Associations among Heavy Metals and Proteinuria and Chronic Kidney Disease

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Abstract: Background: The prevalence of chronic kidney disease (CKD) is increasing annually in Taiwan. In addition to traditional risk factors, heavy metals contribute to the development of CKD. The aim of this study was to investigate associations among heavy metals and proteinuria and CKD in the general population in Southern Taiwan. We also explored the interaction and synergistic effects among heavy metals on proteinuria. Methods: We conducted a health survey in the general population living in Southern Taiwan between June 2016 and September 2018. Seven heavy metals were measured: blood lead (Pb) and urine nickel (Ni), chromium (Cr), manganese (Mn), arsenic (As), copper (Cu), and cadmium (Cd). Proteinuria was measured using reagent strips. CKD was defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m². Results: The mean age of the participants was 55.1 ± 13.2 years and included 977 males and 1470 females. Participants with high blood Pb and high urine Ni, Mn, Cu, and Cd were significantly associated with proteinuria. Interactions between blood Pb and urine Cr, and between urine Cd and Cu, had significant effects on proteinuria. The participants with high blood Pb and high urine Cu were significantly associated with an eGFR of <60 mL/min/1.73 m². Conclusion: High blood Pb and high urine Cu may be associated with proteinuria and an eGFR of <60 mL/min/1.73 m². High urine Ni, Mn, and Cd were significantly associated with proteinuria. Co-exposure to Cd and Cu, and Pb and Cr, may have synergistic effects on proteinuria.

Keywords: heavy metals; proteinuria; chronic kidney disease; estimated glomerular filtration rate; renal function
1. Introduction

Environmental pollution such as heavy metals, air pollutants, agricultural chemicals, and contaminated drinking water and food is a major cause of disease, disability, and death worldwide [1], particularly as the global environment continues to worsen. Exposure to endocrine-disrupting or toxic chemicals plays an important role in disease initiation and progression [2], including respiratory diseases (such as asthma and chronic obstructive pulmonary disorder), neurobehavioral disorders (such as attention-deficit/hyperactivity disorder [3], depression, and other mental disorders), obesity and type 2 diabetes mellitus (DM) [4,5], and cancer [6]. Heavy metal exposure can cause various serious human diseases, such as respiratory problems, neurological disorders, and cancers [7]. Heavy metal exposure is also known to be a cause of acute and chronic kidney disease (CKD) [8], as are high levels of occupational exposure [9]. The widespread use of heavy metals in industrial processes has resulted in environmental contamination of drinking water and soil, thereby increasing potential exposure among the general population [10]. However, the association between heavy metals and kidney problems in the general population remains poorly defined.

CKD is a major public health issue, and it can progress to end-stage renal disease (ESRD), with a reported prevalence ranging between 10.5% and 13.1% [11–13]. Patients with CKD have poor cardiovascular outcomes and a higher risk of mortality [14]. CKD is defined as evidence of kidney damage (such as albuminuria or proteinuria) and/or reduced kidney function (an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m²) for a period of at least three months [15]. Proteinuria is considered to be an early and essential diagnostic tool to evaluate disease severity and to monitor treatment response in several kidney diseases [16], and it is associated with an increased risk of ESRD and early death [17]. Taiwan has been reported to have the highest global incidence and prevalence rates of ESRD [18], and the annual incidence and prevalence rates increased by approximately three- and sevenfold, respectively, from 1990 to 2010 [18,19]. CKD is considered to be a multifactorial disease related to sex, age, obesity and smoking; chronic diseases such as metabolic diseases, DM, hyperlipidemia, hyperuricemia, hypertension and cardiovascular diseases; and also to genetic and environmental factors [7]. In addition to these tradition risk factors, numerous studies have suggested that heavy metals such as cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), uranium, and chromium (Cr) accumulate in the kidneys and that even low levels can induce CKD and proteinuria [9,20].

The aim of this study was to investigate the relationships among serum Pb, urine nickel (Ni), Cr, manganese (Mn), As, copper (Cu), and Cd and proteinuria and CKD in the general population in Southern Taiwan. We also explored the interactions and synergistic effects among these heavy metals on proteinuria.

2. Materials and Methods

2.1. Subject Recruitment

We conducted a health survey of the general population living in Southern Taiwan from June 2016 to September 2018. Participants were recruited through advertisements, and those who were willing to attend the study were included. All participants had face-to-face interviews during which anthropometric variables (weight and height) were measured, physical examinations were performed, and medical histories were recorded by an experienced physician.

