Nine kinds of novel cytokines identified in children with lead exposure

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Abstract. Lead (Pb) is a neurotoxic heavy metal element with many recognized adverse health side effects, and its main target of lead toxicity is the central nervous system. The mechanism of lead toxicity is still uncertain. However, there are few studies investigated the cytokines changes caused by lead exposure in children. The BLLs was quantified using flame atomic absorption spectroscopy. The novel cytokines were detected by RayBio@ Human Cytokine Antibody Array. A total of 4 children with elevated blood lead levels (BLLs) and 4 children with low BLLs were chosen in the study. Volcano plot analysis was performed to identify significant proteins, with the criteria: P value ＜0.05 and log2 fold change ＞1. The mean BLLs of children with elevated BLLs (5.675±1.018 μg/dL) has significant difference compared with those with low BLLs (1.975±0.3966 μg/dL) (P=0.0148, t=3.385). And 9 kinds of novel cytokines were identified. The expression of IL-6, IL-8 and IL-17 was significantly up-regulated, while the expression of BDNF, BMP-4, IGF-1, IL-7, IL-10 and Leptin was significantly down-regulated.

1. Introduction

Lead (Pb) is a neurotoxic heavy metal element derived from anthropogenic activities, including mining, manufacturing, fabrication of batteries, burning fossil fuels and many other human industrial activities[1-2]. Children are the main victims of lead exposure because of more hand-mouth activities, higher lead absorption rate in the digestive tract, lower lead excretion ability than adults, and undeveloped blood-brain barrier[3]. The epidemiological survey of children's blood lead levels (BLLs) showed that the average rate of children with lead poisoning was high[4-5]. It can be seen that the lead exposure of children in the world cannot be ignored. According to epidemiological Meta-analysis, for every 10ug/dl increase in blood lead level, IQ will decrease by 2-3 points[6]. Studies have found that when the blood lead concentration is <5ug/dl, children's cognitive abilities are impaired, and children's arithmetic and reading performance are negatively correlated with blood lead concentration[2-3,7]. These studies show that there is no safe blood lead threshold in the human body. Low blood lead levels can also lead to impairment of children's IQ and cognitive abilities. Even if children who have been diagnosed with lead poisoning are no longer exposed to lead exposure, there is still no significant improvement in IQ after effectively reducing their blood lead levels.

Although many biomarkers, such as bone lead and blood lead, have been used to monitor lead exposure, none of the biomarkers that can organically link cumulative lead exposure and lead toxicity has been generally recognized in the industry. Due to the availability of blood lead level, blood lead level has become the most widely used lead exposure monitoring indicator. In 2012, CDC recommended setting the blood lead levels at 5μg/dL[8]. Recently, a new blood lead levels threshold of 3.5μg/dL was updated by the CDC in October 2021[9]. There is no safe level of lead in the blood. The blood lead level of zero is considered safe.

In the study, we aimed to identify novel cytokines change in children with lead exposure based on the new updated blood lead level of CDC.

2. Methods

2.1. Study population

For the study，8 healthy children aged 1 to 5 years were recruited. These children were divided into two groups-elevated BLLs group (BLLS≥ 3.5μg/dL, n=4) and low BLLs group (BLLS＜3.5μg/dL, n=4). All information on basic demographics, behavioral and environment risk factors were collected by interviews with parents. After testing of 30 min, a venous blood sample was obtained of every subject of the study. The peripheral blood samples (5mL) were collected into vacuum tubes (BD, USA) containing heparin lithium at the baseline for lead. An-

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other peripheral blood sample was drawn into a vacuum tube (BD, USA) containing EDTA, centrifuged for 15 min to collect plasma, and stored at -80℃.

2.2. Blood lead levels (BLLs)

After samples collection, BLLs was quantified using flame atomic absorption spectroscopy (BH-2100 atomic absorption spectrometer, Beijing, China). Calibration and quality control were run daily according to the manufacturer's recommendations.

