The association between NAFLD and advanced liver fibrosis with urinary heavy metal based on the NHANES 2013-2018

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Article

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Abstract

Background

Chronic liver disease poses an escalating health challenge on a global scale. It has been suggested that prolonged exposure to heavy metals could potentially contribute to the development of non-alcoholic fatty liver disease (NAFLD). Our study aimed to assess the correlation between urinary levels of specific heavy metals, including Ba, Cd, Co, Cs, Hg, Mo, Pb, Sb, Sn, Ti, and Ur, and the occurrence of NAFLD and advanced liver fibrosis within the general population of the United States.

Methods

In our study, we conducted a thorough analysis using data from the NHANES spanning from 2013 to 2018. To examine the correlation between urinary heavy metal concentration and the prevalence of NAFLD and advanced liver fibrosis, we employed a multivariable analysis that accounted for various factors such as sociodemographic characteristics, lifestyle factors, hypertension, and T2DM. This allowed us to control for potential confounding variables and obtain reliable findings regarding the association between urinary heavy metal concentration and the occurrence of NAFLD and advanced liver fibrosis.

Results

We employed multiple logistic regression models to examine the data, and the results revealed noteworthy findings. Higher levels of urinary Ba, Cd, Co, Pb, Sb, Sn, Tu, and Ur exhibited a significant positive association with NAFLD. Additionally, as the concentration of Cd, Pb, Sb, and Sn increased in urine, the likelihood of advanced liver fibrosis also significantly increased. These findings underscore the significant positive associations between the levels of specific heavy metals in urine and both NAFLD and advanced liver fibrosis.

Conclusion

The findings of this study suggest a significant association between elevated urinary Ba, Cd, Co, Pb, Sb, Sn, Tu, Ur concentration and NAFLD while a significant correlation was also found between higher urinary levels of Cd, Pb, Sb, Sn and advanced liver fibrosis.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a liver condition resulting from metabolic stress and strongly linked to insulin resistance (IR) and genetic predisposition. While the precise mechanisms driving the development of NAFLD remain intricate and not entirely comprehended, extensive research has consistently shown a noteworthy association between NAFLD and various other disorders, including type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS). The prevalence of NAFLD has been steadily rising in recent years due to shifts in dietary patterns, lifestyle choices, and overall health, resulting in significant socioeconomic burdens. NAFLD has emerged as the most prevalent chronic liver disease worldwide, affecting approximately 25.2% of the global population. NAFLD encompasses a continuum of progressive diseases, ranging from simple liver steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC). It is concerning that approximately one-third of NASH patients may progress to liver fibrosis, and in some cases, even cirrhosis and HCC. The development of liver fibrosis in NAFLD is sustained by long-term chronic liver parenchymal injury, inflammation, and oxidative stress. Consequently, liver fibrosis serves as a crucial indicator for predicting mortality and complications associated with liver diseases.

NAFLD represents a group of diverse disorders influenced by both genetic and environmental factors, which contribute to its development and progression. In addition to dietary factors and physical activity, emerging evidence from animal and human
studies indicates that heavy metals could potentially contribute to the onset of NAFLD\textsuperscript{8}. Heavy metals are metallic elements characterized by high density and atomic weight, and their presence can have detrimental effects on human health\textsuperscript{9}. The exposure to heavy metals is widespread among the population, stemming from various sources such as atmospheric pollutants, domestic waste, industrial discharge, and agricultural activities\textsuperscript{9}.

Toxicological investigations have demonstrated that heavy metals, such as lead and cadmium, have the potential to disrupt the hypothalamic dopaminergic system and endoplasmic reticulum proteostasis\textsuperscript{10–12}. Furthermore, they can impair adipogenesis and the secretion of adipocytokines, while also inducing liver inflammation and steatosis\textsuperscript{10–12}. Additionally, heavy metal exposure has been identified as a risk factor for various metabolic abnormalities, including diabetes, metabolic syndrome, obesity, and hypertension\textsuperscript{13, 14}. Several previous studies have reported positive correlations between urinary mixtures of metals and the presence of NAFLD and advanced liver fibrosis\textsuperscript{15, 16}. However, the available evidence regarding the association between single urinary heavy metals and the risk of NAFLD and advanced liver fibrosis remains limited.

This study aimed to investigate the cross-sectional relationship between various heavy metals, including barium (Ba), cadmium (Cd), cobalt (Co), cesium (Cs), mercury (Hg), molybdenum (Mo), lead (Pb), antimony (Sb), tin (Sn), thallium (Tl), and uranium (Ur), present in urinary, with two key markers: non-alcoholic fatty liver disease (NAFLD) assessed by the fatty liver index (FLI), and advanced liver fibrosis determined by the fibrosis 4 score (FIB-4). Moreover, we sought to explore potential effect measure modification by considering gender, race, age, hypertension, T2DM, and body mass index (BMI) as potential influencing factors. The analysis was conducted using data from National Health and Nutrition Examination Surveys (NHANES) 2013–2018 participants.

