EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE IN PREGNANCY AND LEAD LEVEL IN MATERNAL BLOOD AT DELIVERY

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Abstract
Objectives: The particular purpose of our study was to assess the impact of environmental tobacco smoke (ETS) on the individual variability of blood lead levels in pregnant women as earlier publications demonstrated the increased blood lead in smokers.

Materials and Methods: The material consisted of 240 pregnant women who participated in a prospective cohort study on vulnerability of the fetus and infant to environmental hazards. The enrolment included only non-smoking women with singleton pregnancies between the ages of 18–35 years. Whole blood lead concentrations were determined using inductively coupled plasma mass spectrometry.

Results: On average, blood-lead measured in pregnant women at delivery was low (GM = 1.7 μg/dL; 95% CI:1.6–1.8 μg/dL) and none of them showed levels above 4.0 μg/dL, but persons reporting exposure to ETS had significantly higher blood lead level (GM = 1.9 μg/dL; 95% CI:1.8–2.1 μg/dL) than those free from this exposure (GM = 1.6 μg/dL; 95% CI:1.5–1.7 μg/dL). In order to single out the effect of the ETS exposure from the confounding variables, we used the stepwise multivariate linear regression for log blood-lead in maternal blood as dependent variable and a set of independent variables, such as age, weight of women before pregnancy and their education level. The results of the analysis showed that all the independent variables included in the model explained 11% of total blood-lead variability among the study women. The strongest component of variance was attributed to ETS exposure (5%), age (3%), education level (2%) and weight (1%). Inclusion into the model of other variables, e.g., residence area and traffic intensity did not improve the proportion of explained variability.

Conclusions: The reason for higher levels of blood-lead in the ETS-exposed women may result from the fact that tobacco smoke contains lead. However, it is possible that inhaled tobacco smoke also increases the absorption of lead from particulate matter deposited in the bronchial tree.

Key words: Environmental tobacco smoke, Blood lead, Pregnant women

INTRODUCTION
The level of lead contamination of the ambient air and soil depends on the amount of lead emitted to the environment. Most of the lead in ambient air is in the form of sub-micron-sized particles, and about 30–50% of the inhaled particles are retained in the respiratory system and almost all of the retained lead is absorbed into the body. Particles in the size range of 1–3 μm are also to a great extent de-
posited in the lungs, while larger particles are deposited mainly in the upper respiratory tract with incomplete absorption. All lead particles that are cleared by the lung can be swallowed, which results in a further lead absorption from the gastrointestinal tract. Lead occurring in and on food both naturally and from atmospheric deposition may also contaminate food during harvesting, transportation, processing, packaging, or preparation [1,2].

The usual background environmental lead exposure through air, food, and drinking water, may be increased through additional sources of exposure. These additional exposures can include high lead levels in dust and soil in residential areas near smelters or refineries, high-density traffic, and the consumption of vegetables and fruit grown on high-lead soils or near sources of lead emissions [3–5]. Higher exposure may also result from lifestyle and individual habits, such as alcohol consumption [6] or tobacco smoking [7,8].

The toxicity of lead may largely be explained by its interference with different enzyme systems: lead inactivates these enzymes by binding to SH-groups of its proteins or by displacing other essential metal ions. For this reason many organs or organ systems are potential targets for lead, and a wide range of biological effects of lead have been documented. These include effects on the hem biosynthesis, the nervous system, the kidneys and reproduction as well as on cardiovascular, hepatic, endocrihal and gastrointestinal functions. In conditions of low-level and long-term lead exposure, such as those found in the general population, the most critical effects are exerted on the hem biosynthesis, erythropoiesis, kidneys, nervous system and blood pressure [9,10].

Elimination of lead-based gasoline along with the reduced use of lead in consumer products (e.g., plants), homes (e.g., plumbing system), food-packaging applications (e.g., soldered cans), in conjunction with other public health programs, have brought about a significant reduction of lead levels in the population over the past decade, thereby lead poisoning no longer constitutes a widespread public health threat.

The particular purpose of our study was to assess the impact of environmental tobacco smoke (ETS) on the individual variability of blood-lead among pregnant women. Earlier publications demonstrated the increased lead blood level in smokers, however, no data on possible impact of ETS on blood-lead levels in non-smoking populations, especially those at a higher risk have yet been published. Humans begin to accumulate lead in their bodies already during prenatal development. Since the placenta is no effective biological barrier, pregnant women represent the group at an increased risk because of maternal exposure of the fetus to lead [11–13].

