



Role of Lead and Arsenic Exposure in Oxidative Stress-Mediated Liver Carcinogenesis

Divya Verma, Research Scholar, Department of Chemistry
Shilpa Sharma, Ph.D., Guide, Dipti Chandrakar, Ph.D., Co-Guide, Faculty of Science
Anjaneya University, Raipur, Chhattisgarh, INDIA

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Authors

Divya Verma, Research Scholar
Shilpa Sharma, Ph.D., Guide
Dipti Chandrakar, Ph.D., Co-Guide
E-mail : vermadivya8421@gmail.com

shodhsamagam1@gmail.com

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ABSTRACT

Heavy metal exposure, in particular, lead (Pb) and arsenic (As) exposure, presents a large public health challenge worldwide, and environmental exposure is associated with the development of hepatocellular carcinoma. This is a cross-sectional study aimed to explore the possible mechanism of action behind the induction of liver damage via oxidative stress by lead and arsenic in the region of Raipur, Chhattisgarh, where the ground water and industries are known to be contaminated. 485 participants were evaluated for serum lead and arsenic levels, hepatic oxidative stress biomarkers (malondialdehyde, superoxide dismutase) and liver function parameters. Heavy metal levels were found to be raised in 68.2% of the participants and significant correlation was found between heavy metal levels and transaminase (ALT) levels ($r=0.547$, $p<0.001$), and with decreased antioxidant capacity. After multivariate regression analysis, lead exposure was found to be independently associated with raised ALT ($\beta=2.34$, $p=0.002$) and AST ($\beta=1.89$, $p=0.008$); arsenic was more strongly associated with the markers of oxidative stress (MDA: $\beta=3.12$, $p<0.001$). Histopathology was performed in a subsample ($n=45$) and showed a progression of hepatic fibrosis in 71.1% of those with high exposure. These results highlight the importance of oxidative stress in the pathogenesis of liver damage produced by heavy metals and the need for biomonitoring and remediation plans in the contaminated areas.

KEY WORDS

Lead Exposure, Arsenic, Biomarkers, Oxidative Stress, Hepatocarcinogenesis.

INTRODUCTION

Malignancies acquired through environmental exposures continue to rise, with exposure to heavy metals responsible for approximately 4–6% of all cancer deaths worldwide (International Agency for Research on Cancer [IARC] 2020). The persistent threats are lead and arsenic which are both group 1 cancer causing agents and are focused in areas with high levels of industrial activity, mining and agriculture (Valko et al., 2016). These metalloids do not degrade metabolically and can remain in the soil and groundwater for an unlimited period of time, providing chronic pathways of exposure that can last for decades. Chhattisgarh State capital Raipur is at a crossroads. Rapid industrialization is taking place in the city, due to steelmaking, power generation and chemical production. Its ground water has reported concentration of arsenic between 0.8–145 µg/L in localized hotspots and lead contamination is due to vehicular emission, industrial discharge and legacy contamination from electronic waste recycling process (Sharma & Bhattacharya, 2017). The Sheonath River that runs through the city also carries untreated effluents from tanneries and foundries that pollute agriculture water used for irrigation and the fish population, a staple food item for the people.

Liver carcinogenesis is a multistep process in which oxidative stress plays a key role as a mechanistic factor. Heavy metals lead to NPH oxidase activation and mitochondrial dysfunction, which leads to a decrease in the endogenous antioxidant pool and an increase in reactive oxygen species (ROS) causing damage to DNA, inflammation and epigenetic changes (Sies et al., 2017). Lead binds to the hepatocytes, displacing zinc from metalloproteins and interfering with the function of superoxide dismutase, and arsenic promotes the complexation of arsenic-binding proteins and depletes glutathione, thereby increasing the oxidative load (Patrick, 2006). There are still limited population level exposure studies in Raipur and mechanistic connections between ambient exposure to heavy metal and progression of liver injury are not fully characterized. The aim of this investigation was to quantify the exposure to both the heavy metals (lead and arsenic) and their respective contribution to liver dysfunction via oxidative stress in the adult population of Raipur by using integrated biomonitoring, biochemical analysis and clinical assessments.

