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Leptin and IL-8: Two novel cytokines screened out in childhood lead exposure

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HIGHLIGHTS

- Children were the vulnerable victim of lead poison, and the central nervous system is the main target.
- 88 children (44 high BLL vs. 44 low BLL) from lead poison area were collected.
- Novel cytokines were detected by RayBio® Human Cytokine Antibody Array and validated by enzyme-linked immunosorbent assay.
- We report two cytokines which may help us to further find the mechanisms of lead-induced neurotoxicity.

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ABSTRACT

Lead is a toxic heavy metal with many recognized adverse health side effects. The central nervous system is the main target of lead toxicity. Although many studies on lead toxicity were conducted, the mechanism of lead toxicity remains uncertain. One possible attribution is the immature blood–brain barrier that causes lead exposure in children. Few studies have investigated the cytokine changes caused by this exposure. Novel cytokines were detected by RayBio® Human Cytokine Antibody Array and validated by enzyme-linked immunosorbent assay. Several children were admitted to West China Second University Hospital, after a serious lead pollution event in longchang, Sichuan, China. A total of 4 children with elevated blood lead levels (BLLs) and 4 children with low BLLs were randomly chosen in the discovery set, and 40 children with elevated BLLs and 40 children with low BLLs were included in the validation set. Leptin and interleukin-8 (IL-8) were identified to be significantly different between children with elevated and low BLLs via RayBio® Human Cytokine Antibody Array. In the validation set, IL-8 was higher in children with elevated BLLs [median(P_{25}–P_{75}), 117.69(52.31–233.63) pg/mL] than in children with low BLLs [median(P_{25}–P_{75}): 17.70(10.75–26.52) pg/mL] ($p = 0.000$). Leptin was lower in children with elevated BLLs [median(P_{25}–P_{75}): 1658.23(1421.86–2606.55) pg/mL] than in children with low BLLs [median(P_{25}–P_{75}): 4168.68(3246.32–4744.94) pg/mL] ($p = 0.000$). In children with low BLLs, leptin was higher in children with BLLs < 3 μg/dL ($N = 7$) [median(P25–P75): 7220.86(4265.72–7555.15) pg/mL] than in children with BLL ≥ 3 μg/dL ($N = 33$) [median(P_{25}–P_{75}): 4103.86(3163.40–4678.34) pg/mL] ($p = 0.026$); IL-8 was significantly different in children with BLL < 4 μg/dL ($N = 13$) [median(P_{25}–P_{75}): 12.49(8.25–14.86) pg/mL] than in children with BLL ≥ 4 μg/dL ($N = 27$) [median(P_{25}–P_{75}): 21.98(13.64–33.50) pg/mL] ($p = 0.013$). The results defined specific changes in cytokine expressions to lead exposure, which can be used to explore the mechanism of lead toxicity and monitor lead exposure.

1. Introduction

Lead is a highly toxic heavy metal primarily derived from human activities, such as mining, manufacturing, and burning fossil fuels (Sanders et al., 2009). The toxic effects of lead include neurotoxicity, as well as developmental, reproductive, hematological, and cardiovascular effects (Al-Neamy et al., 2001; Gidlow, 2004; Kuo et al., 2001; Martin et al., 2006; Sanders et al., 2009; Weaver et al., 2005). Among these toxic effects, the neurotoxic effect is dominant. Lead exposure is a common health concern for people of all ages, especially children (Sanders et al., 2009). Given that the main target of lead toxicity is the central nervous system (CNS), studies on lead toxicity focused on the brain. Epidemiological studies have shown

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the association between blood lead levels (BLLs) and IQ in children (Baghurst et al., 1992; Lanphear et al., 2005; Schwartz, 1994).

Although various tissues (e.g., bone, tooth, hair, and nail) and fluids (e.g., blood and urine) have been used to test for lead exposure, the diagnosis of lead poisoning has traditionally relied on measuring blood lead (Roots, 1979; Sanders et al., 2009). U.S. Centers for Disease Control (CDC) established 10 μg/dL lead concentration in blood as the limit for exposure in children (Roper et al., 1991). Zero BLL is considered the safe level in children.

Studies have shown that lead exposure causes a significant shift in the production of many cytokines (Hayashi et al., 2005; Iavicoli et al., 2006; Singh et al., 2003; Tian and Lawrence, 1995). The production of IFN-γ and TGF-β was reduced in rats and adult humans. By contrast, the production of IL-4, IL-6, and IL-10 was elevated. Although children are highly susceptible to lead exposure, few studies have investigated the cytokine changes caused by lead exposure in children. RayBio® Human Cytokine Antibody Array is the first commercially available protein array system (Huang, 2007; Huang et al., 2005; Huang et al., 2010). Using the RayBiotech system, the expression profiles of multiple cytokines can be rapidly and accurately identified in several hours. To date, the application of antibody microarrays to search for the plasma cytokines of lead poisoning remains unassessed.

We select novel cytokines from children suffering from lead poisoning by using antibody microarray techniques.

