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Original Article

Correlation between serum IGF-1 and blood lead level in short stature children and adolescent with growth hormone deficiency

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Abstract: This study aimed to investigate correlation between serum insulin-like growth factor-1 (IGF-1) and blood lead level in short stature children with growth hormone deficiency (GHD), and IGF-1 signal molecules were investigated in lead exposed rats. Our findings may provide evidence for clarifying pathogenesis of lead induced short stature in children. Methods: 880 short stature children were recruited from clinics and divided into GHD group and idiopathic short stature (ISS) group according to the GH peak in growth hormone stimulation test. The height, body weight, serum IGF-1 level and blood lead level were determined. A rat model of lead poisoning was used to establish and western blot assay was employed to detect the phosphorylation of signaling molecules (MAPK and PI3K/Akt) related to IGF-1 signaling pathway. Results: In GHD group, the height, body weight and serum IGF-1 level were significantly lower, but the blood lead level was significantly higher than those in ISS group ($P<0.05$). Western blot assay confirmed that the protein expression of phosphorylated ERK1/2, JNK, p38, Akt473 and Akt308 increased significantly ($P<0.01$) in lead exposure rats. Conclusion: Our study suggesting that reduction in IGF-1 in children with GHD is associated with blood lead level. Lead exposure may induce expression of phosphorylated MAPK and Akt signaling molecules. The activation of these molecules may influence binding of IGF-1 and tyrosine kinase receptor IGFIR to regulate cell growth via the MAPK and Akt signaling pathways, which then interfere with growth-promoting effect of IGF-1 in short children.

Keywords: Growth hormone deficiency, insulin-like growth factor I, lead exposure animal model, short stature, signaling pathway

Background

According to world health organization (WHO) report in 2000, the incidence of short stature in developing countries is as high as 32.5% [1]. Some factors have been found to influence the child growth and the mechanisms are complex. Generally, the growth is affected by genetics, diseases, nutrition, etc. Its occurrence is closely related to the integrity and coordination of growth hormone and hormones in thyroid hormone axis and gonadal axis. Growth hormone deficiency (GHD) is an important cause of short stature, and children with GHD account for 6-10% of short children. To understand the pathogenesis of short stature will help to improve the diagnosis and treatment of short stature [2].

Children are susceptible to lead poisoning, which may cause cognitive impairment and abnormalities in psychology and behaviors, and usually harm the growth and development of children [3]. The height reduces by 1.3 cm if the lead level increases by 0.483 μmol/L, suggesting that lead poisoning has significantly influenced the height of children [4]. As a known environmental toxic, lead has been an invisible

killer harming the health of children, and leading poisoning has been a major risk factor of public health and an important social issue.

Children have a high lead absorption rate, and the lead is often excreted once it enters human body, causing cumulative injury to the body. Currently, the mechanism underling the lead exposure induced injury is still poorly understood. Thus, to investigate the changes in serological parameters and the toxicity of lead in children with lead poisoning is important for exploring the toxicity of lead exposure [5]. Insulin-like growth factors (IGFs) not only mediate the growth-promoting effect of growth hormone (GH), but also exert extensive biological effects via IGF-1 receptor. It has been revealed that IGF-1 is not only indispensable for development of brain, but also able to inhibit cell apoptosis, which is beneficial for functional recovery of injured neurons. Thus, IGF-1, which is protective on some neurological diseases, has been paid attention to by investigators [6].

In our hospital, we screened short children in clinics and they were divided into primary GHD children and idiopathic short stature (ISS) children according to the findings in growth hormone stimulation test (intravenous arginine hydrochloride and oral clonidine). Serum IGF-1 and blood lead level were detected and compared between two groups. The correlation of serum IGF-1 with blood lead level was evaluated. In addition, a lead exposure rat model was established, and the phosphorylation of molecules in the IGF related signaling pathway was determined. Our findings may provide evidence for the elucidation of pathogenesis of lead induced short stature and measures for the prevention and treatment of short stature.

Patients and methods

Source of patients

From June 2011 to March 2013, 880 short children were recruited from the Department of Pediatrics of Xijing Hospital. There were 518 males and 362 females, and the age ranged from 1 year to 18 years.

