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Association of body burden of mercury with liver function test status in the U.S. population

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ABSTRACT

The majority of mercury (Hg) exposure in the US population is from consumption of fish contaminated with methylmercury (MeHg). Since inorganic Hg is the predominant form excreted in the feces and urine, hepatic biotransformation is a critical step in its normal clearance. This study was set to test the hypothesis that compromised liver function is associated with body burden of Hg as indirectly reflected by Hg sampled in blood and urine. From the National Health and Nutrition Examination Survey (NHANES, 2003–2008), 3769 adults aged 20 years and above were selected for analysis. Hepatic function was inferred from the three standard serum liver-related enzyme activities, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT). Multivariate regression models were used to examine the associations of interest. Although urinary Hg was significantly correlated with serum Hg, the blood–urinary Hg relationship was influenced by liver function, which is also a function of demographic and lifestyle factors (e.g., gender). Although the results were only marginally significant for examined enzymes ($p = 0.06$–0.08), urinary Hg tended to be lower among subjects with elevated liver enzymes, as compared to those with normal enzyme measurements. Conversely, MeHg generally represents a higher fraction of the total circulating Hg among those with elevated liver enzyme levels, especially among participants with elevations in all three enzymes ($p = 0.01$). In conclusion, this population-based study identified an association between liver function, serum Hg and urinary Hg. Urinalysis may not be the optimal approach to monitor Hg elimination toxicokinetics or Hg exposure, since the majority of Hg excretion is fecal and the fidelity of urinary excretion may depend on healthy liver function. Future prospective studies are warranted to expand these findings.

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1. Introduction

Mercury (Hg) is a toxic metal that has been associated with a variety of health issues including developmental neurotoxicity, cardiovascular disease, thyroid dysregulation, and impaired liver and renal functions (Clarkson et al., 2007; Houston, 2011; Karagas et al., 2012; Satoh, 2000; Woods and Fowler, 1977). Though there are multiple routes of exposure to Hg, the majority of exposure in the US population is from fish contaminated with methylmercury (MeHg) (U.S. Environmental Protection Agency (EPA), 2014; Mahaffey et al., 2009).

Once Hg enters the body, it is distributed to tissues in the circulation bound to hemoglobin and plasma proteins (e.g., albumin) and slowly eliminated through feces and urine (National Research Council, 2000; Sundberg et al., 1999). In the absence of individual tissue biopsies, one necessarily must rely on circulating Hg measurements and excretion rates as indirect estimates of body burden. Most of the serum Hg is believed to circulate as organic MeHg (Mahaffey et al., 2004). In humans, the average half-life of blood MeHg is 45–70 days (Clarkson, 2002). Evidence suggests that MeHg is converted to inorganic Hg mainly by gut microflora and liver metabolism (Berglund et al., 2005; Carrier et al., 2001), and subsequently excreted from the body into feces and urine (Ballatori and Clarkson, 1984; Clarkson, 2002). These reports suggested that the liver may play a key role in the toxicokinetics of Hg (Dock et al., 1994; Nordenhall et al., 1995). Limited human data are available on this issue. These observations led to the proposed hypothesis that impaired liver function and presumed reduced hepatic biotransformation would be associated with a net increase in body burden as evident in urinary and serum Hg measurements. To test this hypothesis, statistical associations were analyzed between liver function and Hg in blood and urine

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in a cohort of U.S. adults using a national database, the National Health and Nutrition Examination Survey (NHANES), 2003–2008.

2. Materials and methods

2.1. Study design

The data set used in this study was obtained by combining 3 NHANES cycles (2003–2004, 2005–2006, and 2007–2008) (see Statistical analyses section for details). NHANES is a program of studies, conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC), to assess the health and nutritional status of adults and children in the United States using a stratified, multistage, probability sampling design (CDC, 2013a). The protocol was approved by the NCHS Institutional Review Board, and all subjects provided written informed consent. The current study was restricted to 3769 non-Hispanic Whites, non-Hispanic Blacks, and Mexican-American participants aged 20 years or older with valid measurements of blood and urinary Hg and complete data for covariates used in the analyses (e.g., serum biochemistry parameters for estimating liver function status). Detailed sample collection and laboratory analysis were reported in the NHANES Laboratory and Medical Technologists Procedures Manual (CDC, 2013b,c,d).

2.2. Assessment of liver enzyme function

Liver dysfunction is routinely screened through the measurement of liver enzymes that leak into the circulation during liver disease. A standard panel of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) activities were used in the current study. The details of the laboratory assays and quality control methods were reported elsewhere (CDC, 2013b,c,d). In brief, once blood specimens were collected, serum was separated from plasma by centrifugation and aliquots were stored at −70 °C until analysis. Serum liver enzyme activity was measured by the Beckman Synchron System (Beckman Coulter, Brea, CA). Elevated AST and ALT have been widely used as markers of hepatocyte damage, often the result of non-alcoholic fatty liver disease (NAFLD). Elevated serum ALT and AST are defined as: serum ALT >47 U/L for men and >30 U/L for women; serum AST >33 U/L for both men and women. Elevated GGT suggests the presence of hepatobiliary disease (Lee et al., 2004, 2008). Serum GGT >65 U/L for men and >36 U/L for women were considered elevated. The cut-off values for abnormal serum liver enzyme measures were computed based on reported reference ranges from NHANES (CDC, 2013b,c,d).