2.2. Collection of Demographic, Medical, and Laboratory Data

Baseline variables were recorded, including systolic blood pressure (SBP) and diastolic blood pressure (DBP); laboratory data (fasting glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, hemoglobin, eGFR, and uric acid); medical history (DM and hyper-
tension); occupation history; living environment; and demographic characteristics (age and sex). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [21]. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

2.3. Measurement of Blood and Urine Heavy Metals
A total of seven heavy metals were measured: Pb in the blood, and Ni, Cr, Mn, As, Cu, and Cd in the urine. Concentrations of blood Pb (AA800v, PerkinElmer) and the other heavy metals in urine (ICP-MS, NexION 300 Series, Perkin Elmer) were analyzed using graphite furnace atomic absorption spectrometry as described in the National Institute of Environmental Research.

2.4. Definition of Proteinuria and CKD
Proteinuria was measured using reagent strips (Hema-Combistix, Bayer Diagnostics). A test result of 1+ or higher was defined as being positive. CKD was defined as an eGFR of <60 mL/min/1.73 m² depending on the KDOQI/DOQI clinical practice guidelines [22].

2.5. Ethics Statement
The Institutional Review Board of Kaohsiung Medical University Hospital approved the study protocol (number: KMUHIRB-G(II)-20190011). All participants provided informed consent before participating in the study.

2.6. Statistical Analysis
Data are presented as percentages, means ± standard deviations (SD), or medians (25th–75th percentile) for heavy metals and triglycerides. The chi-square test was used to test between-group differences for categorical variables, and the independent t-test was used for continuous variables. Multivariable logistic regression analysis was used to examine associations among the heavy metals and proteinuria and CKD. Interactions among heavy metals and their effects on proteinuria were analyzed using logistic regression analysis. The interaction effects of the heavy metals on proteinuria were illustrated using the SGPLOT procedure. Multivariable linear regression analysis was used to identify associations between heavy metals and eGFR. Receiver operating characteristic (ROC) curves and areas under the curves (AUCs) were used to assess the performance and predictive abilities, respectively, of the heavy metals in identifying proteinuria and an eGFR of <60 mL/min/1.73 m². For all heavy metals (in both blood and urine), natural logarithms were used. A p value of less than 0.05 was considered to indicate a statistically significant difference. All statistical analyses were conducted using SPSS version 19.0 for Windows (SPSS Inc. Chicago, USA).

3. Results
The mean age of the 2447 participants was 55.1 ± 13.2 years and included 977 males and 1470 females. The overall prevalence rates of proteinuria and eGFR <60 mL/min/1.73 m² were 10.3% and 6.3%, respectively. A comparison of the clinical characteristics between the participants with and without proteinuria is shown in Table 1. Compared to the participants without proteinuria, those with proteinuria were older, more frequently male, and more likely to be jobless; had higher prevalence rates of DM and hypertension; and had higher values of BMI, SBP, DBP, fasting glucose, triglycerides, and uric acid. However, those without proteinuria had lower values of total cholesterol, HDL-cholesterol, LDL-cholesterol, and eGFR, and they were less likely to decorate their house in the past six months. Regarding heavy metals, the participants with proteinuria had higher levels of blood Pb and urine Ni, Mn, Cu, and Cd.
3.1. Determinants of Proteinuria

Table 2 shows the determinants of proteinuria in the study participants. After adjusting for each heavy metal and for age, sex, DM, hypertension, BMI, DBP, SBP, occupation, house decoration in the past six months, log triglycerides, fasting glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, eGFR, and uric acid (significant variables in Table 1), the participants with high blood Pb (log per 1 μg/dL; odds ratio (OR), 3.089; 95% confidence interval (CI), 1.630 to 5.853; p = 0.001), high urine Ni (log per 1 μg/L; OR, 3.642; 95% CI, 2.285 to 5.807; p < 0.001), high urine Mn (log per 1 μg/L; OR, 2.443; 95% CI, 1.649 to 3.619; p < 0.001), high urine Cu (log per 0.1 μg/dL; OR, 1.945; 95% CI, 1.750 to 2.162; p < 0.001), and high urine Cd (log per 1 μg/L; OR, 2.671; 95% CI, 1.733 to 4.118; p < 0.001) were significantly associated with proteinuria. However, urine Cr and As were not significantly associated with proteinuria.