Literature Review

The pathways of metal hepatotoxicity are unified, with oxidative stress, inflammatory activation and cell turnover regulation all contributing to an increased risk of cancer (Nordberg et al., 2015). Lead-induced damage begins in the liver with the production of free radicals (non-enzymatic), inhibition of catalase and superoxide dismutase, and lipid peroxidation leading to damage of cellular membranes and organelles (Winder & Stacey, 2011). Epidemiological data indicate that chronic exposure (>10 µg/dL) is linked to progression of liver fibrosis and higher mortality due to cirrhosis (Nawrot et al., 2015), although there are few prospective studies in occupational and residential settings. A meta-analysis of occupational cohorts found that there was a 1.4-fold increase in liver-related mortality for each 10 µg/dL rise in blood lead concentration (Schwartz & Reis, 2000).

This hepatocarcinogenic mechanism of arsenic is quite different. Both trivalent and pentavalent arsenic can cause oxidative stress in cells, through metabolism by cytochrome P450, and pentavalent arsenic can cause activation of transcription factors that are sensitive to arsenic and of proto-oncogenes (Kitchin & Wallace, 2008). Dose-response relationships between ingestion of arsenic (>50 µg/day) and hepatocellular carcinoma incidence have been consistently observed in population-based studies conducted in Taiwan, Bangladesh and West Bengal with relative risks of 2.5–4.8 in high exposure group (Chen et al., 2010). Interestingly, arsenic has been shown to induce hepatic angiogenesis and cirrhotic transformation, which could trigger malignant transformation (Aggarwal et al., 2012).

Biomarkers of oxidative stress (including malondialdehyde [MDA], 8-hydroxydeoxyguanosine, and protein carbonyls) are indicative of the continuous process of lipid peroxidation and oxidative damage of DNA. Heavy metal accumulation causes a progressive induction of the loss of antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase), leading to a metabolic imbalance between the production of ROS and their neutralization. Liver function enzymes (ALT, AST, GGT, ALP) are sensitive, but non-specific markers of hepatocyte necrosis and cholestasis, and their levels are correlated with the degree of histopathological inflammation and fibrosis stage (Giannini et al., 2005).

Environmental exposure studies in India show significant variations in the pattern of exposure to heavy metals. The National Chemical Laboratories survey found concentrations of arsenic in groundwater that were above the WHO limits (10 µg/L) in 25 districts across Uttar Pradesh, Bihar, and West Bengal (Chakraborti et al., 2013). Lead levels in urban soils in central India are as high as 320mg/kg, more than 6 times the baseline geogenic concentration in soil (Sharma et al., 2014). Exposure studies in Indian metros among battery workers and e-waste handlers indicate an average blood lead concentration of 35-45 µg/dL, which is significantly higher than the safe levels of exposure (Shenoy et al., 2017).

Lead and arsenic exposure (co-exposure scenarios) exacerbates hepatic injury with synergistic mechanisms. Exposure to these together can allow for activation of complementary pathways of oxidative stress that may lead to a faster and more overwhelming response to the toxicant than exposure to one toxicant alone (Giacconi et al., 2011). But epidemiological data describing the combined exposure of various heavy metals in community populations is sparse, especially in areas where industrial sources of heavy metals are present in the same area as geogenic sources of arsenic.

Objectives

1. To quantify serum lead and arsenic concentrations in adult residents of Raipur and characterize exposure distribution across occupational and residential gradients.
2. To examine associations between heavy metal exposure and oxidative stress biomarkers (MDA, SOD activity) and liver function parameters, identifying mechanistic pathways linking environmental contamination to hepatic injury.

Methodology

This cross-sectional study was conducted in the capital of Raipur, Chhattisgarh (21.2358N, 81.6304E) from June 2023 to March 2025. Raipur was chosen because of reported arsenic concentrations in groundwater (0.8 – 145 µg/L) in certain areas and because of industrial point sources of lead such as steel production, battery recycling and vehicular emissions from main roads. The city is divided into seven administrative zones and stratified sampling was used to provide representation from three different zones, industrial manufacturing zone (Zone 1: Bhilai-adjacent manufacturing corridor), urban residential zone (Zones 2 to 4: central Raipur), and peri-urban agricultural zone (Zones 5 to 7).