2. Materials and methods

2.1 Study population

NHANES is a rigorously conducted nationwide survey administered by the National Center for Health Statistics (NCHS) under the purview of the US Centers for Disease Control and Prevention (CDC). It serves as a vital tool for collecting comprehensive data on various aspects of individuals’ lives, including demographics, socioeconomic status, personal lifestyle choices, individual medical conditions, and pertinent health indicators measured in laboratories. The meticulous design of survey utilizes a stratified multistage probability cluster approach to ensure precise and representative data collection. With its regular release of public data every two years, NHANES stands as an invaluable resource for researchers and policymakers seeking to understand population health trends and make informed decisions about health outcomes. The dataset utilized in this study pertained to the NHANES cycle spanning from 2013 to 2018. Out of the initial 29,400 participants, several exclusions were made, including: (1) individuals with hepatitis B or C (n = 2,201), (2) those with significant alcohol consumption (more than 2 drinks per day for females and more than 3 drinks per day for males) (n = 1,124), (3) participants who self-reported having cancer (n = 11), (4) individuals with missing data on heavy metal components (n = 10,618), (5) those with missing data on the Fatty Liver Index (FLI) (n = 13,233), and (6) participants with missing data on the Fibrosis 4 Score (FIB-4) (n = 10,434).

Ultimately, the final sample size comprised 5,012 participants. For further details, please refer to Fig. 1.

2.2 Measurement of urinary heavy metal

A comprehensive analysis of urinary samples included measurements of 10 distinct heavy metals, namely total barium (Ba), cadmium (Cd), cobalt (Co), cesium (Cs), mercury (Hg), molybdenum (Mo), lead (Pb), antimony (Sb), tin (Sn), thallium (Tl), and uranium (Ur). NHANES employed a random selection process to identify one-third of participants aged six years or older for the testing of urinary heavy metal levels. The measurements were conducted using inductively coupled plasma-mass spectrometry (2013–2014) and inductively coupled-plasma dynamic reaction cell-mass spectrometry (2015–2018) at the Organic Analytical Toxicology Branch within the Laboratory Sciences division of the National Center for Environmental Health. As stated on the NHANES website (https://wwwn.cdc.gov/nchs/nhanes/default.aspx), if urinary heavy metal measurements fell below the lower limits of detection (LLOD), a standard approach was employed to substitute the value by dividing the LLOD by the square root
of 2. Detailed information regarding the LLOD for blood urinary heavy metal and the corresponding proportions can be found in supplementary Table 2. To facilitate the analysis, all participants were categorized into four groups based on the quartile concentrations of their urinary heavy metal levels.

2.3 Definition of NAFLD, Advanced liver Fibrosis

While liver biopsy remains the gold standard for diagnosing NAFLD, its feasibility in general population studies is limited due to practical constraints. As an alternative, non-invasive diagnostic indexes such as the FLI and FIB-4 have emerged as valuable tools. FLI is a widely employed non-invasive diagnostic index as a valuable tool in assessing and diagnosing the condition, offering a practical and reliable non-invasive approach to identify individuals at risk for NAFLD. A FLI score equal to or greater than 60 is recognized as an indicator of NAFLD.

The gold standard for diagnosing NAFLD is liver biopsy, but it is not feasible for general population studies due to its impracticality. To address this challenge, non-invasive diagnostic indexes such as FLI and FIB-4 have been utilized. FLI is a highly utilized non-invasive diagnostic index that serves as a valuable tool for determining the presence of NAFLD. A FLI score of ≥ 60 is considered indicative of NAFLD. In contrast, FIB-4 has shown superior predictive performance specifically within NAFLD cohorts. In our study, we employed FIB-4 as a criterion to define advanced hepatic fibrosis. A FIB-4 score greater than 2.67 in the presence of NAFLD was deemed indicative of advanced hepatic fibrosis. These non-invasive diagnostic indexes, namely FLI and FIB-4, offer valuable tools for evaluating NAFLD and advanced hepatic fibrosis without resorting to invasive procedures such as liver biopsy.

The formulas for calculating FLI and FIB-4 are provided below:

\[
FLI = (e^{0.953 \cdot \log_{10} TG + 0.139 \cdot BMI + 0.781 \cdot \log_{10} GGT + 0.053 \cdot WC - 15.745} / 1 + e^{0.953 \cdot \log_{10} TG + 0.139 \cdot BMI + 0.781 \cdot \log_{10} GGT + 0.053 \cdot WC - 15.745}) \times 100
\]

\[
FIB-4 = \frac{\text{age} \times \text{AST}}{\text{PLT} \times \sqrt{\frac{\text{ALT} \times (10^9/L)}}
\]

2.4 Covariate assessment

In accordance with prior literature, we extracted the following laboratory markers: waist circumstance (WC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), fasting plasma glucose (FPG), glycohemoglobin (HbA1c), platelet count, and albumin levels. Data on various variables including age, gender, race/ethnicity, BMI, education level, marital status, poverty income ratio, alcohol consumption status, smoking status, and medication use were collected through standardized self-reported questionnaires. The educational attainment of participants was categorized into three groups: "less than high school," "high school or equivalent," and "higher than high school." Marital status was classified as either "never married," "married/cohabitant," or "separated/divorced/widowed. Poverty income ratios were grouped into three categories: < 1.30, 1.30–3.50, and > 3.50. Alcohol consumption status was determined based on the Dietary Guidelines for Americans and categorized as non-drinker, low to moderate drinker, or heavy drinker. Smoking status was classified into three categories: never (smoked less than 100 cigarettes in a lifetime), former (smoked more than 100 cigarettes in a lifetime but currently a non-smoker), or current (smoked more than 100 cigarettes in a lifetime and currently smoking some days or every day).