Table 1. Comparison of clinical characteristics among participants with and without proteinuria.

| Characteristics | All (n = 2447) | Without Proteinuria (n = 2194) | With Proteinuria (n = 253) | p     |
|-----------------|---------------|-------------------------------|---------------------------|-------|
| Age (year)      | 55.1 ± 13.2   | 54.6 ± 13.0                   | 59.7 ± 14.0               | <0.001|
| Male gender (%) | 39.9          | 39.0                          | 48.2                      | 0.004 |
| DM (%)          | 10.5          | 8.4                           | 28.5                      | <0.001|
| Hypertension (%)| 25.3          | 23.0                          | 45.5                      | <0.001|
| BMI (kg/m²)     | 25.0 ± 4.0    | 24.8 ± 3.9                    | 26.2 ± 4.6                | <0.001|
| SBP (mmHg)      | 132.1 ± 19.8  | 131.1 ± 19.2                  | 140.0 ± 22.4              | <0.001|
| DBP (mmHg)      | 77.5 ± 11.7   | 77.2 ± 11.4                   | 80.1 ± 13.6               | 0.001 |
| Occupation (%)  | 4.9           | 4.9                           | 5.1                       | 0.041 |
| Agriculture, forestry, fishing, and animal husbandry | 20.8          | 21.1                          | 18.4                      |       |
| Commerce        | 11.3          | 11.9                          | 6.1                       |       |
| Industry        | 23.0          | 22.9                          | 23.5                      |       |
| Government employees | 6.2           | 6.3                           | 4.6                       |       |
| Service industry| 33.8          | 32.8                          | 42.3                      |       |
| None            |               |                               |                           |       |
| Living environment (%) | 6.3           | 6.2                           | 7.2                       | 0.556 |
| Oil-painted in the past six months | 4.1           | 4.4                           | 1.4                       | 0.041 |
| House decoration in the past six months | 74.2          | 73.9                          | 76.9                      | 0.350 |
| Burned incense  |               |                               |                           |       |
| Laboratory parameters |              |                               |                           |       |
| Fasting glucose (mg/dL) | 99.9 ± 27.4  | 97.6 ± 23.8                   | 119.7 ± 43.4              | <0.001|
| Triglyceride (mg/dL) | 105.0 (73.0–150.0) | 102.0 (72.0–146.0) | 129.0 (90.0–192.5) | <0.001|
| Total cholesterol (mg/dL) | 199.6 ± 37.4 | 200.2 ± 37.2                 | 194.9 ± 39.4              | 0.043 |
| HDL-cholesterol (mg/dL) | 53.0 ± 13.6  | 53.3 ± 13.6                   | 49.9 ± 13.9               | <0.001|
| LDL-cholesterol (mg/dL) | 119.2 ± 34.0 | 119.8 ± 34.0                 | 113.5 ± 34.0              | 0.005 |
| Hemoglobin (g/dL) | 14.0 ± 1.6    | 14.0 ± 1.6                    | 14.0 ± 1.9                | 0.887 |
| eGFR (mL/min/1.73 m²) | 89.1 ± 16.3  | 90.2 ± 15.0                   | 79.3 ± 22.8               | <0.001|
| Uric acid (mg/dL) | 5.7 ± 1.6     | 5.7 ± 1.5                     | 6.2 ± 1.7                 | <0.001|
| Heavy metals     |               |                               |                           |       |
| Blood            |               |                               |                           |       |
| Pb (μg/dL)       | 1.6 (1.0–2.2) | 1.5 (1.0–2.2)                 | 1.9 (1.3–2.7)             | <0.001|
| Urine            |               |                               |                           |       |
| Ni (μg/L)        | 2.4 (1.5–3.7) | 2.3 (1.5–3.5)                 | 3.3 (2.3–5.5)             | <0.001|
| Cr (μg/L)        | 0.1 (0.1–0.1) | 0.1 (0.1–0.1)                 | 0.1 (0.1–0.1)             | 0.134 |
| Mn (μg/L)        | 1.7 (0.9–3.0) | 1.7 (0.9–2.9)                 | 2.2 (1.2–3.5)             | <0.001|
| As (μg/L)        | 78.9 (45.6–142.0) | 78.2 (45.3–142.5) | 82.7 (48.6–138.9) | 0.760 |
| Cu (μg/dL)       | 1.5 (1.0–2.0) | 1.4 (1.0–1.8)                 | 2.4 (1.9–3.5)             | <0.001|
| Cd (μg/L)        | 0.8 (0.5–1.4) | 0.8 (0.3–1.5)                 | 1.1 (0.6–2.0)             | <0.001|