Inclusion criteria for short stature

According to the height and body weight distribution in children aged 0-18 years, which is determined in the children's physical development survey performed in 9 provinces/cities in 2005, short stature was diagnosed. The height and body weight of short children was 2 standard deviations shorter than that in age, gender and race matched children (Height and weight standardized growth charts for Chinese children and adolescents aged 0-18 years in 2009) [7]. The height of recruited children was balanced, patients had normal intelligence and the bone age was 2 or more years younger than that in health children. Children with abnormal liver and kidney function, hypothyroidism, small gestational age, Turners syndrome, premature, genetic diseases or metabolic diseases were excluded [8].

Test and detection

Growth hormone stimulation test was performed in fasting children in the morning. In brief, 20% arginine (2 ml/kg) was dissolved in normal saline (3 ml/kg), and this solution was intravenously administered within 30 min. At the same time, clonidine was orally given. Before treatment and at 30, 60, 90 and 120 min after treatment, venous blood was collected for detection. Children were monitored during this test. Automatic chemiluminescence assay (e601, Roche) was employed to detect the GH in these children according to manufacturer's instructions, and the quality was controlled. IGF-1 level was measured with radioimmunoassay kit (DIAsource ImmunoAssays, Belgium) according to manufacturer's instructions. Atomic absorption spectrophotometer (Shimadzu, Japan) was used to detect the blood lead of these children.

Diagnostic criteria

It is still a controversial question how to conduct the GH stimulation test. The GHRH plus arginine test combining IGF-I test or insulin tolerance test (ITT) is preferred in foreign countries recently. While clonidine plus arginine test is applied to conduct GH stimulation test in our hospital up to now. Patients with thyroid dysfunction, a familial history of delayed puberty and chromosomal abnormalities, familial short stature, intrauterine growth retardation (IUGR) and small gestational age were removed. The diagnosis of short stature was done according to following criteria: 1) The GH peak was measured after growth hormone stimulation test. When the GH peak was <10 ng/ml, the bone

Table 1. Distribution of children in GHD group and ISS group

Age	GHD group		ISS group	
	Female	Male	Female	Male
1	3		2	
2	1		1	
3	0	3	1	1
4	5	3	6	6
5	9	17	9	17
6	15	16	15	19
7	7	14	13	14
8	6	26	17	22
9	16	23	30	25
10	17	33	36	21
11	20	26	35	41
12	9	28	36	33
13	6	21	16	36
14	2	13	13	23
15	3	6	6	15
16	2	5	3	2
17	1	2		1
18	1	3		3
Sum	123	239	239	279

age was ≤2 years younger than the normal, the annual increment of height was <4 cm and cranial CT or MRI excluded intracranial tumor, children were diagnosed with primary GHD. 2) When the height was 2 standard deviations lower than the normal, the annual increment of height was <4 cm, the height and body weight at birth were normal, there was no chronic disease or skeletal developmental disorders, bone age was younger or normal, the karyotype was normal and the GH peak was ≥10 ng/ml, children were diagnosed with ISS [9, 10].

Establishment of the lead exposure rat model

Animals and grouping: Male Sprague Dawley (SD) rats (21 days old; n=80) weighing 50-70 g were purchased and housed in a calm environment for 48 h, and adverse stress was avoided. The temperature, humidity and illumination were consistent with the Guidelines on the Care and Use of Animals for Scientific Purposes. Animals were randomly assigned into 4 groups: control group (n=20), 100 ppm lead exposure group (n=20), 200 ppm lead exposure group (n=20) and 300 ppm lead exposure group (n=20). Animals were given ad libitum access to food and water. The $C_4H_6O_4Pb{\cdot}3H_2O$ at 500 ppm was diluted into 100 ppm, 200 ppm and 300 ppm lead solution which was then used as a drink for rats. The volume of this solution drunk by these animals was monitored. In the control group, deionized water was used as a drink for rats. Animals were housed independently and treatment was performed for 6 weeks.

Detection of blood lead level

At different time points (1, 2, 3, 4, 5 and 6 weeks), the blood was collected from the tail vein and the blood lead was determined.

Influence of lead exposure on molecules related to IGF-1 signaling pathway

Western blot assay of molecules related to IGF-1 signaling pathway was carried out. SDS-PAGE and Western blot assay: Proteins (100 μg) were subjected to SDS-PAGE (12% separating gel) and then transferred onto PVDF membrane. The membrane was blocked in 5% non-fat mild at room temperature for 1 h, and then treated with antibodies against PKC (PKC-α, PKC-β), Akt (Akt-473/Akt-308) and MAPK (ERK, JNK, p-38) in dependently (1:500) at 4°C over night. After washing in TBST thrice (10 min for each), the membrane was treated with secondary antibody (1:1000) at room temperature for 2 h. After washing in TBST thrice (10 min for each), visualization was done, and optical density was determined. β-actin was used as an internal reference (1:3000). After detection, the NC membrane was placed in strip buffer followed by incubation for 8 min. Following washing in TBST thrice, the membrane was then blocked in 5% non-fat milk at room temperature for 1 h and treated with primary antibodies against PKC, Akt, MAPK (ERK, JNK, p-38) (1:500) independently at 4°C over night. After washing in TBST thrice (10 min for each), the membrane was treated with secondary antibody (1:1000) at room temperature for 1 h. Following washing in TBST thrice (10 min for each), visualization was done, and optical density was determined.

