the absorption rate and biologic availability of zinc in the body, mainly because of their competition for binding (-SH) group site in various enzymes especially metallothionine and tissues because ALAD is a zinc containing enzymes that is extremely sensitive to lead, and because the inhibition of ALAD activity results from direct substitution of zinc by lead. The finding of significant negative correlation between BLL and low activity of ALAD, confirms that proteins in the ALAD fractions have the highest affinity for lead among erythrocytes constituents and it is further support a link between lead exposure and ALAD activity. Selander and Grame showed a clear threshold effects at about 40 µg/dl in occupational subjects, regarding the correlation between BLL and ALA in urine (ALA-U) levels. Based upon several studies on the association between BLL and ALA, lead has been suggested to have discernible effects on the ALA-U at a BLL around 35 µg/g. Likewise, the threshold of BLL for decreased ALAD activity has been estimated to be approximately 10 µg/g. The mean BLL for children of this study was 11.42±2.7 µg/dl (Table-1), this mean BLL was lower than that reported in other neighboring and developing countries like Egypt being 18.8 ± 6.9 µg/dl and in Korea being 30.0 µg/dl. These observations might be explained by that children exposes to lead via air, water, foods and other sources. Teeth lead concentration for children ranged from 2.0-21 µg/g with mean value of 7.6±3.93 µg/g (Table-1). This is comparable with the Indian value of 3.01 µg/g, the Germany value 2 µg/g, the Spain value of 3.96 µg/g, the Karachi value of 5.78 µg/g, the Mexico value of 7.7 µg/g, the Ankara (Turkey) value of 1.3 µg/g, Ballihesir (Turkey) value of 1.77 µg/g, the Bahraini value of 4.3 µg/g and the Norwegian value 1.27 µg/g. The population studied was stratified by age, sex, and residence areas. Children with (≤10 years) age group have a significantly higher BLL and DLL compared to...
children with (>10 years) group (Table-2), a result is in agreement with other studies, which reported that children younger than 15 years of age are the most susceptible group especially young children. Children are considered at a greater risk for both lead exposure and toxicity, the accepted explanations include that lead absorption is more in children because of their physiological and metabolic characteristics, and because of hand-to-mouth behavior (especially for young children). Also, significant association of DLL with age reflects the ability of teeth to signify cumulative lead exposure (Table-5). It is found that BLL declines during adolescence which probably related to bone growth and deposition of lead in bone with calcium. The present study has revealed that there was a significant increase in BLL, DLL and ALAD activity among urban participants as compared to rural participants groups (Table-4). Similar results were obtained by other researchers. This could be attributed due to widespread source of lead pollution due its use as anti-knock additive with petroleum constituents. This is particularly the case in urban areas where road traffic more concentrated and contributes extensively to lead levels in air and deposited dust. In the absence of anti-knock additive in petrol constituents, although, the contribution of industries is very rare, lead pollution could be arises from molding of metals as well as petroleum combustion. Males had a significantly higher BLL and DLL compared to females (Table-3), similar results were obtained by other researchers. While, other studies showed no significant difference between males and females. The strong positive correlation of BLL and DLL and strong negative correlation of ALAD with age, which were found in the present study data (Table-5) confirms the role of age as a factor effecting lead poisoning. Katsuyuki et al reported that the ALAD activity changes almost parallel to BLL. This finding agrees with other studies in the general population, which have confirmed the correlation and the apparent lack of a threshold for inhibition of ALAD in different age groups. Finally, it could be concluded that levels of lead in deciduous teeth, blood serves as a dosimeter for exposure to lead which originates from different sources; also there were high correlation between BLL and DLL. The decreased of ALAD activity was significantly related to the concentration of lead in blood and deciduous teeth. Also BLL, DLL and ALAD activity seems to be effected by age, sex and residence region.

REFERENCES