Permanent residents of Raipur, aged 18-65 years with continuous stay of ≥ 2 years were considered eligible. Exclusion criteria were: acute viral hepatitis (HBsAg positive or positive anti-HCV), cirrhosis (Child-Pugh score >6), pregnancy, recent chemotherapy or immunosuppressive therapy, and self-reported occupational exposure to heavy metals (which was studied separately). The participants were obtained by stratified random sampling and the response rate was 73.2% (485/663). Age-stratified quotas ensured representation: 18–30 years (n=121), 31–45 years (n=156), 46–60 years (n=148), >60 years (n=60). There was an even split between the genders (52.2% female).

Sample Collection and Biochemical Analysis

Venous blood (8 mL) was drawn from the arm at a single study clinic in the morning hours between 0700–1000 hours and was fasting. After centrifugation ($1,500 \times g$ for 10 minutes), serum was pipetted off and kept at -80°C until analyzed. For the quantification of lead and arsenic, an inductively coupled plasma mass spectrometer (ICP-MS) was used (Agilent 7700x, Agilent Technologies, Santa Clara, CA). The method detection limits were 0.5 µg/dL (Pb) and 1 µg/L (As), and the inter-assay coefficient of variation was $<5\%$ for both analytes.

Quality assurance consisted of the analysis of certified reference materials (SERONORM Trace Elements Serum L1 and L2) with each analytical run. Standard enzymatic assays (alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma-glutamyl transferase [GGT] and alkaline phosphatase [ALP]) were performed on automated analyzer (Cobas 8000, Roche Diagnostics, Basel, Switzerland) to determine liver function enzymes. Malondialdehyde (MDA) a marker for lipid peroxidation, was measured by TBARS assay, absorbance at 532nm. Superoxide dismutase (SOD) activity was measured according to the pyrogallol autoxidation method (enzymatic units/mL). All analyses were repeated twice.

Covariates and Exposure Assessment

The information obtained included age, sex, occupation, place of residence, source of drinking water (private well, municipal supply, tanker-supplied), frequency of fish consumption, vegetable consumption, frequency of rice consumption (rice is known to be a bio accumulator of arsenic), smoking status, and alcohol consumption, all of which were recorded through structured questionnaires. Participants were divided into non-occupationally exposed, suspected indirect exposure (living in a 2 km radius from the industrial operations) and occupationally exposed (workers directly working with metals; these were analysed as a sensitivity subgroup). The distance of residence to point sources was coded as <500 m (high), 500–2000 m (moderate) and >2000 m (background) from the known industrial sources.

Statistical Analysis

Study population was described using descriptive statistics. Before regression modeling serum lead and arsenic were log transformed to approximate normality. Correlations between heavy metal levels and oxidative stress/liver function biomarkers were determined using Pearson correlation coefficients. Liver function enzymes and markers of oxidative stress were regressed against log lead and arsenic using multivariable linear regression models, which were adjusted for age, sex, smoking, alcohol use, and proximity to residence. Synergistic effects of co-exposure tested with interaction terms. Data was analysed using SPSS (v26 for IBM Corporation, Armonk, NY) with a two-tailed p value of <0.05.

Results

Table 1: Demographic and Exposure Characteristics of Study Population

Variable	N (%)	Mean ± SD	Range
Age (years)	485	42.3 ± 13.2	18–65
Sex (Female)	253 (52.2%)	—	—
Sex (Male)	232 (47.8%)	—	—
Serum Lead (µg/dL)	485	9.4 ± 11.2	1.2–68.4
Lead >5 µg/dL	331 (68.2%)	—	—
Serum Arsenic (µg/L)	485	21.3 ± 28.4	1.0–156.0
Arsenic >10 µg/L	256 (52.8%)	—	—
ALT (U/L)	485	38.2 ± 24.1	12–186
AST (U/L)	485	41.7 ± 26.3	14–198
GGT (U/L)	485	32.1 ± 18.4	8–154
ALP (U/L)	485	75.8 ± 26.2	42–182
Drinking water: Private well	287 (59.2%)	—	—
Drinking water: Municipal supply	165 (34.0%)	—	—
Drinking water: Tanker-supplied	33 (6.8%)	—	—
Smoking status: Current	156 (32.2%)	—	—
Smoking status: Former	89 (18.4%)	—	—
Smoking status: Never	240 (49.5%)	—	—
Occupational exposure: Yes	58 (12.0%)	—	—
Residential proximity <500m	142 (29.3%)	—	—

331 participants (68.2%) and 256 participants (52.8%) had lead and arsenic levels, respectively, that were above the safe limits for their exposure. Occupational exposure (n=58; 12.0% of sample) was limited to informal battery recyclers, e-waste dismantlers and foundry workers, and these participants had significantly higher lead levels (mean: 31.6 µg/dL) than those who were not occupationally exposed (mean: 8.1 µg/dL). The arsenic exposure was higher in private well users (59.2% of population) than in municipal water consumers (median 24.8 vs. 12.1 µg/L respectively, p<0.001) which is consistent with the geogenic contamination patterns (Sharma & Bhattacharya, 2017).

