The association between body lead levels and childhood rickets
A meta-analysis based on Chinese cohort
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Abstract
China has serious lead pollution and a high incidence of childhood rickets. High lead levels have been reported in childhood rickets, but the results were inconsistent.

To evaluate the association between body lead levels and childhood rickets.

After a systematic literature search, we identified 15 studies determining body lead levels between rickets children and healthy controls, and 4 studies focusing on the cases of different disease severity. Standard mean differences (SMD) and the corresponding 95% confidence intervals (CI) were pooled to compare the lead levels between different groups.

Sixteen case-control studies were included with a total of 5082 cases and 6054 controls. Compared with healthy controls, the body lead levels in rickets children were significantly higher (SMD (95%CI): 0.67 (0.41–0.93)), and subgroup analyses showed consistent results. The cases with moderate-to-severe disease activity also had a significantly higher lead level than mild-to-moderate cases (SMD (95%CI): 0.64 (0.31–0.97)).

This meta-analysis suggested an association between body lead levels and childhood rickets, and lead exposure might be a risk factor for rickets.

Abbreviations: AAS = atomic absorption spectrometry, BALP = bone alkaline phosphatase, BMD = bone mineral density, ICP-AES = inductively coupled plasma-atomic emission spectrometry, NOS = Newcastle-Ottawa Scale, SMD = standard mean differences (SMD).

Keywords: body lead, childhood rickets, meta-analysis

1. Introduction
Rickets is a common pediatric disease characterized by skeleton deformity and hypoevolutism. It is prevalent among the Chinese children under 2 years old, with an incidence of 19.7–35.8%.[1] Most cases were caused by vitamin D deficiency, which could lead to abnormal calcium and phosphors metabolism and subsequent osteodysplasty.[2] In addition to inadequate dietary intake, multiple factors were also related with the etiology, especially the pollution of heavy metals.[3–5] Chinese decades of rapid industrialization and urbanization coincided with serious water, food and air pollution, and the children were easily exposed to the environmental heavy metals.[6,7] On the other hand, considering the unique ways to interact with the environment, children are likely to receive larger doses of heavy metals than adults.[8] Thus, Chinese children are at a high risk of the harm from environment heavy metals.[9] As one of the most commonly encountered heavy metals, lead exposure has become a major health hazard for Chinese children.[10]

Lead exposure has been reported in association with the damage in multiple organs. In Reilly et al study, chronic lead exposure was associated with worsening kidney function in both African American male and female residents, as well as male workers in Dallas smelter communities.[11] Blood lead levels was also adversely associated with anthropometric measures among Mexican children.[12] Moreover, recent epidemiological and experimental studies have validated the association between lead exposure and low bone mineral density (BMD), which could eventually lead to osteoporosis, osteopenia and fracture.[13,14] Lead exposure rats showed a decreased in trabecular bone surface and distribution while trabecular thickness and cortical area increased.

As with the rapid industrialization for decades, lead pollution was extremely serious in China. In the Chinese town with serious pollution of heavy metals, blood lead levels in kindergarten children were negatively correlated with both height and weight, but positively correlated with bone resorption biomarkers, indicating the effects of lead exposure in children’s skeletal development.[15] Several Chinese studies have reported the association between body lead levels and rickets, but the results were inconsistent.[16–31] Thus, we conducted a meta-analysis to investigate the association between body lead levels and childhood rickets.

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Y-FZ and J-WX contributed equally to this study.
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2. Methods

2.1. Literature search

A comprehensive literature search was conducted in PubMed, China Knowledge Resource Integrated Database (CNKI), China Wanfang Database and China SinoMed Database from inception to October 2018, using the key words including: (“lead” OR “plumbum” OR “trace element” [MeSH Terms]) and (“rickets” OR “rhachitis” OR “rachitis” [MeSH Terms]). Moreover, we also reviewed the references of related studies and reviews for undetected studies. This study was approved by the ethics committee of The Central Hospital of Enshi Autonomous Prefecture.

2.2. Study selection and exclusion

The studies were included if meeting the following criteria: (i) all patients were diagnosed as childhood rickets according to certain criteria; (ii) assessed serum or hair lead levels in cases and healthy controls or in the cases with different disease severity; (iii) similar age and sex distribution in 2 groups. The exclusion criteria were as follows: animal studies, reviews, or case reports.