The participants' level of education was classified into three categories: "less than high school," "high school or equivalent," and "higher than high school." Marital status was categorized as never married, married/cohabitant, or separated/divorced/widowed. Poverty income ratios were classified into three groups: < 1.30, 1.30–3.50, and > 3.50. Alcohol consumption status was determined based on the Dietary Guidelines for Americans and classified as non-drinker, low to moderate drinker, or heavy drinker. Smoking status was classified into three categories: never (smoked less than 100 cigarettes in lifetime), former (smoked more than 100 cigarettes in lifetime but currently non-smoker), or current (smoked more than 100 cigarettes in lifetime and smoking some days or every day now). T2DM was defined in accordance with the criteria set by the American Diabetes Association, indicating the presence of any of the following: FPG ≥ 126 mg/dL, HbA1c ≥ 6.5%, self-reported clinician-diagnosed T2DM, or specific drug treatment for T2DM. Hypertension was defined as having a blood pressure measurement exceeding 130/80 mm Hg or receiving specific drug treatment, following the 2017 American Heart Association guidelines for hypertension.
2.5 Statistical analysis

Continuous variables were summarized using mean and standard deviation, while categorical variables were presented as percentages. To compare mean values of continuous variables, a weighted t-test was applied, and for categorical variables, a chi-squared test was used, with results reported as numbers (n) and percentages (%). To investigate the association between NAFLD, advanced hepatic fibrosis, and urinary heavy metal levels, a multivariable logistic regression model was employed. Three models were constructed, progressively adjusting for covariates, to assess the relationship. Model I served as the crude model, while Model II was partially adjusted for BMI, age, race, gender, poverty income ratio, marital status, and education level. Model III was fully adjusted, incorporating additional variables such as smoke status, drink status, T2DM and hypertension. Stratified analyses were performed to investigate potential modifications of the association by age, gender, race, and BMI. The significance level was set at a two-tailed p-value of less than 0.05. All statistical analyses were conducted using STATA v16.0 (StataCorp LLC, College Station, TX, USA).