Abbreviations: DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; Pb, lead; Ni, nickel; Cr, chromium; Mn, manganese; As, arsenic; Cu, copper; Cd, cadmium.
Table 2. Association of heavy metals with proteinuria using multivariable logistic regression analysis.

| Heavy Metals | OR (95% CI) | p      |
|--------------|------------|--------|
| Blood        |            |        |
| Pb (log per 1 μg/dL) | 3.089 (1.630–5.853) | 0.001  |
| Urine        |            |        |
| Ni (log per 1 μg/L)  | 3.642 (2.285–5.807) | <0.001 |
| Cr (log per 1 μg/L)  | 1.810 (0.793–4.128) | 0.159  |
| Mn (log per 1 μg/L)  | 2.443 (1.649–3.619) | <0.001 |
| As (log per 1 μg/L)  | 0.765 (0.473–1.239) | 0.277  |
| Cu (log per 0.1 μg/dL) | 1.945 (1.750–2.162) | <0.001 |
| Cd (log per 1 μg/L)  | 2.671 (1.733–4.118) | <0.001 |

Values expressed as odds ratios (OR) and 95% confidence intervals (CI). Covariates in the multivariable model included age, sex, DM, hypertension, BMI, SBP, DBP, occupation, house decoration in the past six months, fasting glucose, log triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, eGFR, and uric acid (significant variables in Table 1).

We analyzed the effects of interactions between the heavy metals on proteinuria using logistic regression analysis. The results showed that the effects of interactions between blood Pb and urine Cr (OR, 14.846; 95% CI, 1.032 to 213.663; p = 0.047) and urine Cd and Cu (OR, 1.226; 95% CI, 1.017 to 1.478; p = 0.033) on proteinuria were statistically significant. However, interactions of other combinations did not achieve significance. Figure 1 illustrates the synergistic effect of blood Pb and urine Cr on proteinuria. When log Pb = 0.70, every additional unit of log Cr increased the risk of proteinuria by 2.43 times (p = 0.0460, Supplement Table S1). Figure 2 illustrates the synergistic effect of urine Cd and Cu on proteinuria. When log Cd = 0.0, every additional unit of log Cr increased the risk of proteinuria by 2.30 times (p < 0.001, Supplement Table S2).

Figure 1. Synergistic effect of blood Pb and urine Cr on proteinuria. The interaction between blood Pb and urine Cr on proteinuria was statistically significant (p = 0.047).
**Figure 2.** Synergistic effect of urine Cd and Cu on proteinuria. The interaction between urine Cd and Cu on proteinuria was statistically significant ($p = 0.033$).

### 3.2. Determinants of CKD

Table 3 shows a comparison of the clinical characteristics among the participants with and without an eGFR of $<60$ mL/min/1.73 m$^2$. Compared to the participants without an eGFR of $<60$ mL/min/1.73 m$^2$, those with an eGFR of $<60$ mL/min/1.73 m$^2$ were older, more frequently female, more likely to be jobless, and more likely to burn incense; had higher prevalence rates of DM and hypertension; and had higher values of SBP, fasting glucose, triglycerides, and uric acid. However, those with an eGFR of $<60$ mL/min/1.73 m$^2$ had lower values of hemoglobin and were less likely to decorate their house in the past six months. Regarding heavy metals, the participants with an eGFR of $<60$ mL/min/1.73 m$^2$ had higher levels of blood Pb and urine Ni, As, and Cu.