2.3. Data extraction and quality assessment

Two authors extracted the data by a standardized collection form. All differences were resolved by discussion. In each study, the following information was extracted: first author, publication year, study area, lead measurement, diagnostic criteria, sample types, age distribution, number of cases and controls, disease severity, and lead levels. The Newcastle-Ottawa Scale (NOS) was used to assess the methodological quality of included studies.[32]

2.4. Statistical analysis

Standard mean differences (SMD) and the corresponding 95% confidence intervals (CI) were pooled to compare body lead levels between rickets and controls, as well as in the cases with different disease severity. The heterogeneity among studies was estimated by Q test and I² statistic.[33] I² > 50% represented substantial heterogeneity, and the summary estimate was analyzed by a random-effects model. Otherwise, a fixed-effects model was applied. Sensitivity analysis was conducted to estimate the stability of the meta-analysis by omitting 1 study at a time during repeated analyses. Publication bias was assessed by using funnel plots and Begg test. All statistical analyses were performed using software STATA version 12.0 (StataCorp LP, College Station, TX).

3. Results

3.1. Characteristics of the included studies

The search strategy identified 596 records: 178 from PubMed, 139 from CNKI, 157 from Wangfan, and 122 from SinoMed (Fig. 1). After excluding duplicated and irrelevant records, 16
### Table 1
Characteristics of included studies on the lead levels between childhood rickets and healthy controls.

| Study          | Area        | Method     | Diagnostic criteria                              | Sample | Rickets | Healthy controls |
|----------------|-------------|------------|--------------------------------------------------|--------|---------|------------------|
| Luo LR 1994    | Guangdong   | ICP-AES    | Disease history, symptom, force line, biochemical examination | Blood 22±1.5y 71  4.21±1.90  | 4m–3y 60  2.52±1.61 | µmol/l             |
| He SL 1999     | Jiangxi     | Polarography | The Chinese criteria in 1986                      | Hair 0–1y 56  15.29±5.14  | 0–1y 36  11.30±4.20 | NA                |
| Ye X 2001      | Guangdong   | ICP-AES    | The Chinese criteria in 1986                      | Hair 0–1y 52  12.81±3.13  | 0–1y 32  9.75±1.70 | NA                |
| Pan H 2002     | Guangdong   | ICP-AES    | The Chinese criteria in 1986                      | Blood 0–3y 103  0.22±0.07  | 0–3y 103  0.23±0.04 | µg/ml              |
| Wang XF 2003   | Henan       | AAS        | The Chinese criteria in 1986                      | Blood 6m–3y 46  109.28±50.10 | 6m–3y 66  89.65±28.36 | µg/l              |
| Zhu Y 2006     | Shandong    | AAS        | The Chinese criteria in 1986                      | Blood 3m–3y 312  0.278±0.143 | 2.3y 297  0.157±0.120 | µmol/l            |
| Tan MZ 2006    | Guangdong   | AAS        | The Chinese criteria in 1986                      | Blood 0–3y 120  65.00±18.25 | 0–3y 100  31.00±20.21 | µl/ml              |
| Liu AP 2007    | Gansu       | AAS        | Chinese Textbook of Pediatrics (1979)              | Blood 0–3y 30  14.00±5.94  | 0–3y 80  8.90±1.69 | µg/dl             |
| Zeng GZ 2007   | Fujian      | AAS        | The Chinese criteria in 1986                      | Blood 0–3y 35  14.60±6.51  | 0–3y 21  7.30±2.14 | µg/ml              |
| Yang ZC 2007   | Jiangsu     | AAS        | The Chinese criteria in 1986                      | Blood 0–3y 120  0.42±0.14  | 0–3y 100  0.26±0.14 | µmol/l            |
| Hu HD 2007     | Jiangxi     | AAS        | Disease history, symptom, physical examination, BALP | Blood 0–6m 121  48.12±27.90 | 0–6m 122  47.23±20.96 | µg/ml              |
| Gan Y 2011     | Guangxi     | AAS        | The Chinese criteria in 1986                      | Blood 3m–3y 117  0.268±0.145 | 3m–3y 108  0.162±0.128 | µmol/l            |
| Liao ZG 2011   | Jiangxi     | AAS        | The Chinese criteria in 1986                      | Blood 3m–3y 216  27.48±15.27 | 4m–3y 220  19.95±12.90 | µmol/l            |
| Du WR 2012     | Hebei       | DPSA       | The Chinese criteria in 1986                      | BALP 0–6y 2897  63.15±26.56 | 0–6y 4014  61.83±31.00 | µg/ml              |
| Zhang XP 2014  | Hebei       | Polarography | The Chinese criteria in 1986                      | BALP 0–6y 96  200±50  | 0–3y 88  100±22 | µg/dl              |

AAS = atomic absorption spectrometry, BALP = bone alkaline phosphatase, DPSA = differential polarimetric stripping analysis, ICP-AES = inductively coupled plasma-atomic emission spectrometry, NA = not available.

