Blood lead levels in iron-deficient and noniron-deficient adults

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Summary
Iron deficiency (ID) has been reported to increase lead absorption. This relationship has been investigated in detail in children but not in adults. This study was designed to investigate whether blood lead levels are significantly higher in iron-deficient adults. ID-parameters (haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, serum iron, total iron-binding capacity, iron-binding saturation, soluble transferrin receptors, washed zinc protoporphyrin and ferritin) together with whole blood lead were measured in three different adult groups – blood donors (n = 73), pregnant women (n = 74) and haemodialysis patients (n = 72). Of a total of 219 subjects tested, 7.7% was found to have a lead level above 10 μg/dl (maximum 16 μg/dl). No association was found between blood lead level and ID (iron-deficient subjects (n: 139), mean: 5.6 μg/dl (SD: 3 μg/dl) and noniron-deficient subjects (n: 80), mean: 5.4 μg/dl (SD: 3 μg/dl)). The results suggest that the inverse association between blood lead and serum iron in studies carried out on children does not occur in adults.

Keywords
Lead, iron, ferritin, zinc protoporphyrin, transferrin receptors, blood donors, pregnancy, haemodialysis, anaemia, zinc

Introduction
Increased lead absorption from environmental exposure has a serious implication for child health that is known and well documented (Nordberg, 1976; Otto et al., 1982; Mahaffey, 1983; Mahaffey, Gartside & Glueck, 1986; Campagna et al., 1999). However, adults, can be similarly affected by chronic environmental exposure to lead, and that includes groups that are susceptible to iron deficiency (ID) particularly pregnant women where it may affect the neurodevelopment of the foetus in utero (Tang et al., 1999). Environmental contamination with lead is still a risk even in industrialized countries, particularly within large crowded inner cities. According to the second National Health and Nutrition Examination Survey, conducted between 1976 and 1980, 78% of the US population had blood lead levels >10 μg/dl (Mahaffey et al., 1982). A report (in 1998) from the US, measuring blood lead in 20 000 individuals, indicated that blood lead levels were still >10 μg/dl in 20% (Anonymous, 1999). Nonindustrialized areas such as the populated areas of the Himalayas gave average blood lead levels around 3 μg/dl (Piomelli et al., 1980).

Iron deficiency is reported to encourage lead absorption and therefore ID and lead toxicity are likely to coexist (Mahaffey & Annest, 1986; Bradman et al., 2001; Wolf, Jimenez & Lozoff, 2003; Wright et al., 2003). The uptake of lead from the gut is influenced by deficiencies of phosphorus, zinc, iron and/or calcium in the diet (Mahaffey, 1983). This association may result from enhancement of lead absorption in patients deficient in iron, zinc, etc. or because of the coexistence of increased lead exposure in those individuals with a diet poor in these elements. Many of the previous studies have been carried out in children. The effect in adults has not been investigated. Therefore this study was conducted on iron-deficient adults from an inner city population with a large ethnic mix in which opportunities for lead exposure are likely to be high. Although environmental lead levels have reduced in recent years it would be expected that even minimal levels would be higher in the iron-deficient patient if lead absorption through the intestine in adults is increased because of ID. As lead absorption may also be increased by zinc deficiency (Mahaffey, 1983) this element was also measured to exclude its effect.
Materials and methods

This study was conducted with the approval of the local hospital ethics committee. Written consent was obtained from all the patients and control subjects.

Three population groups were selected – blood donors attending the regional blood donor centre, pregnant women attending the hospital antenatal clinic and haemodialysis patients attending the dialysis centre. Iron-deficient individuals were identified by various ID indicators and compared with noniron-deficient controls selected from the same groups.

Iron-deficient individuals in the blood donors and pregnant women groups were defined as those who had at least four of the following five parameters positive; >5% hypochromic red blood cells, serum ferritin <20 µg/l, iron binding saturation (IBS) <15%, serum transferrin receptor >28.1 nmol/l, or washed zinc protoporphyrin >2.5 µg/g Hb. Noniron-deficient controls had to have at least four of five parameters negative. In a separate study it was found that normal parameters of ID bore little relationship with body iron stores in haemodialysis patients and serum transferrin receptor levels was the most reliable marker (Allen et al., 1998). Therefore, in the haemodialysis group, ID was defined as soluble transferrin receptors (sTfR) >28.1 nmol/l.

Haemoglobin (Hb), haematocrit (PCV), red cell indices including mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) and percentage of hypochromic red blood cells were estimated using a Bayer Technicon H2 instrument (Bayer Diagnostics, Dublin, Ireland) (normal range for percentage hypochromic red blood cell is <5%).