2. Materials and methods

2.1. Study population

New cases of lead poisoning have been reported in many cities of China since August, 2009 (Ji et al., 2011). Longchang, one of the cities in Sichuan, China, which was reported about several lead poisoning events. Children who lived near the lead refine factory came to our hospital for examination. According to the guidelines of U.S. CDC, elevated BLLs were defined as greater than 10 μg/dL (Roper et al., 1991). And finally, a total of 88 unrelated healthy children were included. A total of 44 children with BLL ≥10 μg/dL and 44 children with BLL < 10 μg/dL were recruited into the elevated and low BLL groups, respectively. Among the 88 children enrolled in this study, 4 children with elevated BLLs (3 boys and 1 girl) and 4 children with low BLLs (2 boys and 2 girls) were randomly chosen in the discovery set; by contrast, 40 children with elevated BLLs (23 boys and 17 girls) and 40 children with low BLLs (21 boys and 19 girls) were included in the validation set. During hospitalization of the child, the parents were interviewed about basic, behavioral and environmental factors from children were collected. The fasting venous blood samples of all subjects were obtained after a 30 min rest in a sitting position. The samples were collected in a vacuum tube (BD, USA) containing heparin lithium for lead. Another blood sample was collected in a vacuum tube (BD, USA) containing EDTA and then centrifuged; plasma was removed and frozen at −80 °C via a single freeze–thaw cycle.

2.2. Basic characteristics of studied children

Table 1 lists the characteristics of the recruited children. The age distribution was similar among children with elevated and low BLLs in the discovery and validation sets.

2.3. Ethics statement

Written informed consents were obtained from all parents; the study was approved by the Institutional Ethics Committee of West China Second University Hospital, Sichuan University, Chengdu, China.

2.4. Blood lead test

BLL was measured by flame atomic absorption spectroscopy using BH-2100-type tungsten boat atomic absorption spectrometer (Beijing Bohui Innovation Technology Co. Ltd., Beijing, China). Daily internal quality control was performed using Norway Seronorm TM trace elements whole blood quality control (Model No. LOTMR9607).

2.5. Human Cytokine Antibody Array

The collected plasma was analyzed using the RayBio® Human Cytokine Antibody Array G series 2000 for detection of 174 human cytokines.

2.6. Cytokine assay

Cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA). IL-8 was detected by a commercially available specific ELISA kit (NeoBioscience Technology Co., Ltd., China), and leptin was tested by an ELISA kit (Wuhan Boster Company, China). Briefly, an eight-point calibration curve was prepared by diluting the standard solution provided in the kit. The absorbance of each sample was measured against the blank at 450 nm with an ELISA plate reader (Rayto RT-6100, Rayto Corporation, Shenzhen, China). Results were plotted against the calibration curve to obtain the amount of leptin and IL-8.

2.7. Statistical analysis

Significance analysis of microarrays (SAM), a statistical technique for determining the statistical significance of the changes in gene expression, was conducted. SAM results show that the differences in cytokines and other protein expressions detected by RayBio® Human Cytokine Antibody Array between children with elevated and low BLLs were significant. The changes were statistically significant when the SAM score was above 1.5 and when the signal intensity increased ≥1.5-fold or decreased ≤0.65-fold ($p < 0.05$). The clustering analysis method was used to analyze the data on Human Cytokine Antibody Array. The differences in age, BMI and BLL between children with elevated and low BLLs were compared using t test, while difference in gender using Chi-square test. The correlation between selected cytokines and age, BMI and BLL using Pearson correlation; between distribution of gender using Spearman rank correlation. Multivariate Logistic regressive analysis is performed to find the relationship between the selected cytokines and basic characteristics. The levels of leptin and IL-8 were presented as median (P_{25}–P_{75}), and All analyses were performed using SPSS 17.0 (Chicago, IL, USA); statistical significance is denoted by $p < 0.05$.

3. Results

3.1. Basic characteristics of studied children

Table 1 lists the characteristics of the recruited children. In the discovery set, the average age of children with elevated BLLs (48.50 ± 5.00 mos) has insignificant difference compared with those with low BLLs (49.03 ± 11.03 mos) ($t = 0.029$, $p = 0.978$). The mean BLL of children in the discovery set with elevated BLLs (19.93 ± 2.22 μg/dL) was higher than those with low BLLs (5.75 ± 1.35 μg/dL) ($p < 0.001$). In the validation set, the average ages of children with elevated and low BLLs were 64.8 and 54 mos ($p > 0.05$), respectively. The average BMI (body mass index, BMI) of children with elevated BLLs (14.84 ± 1.565 kg/m^2) has insignificant difference compared with those with low BLLs(15.23 ± 1.59 15.23 ± 1.59 kg/m^2) ($p > 0.05$).The mean BLL of children with elevated BLLs (15.61 ± 7.05 μg/dL) was significantly different from those with low BLLs (5.04 ± 1.84 μg/dL) ($p < 0.001$).

3.2. Identification of novel cytokines of lead exposure

The plasma samples from four children with low and elevated BLLs (Nos. 1–4 and 5–8, respectively) were spotted on the membranes comprising 174 cytokines (www.raybiotech.com). The cytokines were analyzed by cluster analysis to detect the differences in concentrations between the two groups (Fig. 1). Comparison of the SAM score reveals two cytokines, namely, leptin (SAM score, −2.10; change in signal intensity, 0.21) and IL-8 (SAM score, 1.80; change in signal intensity, 2.35). These cytokines were identified to be significantly different between children with elevated and low BLLs. Fig. 2 shows the signal intensities of IL-8 and leptin.

3.3. Validation of changes in leptin and IL-8 by ELISA

Given that cytokine microarray analysis was performed using the plasma samples in the discovery set, we decided to validate the two pre-selected cytokines (IL-8 and leptin) by ELISA in additional 40 children with elevated BLLs and 40