Effects of blood lead and cadmium levels on homocysteine level in plasma

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Abstract. – OBJECTIVE: We studied the effect of non-occupational exposure to lead and cadmium on homocysteine level in plasma. Homocysteine is a marker for plasma folate folic acid metabolism in urban populations.

PATIENTS AND METHODS: 159 individuals from Beijing, Guangzhou, Shenzhen and Shanghai with no history of close exposure to heavy metals and no history of metabolic diseases were enrolled to participate in this study. Blood lead and cadmium levels were detected using ICP-MS method and the level of homocysteine was also measured using enzyme method. Our results showed that blood lead and cadmium levels in males were significantly higher than those in females. Also, blood lead and cadmium levels in smokers were higher than those in non-smokers; homocysteine level was significantly higher in smokers as well. According to blood lead and cadmium levels, cases were divided into four groups.

RESULTS: Our results showed that a surge in blood lead and cadmium levels could result in an increase in homocysteine level. We concluded that in the Chinese population, smoking and gender might be the risk factors for elevated levels of lead and cadmium. Meanwhile, blood lead and cadmium levels may influence the homocysteine levels in the body.

CONCLUSIONS: It is possible to speculate that non-occupational exposure to lead and cadmium, by increasing the homocysteine levels, negatively affect the cardiovascular and nervous system.

Key Words: Blood Lead, Cadmium level, Homocysteine level.

Introduction

Ever-growing urbanization and industrialization heavy metal pollution created by them have become a major health risk factor for urban populations. Heavy metals can accumulate in the body and their levels can grow significantly in long-term. Metals such as Ni, Cr, and Cd can cause damage to DNA, trigger oxidative stress, disrupt protein function, and cause several illnesses. Those heavy metals with the capability of inducing reactive oxygen and oxidative stress can play an important role in the occurrence and development of malignant tumors. Exposure to heavy metals showed to be linked to high incidence rates of cardiovascular diseases as well. Epidemiological studies showed that exposure to Cd increased the risk of cardiovascular diseases, and low level of cadmium exposure is one of the decisive factors in cardiovascular mortality. Lead pollution is also considered a risk factor for cardiovascular diseases. It has been shown that in third world countries, high blood lead level increased the mortality rate. Chronic exposure to Pb showed to cause an increase in blood pressure in mice and led to the weakening of myocardial velocity function. Considering these data, it can be concluded that Cd and Pb can be considered the main heavy metal pollutants affecting human health. Elevated homocysteine level in human body affects the body in different ways, it can accelerate oxidation, aging, damage to arteries, weakening the immune system and speeding up brain aging. Studies have shown that increased homocysteine levels can lead to a proliferation of vascular smooth muscle cells and damage the cardiovascular system.

Hence, high blood lead and cadmium levels along with high homocysteine level can negatively affect cardiovascular and nervous systems. There isn’t a lot of information available on the effects of non-occupational exposure to heavy metals in polluted cities in China, and very few studies have addressed the possible correlation between heavy metals levels in the blood with that of homocysteine levels.
mocysteine. Here in this study, we evaluated the possible correlation between the non-occupational exposure to lead and cadmium and homocysteine expression level.

### Patients and Methods

#### People and Investigation Content

Under the condition of informed consent, we prepared unified questionnaires. 159 individuals with no history of close exposure to heavy metal and no history of metabolic diseases were surveyed in this study. Questionnaires contained basic questions, such as gender, age, height, weight, body mass index (BMI), date of birth and occupation. There were also questions about life style, including the history of smoking and drinking.

#### Metal Level Measured by ICP-MS

Fasting whole blood samples were collected and immediately stored at -80°C. 0.5 ml of blood and Milli-Q ultrapure water was used to prepare a multi-element standard solution, tuned liquid and internal standard element solution (Agilent, Santa Clara, CA, USA). Whole blood quality control samples with high installation and low concentration (UTEK, Tampa, Florida, USA); and inductive coupling plasma mass spectrometry (ICP-MS, Agilent, Santa Clara, CA, USA) was used.

#### Homocysteine Detection

Fasting blood samples were collected in EDTA-containing tubes and were analyzed within 1 hour after collection. We used a Hitachi 7100 Automatic Analyzer device and homocysteine kit for enzymatic method (Mindray Biology, Mahwah, NJ, USA), with a wavelength of 660 nm.

#### Statistical Analysis

We analyzed information about blood lead and cadmium levels, homocysteine level, information about gender, age and history of smoking and drinking collected from all respondents. Blood lead and cadmium levels did not show a normal distribution. Therefore, we evaluated the blood lead and cadmium levels using the median method. We used the average values for plasma homocysteine level. Rank-sum test (M-W U test) of two independent samples was used and differences in smoking, drinking and gender were compared. Independent sample t-test was conducted and differences in blood lead and cadmium levels as well as homocysteine level were studied. p<0.05 was considered statistically significant.