Serum iron (SI), total iron-binding capacity (TIBC) were measured by an Ortho Vitros 950 multianalyser (Ortho-Clinical Diagnostics, Strasbourg, France); IBS was calculated from the equation: [total serum iron/total iron binding capacity x 100 (normal level for IBS: >15%)]. Serum ferritin (Fer) was measured by ACS: 180-ferritin assay, a two-site sandwich immunoassay using direct chemiluminesometric technology (Bayer, Tarrytown, NY, USA) (normal range for serum ferritin: 20–300 µg/l). Blood samples for zinc protoporphyrin (ZPP) measurement was collected in EDTA. Measurement was carried out within 2 days of blood sampling. Samples that were not examined on the same day were stored in a refrigerator at 4 °C. An Aviv front-face haematofluorometer (Aviv Instruments, Lakewood, NJ, USA) was used to measure ZPP with suitable cover slides (Becton Dickenson, Oxford, UK), and Aviv erythrocyte controls for calibration. In erythrocytes, ZPP was measured, washed three times and during the washing procedure, the samples were centrifuged for 4 min at 100 g just before measurement and the plasma was removed and replaced with isotonic saline (NaCl 9 g/l) to restore the original volume. After repeating the washing process three times, ZPP was measured in 20 µl of erythrocyte suspension after being fully oxygenated. Visibly haemolysed and jaundiced samples was excluded (normal range <2.5 µg/g Hb). Soluble transferrin receptors were measured according to the protocol of R & D Systems Europe, Abingdon, UK [human sTfR immunoassay (normal range for sTfR: 8.7–28.1 nmol/l)].

Samples for lead were taken into EDTA, stored in the refrigerator at 4 °C and analysed in batches with appropriate quality controls. Lead was measured by electrothermal atomic absorption spectrometry (ETAAS) [blood lead in nonoccupationally exposed adults is <10 µg/dl (SAS Trace Element Laboratory 1998)].

Samples for zinc analysis were taken from the same tube as those for ferritin analysis, serum was stored frozen until analysed in batches and the analysis was carried out by flame atomic absorption spectrometry (FAAS). Less than 0.5 mg/l indicates zinc deficiency; 0.5–0.6 mg/l, borderline zinc deficiency; 0.6–1.6 mg/l (11–24 µmol/l), normal range (SAS Trace Element Laboratory, 1998).

Statistics

The relationship between the different ID indicators and whole blood lead, and between lead and serum zinc were examined by linear regression analysis; calculating the correlation coefficient (r) and the significance of correlation (P). Also, P-value was used to measure the distribution of the values of the variables (twos test) around the geometric mean when comparison was made between two similar variables in two subgroups and this analysis was performed for the three groups and their subgroups. In addition descriptive statistics and normality tests were used. All statistics analyses were accomplished by MiniTab statistical package for Windows, version 12 (Microsoft, Redmond, WA, USA).

Results

Results were analysed for each group separately as well as a total.

Blood donor group

Seventy-three subjects were initially recruited according to the National Blood Service copper sulphate (CS) test to identify anaemia. Forty-three donors who did not pass the
Table 1. Geometric mean blood lead levels and iron deficiency parameters in blood donor group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Iron-deficient subgroup [mean (SD)]</th>
<th>Control subgroup [mean (SD)]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>11.6 (1.3)</td>
<td>13.5 (1.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypo RBC (%)</td>
<td>19 (15)</td>
<td>1.7 (2.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IBS (%)</td>
<td>12 (8.8)</td>
<td>27.5 (10.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fer (µg/l)</td>
<td>9.4 (5.0)</td>
<td>74.6 (83.3)</td>
<td>=0.0002</td>
</tr>
<tr>
<td>WZPP (µg/g Hb)</td>
<td>4.2 (2.2)</td>
<td>1.3 (0.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>STIR (nmol/l)</td>
<td>44 (19.8)</td>
<td>22.2 (3.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood lead (µg/dl)</td>
<td>4.8 (1.9)</td>
<td>4.8 (2.4)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Hb, haemoglobin; RBC, red blood cell; IBS, iron binding saturation; Fer, ferritin; STIR, soluble transferrin receptor; WZPP, zinc protoporphyrin after washing red blood cells; SD, standard deviation; ID, iron deficient; P, values of twos (normal distribution).

Table 2. Geometric mean blood lead levels and iron deficiency parameters in pregnant women group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Iron-deficient subgroup [mean (SD)]</th>
<th>Control subgroup [mean (SD)]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>10.5 (1.7)</td>
<td>12.1 (1.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypo RBC (%)</td>
<td>19.5 (17)</td>
<td>9.3 (11.7)</td>
<td>0.0037</td>
</tr>
<tr>
<td>IBS (%)</td>
<td>10.4 (10)</td>
<td>23 (11.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fer (µg/l)</td>
<td>10 (5.2)</td>
<td>50 (31)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WZPP (µg/g Hb)</td>
<td>3.9 (2.2)</td>
<td>1.7 (1.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>STIR (nmol/l)</td>
<td>52 (25.2)</td>
<td>25.4 (6.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood lead (µg/dl)</td>
<td>4.0 (1.9)</td>
<td>4.0 (2.8)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Hb, haemoglobin; RBC, red blood cell; IBS, iron binding saturation; Fer, ferritin; STIR, soluble transferrin receptor; WZPP, zinc protoporphyrin after washing red blood cells; SD, standard deviation; ID, iron deficient; P, values of twos (normal distribution).

