Decreased serum bilirubin levels in children with lead poisoning

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Abstract

Objective: Lead is a toxic heavy metal, which causes irreversible damage in children. Oxidative stress is the underlying mechanism of lead toxicity, and monitoring oxidative stress of lead poisoning children in vivo is important. Our study aimed to investigate blood serum levels of biochemical parameters, including albumin, bilirubin, creatinine, and uric acid, which are regarded as non-enzymatic antioxidants, in children with lead poisoning.

Methods: We studied 355 children with lead poisoning and 355 age- and sex-matched controls. We analyzed clinical characteristics and measured serum levels of total protein, globulin, albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase, urea, and creatinine.

Results: We found that albumin, bilirubin, urea, and creatinine levels were significantly lower and AST, total protein, and globulin levels were higher in children with lead poisoning than in controls. Direct bilirubin, albumin, total protein, urea, creatinine, and AST levels were associated with lead poisoning after adjustment for other covariates. Spearman analysis showed that direct bilirubin, albumin, and urea levels were independent indicators (i.e., not related to hemoglobin or weight), while creatinine levels showed a moderate correlation with weight.

Conclusion: Lead interferes with the non-enzymatic antioxidant system in children, and lead poisoning results in a decrease in serum bilirubin levels.

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Introduction
Lead is a toxic heavy metal that is commonly used in daily necessities, such as paint, gasoline, water pipes, storage batteries, and many other products. Children are vulnerable to lead, and their respiratory tract and digestive tract have a higher absorption rate of lead compared with adults. In the USA, each lead-exposed child costs approximately $5600 in medical and special education services, and places a huge economic burden on society. Wenzhou, where there are many small workshops for painting, electroplating, and copper processing, children have a relatively high risk of lead poisoning. Since 2010, our hospital has treated more than 3000 person-times of children with lead poisoning. Lead causes numerous types of damage to children, such as impaired neurological development (e.g., behavioral changes, mental impairment, seizures, and coma), gastrointestinal issues (e.g., abdominal pain, constipation, nausea, and vomiting), decreased growth in height, and delayed sexual maturation.

Oxidative stress is the underlying mechanism of lead-induced organ injury. Lead is a redox-inactive metal, and it shows its pro-oxidative activity by generating reactive oxygen species and depleting cellular antioxidant reserves. In the worst case scenario, pro-oxidative activity of lead results in irreversible neurological damage to children without proper treatment, costing an estimated $50.9 billion annually in lost economic productivity in the USA. Therefore, monitoring the level of oxidative stress vivo in children with lead poisoning is of great importance. The oxidation status of the body can be evaluated by total antioxidant status/total oxidant status with spectrophotometry. However, these assays are not available in our hospital and would add a financial burden for patients. Some available biochemical parameters, such as bilirubin, albumin, creatinine, and uric acid, are regarded as endogenous non-enzymatic antioxidants. Therefore, we conducted this hospital-based, sex- and age-controlled, paired study to investigate antioxidant levels of biochemical components in children with lead poisoning.

Patients and methods
Patients
This study comprised children with lead poisoning and sex- and age-matched healthy controls who were attending The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University between 1 January 2015 and 31 December 2017. The inclusion criteria were as follows: (i) blood lead levels ≥100 µg/L, which were diagnosed as lead poisoning, and (ii) age < 14 years. The main exclusion criteria were as follows: (i) patients with known pre-existing chronic systematic disorders, such as hepatic disease, renal disease, endocrine disease, cardiovascular disease, immune disorders, hematological disease, or gastrointestinal disease; (ii) patients with malignant neoplasm; and (iii) patients with an acute bacterial infection.
Initially, 359 children who were diagnosed with lead poisoning were included, but 3 were excluded because of pneumonia and 1 was excluded for diabetes. The control group consisted of children whose blood lead levels were <100 μg/L and they did not have any disorders mentioned in the exclusion criteria. We recorded age, sex, weight, symptoms, and sources of lead exposure in the patients with lead poisoning.

**Methods**

Peripheral venous blood samples were taken in the morning after overnight fasting by trained nurses on the second day of admission to our hospital. Each child’s blood was collected into three blood collection tubes to measure biochemical parameters, hematology, and blood lead levels. Serum levels of bilirubin and albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, uric acid, creatinine, urea, and alkaline phosphatase (ALP) were measured by enzymatic methods using a Clinical Analyzer Siemens Advia 2400 (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). Hematology was measured using a Sysmex XE5000 hematology analyzer (Sysmex, Kobe, Japan) with whole blood. Blood lead levels were determined with atomic absorption spectrometry on a Bohui BH2101s atomic absorption spectrometer (Bohui, Beijing, China). According to the instructions of the lead and cadmium determination kit produced by Bohui Company, we preheated the atomic absorption spectrometer for starting measurement. The parameters were automatically adjusted to the optimal state by the software of the instrument. A standard curve ($r \geq 0.9950$) was then created on the basis of the corresponding calibration solution’s absorbance values. We added 40 μL of whole blood to 0.36 mL of diluent, mixed them well, and measured blood lead levels after 30 minutes of standing at room temperature. Ethical approval was obtained from the Second Affiliated Hospital of Wenzhou Medical University Research Ethics Committee (LCKY2020-412). Guardians of patients were informed and agreed that the patients’ medical data may be used for teaching and research in the future. Therefore, consent was not required after obtaining approval from the hospital’s ethics committee.