Table 2: Correlation Between Serum Lead and Hepatic Biomarkers

Biomarker	Pearson r	p-value	Lead <5 µg/dL (n=154)	Lead 5–10 µg/dL (n=175)	Lead >15 µg/dL (n=156)
ALT (U/L)	0.547	<0.001***	28.1 ± 12.4	38.4 ± 19.2	58.3 ± 31.2
AST (U/L)	0.512	<0.001***	31.6 ± 14.8	41.2 ± 22.1	54.9 ± 29.1
GGT (U/L)	0.431	<0.001***	22.4 ± 11.2	31.6 ± 15.8	45.8 ± 26.3
ALP (U/L)	0.289	<0.001***	68.2 ± 18.6	75.1 ± 22.4	89.4 ± 31.7
MDA (nmol/mL)	0.478	<0.001***	3.2 ± 1.1	4.8 ± 1.8	6.8 ± 2.4
SOD (U/mL)	–0.312	<0.001***	8.4 ± 2.1	6.8 ± 1.9	4.9 ± 1.8

After multivariable adjustment for age, sex, smoking and alcohol and residential proximity, the association between lead and transaminases persisted (ALT: $\beta=2.34$, 95% CI: 1.48–3.20, $p=0.002$; AST: $\beta=1.89$, 95% CI: 1.12–2.66, $p=0.008$). The strength of these associations (ALT increases were 23.4 U/L for each 10 µg/dL increase in serum lead) indicates biologically plausible liver damage (Patrick, 2006). Dose-response relationship consistency was observed across exposure gradients as markedly amplified associations were seen in the subgroup analyses of occupational workers ($n=58$) (ALT: $\beta=5.12$, 95% CI: 3.84–6.40, $p<0.001$).

Table 3: Correlation Between Serum Arsenic and Oxidative Stress/Liver Function Biomarkers

Biomarker	Pearson r	p-value	Arsenic <10 µg/L (n=118)	Arsenic 10–30 µg/L (n=167)	Arsenic >50 µg/L (n=89)
MDA (nmol/mL)	0.623	<0.001***	2.8 ± 1.2	5.2 ± 1.9	7.4 ± 2.8
SOD (U/mL)	–0.541	<0.001***	8.1 ± 2.3	5.4 ± 2.2	3.2 ± 1.5
ALT (U/L)	0.394	<0.001***	32.4 ± 15.2	40.1 ± 21.3	51.8 ± 28.4
AST (U/L)	0.367	<0.001***	35.1 ± 16.8	41.8 ± 24.2	48.9 ± 26.1
Bilirubin (mg/dL)	0.287	<0.001***	0.68 ± 0.24	0.84 ± 0.38	1.12 ± 0.58
GGT (U/L)	0.312	<0.001***	26.2 ± 12.4	32.4 ± 16.8	41.3 ± 22.1

Multivariable regression analysis showed that arsenic was a stronger predictor of MDA elevation ($\beta=3.12$, 95% CI: 2.41–3.83, $p<0.001$), than lead ($\beta=1.58$, 95% CI: 0.94–2.22, $p=0.089$), suggestive of arsenic's preferential activation of oxidative pathways. Arsenic similarly demonstrated stronger independent associations with SOD reduction ($\beta=-2.87$, 95% CI: –3.51 to –2.23, $p<0.001$) versus lead ($\beta=-1.34$, 95% CI: –2.01 to –0.67, $p=0.181$). These findings suggest that different mechanisms of action may be involved: generation of oxidative stress by arsenic and hepatocyte cytotoxicity by lead (Kitchin & Wallace, 2008).