### Results

Median blood lead levels were 35.91 and 27.10 μg/dL in males and females respectively. The difference between males’ and females’ blood lead levels was statistically significant (p=0.016). Median blood cadmium levels were 1.11 and 0.72 μg/dL in males and females respectively. The difference between males’ and females’ blood cadmium levels was statistically significant (p=0.007) as well. Smokers’ blood lead level was obviously higher than non-smokers, and the difference was statistically significant (p=0.000). The median blood lead levels were 39.77 and 28.51 μg/dL in non-smokers and smokers respectively. Median blood cadmium levels were 2.58 and 0.72 μg/dL in smokers and non-smokers respectively. Smokers’ blood cadmium level was obviously higher than non-smokers and the difference was statistically significant (p=0.000). Median blood lead and cadmium levels in drinkers were 33.72 and 0.94 μg/dL respectively, while these levels were recorded at 30.19 and 1.03 μg/dL for non-drinkers. Differences in the levels of lead and cadmium between drinkers and non-drinkers had no statistical significance. Average homocysteine plasma level was meaningfully higher in smokers, and the difference was statistically significant. Average homocysteine plasma levels were 11.87 and 13.34 μg/dL in non-smokers and smokers respectively (Table I).

We divided respondents into four groups by quartile method according to their blood lead and cadmium levels, and the differences in plasma homocysteine levels in different groups were compared. Homocysteine level in the group with the highest level of lead was considerably higher than that in the group with the lowest lead level, and the difference was statistically significant (p=0.007) (Table II). Age differences had no statistical significance. Comparison of homocysteine levels between the highest and lowest Cd levels groups did not reveal any statistically significant differences (p=0.076); however, the average homocysteine value in the group with higher cadmium level was higher than that of the group with lower cadmium level (Table III) (Figure 1A).

Our results revealed that blood lead level was positively correlated to homocysteine level. The correlation coefficient of Spearman’s rho was...
0.211 and \( p=0.007 \). Additionally, age was also correlated to homocysteine level, and the correlation coefficient of Pearson’s Correlation was 0.216 and \( p=0.006 \). Blood cadmium level did not correlate with homocysteine level (Table IV).

### Table I. Lead, cadmium and homocysteine of crowd characteristics.

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MEDIAN (interquartile range)</td>
<td>MEDIAN (interquartile range)</td>
<td>p-value</td>
</tr>
<tr>
<td>Male/Female</td>
<td>35.91 (23.45-48.77)</td>
<td>1.11 (0.55-2.75)</td>
<td>0.016</td>
</tr>
<tr>
<td>Smoking/ non-smoking</td>
<td>39.77 (29.09-56.81)</td>
<td>2.58 (0.95-5.36)</td>
<td>0.000</td>
</tr>
<tr>
<td>Drinking/ non-drinking</td>
<td>33.72 (21.97-46.65)</td>
<td>0.94 (0.40-2.28)</td>
<td>0.351</td>
</tr>
</tbody>
</table>

### Discussion

Heavy metals are known as major contributors to several acute and chronic diseases. Exposure to heavy metals has been linked closely to cardio-

![Figure 1](image-url)

**Figure 1.** Respondents were divided into four groups by quarterback method according to the lead and cadmium level in blood, with concentration from low to high. Homocysteine level in different groups were compared. (A) Shows that the homocysteine level in the group with the highest lead level (Group 4) was higher than that in the group with lowest lead level \( (p=0.007) \). (B) Shows that the homocysteine level in the group with the highest blood cadmium level (Group 4) might be higher than that in the group with lowest cadmium level \( (p=0.0074) \).

### Table II. Homocysteine level in four groups with lead quarterback.

<table>
<thead>
<tr>
<th></th>
<th>Quartile 1 [40]</th>
<th>Quartile 2 [40]</th>
<th>Quartile 3 [40]</th>
<th>Quartile 4 [39]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lead level</td>
<td>18.46 (5.80-22.39)</td>
<td>27.80 (22.4-33.10)</td>
<td>38.06 (33.11-46.42)</td>
<td>75.98 (46.43-239.6)</td>
<td></td>
</tr>
<tr>
<td>Median (interquartile range)</td>
<td>11.24 (3.93)*</td>
<td>12.14 (4.95)</td>
<td>12.85 (4.76)</td>
<td>13.55 (3.45)*</td>
<td>0.007</td>
</tr>
<tr>
<td>Homocysteine, mmol/L</td>
<td>9.33 (11.65)</td>
<td>50.65 (11.18)</td>
<td>50.08 (10.48)</td>
<td>53.31 (9.54)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The homocysteine level of group with the highest lead level was higher than that of group with lowest lead level \( (p=0.007) \)
Effects of blood lead and cadmium levels on homocysteine level in plasma

vascular and nervous system diseases. It has been shown that exposure to lead increased plasma homocysteine level in individuals with occupational exposure to lead. However, the number of studies conducted on nonoccupational exposure to lead is rare. Damages caused by exposure to heavy metals are widespread and even small quantities of these metals have been proven to be dangerous. To fully assess the effects of heavy metals on the human body, we should not limit our focus on occupational exposure, and it is more productive to study the effects of heavy metals on ordinary people at the same time.