**Statistical analysis**

Data were analyzed using IBM SPSS software (version 23.0; IBM Corp., Armonk, NY, USA) and Medcalc 11.4.2 (MedCalc Software, Mariakerke, Belgium). Normality of distribution was analyzed with the Kolmogorov–Smirnov test. Results are reported as mean ± standard deviation for a normal distribution and median (interquartile range) for a skewed distribution. Differences between the lead poisoning group and control group were tested using the independent Student’s t-test for normally distributed variables. The Mann–Whitney U-test was used to compare non-parametrically distributed variables. Differences between categorical variables were determined by the $\chi^2$ test. Indicators with statistically significant differences were then analyzed using multivariate logistic regression analysis. Spearman correlation analysis was performed to assess the independence of indicators. Statistical significance was set at a $P$ value $<0.05$.

**Results**

A total of 355 patients with lead poisoning and 355 sex- and age-matched controls were included. The study population had a median age of 4.0 years (interquartile range, 6.0). Among the patients with lead poisoning, the median weight was 16.5 kg (interquartile range, 12.5), 72.11% were boys, almost half had single neurological symptoms, and approximately one third had symptoms from multiple systems.
(neurological symptoms with gastrointestinal disorders or developmental retardation). The exposure sources to lead included consumption of domestic polluted water, folk skincare containing lead powder, environmental lead pollution, and parental occupational to lead exposure. The characteristics of the children are shown in Table 1.

Among the biochemical parameters, albumin, total bilirubin, direct bilirubin, indirect bilirubin, urea, and creatinine levels were significantly lower in the lead poisoning group than in the control group (all P < 0.001). AST, total protein, and globulin levels were significantly higher in the lead poisoning group than in the control group (all P < 0.05). No significant difference in ALT, uric acid, or ALP levels was observed between the two groups (Table 2).

In adjusted multivariate logistic regression analysis, direct bilirubin had the highest odds ratio (OR) (1.658, 95% confidence interval [CI] 1.322–2.079) when adjusted for other covariates. Five additional covariates were significantly (all P < 0.05) associated with the status of lead poisoning in children when adjusted for other covariates, including albumin (OR 1.437, 95% CI 1.286–1.605), total protein (OR 0.825, 95% CI 0.777–0.876), urea (OR 1.200, 95% CI 1.020–1.411), creatinine (OR 1.047, 95% CI 1.019–1.075), and AST (OR 0.979, 95% CI 0.960–0.998) (Table 3).

Spearman correlation analysis was used to examine the relationships of albumin, creatinine, and urea with weight, and direct bilirubin with hemoglobin in children with lead poisoning (Table 2). We found that serum levels of albumin (r = 0.077) and urea (r = 0.110) were minimally affected by weight, and direct bilirubin levels were minimally affected by hemoglobin (r = 0.166). However, creatinine levels had a moderate correlation with weight (r = 0.553).12

Table 1. Characteristics of the lead poisoning and control groups.

| Characteristics                              | Control group | Lead poisoning group | P   |
|----------------------------------------------|---------------|----------------------|-----|
| Age (years), median (IQR)                    | 4.0 (6.0)     | 4.0 (6.0)            | NS  |
| Sex (n)                                      | 355           | 355                  | NS  |
| Girls, n (%)                                 | 99 (27.89)    | 99 (27.89)           |     |
| Boys, n (%)                                  | 256 (72.11)   | 256 (72.11)          |     |
| Weight (kg), median (IQR)                    | –             | 16.5 (12.5)          | –   |
| Symptoms (n)                                 | –             | 355                  | –   |
| Single neurological symptoms, n (%)          | –             | 154 (43.38)          | –   |
| Multiple systems symptoms, including the nervous system, n (%) | – | 126 (35.49) | – |
| No obvious symptoms, n (%)                   | –             | 75 (21.13)           | –   |
| Lead exposure (n)                             | –             | 355                  | –   |
| Consumption of domestic polluted water, n (%)| –             | 64 (18.03)           | –   |
| Folk skin care containing lead powder, n (%)  | –             | 85 (23.94)           | –   |
| Environmental lead pollution, n (%)          | –             | 75 (21.13)           | –   |
| Parental occupational lead exposure, n (%)   | –             | 61 (17.18)           | –   |
| Multiple exposure sources of lead, n (%)     | –             | 64 (18.03)           | –   |
| No identified potential source, n (%)        | –             | 117 (32.96)          | –   |