Table 3. Geometric mean blood lead levels and iron deficiency parameters in haemodialysis patient group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Iron-deficient subgroup [mean (SD)]</th>
<th>Control subgroup [mean (SD)]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo RBC (%)</td>
<td>16.5 (11.9)</td>
<td>9.7 (8.4)</td>
<td>0.0084</td>
</tr>
<tr>
<td>IBS (%)</td>
<td>22.9 (11.3)</td>
<td>27 (10.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>Fer (µg/l)</td>
<td>493 (530)</td>
<td>574 (470)</td>
<td>0.51</td>
</tr>
<tr>
<td>WZPP (µg/g Hb)</td>
<td>2.6 (1.6)</td>
<td>1.7 (0.8)</td>
<td>0.0020</td>
</tr>
<tr>
<td>STIR (nmol/l)</td>
<td>42.4 (12.8)</td>
<td>23.2 (5.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood lead (µg/dl)</td>
<td>7.5 (3.0)</td>
<td>7.0 (3.0)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Hb, haemoglobin; RBC, red blood cell; IBS, iron binding saturation; Fer, ferritin; STIR, soluble transferrin receptor; WZPP, zinc protoporphyrin after washing red blood cells; SD, standard deviation; ID, iron deficient; P, values of twos (normal distribution).

CS test and as a result were not able to give blood, and were found to have ID indicators mentioned previously were selected; 30 donors who passed the CS test were taken as noniron deficient (Table 1).

Mean blood lead levels for controls were 4.8 µg/dl (SD: 2.4) and that for iron-deficient blood donors was 4.8 µg/dl (SD: 1.9 µg/dl). Blood lead levels were slightly lower in iron-deficient females (mean 4.3 µg/dl, SD: 1.6) compared with iron-deficient males (mean 5.6 µg/dl, SD: 2.3), although this was not statistically significant. Only one subject was found to have a blood lead level >10 µg/dl and this was 13 µg/dl and from the control group. No significant association was found between blood lead and serum ferritin concentrations (r = 0.082, P = 0.491). Also, no significant association was found between blood lead and serum zinc (r = -0.221, P = 0.060).

Pregnant women group

Seventy-four pregnant women were recruited, 48 iron deficient and 26 controls (Table 2). Mean blood lead concentration for controls was 4.0 µg/dl (SD: 2.8), com-
compared with 4.0 μg/dl (SD: 1.9) in those who were iron deficient. No significant association was found between blood lead and serum ferritin (r = 0.124, P = 0.294). In addition, the same was true between blood lead and serum zinc (r = 0.072, P = 0.540) respectively. However, mean serum zinc level for controls was 0.73 mg/l (SD: 0.15) compared with 0.66 mg/l (SD: 0.18) in the iron-deficient individuals.

**Discussion**

These results indicate that blood lead levels in the study population are now low relative to concentrations experienced in the past confirming a previous study carried out in Birmingham in 1996 (Wang, Thornton & Farago, 1997). Lead levels in the pregnant women group were also similar to those measured in another study from France, in which the geometric mean of blood lead level was 6.4 μg/dl in pregnant women (Campagna et al., 1999), while another study from the US on blood lead levels in adult medical inpatients gave a level of 6.7 μg/dl (Kilmarx, Allen & Brancati, 1996). It would appear that the relatively low blood lead levels are results of the reduction policies that related to lead emissions in the UK since the 1980s.

The results showed no significant difference in blood lead level between iron-deficient adult subjects and noniron-deficient controls. Indeed, in the total number of subjects (219), the level was almost identical in both groups (5.6 μg/dl in the iron-deficient group compared with 5.4 μg/dl in the controls). Seventeen subjects were found to have blood lead levels between 11 to 16 μg/dl. Five of those subjects were noniron deficient (5.7%), whereas 12 were iron deficient (9%), and this difference did not reach statistical significance.

In contrast to the studies of blood lead levels in adults from environmental exposure, those reported in children have tended to show higher lead levels and this appears to be associated with ID (Mahaffey, 1983; Bradman et al., 2001; Wolf et al., 2003; Wright et al., 2003). There is a theoretical basis for this observation. Iron extraction from the diet is small and limited, as humans have no physiological pathway for excretion. Duodenal enterocytes are responsible for iron absorption. Iron is transferred across the apical membrane of the enterocyte into the cell using a protein named divalent metal transporter 1 (DMT1) (Gunshin et al., 1997). DMT1 is not specific for iron; it can transport a wide variety of divalent metal ions, including copper, zinc and lead. Therefore if the iron content of the diet is low the other divalent metal ions may be absorbed instead including trace quantities of lead. As ID is associated with pica, a bizarre behavioural symptom characterized by the inappropriate consumption of non-nutritive substances (Moore & Sears, 1994) there is opportunity for lead to be absorbed if present because of increased exposure rather than increased absorption. This study has shown that adults with ID had no higher blood lead levels than noniron-deficient adults living in the same environment. Similarly, there was no inverse correlation of zinc level with blood lead. This would argue against the theory that DMT1 would preferably absorb other divalent metal ions in the presence of a shortage of iron in adults although this may still be a factor in children.

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References