Statistical analysis

Statistical analysis was done with SPSS version 16.0. Data were expressed as $x\pm s$. When the number of patients were >30, t test was

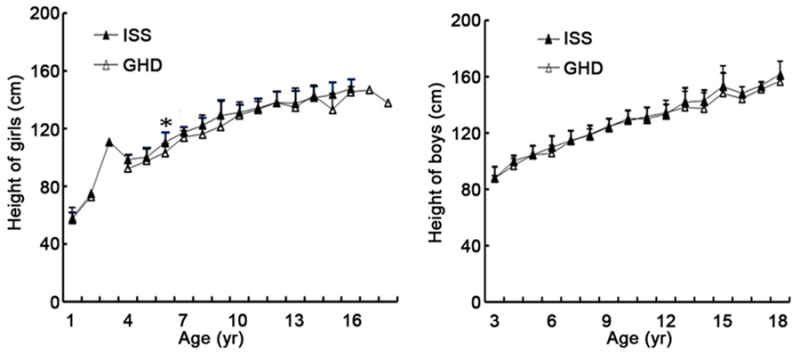

Figure 1. Height of girls and boys in two groups; *P<0.05 vs. GHD group. The height was compared between GHD group and ISS group in children of different age groups. Results showed significant difference in girls aged 6 years between two groups.

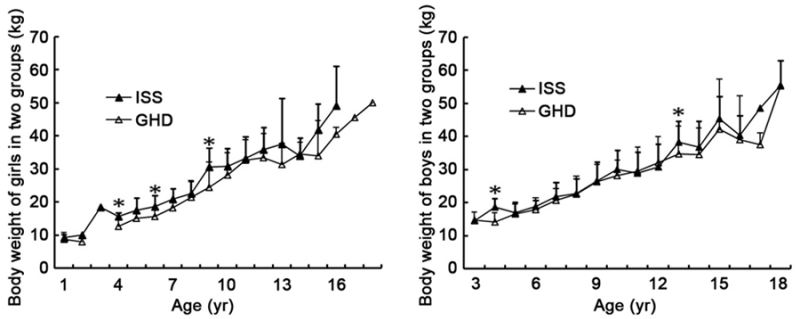

Figure 2. Body weight of girls and boys in two groups; *P<0.05 vs. GHD group. Results showed significant difference in girls aged 4, 6 and 9 years between two groups (P<0.05). Significant difference in body weight was found between two groups in boys aged 4 and 13 years (P<0.05).

employed for comparisons, or non-parametric Mann-Whitney test was used.

Results

Number and age of children

The number of children in ISS group was larger than that in GHD group (58.86%), and the proportion of boys in ISS group was significantly higher than that in GHD group (58.86%).

The short stature children aged 9-12 years accounted for 48.75%, which was markedly higher than that in GHD group. The number of children in different age is shown in **Table 1**.

Height and body weight of children in different groups

The height was compared between GHD group and ISS group in children of different age groups. Results showed significant difference in girls aged 6 years between two groups (P<0.05). However, there was no significant difference between two groups in boys (**Figure 1**).

Body weight of children in different groups

The body weight was compared between GHD group and ISS group in children of different age groups. Results showed significant difference in girls aged 4, 6 and 9 years between two groups (P<0.05). Significant difference in body weight was found between two groups in boys aged 4 and 13 years (P<0.05) (**Figure 2**).

Serum IGF level in GHD group and ISS group

The serum IGF-1 level was compared between GHD group and ISS group in children of different age groups. Results showed significant difference in serum IGF-1 level between two groups in girls aged 8-15 years (P<0.05). However, in boys aged 5-15 years, significant difference in serum IGF-1 level was found between two groups (P<0.05) (**Figure 3**).