1 day = day, m = month, y = year.

3 moderate disease.

4 severe disease.

### Table 2
Characteristics of included studies on the association between body lead levels and rickets severity in children.

| Study          | Area        | Measurement | Diagnostic criteria | Sample | Disease severity | Age | Cases | lead levels | Unit |
|----------------|-------------|-------------|---------------------|--------|------------------|-----|-------|-------------|------|
| He SL 1999     | Jiangxi     | Polarography | The Chinese criteria in 1986 | Hair  | Mild            | 0–1y | 34    | 13.65±5.62 | NA   |
|                 |             |             |                     |        | Moderate         | 1–3y | 35    | 13.76±5.23 | NA   |
|                 |             |             |                     |        |                  |      |       |             |      |
| Ye X 2001      | Guangdong   | ICP-AES     | The Chinese criteria in 1986 | Hair  | Mild            | 0–1y | 40    | 12.29±2.51 | NA   |
|                 |             |             |                     |        | Moderate         | 1–3y | 42    | 10.90±2.06 | NA   |
|                 |             |             |                     |        |                  |      |       |             |      |
| Lin L 2004     | Guangdong   | ICP-AES     | The Chinese criteria in 1986 | Blood | Mild            | >1y  | 106   | 135.70±71.24 | µg/ml |
|                 |             |             |                     |        | Moderate to severe | >1y  | 33    | 185.45±68.83 | µg/dl |
| Liu AP 2007    | Gansu       | AAS         | Chinese Textbook of Pediatrics (1979) | Blood | Mild            | 0–3y | 30    | 14.00±5.94 | µg/dl |
|                 |             |             |                     |        | Moderate         | 0–3y | 35    | 14.60±6.51 | µg/dl |

AAS = atomic absorption spectrometry, ICP-AES = inductively coupled plasma-atomic emission spectrometry, NA = not available, y = year.
3.2. Body lead levels and rickets

Fifteen studies investigated the difference of body lead levels between rickets and controls, which were expanded into 24 studies in the meta-analysis according to age distribution. It was found that body lead levels in rickets cases were significantly higher than in controls (SMD (95%CI): 0.67 (0.41–0.93); $I^2$=96.3%, $P_{het}$<0.001) (Fig. 2). Sensitivity analysis showed the result was robust (Fig. S1). Begg’s test detected significant publication bias ($P=0.170$) (Fig. S2, http://links.lww.com/MD/C845). Subgroup analysis was conducted on area, measurement, type of samples, age distribution, diagnosis criteria, and developed area. No substantial changes of the primary result were found between subgroups (Table 3).

3.3. Body lead levels and rickets severity

Four studies investigated the difference of body lead levels between mild-to-moderate cases and moderate-to-severe cases, which were expanded into 6 studies in the meta-analysis according to age distribution. The disease severity was classified into mild, moderate and severe according to clinical symptoms (e.g., apopistia, night terror, hidrosis, dysphoria), skeletal change and blood levels of calcium, phosphorus and bone alkaline phosphatase (BALP). It was found that body lead levels in moderate-to-severe cases were significantly higher than in mild-to-moderate cases (SMD (95%CI): 0.64 (0.31–0.97); $I^2$=57.1%, $P_{het}$=.040) (Fig. 3). Sensitivity analysis showed the result was robust (Fig. S3, http://links.lww.com/MD/C845). Begg’s test detected significant publication bias ($P=.750$) (Fig. S4, http://links.lww.com/MD/C845).