| Characteristics | eGFR $\geq 60$ ($n = 2292$) | eGFR $< 60$ ($n = 155$) | $p$  |
|-----------------|-----------------------------|--------------------------|------|
| Heavy metals    |                             |                          |      |
| Blood           |                             |                          |      |
| Pb (μg/dL)      | 1.5 (1.0–2.2)               | 1.8 (1.2–2.5)            | 0.002|
| Urine           |                             |                          |      |
| Ni (μg/L)       | 2.4 (1.5–3.7)               | 2.7 (1.8–4.3)            | <0.001|
| Cr (μg/L)       | 0.1 (0.1–0.1)               | 0.1 (0.1–0.1)            | 0.371|
| Mn (μg/L)       | 1.7 (0.9–3.0)               | 1.6 (0.7–2.7)            | 0.249|
| As (μg/L)       | 76.3 (44.1–139.0)           | 107.4 (73.0–177.1)       | <0.001|
| Cu (μg/dL)      | 1.4 (1.0–1.9)               | 1.7 (1.3–2.4)            | <0.001|
| Cd (μg/L)       | 0.8 (0.5–1.4)               | 0.8 (0.5–1.5)            | 0.352|

Abbreviations are the same as in Table 1.

Table 4 shows the determinants of an eGFR of $<60$ mL/min/1.73 m$^2$ in the study participants. After adjusting for each heavy metal and for age, sex, DM, hypertension, SBP, occupation, house decoration in the past six months, burned incense, fasting glucose, log triglycerides, hemoglobin, and uric acid (significant variables in Table 3), the participants with high blood Pb (log per 1 μg/dL; OR, 3.727; 95% CI, 1.207 to 11.510; $p = 0.022$) and
high urine Cu (log per 0.1 μg/dL; OR, 1.163; 95% CI, 1.038 to 1.303; \( p = 0.009 \)) were significantly associated with an eGFR of <60 mL/min/1.73 m². However, urine Ni, Cr, Mn, As, and Cd were not significantly associated with an eGFR of <60 mL/min/1.73 m².

Table 4. Association of heavy metals with an eGFR of <60 mL/min/1.73 m² using multivariable logistic regression analysis.

| Heavy Metals | Multivariable OR (95% CI) | p     |
|--------------|---------------------------|-------|
| Blood        |                           |       |
| Pb (log per 1 μg/dL) | 3.727 (1.207–11.510) | 0.022 |
| Urine        |                           |       |
| Ni (log per 1 μg/L)   | 1.315 (0.779–2.220)     | 0.305 |
| Cr (log per 1 μg/L)   | 0.653 (0.128–3.329)     | 0.608 |
| Mn (log per 1 μg/L)   | 0.894 (0.539–1.482)     | 0.663 |
| As (log per 1 μg/L)   | 0.775 (0.369–1.629)     | 0.502 |
| Cu (log per 0.1 μg/dL) | 1.163 (1.038–1.303)   | 0.009 |
| Cd (log per 1 μg/L)   | 0.758 (0.404–1.423)     | 0.389 |

Values expressed as odds ratios (OR) and 95% confidence intervals (CI). Covariates in the multivariable model included age, sex, DM, hypertension, SBP, occupation, house decoration in the past six months, burned incense, fasting glucose, log triglyceride, hemoglobin, and uric acid (significant variables in Table 3).

3.3. ROC Curve Analysis for Heavy Metals in Identifying Proteinuria and eGFR < 60 mL/min/1.73 m²

Figure 3a demonstrates the ROC analysis and AUCs of seven heavy metals in identifying proteinuria. Among these heavy metals, Cu had the greatest AUC (AUC = 0.842), followed by Ni (AUC = 0.667), Pb (AUC = 0.612), Cd (AUC = 0.599), Mn (AUC = 0.583), Cr (AUC = 0.513), and As (AUC = 0.509). Table 5 demonstrates the ROC analysis and AUCs, the cutoff values, the Youden index values, and the sensitivity and specificity of seven heavy metals for proteinuria.

Figure 3b demonstrates the ROC analysis and AUCs of seven heavy metals in identifying eGFR < 60 mL/min/1.73 m². Among these heavy metals, As had the greatest AUC (AUC = 0.623), followed by Cu (AUC = 0.63), Pb (AUC = 0.580), Ni (AUC = 0.559), Cd (AUC = 0.515), Cr (AUC = 0.500), and Mn (AUC = 0.459). Table 6 demonstrates the ROC analysis and AUCs, the cutoff values, the Youden index values, and the sensitivity and specificity of seven heavy metals for eGFR <60 mL/min/1.73 m².
Figure 3. Comparison of the AUCs of seven heavy metals for identifying (a) proteinuria and (b) eGFR < 60 mL/min/1.73 m².