Blood lead level in GHD group and ISS group

In children of different age groups, the blood lead level was compared between GHD group and ISS group. Results showed, in girls aged 4-6 years and 8-14 years, the blood lead level in GHD group was significantly higher than that in ISS group (P<0.05). In boys aged 5-9 years, 11-13 years and 15 years, the blood lead level in GHD group significantly increased when compared with ISS group (P<0.05) (**Figure 4**).

Height, body weight, serum IGF-1 and blood lead of children in two groups

Overall, the height, body weight, IGF-1 and blood lead level were significantly different in

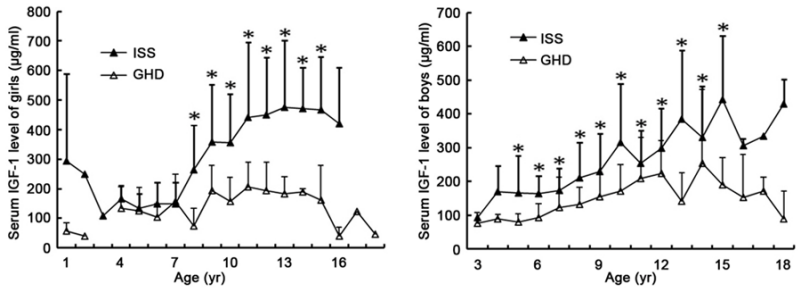

Figure 3. Serum IGF-1 level of girls and boys of two groups; *P<0.05 vs. GHD group. Results showed significant difference in serum IGF-1 level between two groups in girls aged 8-15 years (P<0.05). However, in boys aged 5-15 years, significant difference in serum IGF-1 level was found between two groups (P<0.05).

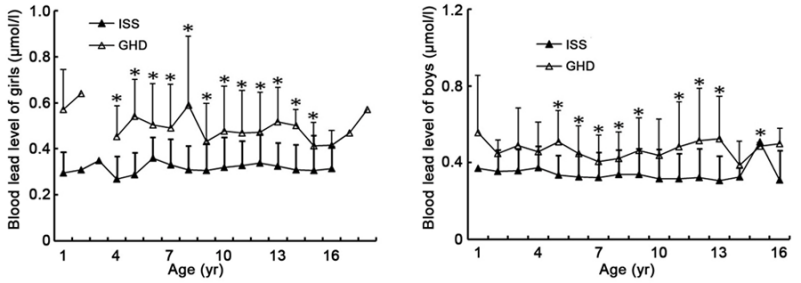

Figure 4. Blood lead level of girls and boys in two groups; *P<0.05 vs. GHD group. Results showed, in girls aged 4-6 years and 8-14 years, the blood lead level in GHD group was significantly higher than that in ISS group (P<0.05). In boys aged 5-9 years, 11-13 years and 15 years, the blood lead level in GHD group significantly increased when compared with ISS group (P<0.05).

Table 2. Height, body weight, serum IGF-1 and blood lead of children in two groups

Parameters	Girls		Boys	
	p	F	*p*	F
Height	0.000*	16.710	0.032*	0.705
Body weight	0.000*	1.150	0.017*	2.478
IGF-I	0.000*	70.256	0.000*	29.050
lead	0.000*	7.624	0.000*	27.195

*P<0.05 vs. GHD group.

both girls and boys between ISS group and GHD group (P<0.01 and 0.05, respectively) (**Table 2**).

Influence of lead exposure on rat blood lead level

Atomic absorption spectrophotometry showed the blood lead level in rats treated with lead containing water for 6 weeks significantly increased when compared with control group (P<0.05). One-way analysis of variance showed significant difference in the blood lead level between control group and 100 ppm, 200 ppm and 300 ppm groups (P<0.05). Moreover, marked difference was also noted among 100 ppm, 200 ppm and 300 ppm groups (P<0.05) (**Table 3** and **Figure 5**).

Influence of lead exposure on molecules related to IGF-1 signaling pathway

Western blot assay showed the protein expression of phosphorylated ERK1/2, JNK, p38, Akt473 and Akt308 increased markedly in rats after lead exposure (**Figure 6**).

Discussion

Short stature is a common disease of children in Department of Pediatric Endocrinology. Abnormal bone metabolism, chronic diseases, abnormal endocrine system, intrauterine growth retardation, malnutrition and familial short stature are found to cause short stature in children. Of these causes, GHD and ISS are major causes of short stature in children and accounts for about 1/3 of causes [11]. In the present study, results showed endocrinological diseases are the major cause of short stature, of which GDH was the most common (41.14%), slightly higher than previously reported [12, 13]. This might be attributed to the source of children and the blood lead level.