2.3. Human cytokines antibody array

The RayBio Human Cytokine Antibody Arrays G Series 2000 kit (RayBiotech, Norcross, GA) with detection of 174 human cytokines were used to analyze the collected plasma of recruited children.

2.4. Ethical standards

Written informed consent was obtained from all parents. The overall study plan was approved by the Ethical Committee of Zibo Central Hospital.

2.5. Statistical analysis

Volcano plot analysis was performed to identify significant proteins, with the criteria: *P* value ＜0.05 and log2 fold change ＞1. The T-test was used to compare statistical differences of age, Body Mass Index (BMI), BLLS and selected cytokines between elevated BLLs children and low BLLs children. All statistical analyses were performed with the software Graphpad Prism 7.0. Statistical significance was denoted as follows: *P* value＜0.05.

3. Results

3.1. The basic characteristics of enrolled children

The basic characteristics of enrolled children in the study are shown in Table 1. There was no statistically significant difference in mean age, gender and BMI between children with elevated BLLs and those with low BLLs. The mean BLLs of children with elevated BLLs (5.675±1.018 μg/dL) has significant difference compared with those with low BLLs (1.975±0.3966 μg/dL) (*P*=0.0148, *t*=3.385).

Table 1. The basic characteristics of enrolled children

	elevated BLLs (N=4)	low BLLs (N=4)	*P*
Age (month)	39.00±8.916	37.00±9.065	0.8802
Gender(boys/girls)	1/3	2/2	-
BMI（Kg/m^2）	15.85 ±0.6262	16.02 ±1.206	0.9054
BLLs (μg/dl)	5.675±1.018	1.975 ±0.3966	0.0148

3.2. Identification of abnormal expression of cytokines

After analyzed, a comparison of volcano plot analysis in Fig.1 revealed that there were 3 significantly up-regulated cytokines including IL-6, IL-8 and IL-17, and 6 significantly down-regulated cytokines including BDNF, BMP-4, IGF-1, IL-7, IL-10 and Leptin. And then, the data of identified cytokines was run by the method of hierarchical clustering analysis (Fig.2)

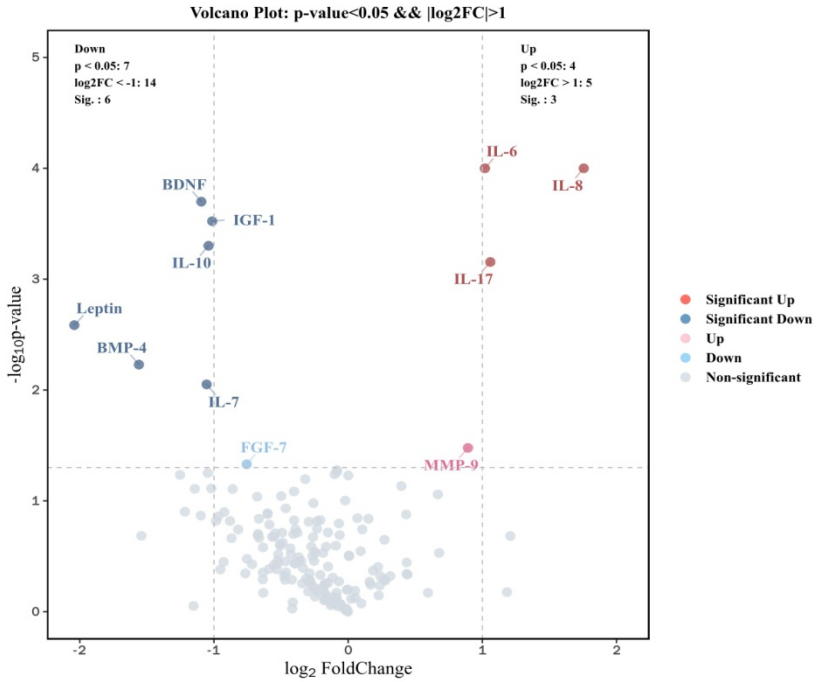

Fig. 1. The 174 cytokines were analyzed by volcano plot analysis with the criteria: P value ＜0.05 and log2 fold change ＞1.