Table 5. Area under the curve (AUC), cutoff value, Youden index, and sensitivity and specificity of seven heavy metals for proteinuria.

| Heavy Metals | AUC (95% Confidence Interval) | Cutoff Value | Sensitivity (%) | Specificity (%) | Youden Index |
|--------------|-------------------------------|--------------|----------------|----------------|--------------|
| Pb           | 0.612 (0.575–0.648) *         | 0.217        | 61.0           | 56.1           | 0.171        |
| Ni           | 0.667 (0.633–0.701) *         | 0.455        | 60.6           | 62.5           | 0.231        |
| Cr           | 0.513 (0.474–0.551)           | −0.850       | 6.8            | 95.7           | 0.025        |
| Mn           | 0.583 (0.546–0.620) *         | 0.267        | 57.4           | 54.9           | 0.123        |
| As           | 0.509 (0.472–0.547)           | 1.907        | 51.4           | 51.4           | 0.028        |
| Cu           | 0.842 (0.816–0.867) *         | 0.271        | 76.7           | 76.9           | 0.536        |
| Cd           | 0.599 (0.560–0.638) *         | −0.023       | 57.0           | 56.4           | 0.134        |

* *p* < 0.05. Abbreviations are the same as in Table 1.

Table 6. Area under the curve (AUC), cutoff value, Youden index, and sensitivity and specificity of seven heavy metals for an eGFR of < 60 mL/min/1.73 m².

| Heavy Metals | AUC (95% Confidence Interval) | Cutoff Value | Sensitivity (%) | Specificity (%) | Youden Index |
|--------------|-------------------------------|--------------|----------------|----------------|--------------|
| Pb           | 0.580 (0.535–0.624) *         | 0.217        | 56.9           | 55.0           | 0.119        |
| Ni           | 0.559 (0.514–0.603) *         | 0.407        | 52.9           | 53.3           | 0.062        |
| Cr           | 0.500 (0.453–0.547)           | −0.850       | 4.6            | 95.5           | 0.001        |
| Mn           | 0.459 (0.413–0.506)           | 0.217        | 45.1           | 47.1           | −0.078       |
| As           | 0.623 (0.582–0.665) *         | 1.984        | 60.8           | 60.8           | 0.216        |
| Cu           | 0.613 (0.568–0.659) *         | 0.192        | 55.6           | 56.8           | 0.124        |
| Cd           | 0.515 (0.469–0.561)           | −0.071       | 49.7           | 49.8           | −0.005       |

* *p* < 0.05. Abbreviations are the same as in Table 1.
4. Discussion

In this study, we found that the participants with high levels of blood Pb and urine Cu may be associated with proteinuria and an eGFR of <60 mL/min/1.73 m². In addition, the participants with high levels of urine Ni, Mn, and Cd were significantly associated with proteinuria. A synergistic effect of urine Cd and Cu on proteinuria was also observed. Although urine Cr was not significantly related to proteinuria, a synergistic effect of blood Pb and urine Cr on proteinuria was observed.

The first important finding of this study is that high blood Pb may be associated with proteinuria and an eGFR of <60 mL/min/1.73 m². In ROC curve analysis among seven heavy metals, blood Pb had the third highest predictive performance to identify proteinuria and an eGFR of <60 mL/min/1.73 m². Factors known to be associated with high Pb levels include the male sex, older age, low socioeconomic status, smoking, living in urban areas and older buildings, and Pb in paint and water pipes [23]. Pb accumulates in and is excreted by the kidneys [24], and there is convincing evidence to support a direct relationship between Pb exposure and several kidney diseases [8,25,26]. Pb exposure can cause oxidative stress in tubular and glomerular cells, leading to the generation of free radicals, which can contribute to cellular apoptosis and subsequent changes in renal structure and function [27]. Acute Pb intoxication can disturb solute and amino acid transport in renal tubules, leading to proximal tubular dysfunction, such as Fanconi syndrome [8,26]. In addition, chronic intoxication can cause progressive tubulointerstitial nephritis, glomerular sclerosis, and tubular atrophy [25]. Epidemiologic studies have shown a positive association between chronic low Pb exposure (blood Pb levels of <5–10 mg/dL) and reduced renal function [23,28]. Our findings suggest that the participants with high blood Pb may be associated with proteinuria and an eGFR of <60 mL/min/1.73 m², which is consistent with previous studies.