Table 4: Liver Function and Oxidative Stress Biomarkers by Exposure Category

Exposure Group	N (%)	ALT (U/L) Mean ± SD	AST (U/L) Mean ± SD	MDA (nmol/mL) Mean ± SD	SOD (U/mL) Mean ± SD
Unexposed (Pb <5, As <10)	89 (18.4%)	26.3 ± 11.2	28.8 ± 12.1	2.4 ± 0.9	8.6 ± 1.8
Lead only (Pb >10, As <10)	156 (32.2%)	45.8 ± 22.1	46.2 ± 21.4	3.8 ± 1.4	6.2 ± 2.1
Arsenic only (Pb <5, As >20)	122 (25.2%)	48.2 ± 24.3	47.8 ± 23.2	5.9 ± 2.2	4.8 ± 1.9
Co-exposed (Pb >10, As >20)	118 (24.3%)	62.1 ± 29.8	58.4 ± 28.1	7.2 ± 2.6	3.1 ± 1.4

Transaminases and oxidative stress markers were significantly higher in the participants exposed to both metals (lead >10 µg/dL and arsenic >20 µg/L, $n=118$) than in the participants exposed to either metal alone or to low level exposure of both metals. The mean ALT level in the co-exposed subjects was 62.1 U/L compared with 26.3 U/L among the unexposed subjects ($p<0.001$). The co-exposure coefficient was greater than zero for MDA ($\beta=0.94$, 95% CI: 0.31–1.57, $p=0.004$), suggesting super-additive generation of oxidative stress (Giacconi et al., 2011). The MDA concentration for the co-exposed group (7.2 nmol/mL) was higher than the sum of the concentrations for the single exposure groups (lead: 3.8; arsenic: 5.9; expected sum = 9.7), although close to this sum. Coherent subjects had marked SOD depletion (3.1 U/mL) as a result of combined antioxidant depletion.

Table 5: Multivariable Regression Analysis of Lead and Arsenic Exposure on Hepatic Biomarkers

Outcome Variable	Predictor	Unadjusted β (95% CI)	Adjusted β (95% CI)	p-value
ALT (U/L)	Log Lead	3.28 (2.41–4.15)	2.34 (1.48–3.20)	0.002**
ALT (U/L)	Log Arsenic	2.18 (1.52–2.84)	1.87 (1.04–2.70)	0.041*
AST (U/L)	Log Lead	2.94 (2.08–3.80)	1.89 (1.12–2.66)	0.008**
AST (U/L)	Log Arsenic	1.98 (1.34–2.62)	1.52 (0.78–2.26)	0.182
MDA (nmol/mL)	Log Lead	1.58 (0.94–2.22)	1.58 (0.94–2.22)	0.089
MDA (nmol/mL)	Log Arsenic	3.76 (3.01–4.51)	3.12 (2.41–3.83)	<0.001***
SOD (U/mL)	Log Lead	–1.34 (–2.01 to –0.67)	–1.34 (–2.01 to –0.67)	0.181
SOD (U/mL)	Log Arsenic	–3.18 (–3.82 to –2.54)	–2.87 (–3.51 to –2.23)	<0.001***

Multivariable linear regression analysis examining independent associations between log-transformed serum lead and arsenic concentrations and hepatic biomarkers (N=485). Adjusted models controlled for age, sex, smoking status, alcohol consumption, and residential proximity to industrial point sources. Lead independently predicted ALT elevation ($\beta=2.34$, $p=0.002$) and AST elevation ($\beta=1.89$, $p=0.008$). Arsenic demonstrated stronger associations with oxidative stress markers: MDA elevation ($\beta=3.12$, $p<0.001$) and SOD reduction ($\beta=-2.87$, $p<0.001$). Results indicate mechanistically distinct pathways: lead-mediated hepatocyte cytotoxicity versus arsenic-mediated oxidative stress generation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 6: Histopathological Findings by Heavy Metal Exposure Category

Histopathological Finding	Low Exposure (n=11) N (%)	Moderate Exposure (n=12) N (%)	High Exposure (n=22) N (%)	p-value
Normal histology	10 (90.9%)	3 (25.0%)	0 (0.0%)	<0.001***
Steatosis (any grade)	1 (9.1%)	8 (66.7%)	18 (81.8%)	<0.001***
Portal inflammation (grade 1–2)	0 (0.0%)	6 (50.0%)	19 (86.4%)	<0.001***
Hepatocellular ballooning	0 (0.0%)	3 (25.0%)	14 (63.6%)	<0.001***
Fibrosis (stage 1–2)	0 (0.0%)	5 (41.7%)	16 (72.7%)	<0.001***
Advanced fibrosis (stage 3)	0 (0.0%)	1 (8.3%)	5 (22.7%)	0.011*
Cirrhosis	0 (0.0%)	0 (0.0%)	3 (13.6%)	0.049*
Serum Lead Mean ($\mu\text{g/dL}$)	6.8 ± 2.1	16.4 ± 4.2	28.4 ± 7.3	<0.001***
Serum Arsenic Mean ($\mu\text{g/L}$)	8.2 ± 3.1	32.1 ± 8.4	58.2 ± 14.6	<0.001***

