Changes in Inflammatory Cytokines, Antioxidants and Liver Stiffness after Chelation Therapy in Individuals with Chronic Lead Poisoning

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Abstract

Background Chronic exposure to lead accumulates mainly in the liver. In vivo studies showed that lead toxicities are related to alterations of the inflammatory response. We aimed to evaluate the association between lead poisoning and liver fibrosis as well as the change in degree of liver fibrosis, levels of inflammatory mediators and glutathione (GSH) after chelation therapy.

Methods Workers from a battery factory who were exposed to lead for > 12 months and had blood lead level (BLL) > 70 µg/dL were enrolled (n=86) into the study. They received chelation therapy with intravenous CaNa2EDTA for 2 days followed by oral D-penicillamine for 90 days. Primary outcome was the change in the degree of liver fibrosis, which was presented as liver stiffness (LS), measured by FibroScan®. Secondary outcomes were the change in the levels of serum GSH and inflammatory mediators such as tumor necrotic factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) after chelation therapy.

Results Of the 86 participants, there was a positive correlation between duration of lead exposure and LS (r=0.249, p=0.021). To avoid the confounder effect of obesity-related steatosis, only 70 individuals who had controlled attenuation parameters < 296 dB/m, BMI ≤ 25 kg/m2 and normal waist circumference were included in the interventional analysis. After chelation, the mean LS significantly decreased from 5.4 ± 0.9 to 4.8 ± 1.4 kPa (p=0.001). Similarly, all of the inflammatory cytokines studied significantly decreased after chelation (p<0.001); TNF-α dropped from 371.6 ± 211.3 to 215.8 ± 142.7; the levels of IL-1β dropped from 29.8 ± 1.7 to 25.9 ± 4.3 and the levels of IL-6 dropped from 46.8 ± 10.2 to 35.0 ± 11.9. On the other hand, the mean GSH level significantly increased from 3.3 ± 3.3 to 13.1 ± 3.7 (p<0.001) after chelation therapy.

Conclusions Our findings suggest that long-term exposure of lead may cause liver fibrosis. Lead poisoning induces chronic inflammatory response and oxidative stress. Treatment by chelation decreases inflammation and replenishes antioxidants, and potentially reduces the degree of LS.

Introduction

Lead is a heavy metal that can be found in the environment at a low concentration level. However, in the industrialization era, lead is widely used as a compound for a variety of products such as battery, gasoline, and ceramics[1]. Workers in these manufacturing processes are therefore at higher risk to develop lead toxicity. In a recent article, it's authors were concerned that there was an underestimation of lead poisoning in low- and middle-income countries[2,3]. Individuals with chronic lead poisoning, although uncommon, have non-specific symptoms such as constipation, anorexia and recurrent colicky abdominal pain that have an insidious onset and affect multiple organ systems. Hence, many cases are misdiagnosed or detected late.

Most previously reported cases of lead-induced liver injury had mild and self-limited hepatitis. However, there are limited studies conducted in humans that assessed the effects of lead poisoning on the liver[4-6]. All previous studies focused on the abnormalities of liver function tests which represented only liver injury at one time point. Since the liver is one of the major reservoirs of lead accumulation, this can cause chronic liver injury. Few lead-intoxicated animal studies pathologically investigated liver fibrosis and steatosis. In the animal models, it was shown that there was a reduction in antioxidants, particularly glutathione (GSH), which was the main mechanism for lead-induced hepatoxicity[7]. Aside from that, it was shown that chronic lead-exposed animals had

elevated pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, IFN-γ and tumor necrotic factor (TNF)-α[7].

As a result of this, our primary aim for our study was to evaluate the effects of chronic lead toxicity on the liver, specifically fibrosis and steatosis. This study assessed the levels of hepatic fibrosis and steatosis as well as levels of inflammatory cytokines and antioxidants before and after chelation therapy.

Methods

Study design

This study was conducted in two phases. For the first phase, this was a cross-sectional cohort composed of participants with severe chronic lead poisoning. We determined the associations between chronic lead poisoning and liver injury. For the second phase, this was a prospective interventional cohort that evaluated whether chelation therapy could reduce the lead-related liver injuries. Both studies were conducted from August 1, 2018 to February 1, 2019.