2.3. Measurement of blood and urinary Hg

Blood total Hg is generally regarded a measure of methyl mercury exposure (Berglund et al., 2005; CDC, 2009a; Mahaffey et al., 2004). Blood total mercury was assessed by inductively-coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). In the current analysis, blood MeHg was estimated following the method reported by Mahaffey et al. (2004), since blood MeHg is not directly measured in NHANES. In brief, blood MeHg was estimated as the difference between the levels of blood total Hg and blood inorganic Hg, both of which were measured in the NHANES labs. The limits of detection (LOD) were approximately 0.14–0.3 and 0.4 μg/L for total and inorganic Hg, respectively. It was estimated that about 11% of total Hg and 72% of inorganic Hg detectable measurements are below LOD and thus were assigned with a fill value, which were calculated as LOD divided by the square root of 2. Given that LOD for blood inorganic Hg is larger than LOD for blood total Hg, negative values for estimated blood organic Hg occurred among approximately 17% of the samples when both blood total and inorganic Hg measurements are near or below their respective LODs. As suggested by Mahaffey et al. (2004), a fill value of 0.2 μg/L was assigned to the samples with negative values for estimated blood organic Hg.

Urinary Hg was measured by flow injection cold vapor atomic absorption (CVAA) analysis in NHANES 2003–2004 cycle and by ICP-DRC-MS in the NHANES 2005–2008 cycle. The LOD for urinary LOD ranged from 0.06 to 0.14 μg/L. To adjust for variation in the diluteness of urine, urinary Hg was expressed as urinary Hg/urinary creatinine ratio (μg/g). Of note, urinary creatinine was measured with Beckman Synchron CX3 clinical analyzer by the modified kinetic Jaffé method in NHANES 2003–2006 cycle and by an enzymatic (creatinase) method in NHANES 2007–08 cycle (CDC, 2009b). Because the Jaffe method is subject to more interference, urinary creatinine determinations before 2007 were corrected following the analytic approach recommended by NHANES (CDC, 2009b).

2.4. Collection of demographic and clinical data

Self-reported demographic characteristics including age, gender, body mass index (BMI), alcohol consumption, and race/ethnicity were obtained from the survey interviews. BMI was calculated from measured height and weight, and then grouped in the generally accepted categories: <25, 25.0–29.9, and ≥30 kg/m^2. Average daily alcohol consumption was estimated by multiplying the number of drinking days over the past 12 months by the average number of drinks divided by 365. Subjects with serum cotinine values greater than 14 ng/ml were classified as smokers, while all others were considered as nonsmokers (Wei et al., 2001). The glomerular filtration rate (eGFR) was estimated using the Modification of Diet in Renal Disease (MDRD) Study equation: GFR (mL/min/1.73 m^2) = 175 × (Scr)$^{-1.154}$× (age)$^{-0.203}$× (0.742 if female)× (1.210 if African American), where Scr is serum creatinine (mg/dL) (Levey et al., 2006).

2.5. Statistical analyses

To investigate the role of liver function in the body burden of Hg, blood MeHg and urinary Hg were normalized to the total amount of blood Hg in the regression analyses. Natural logarithmic transformations were applied to normalize the distributions of the continuous data whenever necessary. As the outcomes of interest might be confounded by other factors, both simple and multiple linear regression models were used to investigate crude and adjusted relationships of interest. For instance, the fraction of blood total Hg as MeHg has been shown to vary with differing amounts of total blood Hg (Mahaffey et al., 2004). Except for blood Hg, age, and estimated GFR, all variables were fit as categorical terms. We also ran sensitivity analyses by excluding participants with blood total Hg >15 μg/L (n = 8) to determine whether they had excessive influence on the model results. The examination participation rates among adults aged 20 years or older were approximately 68.1%, 69.8%, and 69.6% for NHANES 2003–2004, 2005–2006, and 2007–2008, respectively (CDC, 2013e). NHANES provided sampling weights to account for the complex survey design (including oversampling of minorities and young children), survey non-response, and post-stratification in order to ensure that calculated estimates are representative of the U.S. civilian non-institutionalized population. In the current analysis, sample weights for NHANES 2003–2008 analyses were computed by combining the sample weights for each individual survey cycle (2003–2004, 2005–2006, and 2007–2008) following the NCHS analytical method guideline (Johnson et al., 2013). Thus, weighted analyses were performed using SUDAAN 10.01 (Research Triangle Institute, 2009) to produce unbiased variance estimates using the Taylor-series linearization method. Statistical significance is set at 0.05.