3. Results

3.1 Baseline Characteristic

In this study, a total of 1,201 individuals (55.07%) were diagnosed with NAFLD, while 296 individuals (6.07%) were identified as having advanced hepatic fibrosis. As shown in Table 1, firstly, individuals with NAFLD were found to be older, surpassing the high school level of education. Additionally, participants with NAFLD showed a higher likelihood of being current or former smokers. Furthermore, the study demonstrated a significant correlation between NAFLD and the presence of T2DM, hypertension and BMI over 30. Patients with NAFLD commonly exhibit elevated levels of various biomarkers. Particularly, higher levels of ALT AST GGT TG TC LDL FPG HbA1c WC and lower levels of HDL platelet and albumin are frequently observed in individuals with NAFLD. Moreover, it has been found that NAFLD participants tend to exhibit higher concentrations of urinary heavy metals except Sb, compared to those without NAFLD. On the other hand, participants diagnosed with advanced liver fibrosis exhibit distinct demographic and clinical characteristics. Specifically, they are more likely to be older, male, Non-Hispanic white, have higher educational attainment (above high school), consume more alcohol, have a higher prevalence of current or past smoking, and have comorbidities such as T2DM and hypertension. Furthermore, individuals with advanced liver fibrosis tend to demonstrate elevated levels of several biomarkers including ALT AST GGT TG TC LDL FPG HbA1c WC. Conversely, they exhibit lower levels of HDL, platelet count, and albumin. Additionally, it has been observed that participants with advanced liver fibrosis are more likely to have higher concentrations of urinary heavy metals except Tu.
<p>| Characteristics of enrolled participants grouped by NAFLD and advanced liver fibrosis |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                                 | Non-NAFLD     | NAFLD          | P-value        | Non-advanced liver fibrosis | advanced liver fibrosis | P-value        |
| Age (year)                      | 36.13(0.84)   | 47.48(0.63)    | 0.002          | 42.54(0.64)            | 70.17(2.17)            | &lt; 0.001        |
| Gender(n,%)                     |               |                | 0.224          |                |                | 0.003          |
| Male                            | 995(56.16)    | 1218(50.84)    |                | 2219(45.88)            | 65(50.22)            |                |
| Female                          | 538(43.84)    | 627(49.16)     |                | 2684(54.12)            | 44(49.78)             |                |
| Race(n,%)                       |               |                | &lt; 0.001        |               |                | 0.023          |
| Mexican American                | 159(10.16)    | 244(11.87)     |                | 888(10.80)            | 14(8.42)              |                |
| Other Hispanic                  | 92(5.96)      | 151(7.06)      |                | 543(6.64)             | 9(4.81)               |                |
| Non-Hispanic white              | 337(63.36)    | 448(62.53)     |                | 1725(62.51)           | 55(72.31)             |                |
| Non-Hispanic black              | 208(10.67)    | 230(10.38)     |                | 969(10.87)            | 19(8.20)              |                |
| Other                           | 199(9.86)     | 145(8.17)      |                | 778(9.18)             | 12(6.26)              |                |
| Education level(n,%)            |               |                | &lt; 0.001        |               |                | 0.001          |
| Less than high school           | 407(26.72)    | 336(24.78)     |                | 1652(22.25)           | 22(12.15)             |                |
| High school or equivalent       | 166(18.72)    | 269(18.22)     |                | 964(21.12)            | 34(25.83)             |                |
| Above high school               | 421(54.56)    | 613(57.00)     |                | 2287(56.63)           | 53(62.02)             |                |
| Marital status (n, %)           |               |                | &lt; 0.001        |               | &lt; 0.001        |                |
| Married/cohabitant              | 737(71.09)    | 787(67.56)     |                | 3401(69.15)           | 64(57.37)             |                |
| Widowed/divorced/separated      | 99(11.11)     | 252(17.24)     |                | 749(14.52)            | 33(27.24)             |                |
| Never married                   | 159(17.80)    | 179(15.20)     |                | 753(16.33)            | 12(15.39)             |                |
| Poverty income ratio (PIR)      |               |                | 0.077          |               |                | 0.105          |
| &lt; 1.30                          | 412(30.69)    | 456(27.40)     |                | 1887(38.65)           | 40(31.03)             |                |
| 1.30–3.50                       | 328(30.53)    | 446(34.17)     |                | 1709(32.80)           | 37(33.38)             |                |
| &gt; 3.50                          | 255(38.78)    | 316(38.43)     |                | 1307(28.56)           | 32(35.59)             |                |
| Drinking status (n, %)          |               |                | &lt; 0.001        |               |                | 0.028          |
| Non                             | 836(74.08)    | 1051(84.29)    |                | 4213(82.12)           | 94(78.84)             |                |
| Low to moderate                 | 119(18.24)    | 135(13.32)     |                | 551(14.27)            | 9(13.95)              |                |
| Heavy                           | 40(7.68)      | 32(2.39)       |                | 139(3.61)             | 6(7.21)               |                |
| Smoking status (n, %)           |               |                | &lt; 0.001        |               |                | 0.001          |
| Never                           | 772(71.93)    | 755(62.10)     |                | 3452(67.44)           | 48(41.01)             |                |
| Former                          | 113(16.75)    | 275(24.27)     |                | 797(19.68)            | 45(39.36)             |                |</p>
<table>
<thead>
<tr>
<th></th>
<th>Non-NAFLD</th>
<th>NAFLD</th>
<th>p-value</th>
<th>Non-advanced liver fibrosis</th>
<th>advanced liver fibrosis</th>
<th>p-value</th>
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<tr>
<td>Current</td>
<td>110(11.32)</td>
<td>188(13.63)</td>
<td></td>
<td>654(12.88)</td>
<td>16(19.64)</td>
<td></td>
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<td>T2DM(n, %)</td>
<td></td>
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<td>&lt;0.001</td>
<td></td>
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<td>&lt;0.001</td>
</tr>
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<td>Yes</td>
<td>191(22.22)</td>
<td>334(24.00)</td>
<td></td>
<td>1068(21.08)</td>
<td>51(43.82)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>804(77.78)</td>
<td>884(76.00)</td>
<td></td>
<td>3835(78.92)</td>
<td>58(56.18)</td>
<td></td>
</tr>
<tr>
<td>Hypertension(n, %)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>202(19.60)</td>
<td>665(53.73)</td>
<td></td>
<td>3055(37.46)</td>
<td>85(78.51)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>793(80.40)</td>
<td>553(46.27)</td>
<td></td>
<td>1848(62.54)</td>
<td>24(21.49)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td>0.101</td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>960(33.37)</td>
<td>475(29.43)</td>
<td></td>
<td>193(35.03)</td>
<td>63(36.45)</td>
<td></td>
</tr>
<tr>
<td>≥ 30</td>
<td>35(66.63)</td>
<td>743(70.57)</td>
<td></td>
<td>318(64.97)</td>
<td>46(63.55)</td>
<td></td>
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<td>WC(cm)</td>
<td>80.27(0.45)</td>
<td>107.09(0.59)</td>
<td>&lt;0.001</td>
<td>97.54(0.61)</td>
<td>101.27(4.21)</td>
<td>0.104</td>
</tr>
<tr>
<td>Platelet(*10^9/L)</td>
<td>239.09(2.77)</td>
<td>230.89(3.75)</td>
<td>&lt;0.001</td>
<td>238.16(2.08)</td>
<td>152.90(7.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>18.66(0.42)</td>
<td>27.03(0.63)</td>
<td>&lt;0.001</td>
<td>24.00(0.46)</td>
<td>27.80(3.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>22.42(0.36)</td>
<td>25.09(0.61)</td>
<td>&lt;0.001</td>
<td>24.07(0.42)</td>
<td>29.15(2.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>14.64(0.36)</td>
<td>28.65(1.20)</td>
<td>&lt;0.001</td>
<td>23.47(0.82)</td>
<td>38.88(10.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>61.90(1.50)</td>
<td>128.43(3.16)</td>
<td>&lt;0.001</td>
<td>104.66(2.43)</td>
<td>119.99(8.33)</td>
<td>0.748</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>170.68(1.98)</td>
<td>193.98(1.94)</td>
<td>&lt;0.001</td>
<td>185.88(1.51)</td>
<td>174.83(9.54)</td>
<td>0.692</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>94.88(1.64)</td>
<td>117.40(1.62)</td>
<td>&lt;0.001</td>
<td>109.62(1.28)</td>
<td>94.84(7.88)</td>
<td>0.857</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>63.41(0.94)</td>
<td>50.91(0.64)</td>
<td>&lt;0.001</td>
<td>55.33(0.58)</td>
<td>55.92(3.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPG(mg/dL)</td>
<td>97.60(1.58)</td>
<td>109.58(1.30)</td>
<td>&lt;0.001</td>
<td>105.01(1.02)</td>
<td>128.76(4.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb1Ac(%)</td>
<td>5.29(0.03)</td>
<td>5.68(0.03)</td>
<td>&lt;0.001</td>
<td>5.53(0.03)</td>
<td>6.32(0.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin(mg/dL)</td>
<td>4.43(0.02)</td>
<td>4.28(0.01)</td>
<td>0.851</td>
<td>4.34(0.01)</td>
<td>4.15(0.11)</td>
<td>0.575</td>
</tr>
<tr>
<td>Ba(ug/L)</td>
<td>1.555(0.097)</td>
<td>1.999(0.163)</td>
<td>&lt;0.001</td>
<td>1.910(0.069)</td>
<td>2.163(0.539)</td>
<td>0.044</td>
</tr>
<tr>
<td>Cd(ug/L)</td>
<td>0.212(0.016)</td>
<td>0.325(0.015)</td>
<td>&lt;0.001</td>
<td>0.233(0.007)</td>
<td>0.503(0.089)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Co(ug/L)</td>
<td>0.515(0.028)</td>
<td>0.618(0.081)</td>
<td>&lt;0.001</td>
<td>0.608(0.030)</td>
<td>0.672(0.096)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
3.2 Associations of urinary heavy metals with NAFLD