GH is regulated by multiple hormones including growth hormone releasing hormone from hypothalamus, growth hormone release inhibiting hormone and other hormones. In addition, physiological factors such as hunger, sleep, exercise and blood glucose and environmental factors induced stress may also influence the GH secretion [14]. The pulsatile secretion of GH makes the random detection of GH meaningless. To date, GH stimulation test is still an important tool in the clinical evaluation of GH

Table 3. Blood lead level in rats after lead exposure (μg/L)

Group	1 w	2 w	3 w	4 w	5 w	6 w
Con	12.03±5.01	13.99±4.76	14.19±7.47	15.80±10.37	13.00±1.56	17.35±8.42
100	22.49±3.40*	35.18±7.94*	47.57±19.69*	93.58±29.40*	91.48±10.63*	98.48±22.56*
200	78.90±15.70*	113.25±11.38*	139.35±23.42*	143.63±22.65*	152.28±22.55*	185.60±27.68*
300	122.00±22.30*	157.92±19.73*	171.83±5.14*	186.30±24.63*	197.78±26.83*	247.15±21.67*

*P<0.05 vs. GHD group.

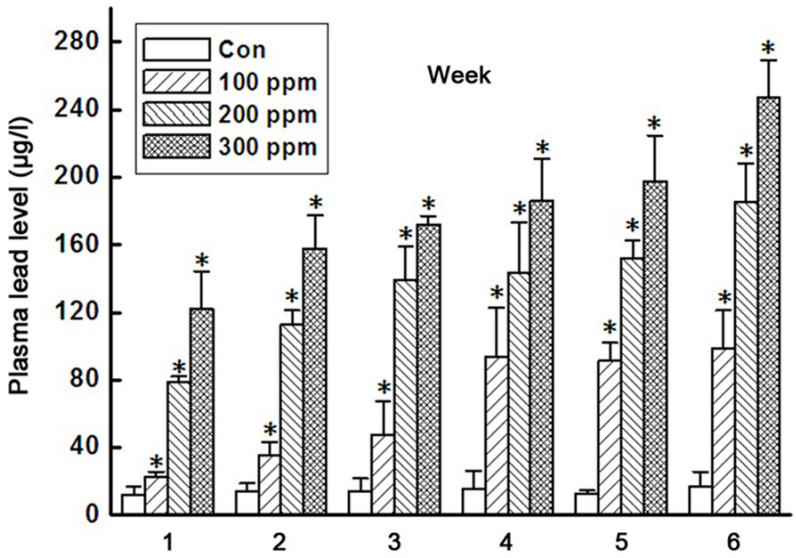

Figure 5. Blood lead level of rats after lead exposure; *P<0.05 vs. Control. Atomic absorption spectrophotometry showed the blood lead level in rats treated with lead containing water for 6 weeks significantly increased when compared with control group (P<0.05). One-way analysis of variance showed significant difference in the blood lead level between control group and 100 ppm, 200 ppm and 300 ppm groups (P<0.05). Moreover, marked difference was also noted among 100 ppm, 200 ppm and 300 ppm groups (P<0.05).

secretion, in which several drugs (such as arginine, clonidine, levodopa and insulin) are used to stimulate the GH secretion [15, 16]. Clonidine is an agonist of á-adrenoceptor and can induce the secretion of growth hormone releasing hormone to increase GH, with high positive rate (31.3%) and peak [17]. Arginine can increase the GH release via inhibiting the secretion of growth hormone release inhibiting hormone. In arginine stimulation test, the GH peak occurs later (60.2% at 30 min after arginine administration), and the positive rate is relatively low. Thus, administration of both clonidine and arginine is an ideal tool for GH stimulation test [18].

GH can promote the differentiation, proliferation and protein synthesis in cells via IGF, to significantly influence the growth and metabolism. IGF-1 is an alkaline single-chain polypeptide molecule composed of 70 amino acids and has the molecular weight of 7649. A lot of cells can secret IGF-1, which then binds to IGF-1 receptor in autocrine, paracrine and endocrine ways exerting physiological effects [6]. Hilczer et al proposed that GH stimulation test has poor repeatability, serum IGF-1 is a protein synthesized and metabolized in the liver, the serum IGF-1 is more stable than the GH and easy to detect, and thus to detect the serum IGF-1 was recommended to be used to evaluate the GH secretion [19]. However, Haghshenas et al proposed that the reduction in IGF-1 and IGFBP-3 was not associated with GHD, evaluation of GHD with IGF and IGFBP-3 had the sensitivity of 35% and 12%, respectively, and thus it was improper to use IGF or IGFBP-3 alone in the diagnosis of GHD [20]. Our results showed serum IGF-1 level was significantly different between GHD group and ISS group (P<0.05), and the reduction in IGF-1 was closely associated with the blood lead in GHD children. Thus, whether IGF-1 can be used as a serum marker for lead exposure is required to be further studied. In the present study, the diagnostic tool of GHD was re-evaluated, and our findings provided evidence on the use of IGF-1 as a new diagnostic tool.