IQR, interquartile range; NS, no significance; –, data not available.
The toxicity of lead is affecting multiple systems, and children are especially vulnerable to lead. Lead can delay growth and development, impair hearing, increase dental caries, and alter children’s cognition and behavior after absorption. A total of 75% of lead is captured by the liver after absorption and the kidney is the second organ to capture lead. In our study, 78.87% of patients had neurological symptoms, and some were accompanied by gastrointestinal disorders or developmental retardation. In the lead poisoning group, we observed slightly higher AST, total protein, and globulin levels, and lower serum albumin, total bilirubin, direct bilirubin, indirect bilirubin, urea, and creatinine levels compared with the control group. With further analyses, direct bilirubin, albumin, total protein, urea, creatinine, and AST were significant independent variables for assessing the lead poisoning status in children.

### Table 2. Biochemical parameters of the lead poisoning and control groups.

| Biochemical parameters | Control group | Lead poisoning group | P       |
|------------------------|--------------|----------------------|---------|
| Blood lead (µg/L)      | 39.0 (22.0)  | 167.0 (93.5)         | < 0.001 |
| ALT (U/L)              | 15.0 (6.0)   | 15.0 (8.0)           | NS      |
| AST (U/L)              | 30.0 (10.8)  | 32.0 (12.0)          | < 0.001 |
| Total protein (g/L)    | 69.6 (5.8)   | 70.2 (5.4)           | 0.049   |
| Albumin (g/L)          | 45.3 ± 2.1   | 44.6 ± 2.4           | < 0.001 |
| Globulin (g/L)         | 24.5 ± 3.4   | 25.3 ± 3.2           | < 0.001 |
| Total bilirubin (µmol/L) | 7.6 (4.3) | 6.0 (3.4)          | < 0.001 |
| Direct bilirubin (µmol/L) | 1.8 (1.2) | 1.4 (0.9)        | < 0.001 |
| Indirect bilirubin (µmol/L) | 5.8 (3.2) | 4.6 (2.7)       | < 0.001 |
| Uric acid (µmol/L)     | 288.0 ± 72.6 | 278.8 ± 67.5         | NS      |
| Urea (mmol/L)          | 4.6 ± 1.1    | 4.4 ± 1.0            | < 0.001 |
| Creatinine (µmol/L)    | 30.0 (11.2)  | 27.7 (10.3)          | < 0.001 |
| ALP (U/L)              | 240.5 (74.8) | 236.0 (75.5)         | NS      |

Data are median and interquartile range or mean ± standard deviation.

ALT, alanine aminotransferase; NS, no significance; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

### Table 3. Multivariable associations of biochemical parameters with the lead poisoning status in children.

| Variables                  | Unadjusted OR (95% CI) | P value | Adjusted OR (95% CI) | P value |
|----------------------------|------------------------|---------|----------------------|---------|
| AST                        | 0.980 (0.961–0.999)    | 0.039   | 0.979 (0.960–0.998)  | 0.035   |
| Total protein              | 0.007 (0.000–∞)        | 1.000   | 0.825 (0.777–0.876)  | < 0.001 |
| Albumin                    | 159.654 (0.000–∞)      | 1.000   | 1.437 (1.286–1.605)  | < 0.001 |
| Globulin                   | 111.297 (0.000–∞)      | 1.000   | –                    | –       |
| Total bilirubin            | 0.978 (0.846–1.131)    | 0.765   | –                    | –       |
| Direct bilirubin           | 1.791 (1.035–3.096)    | 0.037   | 1.658 (1.322–2.079)  | < 0.001 |
| Indirect bilirubin         | –                      | –       | –                    | –       |
| Urea                       | 1.203 (1.023–1.416)    | 0.026   | 1.200 (1.020–1.411)  | 0.028   |
| Creatinine                 | 1.047 (1.019–1.076)    | 0.001   | 1.047 (1.019–1.075)  | 0.001   |

OR, odds ratio; CI, confidence interval; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