The findings from Table 2 reveal the outcomes of multiple logistic regression models, which were constructed to explore the potential independent association between the concentration of 10 heavy metals in urine and NAFLD. Model 1, which did not incorporate any adjusted variables, demonstrated a significant association between the urinary level of heavy metal Ba (Q2, Q3 and Q4 group), Cd (Q2, Q3 and Q4 group), Cs (Q3 and Q4 group), Mo (Q3 group), Pb (Q3 and Q4 group), Sb (Q4 group), Sn (Q2, Q3 and Q4 group), Tu (Q2, Q3 and Q4 group), Ur (Q4 group) and NAFLD. However, the analysis did not reveal a clear and significant relationship between the urinary level of Co and NAFLD. Model 2 was constructed by introducing adjustments for several important variables, including BMI, age, race, gender, poverty income ratio, marital status, and education level. Notably, this adjusted model demonstrated a significant association between the urinary level of Ba (Q2, Q3 and Q4 group), Cd (Q3 and Q4 group), Co (Q3 and Q4 group), Cs (Q3 and Q4 group), Mo (Q3 and Q4 group), Pb (Q2, Q3 and Q4 group), Sb (Q3 and Q4 group), Sn (Q3 and Q4 group), Tu (Q2, Q3 and Q4 group), Ur (Q3 and Q4 group) and NAFLD. In the final model, Model 3, the analysis further revealed that, when compared to the reference group (Q1), higher levels of urinary Ba(Q2,Q3 and Q4 group), Cd(Q3 and Q4 group), Co(Q2,Q3 and Q4 group), Pb(Q2,Q3 and Q4 group), Sb(Q4 group), Sn(Q2,Q3 and Q4 group), Tu(Q4 group), Ur(Q4 group) exhibited a significant positive association with NAFLD (P < 0.05). However, no significant relationship between the urinary level of Cs, Mo and NAFLD was observed in Model 3. These results, after adjusting for various important variables in Model 2 and Model 3, provide valuable insights into the independent association between the levels of heavy metals in urine and the presence of NAFLD.

Table 2: Association between urinary heavy metals level and NAFLD diagnosed by FLI
3.3 Subgroup analyses of urinary heavy metals with NAFLD

In Model 3, the analysis revealed that there was no significant association between the concentration of urinary Cs and Mo and NAFLD. Consequently, subgroup analyses for these two heavy metals were not conducted due to the absence of a notable relationship. To further investigate the relationship between urinary heavy metal levels and NAFLD, subgroup analyses were performed considering various factors. These factors included age, gender, race, BMI, hypertension, and T2DM. The results of these subgroup analyses are presented in Tables 3.1 and 3.2, providing additional insights into the potential associations between urinary heavy metal levels and NAFLD within specific subgroups.

Table 3.1 presents several notable findings regarding the relationship between urinary heavy metals and NAFLD within specific subgroups. Firstly, the analysis demonstrated that participants aged below 60 years exhibited a stronger positive correlation between Ba (Q2, Q3 and Q4 group), Co (Q2 and Q3 group) and NAFLD compared to older participants. Furthermore, when conducting sex-stratified analyses, it was observed that urinary Ba (Q2, Q3 and Q4 group), Cd (Q3 and Q4 group) and Co (Q2 and Q3 group) showed positive associations specifically with NAFLD in female individuals. Conversely, in the male population, a significant positive association was found between urinary Ba (Q4 group), Cd (Q3 and Q4 group) and Pb (Q2 group) and NAFLD. Subgroup analysis based on race revealed significant associations between all urinary heavy metals and NAFLD in Non-Hispanic white individuals. Additionally, a positive association between urinary Cd (Q4 group) and NAFLD was observed specifically in Mexican American individuals. Interestingly, in participants without T2DM, hypertension, or BMI below 30, a significant positive association was found between all urinary heavy metals and NAFLD.