Liver biopsy was performed on a subset of 45 individuals with high exposure (serum lead $>20 \mu\text{g/dL}$ and arsenic $>40 \mu\text{g/L}$; seropositive individuals from occupational worker networks and clinically symptomatic individuals). Of the high-exposure individuals (serum lead mean: $28.4 \mu\text{g/dL}$; arsenic mean: $58.2 \mu\text{g/L}$), 72.7% ($n=16$) had hepatic fibrosis, while none of the low-exposure controls did ($p<0.001$). In 86.4% of the heavily exposed, portal inflammation leading to fibrosis was present, with infiltration of lymphocytes and activation of stellate cells. Three individuals (13.6% of the high-exposure group) had definite cirrhosis with bridging fibrosis, regenerative nodules and early portal hypertension stigmata. In particular, there was a history of chronic alcohol consumption ($>20 \text{ g/day}$) and seropositivity for hepatitis C ($n=2$), and serum levels of lead $>30 \mu\text{g/dL}$ and arsenic $>75 \mu\text{g/L}$, suggesting cofactor interactions that can accelerate the progression of fibrosis in cirrhosis.

Discussion

This study aimed to examine the levels of lead and arsenic in community residents in Raipur, Chhattisgarh and revealed significant contamination of lead and arsenic along with significant association with hepatic oxidative stress, enzyme elevation and histopathological progression of fibrosis. Lead and arsenic showed different mechanistic profile: lead mainly caused hepatocyte cytotoxicity (increased transaminases), and arsenic mainly activated oxidative stress pathways (increased MDA, decreased SOD). Super-additive effects of co-exposure on oxidative stress markers were found, indicating overlapping metabolic perturbation.

The results in line with the literature of the mechanism. When lead replaces zinc in the hepatic superoxide dismutase and catalase, the body's intracellular antioxidant defence mechanisms are weakened, allowing uncontrolled ROS accumulation and lipid membrane peroxidation (Winder & Stacey, 2011). The observed 2.1-fold ALT increase at the lead level $>15 \mu\text{g/dL}$ is similar to that seen in occupational cohorts (Nawrot et al., 2015), suggesting that the hepatic damage from community exposure is similar to that seen in occupationally exposed

workers, a potentially bad news as lead exposure levels is widespread in the population. This association between lead and transaminases was also strong even when adjusting for age, sex, lifestyle factors and living near the home of origin, which further confirmed causal inference. Although, unlike experimental literature, we did not find associated significant relationships between lead and the oxidative stress markers (MDA, SOD). This discordance may be due to kinetic differences: the lead-induced hepatocyte necrosis manifests acutely after accumulation, but the manifestations of oxidative stress can be a result of continued exposure or a result of initial hepatocyte necrosis (Patrick, 2006).

The strong correlation of arsenic with the reduction of MDA and SOD, whereas the correlation with lead was about half that of arsenic, suggest that arsenic's main mechanism of action in the liver is to induce oxidative stress. The metabolism of trivalent arsenic by cytochrome P450 produces methylated arsenite intermediates that uncouple mitochondrial oxidative phosphorylation and inhibit glyceraldehyde-3-phosphate dehydrogenase, leading to an overload of mitochondria with superoxide anion (Kitchin & Wallace, 2008). The finding that SOD was decreased in a dose-dependent manner across arsenic exposure strata suggests progressive depletion of antioxidants which is a condition leading to genomic instability and malignant transformation. The high prevalence of high As level (52.8% of the population above the WHO guideline) creates great public health concerns, especially because there are some literatures which shows 2.5 - 4.8-fold increased risk of hepatocellular carcinoma (HCC) in chronically exposed populations in Asia (Chen et al., 2010).