Children are susceptible to lead contamination. Lead exposure is more harmful for the health of children aged 0-5 years. In the early phase of growth and development (including embryonic phase), lead exposure may influence the growth

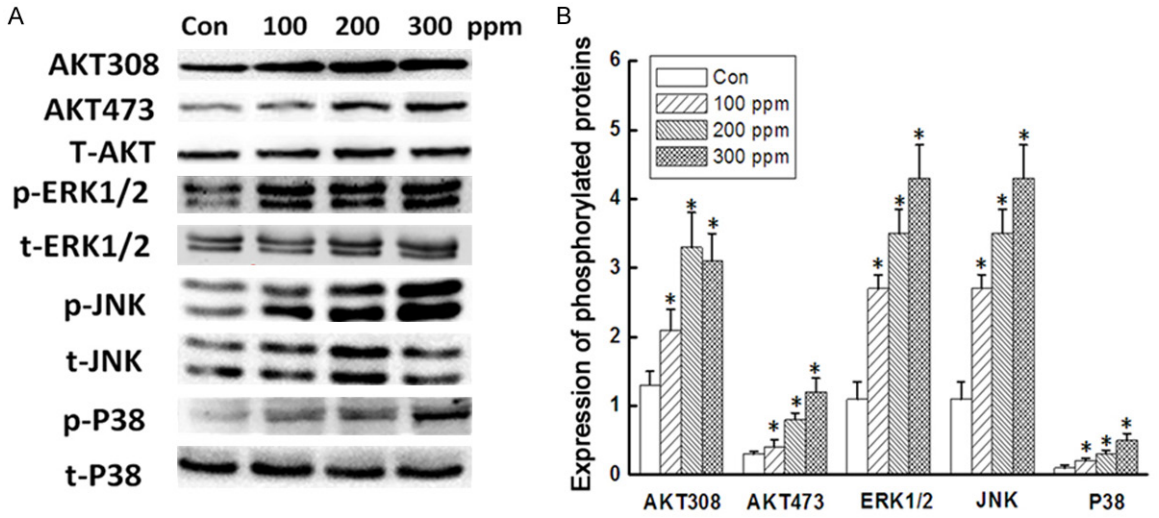

Figure 6. Protein expression of phosphorylated molecules related to IGF-1 pathway; *p<0.05 vs. Control. Western blot assay showed the protein expression of phosphorylated ERK1/2, JNK, p38, Akt473 and Akt308 increased markedly in rats after lead exposure.

and development of children. According to the characteristic of biology and living styles in children, who are the biggest victims of lead pollution, lead absorption rate in children reaches 5-8 times of adults, while the excretion rate is only 30% [21]. The average blood lead level of Chinese children was 0.3772 μmol/L from 1995 to 2003, and the rate of plumbism was 21.05% [22].

Among top 10 polluted cities all over the world, 7 of them were located in China: Taiyuan, Beijing, Wulumuqi, Lanzhou, Chongqing, Jinan and Shijiazhuang. Among them, Taiyuan, Lanzhou, Shijiazhuang, Chongqing and Jinan are next to Shanxi Province. Children in this study were mostly from Shanxi Province and circumjacent Shanxi and Gansu Province, and from cities and countries. There have been several catastrophic heavy metal contamination events in China from 2009. One them was in Aug, 2009, that emissions from the smelter in Fengxiang, Shanxi Province caused blood lead level elevating in 851 children, among whom more than 170 children were hospitalized [23]. Prevention and treatment of lead pollution in children has been regarded as the major social problem associated with the national economy, the people's livelihood and population quality.

To date, the mechanisms underlying the lead exposure induced injury in children is still poorly understood. Our results showed that the blood lead level in GHD children was significantly higher than that in ISS children (P<0.05), but the IGF-1 level was markedly reduced (P<0.05). Whether the blood lead level is related to serum IGF-1 level and what the pathogenesis of lead induced short stature is are still unclear. A variety of studies have shown that GH may regulate the growth of osteoblasts via the MAPKs signaling pathway. IGF can bind to tyrosine kinase receptor IGFIR to initiate two signaling pathways (PI3/Akt and MAPK), resulting in promotion of mitosis, induction of cell proliferation, transdifferentiation and inhibition of apoptosis [24, 25].