Study cohorts

Phase I: Initial cross-sectional cohort

This cohort aimed to evaluate associations between severe chronic lead poisoning parameters and potential adverse effects on the liver. Participants were workers who had worked at a battery factory. Inclusion criteria were: (1) age ≥ 18 years; (2) occupational exposure to lead in the battery factory for ≥ 12 months, and (3) had blood lead level (BLL) ≥ 70 μg/dl, which is the cut-off level for severe occupational chronic lead poisoning and should be treated with intravenous chelation therapy as per the US Occupational Safety and Health Administration (OSHA)[8]. We excluded participants who had chronic liver diseases such as viral hepatitis B or C (HBV or HCV) infection, autoimmune hepatitis, Wilson's disease, hemochromatosis, alcoholic liver disease (i.e. history of alcohol consumption for ≥ 30 gm/day in men or ≥ 20 gm/day in women for at least 3 months within 1 year prior to enrollment) and non-alcoholic fatty liver disease (NAFLD). Participants who had risk factors for NAFLD such as diabetes mellitus and/or serum triglyceride ≥ 200 mg/dL, or waist circumference (WC) ≥ 102 cm in men or ≥ 88 cm in women were also excluded. Other exclusion criteria were history of use of possible liver-toxic medication within 12 monthsrior to study enrollment, previous chelation therapy, and presence of signs or symptoms of acute lead poisoning such as colicky abdominal pain, hemolytic anemia, and polyneuropathy.

All workers from the battery factory were screened for BLL (n=720). Participants who met the inclusion and exclusion criteria were enrolled into the initial cross-sectional cohort (n=86).

Phase II: Prospective interventional cohort

This interventional cohort aimed to evaluate the mechanisms of liver injury from chronic lead poisoning by comparing the change in liver stiffness (LS) and amount of hepatic fat based on the changes of the amount of GSH and pro-inflammatory cytokines. Since fatty liver is a well-known factor that contributes to liver fibrosis, hence we excluded 16 participants with severe fatty liver which is defined as having controlled attenuation

parameters (CAP) of > 296 dB/m or has high risk features for metabolic syndrome such as BMI > 25 kg/m^2 and WC > than the cut-off criteria for metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (i.e., WC > 80 cm for women and WC > 90 cm for men) in order to eliminate the effects of confounding factors for obesity-related steatosis. The remaining eligible 70 participants were then enrolled into the prospective interventional cohort. The participants received 2 grams of $CaNa_2EDTA$ intravenously for 2 days followed by oral D-penicillamine 1 gm/day for 90 days. All participants were advised to avoid potential hepatotoxic medications and alcohol consumption. Primary outcome was the change in LS and steatosis after chelation therapy. Amount of GSH and inflammatory cytokines were prospectively evaluated at the last day of treatment. Secondary outcomes were the correlation between the change of BLL, liver steatosis, liver fibrosis, GSH and inflammatory markers at pre- and post-chelation therapy. The investigators and the participants were blinded to the results of the blood tests and FibroScan® (Echosens, Paris, France).

Data and Specimens Collection

Clinical information, complete blood count, liver function test, and serum samples were collected upon admission. Non-invasive liver assessments were performed by a certified single operator. FibroScan®, transient elastography (TE), was used to assess liver stiffness (LS) and the degree of liver steatosis which was presented as controlled attenuation parameters (CAP).

Serum samples at pre- and post-chelation were stored at -80˚C until analysis. The levels of GSH, TNF-α, IL-1β, and IL-6 were measured using a solid-phase enzyme immunoassay technique using commercially available kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. The absorbance was read at 450 nm. The flow of the study is shown in Figure 1.

Statistical Analysis

For testing a difference of two dependent means, the estimated sample size was based on the results of a pilot study of 10 chronic lead poisoning workers done at our hospital; the mean LS was 5.2 $\pm$ 0.7 kPa. The reference value of 5.5 $\pm$ 3.8 kPa in the control group was derived from a survey among 782 healthy Thai volunteers[7]. The calculated minimum sample size was 58 cases for 80% power. Categorical data were compared using Fisher's exact test. Continuous variables were described as the means and standard deviations. Potential relationships between lead-related parameters and degree of hepatic fibrosis and steatosis were initially assessed using Pearson’s correlation analysis. Statistically significant parameters were subsequently included in the multivariate linear regression analysis. A dependent samples t-test was used to compare the results between pre- and post-treatment. Pearson’s correlation analysis was used to find the correlation between the percentage changes of BLL and the percentage changes in the levels of inflammatory biomarkers.

Results

Phase I: Initial cross-sectional cohort

Baseline characteristics

A total of 720 participants were screened for BLL. 180 participants had BLL of ≥ 40 µg/dl. This level indicated that there was chronic lead toxicity and chelation therapy was required (Figure 1). Among the 180 participants, 86

participants met the inclusion and exclusion criteria and were enrolled into the study. Table 1 shows the baseline characteristics of the 86 participants. Most of the participants were males (n=71, 85%) and the average age was 37.6 ± 7.3 years. The mean BLL was 81.4 ± 9.8 µg/dl. Twenty-six (30.2%) participants had hepatitis; they had SGOT above ULN (>35 mg/dl, n=14) and/or SGPT above ULN (>40 mg/dl, n=17).

The mean LS of the 86 participants was 5.4 ± 0.9 kPa. Notably, 23 (26.7%) participants from this cohort had significant fibrosis (i.e., the LS was > 6.1 kPa). The mean CAP was 225.1 ± 49.3 dB/m. Forty four (51.2%) of the participants had CAP > 213 dB/m, indicating that there was significant liver steatosis. Among those with significant fibrosis, 42 (48.8%) participants had no steatosis (S0), while 30 (34.8%) and 15 (17.4%) participants had mild-moderate steatosis (S1-2) and severe steatosis (S3), respectively. The number of participants with LS and CAP are shown in Figure 2.