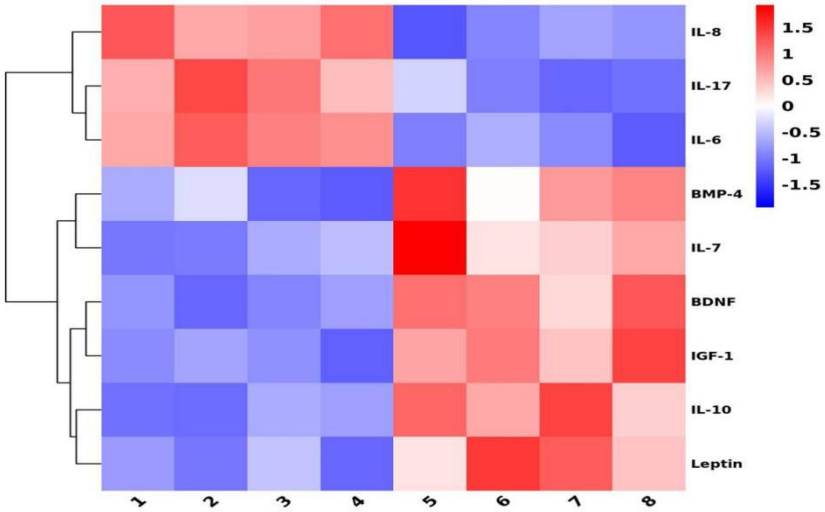

Fig. 2. Hierarchical clustering analysis was used to analyze the 9 identified cytokines between children with elevated blood lead levels (BLLs) (Nos. 1–4) and children with low BLLs (Nos. 5–8)

4. Discussion

The mechanisms of lead neurotoxicity are complex and unclear. Studies have confirmed that lead can affect cognitive function by affecting neurotransmitter systems and calcium channel systems[1-3]. It shows that the formation of lead neurotoxicity involves changes in the function of various special or key proteins related to the nervous system and the changes in the interaction between these proteins. Recent studies have also reported that lead exposure can lead to changes in some cytokines. In studies on rats, rabbits and adults, it was found that lead exposure can lead to a decrease in the levels of cytokines Leptin, IFN-γ and TGF-β, while significantly increasing the levels of cytokines IL-4, IL-6, IL-8 and IL-10[1,3,10]. As children are the main victims of lead exposure, the changes in proteins such as cytokines in children caused by lead exposure have not been studied according to the new updated blood lead level of CDC. While in our study, 3 cytokines namely IL-6, IL-8 and IL-17 were significantly up-regulated, and 6 cytokines namely BDNF, BMP-4, IGF-1, IL-7, IL-10 and Leptin were significantly down-regulated.

Proteins are the real executors of life activities and the real bearers of the occurrence and development of diseases. Direct research on the executive bodies of life activities-proteins and their expression patterns can be more directly related to the functions of genes, and can be closer to revealing the details of life activities. Proteomics is the study of the composition and activity of proteins in cells at the overall level. Protein chip technology can well meet the requirements of comprehensive proteome research, and it has the function of studying the interaction between proteins and a large number of potential related molecules. Therefore, this study intends to use the protein chip method to screen the plasma differential proteins between children with high blood lead and low blood lead, in order to find and screen the signaling proteins related to the mechanism of lead toxicity.

5. Conclusions

In the study, we identified 9 novel cytokines change in children with lead exposure based on the new updated BLLs of CDC. Of the 9 cytokines, expressions of 3 cytokines including IL-6, IL-8 and IL-17 were significantly up-regulated, while expressions of 6 cytokines including BDNF, BMP-4, IGF-1, IL-7, IL-10 and Leptin were significantly down-regulated.

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