Our animal study showed lead exposure significantly increased the protein expression of phosphorylated ERK1/2, JNK, p38, Akt473 and Akt308. Taken together, we speculate that lead may increase the phosphorylation of molecules related to MAPK and Akt signaling pathway to influence the GH and IGF-1 mediated promotion of cell growth in MAPK and PI3/Akt signaling pathway dependent manners. This interferes with the promotive effect of IGF-1 on the proliferation, differentiation and maturation of cells, inhibits the cell apoptosis and suppresses the growth and anabolic metabolism, resulting in growth retardation in children.

In pregnant women, lead may transfer into the fetus via the placenta. In addition, bone lead (an indicator of cumulative lead exposure) as a

source of endogenous contamination may be related into circulation. About 45-50% of blood lead may be released into the circulation in pregnant women, even they has no recent lead exposure [26]. Goyer found that the brain lead level of rats in embryonic phase, rats in breast-feeding phase and rats in weaning phase was 10, 3.5 and 2 times higher than that of adult rats when the dose of lead and duration of lead exposure were identical [27]. It is suggested that lead exposure in the early development phase may cause more severe injury and is more toxic. IGF-1 and IGF-II are two key growth factors for the embryonic development, and their effects are independent of GH in the embryonic development. When there is GH gene or GH receptor gene mutation, the embryonic development is slightly retarded, but embryonic mice with IGF-1 gene mutation show obvious developmental retardation. In 1996, Woods reported a body with homozygous IGF-I gene deficiency who showed obvious embryonic developmental retardation and had the birth weight of 1.4 kg (5.4 s lower than the normal mean) and the birth length of 37.8 cm (3.9 s lower than the normal mean) [28]. Thus, we postulate that lead may influence the physiological function of IGF-1 in the early embryonic developmental phase to inhibit the growth and development of children.

To elucidate the mechanisms underlying the lead induced injury and the molecular functions of IGFs is a hot topic in the field of cell biology. In addition, the role of IGF-1 in the therapy is increasingly emphasized. Currently, IGF-1 is used in the treatment GH-insensitive syndrome (Laron-type dwarfism), diabetes, insulin resistance, osteoporosis, catabolism state, neuromuscular diseases and GH resistance [29]. The extensive physiological functions of IGF-1 are the basis for the therapeutic application of IGF-. Whether IGF-1 can be used in the treatment of lead poisoning in children is required to be studied in future.

Conclusions

Our study suggesting that reduction in IGF-1 in children with GHD is associated with blood lead level. Lead exposure may induce expression of phosphorylated MAPK and Akt signaling molecules. The activation of these molecules may influence binding of IGF-1 and tyrosine kinase receptor IGFIR to regulate cell growth via the MAPK and Akt signaling pathways, which then interfere with growth-promoting effect of IGF-1 in short children.

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Disclosure of conflict of interest

There is no conflict of interest to disclose.

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References

[1] de Onis M, Frongillo EA and Blossner M. Is malnutrition declining? An analysis of changes in levels of child malnutrition since 1980. Bull World Health Organ 2000; 78: 1222-1233.

[2] Zeng JS and Wang DF. Modern Basic and Clinical Science of Pediatric Endocrinology. Shanghai: Shanghai Science and Technology Press, 2001.

[3] Binns HJ, Campbell C and Brown MJ. Interpreting and managing blood lead levels of less than 10 microg/dL in children and reducing childhood exposure to lead: recommendations of the Centers for Disease Control and Prevention Advisory Committee on Childhood Lead Poisoning Prevention. Pediatrics 2007; 120: e1285-1298.

[4] Cen SY, Qu TF and Wu J. Study on correlation between blood lead level and living environment in infants with growth retardation. J Clin Pediatr 2004; 22: 464-466.

[5] Taylor MP, Camenzuli D, Kristensen LJ, Forbes M and Zahran S. Environmental lead exposure risks associated with children's outdoor playgrounds. Environ Pollut 2013; 178: 447-454.

[6] Nitert MD, Chisalita SI, Olsson K, Bornfeldt KE and Arnqvist HJ. IGF-I/insulin hybrid receptors in human endothelial cells. Mol Cell Endocrinol 2005; 229: 31-37.