4. Discussion

As a heavy metal, lead and its compounds are widely used, like ethyl petrol, paint and storage battery. It is non-degradable in the environment, and excessive lead could enter the human body by polluted food, water and air. As with the rapid industrialization for decades, lead pollution was extremely serious in China, and the average lead level among urban children was up to 7.02 μg/dl in blood. Different from adults, children had a higher absorption rate and lower excretion rate for lead, and thus the lead load was heavier than adults. Furthermore, most of the
residual lead deposited in the bone, and it was easily transferred into blood and soft tissues. As a result, high lead levels could cause systemic lesions in children, and the most common was cognitive impairment.\(^{[36]}\)

High lead levels also made an influence on skeletal development. The mechanism was multi-aspect. Firstly, high blood lead levels could decrease children’s appetite, and contribute to inadequate dietary intake of calcium.\(^{[37]}\) Secondly, lead could reduce intestinal absorption of calcium by competitive inhibition. Thirdly, high blood lead levels were related with low blood calcium and 1, 25-dihydroxy-vitamin D levels, which lead to the decrease of active vitamin D.\(^{[38]}\) Forthly, high lead levels could induce the apoptosis of osteoblasts.\(^{[8]}\)

Thus, lead might get involved in the pathogenesis of childhood rickets. In the study of Huang et al, the incidence of rickets was 28.8% (13/45) in the lead poisoning cases under 3 years, while it was 20.8% (23/112) in the cases aging from 3 to 12 years.\(^{[39]}\) In our study, we also found a higher lead level in rickets than in healthy controls, and the levels were also associated with the disease severity. In the recent study, lead-related lesions could also occur in low concentrations of less than 10 μg/dl, indicating the great harm for children.\(^{[40]}\) Thus, it was necessary to take some interventions to prevent lead exposure in children.\(^{[41]}\)

This study had several strengths. Firstly, to the best of our knowledge, this is the first meta-analysis to evaluate the association between body lead levels and childhood rickets. Secondly, we also evaluated the association between body lead levels and rickets severity. However, several limitations in this study should be considered. First, the number of cases in the meta-analysis was relatively small. Second, there existed obvious heterogeneity between studies, although we conducted a sensitivity analysis to evaluate the stability of pooled results. Third, most included studies were case-control designed, and it was more difficult confirm causal relationship between lead exposure and rickets. Forth, the data of vitamin D supplement in the included studies were unavailable. Fifth, it was unknown whether the association between lead and rickets could be generalizable to populations other than Chinese. In spite of these, we thought our robust subgroup analysis and the further analysis between body lead levels and rickets severity could strongly indicate the relationship between body lead levels and childhood rickets. In the future, it is necessary to conduct a prospective study to warrant the association.

In conclusion, this meta-analysis suggested an association between body lead levels and childhood rickets. Lead exposure might be a risk factor for childhood rickets, and high lead levels should been considered in the diagnosis of childhood rickets. Further study was needed to identify the role of lead-discharging in anti-rickets treatment, especially for the children with high lead levels.

| Variable        | Studies | SMD (95% CI) | I\(^2\) (%) |
|-----------------|---------|--------------|-------------|
| Area            |         |              |             |
| Developed       | 8       | 0.96 (0.60–1.31) | 92.5        |
| Developing      | 16      | 0.52 (0.22–0.82) | 95.2        |
| Measurement     |         |              |             |
| AAS             | 15      | 0.53 (0.24–0.83) | 93.5        |
| ICP-AES         | 5       | 0.80 (0.22–1.39) | 92.8        |
| Polarography    | 3       | 1.39 (0.23–2.56) | 95.8        |
| DPSA            | 1       | 0.05 (0.00–0.09) | NA          |
| Sample          |         |              |             |
| Blood           | 20      | 0.62 (0.33–0.90) | 96.8        |
| Hair            | 4       | 0.93 (0.71–1.15) | 0.0         |
| Age (years)     |         |              |             |
| 0–1             | 2       | 0.97 (0.65–1.29) | 0.0         |
| 0–3             | 19      | 0.65 (0.35–0.95) | 95.2        |
| 1–3             | 2       | 0.89 (0.59–1.19) | 0.0         |
| Diagnosis criteria |       |              |             |
| Comprehensive   | 22      | 0.62 (0.38–0.88) | 91.4        |
| Only based on BALP | 2    | 0.08 (0.03–0.13) | 64.4        |
| Developed area  |         |              |             |
| Yes             | 8       | 0.96 (0.60–1.31) | 33.8        |
| No              | 16      | 0.52 (0.23–0.82) | 66.2        |