MATERIALS AND METHODS

Study subjects

The cohort consisted of 240 infants who were born at 33–42 weeks of gestation between January 2001 and March 2003 to mothers participating in an ongoing prospective cohort study on vulnerability of the fetus and infant to environmental hazards. Women attending ambulatory prenatal clinics in the first and second trimesters of pregnancy were eligible for the study. The enrolment included only non-smoking women with singleton pregnancies at the age between 18 and 35 years, and who were free from chronic diseases, such as diabetes and hypertension. Upon enrolment, a detailed questionnaire was administered to each subject to elicit demographic data, medical and reproductive history, occupational exposures, alcohol consumption, and smoking practices of others present in the home. Women who declared that they were living with a smoker in the household or were exposed to passive smoking in the occupational settings were treated as ETS positive.

Blood sample collection and analysis

A maternal blood sample (30–35 ml) at delivery was drawn into the vacutainer tube that had been treated with ethylene diamine tetra-acetate (EDTA). Then the tube was inverted several times to mix the EDTA and the blood to prevent coagulation. Within 12 h of blood collection, the blood samples were transported to the clinical biochemistry laboratory at the University Hospital in Kraków for processing and storage. Blood samples were stored in liquid nitrogen in the laboratory prior to...
shipment to the Centers for Disease Control (CDC), Atlanta, GA, USA, for blood lead analysis. Whole blood lead concentrations were determined using inductively coupled plasma mass spectrometry CLIA'88 method “Blood lead cadmium mercury ICPMS_ITB001A”. This multi-element analytical technique is based on quadrupole ICP-MS technology [14].

**Statistical analysis**

The Chi-square statistics and analysis of variance tested differences in characteristics between ETS exposure groups. The Spearman rank correlation was used to assess the association between mercury levels in maternal blood and predictor variables. The relative contribution of ETS was evaluated using forward stepwise multiple linear regression models, where variables were introduced in decreasing order of significance. The models computed lead blood as dependent variables on the predictor variable (ETS exposure) and accounted for potential confounders (education, maternal age, and weight before pregnancy). All statistical analyses were performed with NCSS and BMDP software for Windows (BMDP).

**RESULTS**

Table 1 presents the characteristics of the study population by the ETS exposure status.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n = 240)</th>
<th>ETS(-) (n = 170)</th>
<th>ETS(+) (n = 70)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>27.91</td>
<td>28.28</td>
<td>27.01</td>
<td>0.0136</td>
</tr>
<tr>
<td>SD</td>
<td>3.61</td>
<td>3.37</td>
<td>4.03</td>
<td></td>
</tr>
<tr>
<td>Education (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>10.0</td>
<td>8.8</td>
<td>12.9</td>
<td>0.0000</td>
</tr>
<tr>
<td>Secondary</td>
<td>35.0</td>
<td>26.5</td>
<td>55.7</td>
<td></td>
</tr>
<tr>
<td>Higher (university)</td>
<td>55.0</td>
<td>64.7</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>Weight before pregnancy (kg)</td>
<td>58.38</td>
<td>58.46</td>
<td>58.19</td>
<td>0.8257</td>
</tr>
<tr>
<td>SD</td>
<td>8.71</td>
<td>8.67</td>
<td>8.85</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.89</td>
<td>164.78</td>
<td>165.14</td>
<td>0.6476</td>
</tr>
<tr>
<td>SD</td>
<td>5.54</td>
<td>5.40</td>
<td>5.88</td>
<td></td>
</tr>
<tr>
<td>Lead in maternal blood (µg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.80</td>
<td>1.71</td>
<td>2.03</td>
<td>0.0008</td>
</tr>
<tr>
<td>SD</td>
<td>0.66</td>
<td>0.61</td>
<td>0.72</td>
<td>0.0006</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>1.69</td>
<td>1.61</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>1.62–1.77</td>
<td>1.52–1.70</td>
<td>1.77–2.07</td>
<td></td>
</tr>
<tr>
<td>Lack of measurements</td>
<td>2</td>
<td>2</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Traffic intensity in the residence area (medium + high) (%)</td>
<td>10.4</td>
<td>8.2</td>
<td>15.7</td>
<td>0.2180</td>
</tr>
<tr>
<td>Presence of bus depot in the residence area (%)</td>
<td>4.2</td>
<td>7.1</td>
<td>2.9</td>
<td>0.2177</td>
</tr>
<tr>
<td>City area (city centre) (%)</td>
<td>18.3</td>
<td>19.4</td>
<td>15.7</td>
<td>0.5010</td>
</tr>
</tbody>
</table>

SD – standard deviation; ETS(-) – no environmental tobacco smoke; ETS(+) – environmental tobacco smoke reported.

Table 1. Characteristics of the study population by the ETS exposure status.