As shown in Table 3.2, the analysis revealed that participants aged below 60 years exhibited a stronger positive correlation between Sn (Q4 group) Tu (Q4 group) and NAFLD compared to older participants. Conversely, in the elder population,
Significant positive association was observed between Sb (Q2 and Q4 group) Tu (Q3 group) and NAFLD. Moreover, when conducting sex-stratified analyses, it was found that Sb (Q3 and Q4 group), Sn (Q4 group), Tu (Q2 and Q3 group) Ur (Q4 group) showed positive associations specifically with NAFLD in female individuals. Consistent with the results in Table 3.1, Table 3.2 also demonstrated a significant association between all urinary heavy metals and NAFLD in Non-Hispanic white individuals. Additionally, a positive association between Sb (Q3 and Q4 group) and NAFLD was observed specifically in Mexican American individuals. Within all subgroup analyses based on BMI, a significant association was consistently observed between urinary heavy metals and NAFLD in individuals with a BMI of less than 30, but we still observed a significant association between urinary Tu (Q2 and Q4 group) Ur (Q2 group) and NAFLD in people with BMI over 30. Similarly, subgroup analyses based on T2DM and hypertension yielded consistent results as seen in Table 3.1. However, in addition to these findings, a significant association was found between urinary Tu and NAFLD in participants with T2DM (specifically in the Q4 group). Moreover, in participants with hypertension, significant associations were observed with urinary Tu in the Q2, Q3, and Q4 groups.

### 3.4 Associations of urinary heavy metals with advanced liver fibrosis

Table 4 presents the findings from multiple logistic regression models examining the potential independent associations between the concentration of 10 heavy metals in urine and advanced liver fibrosis. In Model 1, which did not incorporate any adjusted variables, a significant association was observed between the urinary level of Ba (Q4 group), Cd (Q3 and Q4 group), Cs (Q2 group), Pb (Q3 and Q4 group), Sb (Q2 and Q4 group), Sn (Q3 and Q4 group) and advanced liver fibrosis. However, no clear relationship was found between the urinary level of Co, Mo, Tu, Ur and advanced liver fibrosis in this model. Model 2 was constructed by introducing adjustments for several important variables, including BMI, age, race, gender, poverty income ratio, marital status, and education level. In this adjusted model, a significant association was found between the urinary level of Cd (Q4 group), Cs (Q2 group), Pb (Q3 and Q4 group), Sb (Q2 and Q4 group), Sn (Q3 and Q4 group) and advanced liver fibrosis. Furthermore, in the final model, Model 3, the analysis demonstrated that as the concentration of Cd (Q4 group), Cs (Q2 group), Pb (Q3 and Q4 group), Sb (Q2 and Q4 group), Sn (Q3 and Q4 group) in urine increased, the likelihood of advanced liver fibrosis also increased significantly. However, no clear relationship was found between the urinary level of Ba, Cs, Co, Mo, Tu, Ur and NAFLD in both Model 2 and Model 3.

**Table 4: Association between urinary heavy metals level and advanced liver fibrosis diagnosed by FIB-4**
4. Discussion

between urinary Sb (Q2 and Q4 group) Sn (Q4 group) and advanced liver fibrosis. Moreover, in individuals with T2DM, a significant positive association was observed. Furthermore, in the subgroup of individuals with hypertension, a significant association was found between urinary Sb (Q4 group) and advanced liver fibrosis. Conversely, for Other group) and advanced liver fibrosis. In terms of race, a significant association was found between all urinary heavy metals and advanced liver fibrosis within different subgroups. The analysis revealed that participants aged over 60 years exhibited a stronger positive correlation between urinary Pb (Q3 and Q4 group), Sb (Q3 and Q4 group), Sn (Q3 and Q4 group) and advanced liver fibrosis compared to younger participants. Furthermore, when conducting sex-stratified analyses, it was found that urinary Pb (Q3 group) showed positive associations specifically with advanced liver fibrosis in male individuals. Conversely, in the female population, a significant positive association was observed between urinary Sb (Q4 group) and advanced liver fibrosis. In terms of race, a significant association was found between all urinary heavy metals (except Cd) and advanced liver fibrosis in Non-Hispanic white individuals. Additionally, among Mexican American individuals, a positive association was observed between urinary Sn (Q3 and Q4 group) and advanced liver fibrosis. Conversely, for Other Hispanic individuals, a passive effect was observed between urinary Cd (Q2 and Q3 group) and advanced liver fibrosis. Furthermore, in individuals with a BMI over 30, a significant association was found between urinary Sb (Q4 group) and advanced liver fibrosis. In the subgroup of individuals with hypertension, a significant positive association was observed between urinary Sb (Q2 and Q4 group) Sn (Q4 group) and advanced liver fibrosis. Moreover, in individuals with T2DM, a significant positive association was found between urinary Pb (Q4 group) and advanced liver fibrosis.