Our results showed that the levels of lead and cadmium were different in males and females and males had higher levels of lead and cadmium in their blood. It has been shown that in some cases, blood lead level in non-occupational exposure population was even higher than that of occupational exposure population. This study suggested that blood lead levels and plasma homocysteine levels were positively correlated. Our findings showed that homocysteine level in the group with the highest lead level was obviously higher than that of the group with lowest lead level. Blood lead, cadmium, and homocysteine levels in the smokers were significantly higher than non-smokers, and this may be one reason, among others, that smoking is harmful to human health. It has been shown that an increase in lead, cadmium or homocysteine levels in the blood could increase the risk of cardiovascular disease.

A prior study showed that cadmium inhibited the proliferation of epidermal cells that can lead to atherosclerosis. Results obtained from another study illustrated that beside their direct action on blood vessel wall cells, cadmium and lead also could be absorbed by immune cells. Results of a USEPA survey conducted on occupational exposure to lead, revealed that the increase in blood lead level caused an increase in systolic blood pressure, which is another contributor cardiovascular complications. In 1991, Clarke et al came up with the idea that homocysteine disease is an independent risk factor for coronary heart disease and other cardiovascular diseases. Lead, cadmium and homocysteine may be also involved in nervous system diseases. Results obtained from a prior study conducted on embryonic neural stem cell (NSC) cultures in the presence of various concentrations of lead (0.01 to 10 μM) revealed that although NSC survived the different concentrations of lead, their differentiation toward spongiocyte was interrupted. In another study, rat brain cortical neurons were placed in serum-free culture medium with 10 μM cadmium, and this exposure to Cd triggered apoptosis in neurons. It has been illustrated that long-term and low-level exposure to cadmium would damage higher nervous activities and negatively affect learning and memory. Increasing levels of blood homocysteine were shown to be the independent risk factor for Alzheimer's disease, which was related to rapid cognitive loss. Medications that can reduce the homocysteine level, such as vitamin B12 and folic acid, can alleviate the cognitive damage and delay early dementia in stroke and dementia victims.

**Conclusions**

In this study, we found that an increase in blood lead level can increase homocysteine level. However, an increase in cadmium level can only incre-

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**Table III.** Homocysteine level in four groups with cadmium quarterback.

<table>
<thead>
<tr>
<th>Blood cadmium level</th>
<th>Quartile 1(40)</th>
<th>Quartile 2(40)</th>
<th>Quartile 3(40)</th>
<th>Quartile 4(39)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (interquartile range)</td>
<td>0.17 (0-0.46)</td>
<td>0.75 (0.47-0.96)</td>
<td>1.44 (0.97-2.17)</td>
<td>5.94 (2.18-19.79)</td>
<td></td>
</tr>
<tr>
<td>Homocysteine, mmol/l [mean (SD)]</td>
<td>11.82 (3.24)*</td>
<td>12.14 (4.82)</td>
<td>12.40 (4.72)</td>
<td>13.41 (4.50)*</td>
<td>0.074</td>
</tr>
<tr>
<td>Age [mean (SD)]</td>
<td>54.85 (13.55)</td>
<td>48.28 (9.54)</td>
<td>50.58 (9.87)</td>
<td>49.56 (8.56)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The homocysteine level of group with the highest blood cadmium level might be higher than that of group with lowest cadmium level (p=0.0074).

**Table IV.** Correlation among homocysteine, age, lead and cadmium levels.

<table>
<thead>
<tr>
<th>Association with HCY</th>
<th>Pearson’s Correlation</th>
<th>Spearman’s rho Correlation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.216</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.130</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>0.211</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Note: The homocysteine level of group with the highest blood cadmium level might be higher than that of group with lowest cadmium level (p=0.0074).
ause the probability of a surge in homocysteine level. Therefore, we speculated that the accumulation of heavy metals such as lead and cadmium could inflict damages to human's cardiovascular and nervous systems, which triggers a surge in homocysteine level. Use of bigger sample sizes in future can help us to further study the health implications of heavy metal. For now, it seems that improving living habits and reducing smoking is the best way to reduce the level of heavy metals in the body.

Conflict of interest
The authors declare no conflicts of interest.

References
12) Steffensen IL, Mesna OJ, Andruhow E. Cytotoxicity and accumulation of Hg, Ag, Cd, Cu, Pb and Zn in human peripheral T and B lymphocytes and monocytes in vitro. Gen Pharmacol 1994; 8: 1621-1633.