The second important finding of this study is that high urine Cu was associated with proteinuria and an eGFR of <60 mL/min/1.73 m². In ROC curve analysis among seven heavy metals, urine Cu had the highest predictive performance in identifying proteinuria and the second highest predictive performance to identify an eGFR of <60 mL/min/1.73 m². Cu is the third most abundant essential transition metal in humans, and it is a cofactor of many enzymes involved in a number of physiological pathways. Humans are primarily exposed to Cu through food intake, and it reaches the kidneys via circulation [20]. In the kidneys, Cu catalyzes the generation of highly reactive hydroxyl radicals, and this oxidative stress can cause proximal tubule necrosis [29,30]. Few epidemiological studies have investigated the potential relationship between Cu exposure and renal function. Yang et al. conducted a population-based cross-sectional study in China and found that urine Cu (>20.92 μg/L) was associated with an abnormal eGFR (an eGFR of <60 mL/min/1.73 m²) [31]. Another study enrolled 194 CKD patients in Taiwan and reported a significantly increasing trend of serum Cu with advanced stages of CKD [32]. This study also proposed that high urine Cu may increase the risk of proteinuria and a reduced eGFR.

Previous studies have discussed the relationship between Mn and CKD, however the results have been inconsistent [33,34]. A cross-sectional study in Spain found that predialysis patients with CKD were associated with higher circulating levels of Mn than controls [33]. However, Liu et al. conducted a prospective study to evaluate the associations between plasma metal levels and a decline in kidney function among middle-aged and elderly Chinese and did not find an association between plasma Mn and reduced renal function [34]. Another study from China reported that plasma Mn was negatively associated with CKD in people aged ≥90 years [35]. In the present study, we found that high urine Ni and Mn levels were associated with proteinuria. Nickel mainly accumulates in and is excreted by the kidneys [20]. Excess Ni has been shown to trigger an inflammatory response by activating nuclear factor-κB and tubular apoptosis through the phosphoinositide 3-kinase (PI3k)–RAC serine/threonine-protein kinase (AKT) pathway [20,36]. A retrospective study in Changhua, Taiwan, found that high urine Ni was a risk
could not exclude confounding factors including genetic variations, the use of medical and plastic factors such as...still considered to be a reasonable biomarker for inorganic As exposure in clinical practice. Measured quickly from urine was used to reflect the toxic form of inorganic As exposure. Total urine As concentration of the materials...nal dysfunction causes single measurement to define metal exposure is an important limitation. Min...been shown to have high sensitivity and specificity of >90%, when using a urine albumin-to-creatinine ratio of ≥300 mg/g as the reference standard [50]. Third, the use of a single measurement to define metal exposure is an important limitation. In addition, renal dysfunction causes less clearance of heavy metals, resulting in a high serum concentration of the materials, which may produce an incorrect interpretation. Fourth, total urine was used to reflect the toxic form of inorganic As exposure. Total urine As can be measured quickly for a large number of samples, however it does not account for variations in As uptake and metabolism between individuals. Nevertheless, total urine As is still considered to be a reasonable biomarker for inorganic As exposure in clinical practice. In addition, the living environment questionnaire did not include environments such as gas stations, thermal power plants, incinerators, oil refineries, chemical plants, and plastic factories near home, which might influence heavy metal values. Finally, we could not exclude confounding factors including genetic variations, the use of medical and...
tions, and the effect of other environmental pollutants, which could potentially induce renal dysfunction.

5. Conclusions

In the present study, high blood Pb and high urine Cu may be associated with proteinuria and an eGFR of <60 mL/min/1.73 m². High urine Ni, Mn, and Cd were also associated with proteinuria. Co-exposure to Cd and Cu, and Pb and Cr, may have synergistic effects on the association with proteinuria. Our findings should serve to remind health researchers and the government of the importance of environmental policies and legislative changes to improve human health. Future follow-up studies are necessary to clarify the causal relationships among heavy metals and proteinuria and CKD.

Supplementary Materials: The following are available online at www.mdpi.com/2075-4418/11/2/282/s1, Table S1: The interaction effect of Cr and Pb is associated with an increase in the risk of proteinuria occurrence. Table S2: the interaction effect of Cu and Cd is associated with an increase in the risk of proteinuria occurrence.

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