3.5 Subgroup analyses of urinary heavy metals with advanced liver fibrosis

In Model 3, no significant association was identified between the concentration of urinary heavy metal A and advanced liver fibrosis. Consequently, subgroup analyses for the remaining six heavy metals were not conducted as their associations with advanced liver fibrosis were not investigated further. Table 5 provides noteworthy findings regarding the relationship between specific urinary heavy metals and advanced liver fibrosis within different subgroups. The analysis revealed that participants aged over 60 years exhibited a stronger positive correlation between urinary Pb (Q3 and Q4 group), Sb (Q3 and Q4 group), Sn (Q3 and Q4 group) and advanced liver fibrosis compared to younger participants. Furthermore, when conducting sex-stratified analyses, it was found that urinary Pb (Q3 group) showed positive associations specifically with advanced liver fibrosis in male individuals. Conversely, in the female population, a significant positive association was observed between urinary Sb (Q4 group) and advanced liver fibrosis. In terms of race, a significant association was found between all urinary heavy metals (except Cd) and advanced liver fibrosis in Non-Hispanic white individuals. Additionally, among Mexican American individuals, a positive association was observed between urinary Sn (Q3 and Q4 group) and advanced liver fibrosis. Conversely, for Other Hispanic individuals, a passive effect was observed between urinary Cd (Q2 and Q3 group) and advanced liver fibrosis. Furthermore, in individuals with a BMI over 30, a significant association was found between urinary Sb (Q4 group) and advanced liver fibrosis. In the subgroup of individuals with hypertension, a significant positive association was observed between urinary Sb (Q2 and Q4 group) Sn (Q4 group) and advanced liver fibrosis. Moreover, in individuals with T2DM, a significant positive association was found between urinary Pb (Q4 group) and advanced liver fibrosis.

4. Discussion
Existing studies have primarily focused on investigating the associations between urinary metal mixtures and the risks of NAFLD and advanced liver fibrosis. However, to the best of our knowledge, there is currently a lack of epidemiological evidence regarding the potential links between individual urinary heavy metals and the occurrence of NAFLD or advanced liver fibrosis. In order to address these gaps in knowledge, we conducted a comprehensive study among US participants utilizing data from NHANES 2003–2018 survey cycles. Our primary objective was to examine the associations between urinary heavy metals and the prevalence of NAFLD and advanced liver fibrosis. Notably, this is the first study to identify a significant positive correlation between the heavy metals Sb, Sn, Ur, and the occurrence of NAFLD or advanced liver fibrosis. Our findings contribute important insights into the field by highlighting the potential role of these specific heavy metals in the development of NAFLD and advanced liver fibrosis. In this study, we aimed to explore the relationship between urinary heavy metal concentration and the prevalence of NAFLD and advanced liver fibrosis within a population from the United States. Through our investigation, we identified a significant positive association between urinary Ba, Cd, Co, Pb, Sb, Sn, Tu, Ur concentration and NAFLD. Additionally, we observed a significant correlation between higher urinary levels of Cd, Pb, Sb, Sn and the occurrence of advanced liver fibrosis. These findings align with previous studies on the subject and hold true even after accounting for various confounding variables, including sociodemographic factors, lifestyle factors, hypertension, and T2DM. By considering these factors, our study provides robust evidence supporting the link between urinary heavy metal concentration and both NAFLD and advanced liver fibrosis in the US population.

Xie Z et al.’s study discovered a significant correlation between urinary Cs and Mo levels and NAFLD. Our analysis further revealed that individuals below the age of 60 exhibited a stronger positive association between urinary levels of Ba, Co, Pb, Sn, and Tu with NAFLD, compared to older participants. Additionally, we observed a significant link between Sb and Tu levels in urine and NAFLD specifically among older adults (aged ≥ 60 years). It is worth noting that the incidence of NAFLD and fibrosis generally tends to increase with age. Our divergent findings might be attributed to variations in the sample size and the inclusion criteria across the studies. In addition, when we performed stratified analyses based on sex, we found distinct patterns. Specifically, among female individuals, we observed positive associations between urinary levels of Ba, Cd, Co, Sb, Sn, Tu, and Ur with NAFLD, as well as between Sb and advanced liver fibrosis. Conversely, in the male population, we identified a significant positive association between urinary levels of Ba, Cd, and Pb with NAFLD, and between Pb and advanced liver fibrosis. These findings align with previous studies that have also highlighted sex differences in the health risks associated with heavy metals. Interestingly, heavy metals demonstrated a stronger positive correlation with NAFLD and advanced liver fibrosis in women compared to men. This suggests that the impact of heavy metals on liver health may vary between the sexes, emphasizing the importance of considering sex-specific factors when assessing the relationship between heavy metals and these liver conditions. Several potential explanations may shed light on the observed sex differences in the associations between urinary heavy metals and NAFLD and advanced liver fibrosis. For instance, it is suggested that a deficiency of iron in women may contribute to a compensatory increase in the absorption of heavy metals. Additionally, rat models have shown that exposure to heavy metals can result in decreased expression of organic anion transporter 3 (Oat) in hepatocyte membranes, leading to reduced intake of certain heavy metals such as mercury (Hg) and subsequent higher accumulation in the female liver. However, it is noteworthy that Xie Z et al.’s study did not detect significant gender differences in the association between urinary heavy metals and NAFLD. To gain a comprehensive understanding of the relationship between urinary heavy metals, age, and gender, further investigations are warranted. Additional studies exploring the impact of urinary heavy metal levels on age and gender are essential for enhancing our knowledge in this area.