On average, the blood-lead measured in pregnant women at delivery was very low (GM = 1.7 µg/dL; 95% CI: 1.6–1.8 µg/dL) and none of the women had levels above 4.0 µg/dL, but persons reporting exposure to ETS had a significantly higher blood lead level (GM = 1.9 µg/dL; 95% CI: 1.8–2.1 µg/dL) than those non-exposed (GM = 1.6 µg/dL; 95% CI: 1.5–1.7 µg/dL). Figure 1 shows the cumulative frequency distribution of blood lead levels across the exposure groups. In the univariate analysis of the data the positive correlation was found between the maternal blood lead, age (r = 0.13, p ≤ 0.05) and weight before pregnancy (r = 0.15, p ≤ 0.05), but there was inverse correlation with the maternal education level (r = -0.15, p < 0.05). The blood lead level was not significantly higher in the women who reported their living in houses located near busy roads (GM = 1.8 µg/dL; 95% CI: 1.6–2.1 µg/dL) than in those living in houses situated along the streets with light traffic (GM = 1.7 µg/dL; 95% CI: 1.6–1.8 µg/dL). Similarly, blood lead in the women who lived close to bus depots were not significantly higher than that in women free from potential exposure to diesel exhausts (GM = 1.8 µg/dL; 95% CI: 1.4–2.4 µg/dL vs. 1.7 µg/dL; 95% CI: 1.6–1.8 µg/dL).
In order to single out the effect of the ETS exposure accounted for confounding variables, we used the stepwise multivariate linear regression for log blood lead values in maternal blood as a dependent variable and a set of the independent variables such as age, weight before pregnancy, and maternal education. Traffic variables were not included in the model since they became insignificant in the univariate analysis. The results of the analysis presented in Table 2 show that all the independent variables included in the model explained about 11% of the total blood lead variability among the study subjects. The strongest component of variance was attributed to ETS exposure (5%), and this was followed by age (3%), education (2%), and weight before pregnancy (1%). Inclusion into the model of other variables, such as residence area did not improve the proportion of explained variability.

Table 2. Results of the stepwise multiple linear regression analysis of log concentrations of maternal blood-lead related to potential predictor variables

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Beta Coefficient</th>
<th>SE</th>
<th>P</th>
<th>R</th>
<th>R²</th>
<th>Change in R²</th>
<th>F at entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETS</td>
<td>0.0744</td>
<td>0.0220</td>
<td>0.00</td>
<td>0.220</td>
<td>0.048</td>
<td>0.048</td>
<td>12.01</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.0084</td>
<td>0.0030</td>
<td>0.00</td>
<td>0.283</td>
<td>0.080</td>
<td>0.032</td>
<td>8.15</td>
</tr>
<tr>
<td>Maternal education</td>
<td>-0.0152</td>
<td>0.070</td>
<td>0.03</td>
<td>0.315</td>
<td>0.099</td>
<td>0.019</td>
<td>4.96</td>
</tr>
<tr>
<td>Maternal weight</td>
<td>0.0021</td>
<td>0.0011</td>
<td>0.07</td>
<td>0.334</td>
<td>0.112</td>
<td>0.012</td>
<td>3.23</td>
</tr>
</tbody>
</table>

SE = standard error;  P = level of significance;  R² = coefficient of determination;  F = variance test statistics;  ETS = environmental tobacco smoke.

DISCUSSION AND CONCLUSIONS

Our study showed that blood lead concentrations measured in women at delivery were rather low (GM = 1.7; 95% CI: 1.6–1.9) and all measurements showed levels below 4 μg/dL, i.e., much below the CDC level currently considered to be the benchmark for intervention (10 μg/dL). The study indicated that the persons reporting exposure to ETS had a significantly higher blood lead level (GM = 1.9 μg/dL; 95% CI: 1.8–2.1 μg/dL) than those non-exposed (GM = 1.6 μg/dL; 95% CI: 1.5–1.7 μg/dL). These findings are in agreement with earlier published data showing that the active tobacco smokers had higher level of blood lead level [7,8], however, the effect of passive smoking has not yet been reported in the literature. Our data showed that about 5% of estimated variability among the study subjects could have been explained by ETS exposure. The reason for higher level of lead in the ETS exposed results from the fact that tobacco smoke contains lead. However, it is possible that the inhaled tobacco smoke also increases the absorption of lead from particulate matter deposited in the bronchial tree. Although contribution of ETS to variability of blood lead level was not extensive, it could intensify the detrimental impact of other chemical compounds present in the side-stream smoke being important for the growth and development of fetus. The study confirmed the positive correlation of blood lead level with age in young adult women. The association with age could result from the accumulation of lead over time, but it may also be explained by the fact that the older women were possibly exposed to a higher environmental lead exposure in the past than the younger cohort. We could also show the association between pre-pregnancy weight and the blood lead level. We think that this association results from the higher food intake by those who weigh more. If one eats more food, there is a greater likelihood of ingestion of lead contaminated food products. Out of the lifestyle variables considered in our study, the education level was inversely correlated with blood lead concentration and this may be associated with higher environmental hygiene standards of those who are better educated. The study on the effect of lifestyle factors, per-
formed by Muddoon et al. [8] among white women aged 65–87 years from urban Baltimore and rural Monongahela Valley, Pennsylvania, USA, showed that the urban residence, smoking, alcohol consumption, and years since menopause were positively associated with higher blood lead level, while obesity, prior breast feeding, current estrogen replacement therapy, moderate physical activity, and calcium intake were inversely related.