[7] Li H, Ji CY, Zong XN and Zhang YQ. [Height and weight standardized growth charts for Chinese

children and adolescents aged 0 to 18 years]. Zhonghua Er Ke Za Zhi 2009; 47: 487-492.
[8] Wang WP. Pediatrics. Beijing: Higher Education Press, 2004.
[9] Gasco V, Corneli G, Beccuti G, Prodam F, Rovere S, Bellone J, Grottoli S, Aimaretti G and Ghigo E. Retesting the childhood-onset GH-deficient patient. Eur J Endocrinol 2008; 159 Suppl 1: S45-52.
[10] Smyczynska J, Lewinski A, Hilczer M, Stawerska R and Karasek M. Partial growth hormone deficiency (GHD) in children has more similarities to idiopathic short stature than to severe GHD. Endokrynol Pol 2007; 58: 182-187.
[11] Han B, Zhu ZY, Shi X, Ni SN and Gu YX. Common Etiology of 309 Children with Short Stature. J Appl Clin Pediatr 2008; 23: 604-605, 620.
[12] Zheng SP, Ma BJ, Zeng ZW, Li ZQ and Liu YH. Application and analysis of the provocation test by exercise screening and levodopa in short stature children. J Lab Clin Med 2008; 5: 1047-1049.
[13] Dong ZY, Wang W and Xi RP. Evaluation of the diagnostic value of biochemical tests in children with growth hormone deficiency. Chin J Endocrinol Metab 2005; 21: 341-343.
[14] Giustina A and Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 1998; 19: 717-797.
[15] Cole TJ, Hindmarsh PC and Dunger DB. Growth hormone (GH) provocation tests and the response to GH treatment in GH deficiency. Arch Dis Child 2004; 89: 1024-1027.
[16] Wang LF and Yang ML. Importance of different growth hormone stimulation tests. J Med Dis 2006; 27: 106.
[17] Yang SJ. Pharmacology. Beijing: People's Health Publishing House, 2001.
[18] Li YQ, Li QL and Zhang MH. Comparison of Diagnostic value of growth hormone exercise test and growth hormone provocative test on growth hormone deficiency. J Appl Clin Pediatr 2009; 24: 601-602.
[19] Hilczer M, Smyczynska J, Stawerska R and Lewinski A. Stability of IGF-I concentration despite divergent results of repeated GH stimulating tests indicates poor reproducibility of test results. Endocr Regul 2006; 40: 37-45.
[20] Haghshenas Z, Sotoudeh K, Karamifar H, Karamizadeh Z and Amirhakimi G. The role of insulin like growth factor (IGF)-1 and IGF-binding protein-3 in diagnosis of Growth Hormone Deficiency in short stature children. Indian J Pediatr 2009; 76: 699-703.
[21] Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP). Recommendations for blood lead screening of young children enrolled in medicaid: targeting a group at high risk. MMWR Recomm Rep 2000; 49: 1-13.
[22] Zhang JL, He KM, Wang SQ and Wu YZ. Analysis of Blood Lead Levels and Changing Trend in Children in China. J Environ and Health 2009; 26.
[23] Watts J. Lead poisoning cases spark riots in China. Lancet 2009; 374: 868.
[24] Lai CF, Chaudhary L, Fausto A, Halstead LR, Ory DS, Avioli LV and Cheng SL. Erk is essential for growth, differentiation, integrin expression, and cell function in human osteoblastic cells. J Biol Chem 2001; 276: 14443-14450.
[25] Grey A, Chen Q, Xu X, Callon K and Cornish J. Parallel phosphatidylinositol-3 kinase and p42/44 mitogen-activated protein kinase signaling pathways subserve the mitogenic and antiapoptotic actions of insulin-like growth factor I in osteoblastic cells. Endocrinology 2003; 144: 4886-4893.
[26] Lanphear BP, Burgoon DA, Rust SW, Eberly S and Galke W. Environmental exposures to lead and urban children's blood lead levels. Environ Res 1998; 76: 120-130.
[27] Goyer RA. Results of lead research: prenatal exposure and neurological consequences. Environ Health Perspect 1996; 104: 1050-1054.
[28] Woods KA, Camacho-Hubner C, Savage MO and Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 1996; 335: 1363-1367.
[29] Latrech H, Simon A, Beltrand J, Souberbielle JC, Belmejdoub G and Polak M. Postprandial hyperglycemia corrected by IGF-I (Increlex®) in Laron syndrome. Horm Res Paediatr 2012; 78: 193-200.