AAS = atomic absorption spectrometry, BALP = bone alkaline phosphatase, DPSA = differential potentiometric stripping analysis, ICP-AES = inductively coupled plasma-atomic emission spectrometry, NA = not available.
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References
[1] Qu HX, Xia RM. X-ray diagnosis of vitamin D deficiency rickets. Chin J Pract Radiol 2012;28(12):625–8. Available at: http://d.wanfangdata.com.cn/Periodicals/yyyxzz201208030. Accessed September 24, 2012.
[2] Usah T. Prevalence of classic signs and symptoms of rickets and vitamin D deficiency in Mongolian children and women. J Steroid Biochem Mol Biol 2013;136:207–10.
[3] Tahir S, Demirbüke H, Ozbek MN, et al. Genotype and phenotype characteristics in 22 patients with vitamin D-dependent rickets type I. Horm Res Pediatr 2016;85:309–17.
[4] Unuvar T, Buyukgebiz A. Nutritional rickets and vitamin D deficiency in infants, children and adolescents. Pediatr Endocrinol Rev 2010;7:283–91.
[5] Hamilton JD, O’Flaherty EJ. Influence of lead on mineralization during bone growth. Fundam Appl Toxicol 1995;26:265–71.
[6] Chowdhury S, Mazumder MA, Al-Attas O, et al. Heavy metals in drinking water: occurrences, implications, and future needs in developing countries. Sci Total Environ 2016;569:1075–85.
[7] Bosch AC, O’Neill D, Geurtsen W, et al. The relationship between lead exposure and kidney function among African American infants, children and adolescents. Pediatr Endocrinol Rev 2010;7:283–91.
[8] Heacock M, Kelly CB, Asante KA, et al. E-waste and harm to vulnerable populations: a growing global problem. Environ Health Perspect 2014;122:547–54.
[9] Zeng X, Xu X, Boezen HM, et al. Children with health impairments by heavy metals in an e-waste recycling area. Chemosphere 2016;148:408–14.
[10] Pettifor JM. Rickets and vitamin D deficiency in children and adolescents. Endocrinol Metab Clin North Am 2005;34:537–53.
[11] Huang YP, Xiao CL. Analysis of the blood plumbum levels in 312 children with rachitis in Qinqdao. Chin J Appl Clin Pediatr 2006;21:703–4.
[12] Tan MZ, Chen L, Peng JY, et al. Analysis on the levels of blood trace elements in infants with rickets. Guangdong Trace Elem Sci 2006;13:34–6.
[13] Liu AP. The change of blood calcium and plumbum levels in vitamin D deficient rickets. Gansu Sci Technol 2007;23:148–9.
[14] Zeng GZ. Determination and analysis of blood mineral elements in children with rickets. J Clin Exp Med 2007;6:102.
[15] Yang ZC, Li M. Analysis of blood trace elements in infants with rickets. Stud Trace Elem Health 2007;24:16–7.
[16] Hu HD, Tu L, Gao P, et al. Research on relationship between rickets and blood lead level in babies and infants. Materin Child Health Care China 2007;22:3818–9.
[17] Liao ZG, Li JY, Wang SH, et al. Relationship of blood zinc, iron, lead, levels to rickets in infants and preschool children. J Nanchang Univ (Med Sci) 2011;51:11–3.
[18] Gan Y, Zhang C. Investigation of the relationship between serum trace element levels and rickets in infants. Chin J Postgraduates of Med Sci 2011;34:43–4.
[19] Du WR, Wang P, Cui LH, et al. Relationship between rickets and trace elements in children. Materin Child Health Care China 2012;2:231–3.
[20] Zhang XP, Huang RC, Liu LN, et al. The research on the relationship between trace element and vitamin D deficient rickets. Shanxi Med J 2014;43:2547–8.
[21] Wells GA, Shea B, O’Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses 2011; Available at: http://www.ohri.ca. Accessed October 20, 2011.
[22] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analysis. BMJ 2003;327:557–60.
[23] Wang B, Zhang JL, Zhang YS, et al. Health risk assessment for lead exposure and bone mineral density in Korean adult. J Bone Metab 2018;15:60.
[24] Nussbaumer-Streit B, Yeoh B, Griebler U, et al. Household interventions for preventing domestic lead exposure in children. Cochrane Database Syst Rev 2016;10:CD006047.