Against our expectations we could not confirm any significant relationship between blood lead level and traffic density reported by the respondents. However, this may evidence that in case of lead-free gasoline used for vehicle transportation, the traffic profile and its intensity is no longer a significant predictor for the lead exposure assessment. The low blood lead level in the study subjects is consistent with the air lead concentrations currently observed in the city of Kraków. The environmental data monitoring carried out in 2003 in Kraków demonstrated very low annual lead concentrations in the communal air, which was within the range of 0.07–0.09 μg/m³.

Air lead concentrations in industrial areas and in urban areas with high traffic density across Europe have decreased steadily over the past 15 years, subsequent to abatement of industrial emissions and to reductions in the lead content of petrol or to the increasing use of lead-free petrol. A typical example are annual means of air lead concentrations reported for the industrialized urban Rhine–Ruhr region in Germany. Whereas annual means were 0.81–1.37 μg/m³ in 1974, they were only 0.17–0.19 μg/m³ in 1988; for traffic-dominated cities (Cologne and Düsseldorf) they decreased from 0.81 and 0.96 μg/m³ to 0.17 and 0.18 μg/m³, respectively, whereas for more industrialized areas (Essen and Dortmund) from 1.30 and 1.37 μg/m³ to 0.17 and 0.19 μg/m³, respectively [15]. Even more pronounced downward trends, from above 3 μg/m³ in 1974 to about 0.5 μg/m³ in 1988, have been reported for areas with high traffic density in Belgium [16]. Higher air lead concentrations may still be found in the vicinity of primary or secondary lead smelters. In 1984–1985, spot measurements in French and American cities reported air lead levels ranging from 0.005 to 0.44 μg/m³, with the highest value in Paris [17,18]. Similarly, data from that survey (NHANES II) revealed a decline in blood lead levels that was closely correlated with declines in the use of leaded gasoline during those years [19].

The data obtained from our study may suggest that no apparent toxic impact of lead should be expected in Kraków residents, since to our current knowledge, frank anemia may occur at 80 μg/dL of blood lead, decreased hemoglobin production below 40 μg/dL, neurotoxicity and chronic kidney toxicity at blood lead levels of 30 μg/dL. Reduced birth weight of newborns were noted in maternal blood lead levels above 15 μg/dL or increased preterm delivery or spontaneous abortions are not apparent at maternal blood lead levels lower than 30 μg/dL [11–13].

Some weakness of our study results from the fact that the classification of smoking status at the recruitment was based on interviewing women about smoking practices. Women who denied smoking since the beginning of the pregnancy were classified as non-smokers. This may be a source of misclassification bias in defining the exposure status since the study sample could not be homogeneous in respect of the non-smoking status. The sample consisted of never-smokers as well as of women who stopped to smoke just before the pregnancy. It is possible, however, that a number of smoking women could declare non-smoking status. All the aforesaid factors could lead to underestimation of the relationship between ETS and blood lead level. However, we do not believe that this could have changed the direction of estimates. Moreover, there was a significant correlation between the reported number of cigarettes smoked in the presence of women over the pregnancy period and the lead level in maternal blood (r = 0.154, 95%CI = 0.06–0.25, p = 0.002). Due to the particular design of the study we were not able to provide additional evidence of the impact of active smoking on the lead levels in maternal and cord blood.

To sum up, the Kraków study showed lower than expected blood lead level in pregnant women, which may be explained by the successful implementation of lead-free gasoline program not only in Poland but also in the neighboring countries. It is worth noticing, however, that the study highlighted the importance of lifestyle factors, such as the environmental tobacco smoke, education level and pre-
pregnancy body weight of women in the health risk assessment. As demonstrated, blood levels in pregnant women might in part be attributed to ETS exposure, the revision of public health policy should also aim at the promotion of smoking cessation among all household members during pregnancy.

REFERENCES