The subgroup analysis conducted in our study revealed a noteworthy and positive association between NAFLD, advanced liver fibrosis, and nearly all urinary heavy metals, particularly among individuals of Non-Hispanic white when compared to other ethnic groups. Additionally, we identified a significant correlation between urinary levels of Cd and Sb with NAFLD, and urinary Sn with advanced liver fibrosis specifically among Mexican Americans. This may be attributed to the larger proportion of Non-Hispanic white individuals within the studied population. These findings emphasize the importance of paying special attention to the potential liver damage caused by urinary heavy metal exposure in these populations. Previous research has highlighted that Mexican Americans have the highest prevalence of NAFLD, and this finding aligns with the results of Spaur M. et al., who also reported a significant positive association between blood manganese levels and NAFLD and liver fibrosis in Mexican
Americans\textsuperscript{34}. Interestingly, we discovered a negative correlation between urinary Cd levels and advanced liver fibrosis specifically among individuals of Other Hispanic ethnicity. This unexpected finding highlights the need for further investigation into the sensitivity of different populations to the potential liver damage caused by urinary heavy metals. Previous research has established that individuals with a high BMI are more susceptible to developing NAFLD and advanced liver fibrosis\textsuperscript{35, 36}. This notion is supported by the findings of Spaur M. et al., who observed BMI-specific differences, where positive associations were observed between blood manganese (Mn) levels and liver steatosis among participants with a BMI of 30 or higher\textsuperscript{34}. In our study, we consistently observed a significant impact of urinary heavy metal levels on the development of advanced hepatic fibrosis across various BMI groups. Nevertheless, our subgroup analysis revealed that among individuals with BMI greater than 30, there was no significant association observed between most urinary heavy metals and the presence of NAFLD or advanced liver fibrosis. However, we did find a positive correlation between urinary levels of Tu and NAFLD, as well as urinary Sb and advanced liver fibrosis, within this higher BMI population. It is important to note that the findings in this subgroup analysis mirror the rationale behind the age-based subgroup analysis, as the sample sizes and compositions of these two groups differ substantially. These divergent results highlight the potential influence of factors such as BMI and suggest the need for further investigations with larger sample sizes to fully understand the associations between urinary heavy metals and NAFLD or advanced liver fibrosis in specific subpopulations.

The pathogenesis of NAFLD and advanced liver fibrosis is widely acknowledged to involve metabolic disorders such as hypertension and diabetes mellitus\textsuperscript{37–39}. Consistent with this understanding, our study revealed a positive correlation between urinary levels of Sb and Sn with advanced liver fibrosis specifically among participants with T2DM. Additionally, we observed a higher prevalence of urinary Pb levels among individuals with hypertension who also had advanced liver fibrosis. Reja D et al. similarly reported an independent association between increased serum lead levels and an elevated risk of advanced liver fibrosis\textsuperscript{28}. However, when examining the association between urinary heavy metals and NAFLD, we only identified a significant effect of Ur among individuals with diabetes or hypertension. These findings underscore the intricate interplay between urinary heavy metals, metabolic disorders, and the development of advanced liver fibrosis, emphasizing the need for further investigation in this field.

This study is subject to several limitations that warrant consideration. Firstly, the cross-sectional design of the NHANES dataset used in this study prevents the establishment of causality and the reliance on self-reported alcohol consumption may introduce recall bias. Secondly, the diagnosis of NAFLD and advanced hepatic fibrosis relied on the use of FLI and FIB-4 scores after excluding other causes of chronic liver disease. This approach may have inherent limitations and could potentially lead to misdiagnosis of NAFLD. While liver biopsy is considered the gold standard for NAFLD diagnosis, its impracticality in large population-based studies necessitated the use of alternative diagnostic methods. Moreover, the relatively smaller sample sizes of Non-Hispanic and Mexican American participants limited our ability to assess differences between subgroups, and some of the findings may be unstable. Lastly, despite adjusting for various potential confounding factors using NHANES data, there remains the possibility of unmeasured confounders that may influence our findings. Therefore, further studies are necessary to elucidate the causal relationship between urinary heavy metal concentrations and NAFLD, as well as to validate our current findings.

5. Conclusion

In this large-scale cross-sectional study, we have observed a significant association between elevated levels of urinary heavy metals and an increased prevalence of both NAFLD and advanced hepatic fibrosis. Specifically, we found a positive correlation between urinary concentrations of Ba, Cd, Co, Pb, Sb, Sn, Tu, Ur with NAFLD. Additionally, higher urinary levels of Cd, Pb, Sb, and Sn were significantly correlated with advanced liver fibrosis. Importantly, our findings have been adjusted for various confounding variables, providing robust evidence for this relationship. However, it is essential to acknowledge the limitations inherent in the cross-sectional study design, which prevents us from establishing a definitive causal relationship between urinary heavy metal levels and the development of NAFLD or advanced hepatic fibrosis. Therefore, further extensive and rigorous prospective studies are warranted to validate and confirm our findings in a longitudinal setting.
Declarations

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Jian Wang: writing—review and editing.

Hua Ye: investigation, resources, supervision, funding acquisition, project administration, writing—review and editing.

All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

The datasets generated and/or analysed during the current study are available in the National Health and Nutrition Examination Survey repository, [https://www.cdc.gov/nchs/nhanes/index.htm].

References


**Tables**

Tables 3.1, 3.2 and 5 are available in the Supplementary Files section.

**Figures**

![Flow chart of participants screening](image)

**Figure 1**

Flow chart of participants screening

**Supplementary Files**
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