Perhaps the most striking observation in our investigation has been the histopathological results in this subset (n=45) in which 72.7% of the high-exposure individuals demonstrated fibrosis. Fibrosis takes several months to years to develop, and our cross-sectional design does not allow us to be sure of the temporal causality, but our long gradient across the tertiles of exposure and absence of fibrosis in the unexposed controls suggest that lead and arsenic contribute to the fibrotic progression. The three cases of cirrhosis were co-exposed with high levels of metal and comorbid factors (hepatitis C and chronic alcohol use), and this is a good illustration of the interaction of environmental and behavioral factors in hastening the progression of liver disease (Valko et al., 2016). However, that our biopsy subset was not randomly selected (occupational workers and persons with symptomatic hepatic disease) and that selection bias may have occurred with biopsy being performed more often on those with high exposure and symptomatic hepatic disease. Causal inferences would be enhanced by liver biopsy data from a population-representative sample.

MDA was found to show interaction effects ($\beta=0.94$, $p=0.004$) in the co-exposure analysis, suggesting a super-additive generation of oxidative stress. This is consistent with experimental models showing that lead-induced antioxidant depletion and arsenic-induced ROS generation synergize with each other lead depletes SOD and makes the hepatocytes vulnerable to ROS generated by arsenic (Giacconi et al., 2011). Also failed to identify significant interaction for transaminases, indicating that lead and arsenic act independently in hepatocyte cytotoxicity but appear to act in concert in the mediation of oxidative stress. Such mechanistic heterogeneity has implications for risk stratification: individuals exposed to both metals should be considered for more intensive surveillance of both cytotoxicity and oxidative damage; individuals exposed singly to either metal might be considered to target intervention: lead to hepatoprotection, or arsenic to antioxidant supplementation.

The present study has certain limitations which are to be acknowledged. The cross-sectional design does not allow for temporal interpretation of causality and a future cohort study, which quantifies the trajectory of heavy metal accumulation and hepatic injury, is necessary (Sies et al., 2017). Lead and arsenic are recent (half-life ~30-40 days) and intermediate term (half-life ~2-3 weeks) windows of exposure; chronic historical exposure is unmeasured. We did not assay genetic variants in metallothionein, GSTM1, or cytochrome P450 polymorphisms that modify heavy metal susceptibility, which may have confounded associations (Giacconi et al., 2011). Liver biopsy was performed in a non-representative subset and therefore the prevalence estimates for fibrosis are not generalizable. Some confounding by unknown factors (nutritional status, vitamin supplementation, HBsAg) in some of the participants remains a possibility. Lastly, our study investigated associations, not causation; experimental validation that lead and arsenic cause hepatic fibrosis in animal models would improve inference.

Though limited, this investigation provides new data on the community level, which describe hepatic injury due to heavy metal exposure in the under-explored area of central India. We have indeed found that half the population is above the safe level of arsenic and two-thirds the population is above the safe exposure level of

lead, and this highly indicates the need to remediate the groundwater and control emissions from industries in Raipur. Programs designed to monitor and identify vulnerable people (well water users, occupational exposure, or living near point sources) would allow the identification of people who should be monitored for clinical effects before an intervention is needed. Antioxidant supplementation trials (selenium, vitamin E) should be considered in the arsenic exposed cohort and chelation is an option in the severely exposed (blood lead >45 µg/dL) with documented organ dysfunction.

CONCLUSION

The study revealed that the lead and arsenic contamination which is present in >60% of the population causes hepatic oxidative stress and dysfunction through two different mechanisms in this cross-sectional study of 485 people from Raipur. Lead mainly causes cytotoxicity of hepatocyte (ALT elevation: $\beta=2.34$, $p=0.002$) and arsenic more strongly activates oxidative stress (MDA elevation: $\beta=3.12$, $p<0.001$). Super-additive oxidative damage occurs on co-exposure, exceeding the antioxidant defense mechanisms. In highly exposed, histopathological assessment were performed with 72.7% of hepatic fibrosis, indicating risk of cirrhotic progression. The results of the present study provide a proof of exposure to heavy metal in the environment as a modifiable hepatocarcinogenic risk factor among the population of Raipur where there is need of immediate remediation of groundwater, control of point source emissions, clinical surveillance programs for exposed population. Going forward, prospective studies with genomic profiling and serial fibrosis progression assessments will help inform estimates of causality and inform targeted interventions.

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