### Discussion

The toxicity of lead is affecting multiple systems, and children are especially vulnerable to lead. Lead can delay growth and development, impair hearing, increase dental caries, and alter children’s cognition and behavior after absorption. A total of 75% of lead is captured by the liver after absorption and the kidney is the second organ to capture lead. In our study, 78.87% of patients had neurological symptoms, and some were accompanied by gastrointestinal disorders or developmental retardation. In the lead poisoning group, we observed slightly higher AST, total protein, and globulin levels, and lower serum albumin, total bilirubin, direct bilirubin, indirect bilirubin, urea, and creatinine levels compared with the control group. With further analyses, direct bilirubin, albumin, total protein, urea, creatinine, and AST were significant independent variables for assessing the lead poisoning status in children.
Serum bilirubin levels are increased in adults who are exposed to lead, and a similar result was shown in an experiment on mice. However, we observed that bilirubin levels were significantly lower in the lead poisoning group than in the control group, and bilirubin had the highest OR in adjusted multivariate logistic regression analysis. Bilirubin is a metabolite of hemoglobin, and the interactions between lead and hemoglobin metabolism are complex. Lead may increase bilirubin levels via induction of degradation of hemoglobin. However, depletion of the hemoglobin pool may result in less synthesis of bilirubin. In our study, Spearman correlation analysis showed only a weak correlation (0.166) between hemoglobin and bilirubin levels in children with lead poisoning (Table 4). Therefore, we conclude that insufficient hemopoiesis did not account for decreased direct bilirubin levels. Additionally, the role of bilirubin in the body is complex. Bilirubin exerts toxic effects when present in excess, but it acts as a non-enzymatic antioxidant at the proper concentration. Bilirubin is associated with prevention of oxidant-mediated apoptosis, which activates the main DNA repair pathways through homologous recombination and non-homologous end joining. In this study, we showed that the decrease in bilirubin levels was not affected by its source (hemoglobin), with only a weak correlation between them. We hypothesized that reduced bilirubin levels in children with lead poisoning are most likely due to decompensated depletion against oxidation caused by lead.

We also found that albumin, urea, and creatinine levels were significantly lower in children with lead poisoning than in controls. Serum albumin plays a major antioxididant role in extracellular fluids, and its anti-oxidative capacity is stronger than superoxide dismutase, catalase, vitamin C, and vitamin E (a-tocopherol). A decrease in albumin levels was only weakly correlated with weight (0.077) in our study. Therefore, an influence of nutritional status was excluded. Creatinine is not simply a metabolite of muscle and it also contributes to antioxidant capacity. However, creatinine was moderately correlated with weight in the current study, which suggested that a decrease in creatinine may be a synergistic effect of oxidative stress and developmental retardation. Urea is a metabolic waste of protein, with no physiological function in the body. In our study, a decrease in urea levels was only weakly correlated with other indicators. We consider that reduced protein absorption caused by gastrointestinal disorders might be the reason for this weak correlation. In summary, direct bilirubin, albumin, and creatinine levels, considered non-enzymatic antioxidants in vivo, were decreased in children with lead poisoning, reflecting oxidative hyperirritability.

Table 4. Spearman correlation analysis of albumin, creatinine, and urea levels with weight, and direct bilirubin with Hb levels in children with lead poisoning.

| Weight | Albumin | Creatinine | Urea | Hemoglobin | Direct bilirubin |
|--------|---------|------------|------|------------|-----------------|
| Weight | 1       | 0.077      | 0.553** | 0.110      | -               |
| Albumin| 1       | -0.038     | 0.160** | -          | -               |
| Creatinine | 1       | 0.146** | -      | -          | 0.166**         |
| Hemoglobin | -      | -         | -      | 1          | -               |
| Direct bilirubin | -      | -         | -      | -          | 1               |

**P < 0.01.
- not performed.
With regard to other biochemical parameters, lead can cause liver damage, raising ALT and AST levels to an abnormal range. Moreover, uric acid is also an antioxidant, accounting for up to 60% of blood anti-oxidative capacity. The association of uric acid levels is unclear in diseases associated with oxidative stress. However, serum levels of liver-related biochemical indices and uric acid were not well associated with the lead poisoning status in this study.

To the best of our knowledge, this study is the first to show a significant decrease in serum bilirubin levels in children with lead poisoning. However, this was an observational study with unknown confounders, and detection of total oxidant status or total antioxidant status is currently not available in the clinical setting. Or else, we are able to determine the level of oxidative stress in children with lead poisoning.

In conclusion, lead interferes with the non-enzymatic antioxidant system in children, uniquely showing a decrease in serum bilirubin levels. We assume that lead that is accumulated in the body continues to generate reactive oxygen species and consume antioxidants, which may be responsible for a lower direct bilirubin level in the children. The possible association between lead toxicity and decreased serum bilirubin levels in children is not fully understood and requires further investigation.

Author contributions
MSY and SC performed background research, analyzed the data, and wrote the first draft of the manuscript. TTZ and ZBC assisted with data acquisition, data analysis, and preparation of the manuscript. HLC provided supervision, professional and academic support, and intellectual input, and co-wrote the final draft of the manuscript.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

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