Table 1 Baseline pre-treatment laboratory profiles of 86 participants in the initial cross-sectional cohort

Parameters	Mean ± SD
Age (year)	37.6 ± 7.3
Waist (cm)	80.2 ± 12.0
BMI (kg/m^2)	24.2 ± 4.9
Blood lead level (µg/dL)	81.4 ± 9.8
Hemoglobin (g/dL)	13.4 ± 1.6
White blood cell count ($10^3/\mu L$)	8.0 ± 1.8
Platelet ($10^{12}/L$)	285.6 ± 58.4
Creatinine (mg/dL)	0.9 ± 0.3
Total bilirubin (mg/dL)	0.8 ± 0.4
Direct bilirubin (mg/dL)	0.3 ± 0.1
Alkaline phosphatase (U/L)	68.8 ± 19.5
SGOT (U/L)	30.7 ± 28.9
SGPT (U/L)	33.3 ± 33.2
Liver stiffness (kPa)	5.4 ± 0.9
CAP (dB/m)	225.1 ± 49.3

SGOT; serum glutamate oxaloacetate transaminase, SGPT; serum glutamate pyruvate aminotransferase, CAP; controlled attenuation parameters

Factors associated with liver fibrosis and steatosis

In the univariate analysis, duration of lead exposure, but not BLL, was significantly associated with the degree of hepatic fibrosis (Pearson's r=0.249, p=0.021). Other factors associated with liver fibrosis included age, BMI, liver

steatosis, SGPT, and ALP (Table 2). In the multivariate analysis, only duration of lead exposure and SGPT level remained independently associated with liver fibrosis, with Pearson's r of 0.229 and 0.317, p= 0.026 and 0.002, respectively.

As for liver steatosis, the following variables were found to be significantly associated with the presence of liver steatosis in the univariate model: sex, WC, BMI, liver fibrosis, SGOT, and SGPT. WC and SGPT were independently associated with liver steatosis, with Pearson's r of 0.524 and 0.397, p<0.001 and 0.018, respectively. We did not detect any association between lead-related parameters and liver steatosis (Table 2).

Table 2 Potential factors that may be associated with liver fibrosis and steatosis from the initial cross-sectional cohort

Parameters	**Factors associated with liver fibrosis**				**Factors associated with liver steatosis**			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Pearson Correlation	P-value	Pearson Correlation	P-value	Pearson Correlation	P-value	Pearson Correlation	P-value
Age	0.215	0.047	0.195	0.060	-0.042	0.703	-	-
Sex	-0.155	0.153	-	-	-0.237	0.028	-0.161	0.062
BLL	-0.086	0.430	-	-	-0.075	0.493	-	-
Duration of lead exposure	0.249	0.021	0.229	0.026	0.020	0.854	-	-
Waist circumference	0.220	0.076	-	-	0.702	<0.001	0.524	<0.001
BMI	0.226	0.037	0.107	0.350	0.660	<0.001	2.064	0.360
Liver Steatosis	0.242	0.025	0.080	0.525	1	-	-	-
Liver Fibrosis	1	-	-	-	0.242	0.025	2.155	0.656
SGOT	0.161	0.142	-	-	0.329	0.002	-0.531	0.273
SGPT	0.332	0.002	0.317	0.002	0.566	<0.001	0.397	0.018
ALP	0.222	0.041	0.107	0.347	0.154	0.161	-	-

BLL; blood lead level, BMI; body mass index, SGOT; serum glutamate oxaloacetate transaminase, SGPT; serum glutamate pyruvate aminotransferase, ALP; alkaline phosphatase

Phase II. Interventional prospective cohort

Effects of chelation therapy on liver fibrosis and steatosis

Association between liver fibrosis and steatosis was detected from our initial cross-sectional cohort (Pearson's r =0.242, p=0.025). After excluding participants with severe fatty liver or those with high risk features for metabolic syndrome as previously described**,** a total of 70 participants were enrolled into this study. Correlation analyses were repeated to confirm the independent effects between degree of liver fibrosis and steatosis. We found that there was no significant association between the degree of fibrosis and steatosis. The Pearson's r between level of LS and CAP at pre-chelation phase was -0.039 (p = 0.75) and at post-chelation phase, it was 0.151 (p = 0.21). Pearson's correlation analysis between degree of post-chelation LS reduction and degree of post-chelation CAP reduction was 0.160, (p = 0.19) (Supplementary table 1).

After 3 months of chelation therapy, the mean BLL decreased from 81.8 ± 9.9 to 56.6 ± 16.8 µg/dL (30.8%). After treatment, the degree of LS significantly dropped from 5.33 ± 0.9 to 4.8 ± 1.4 kPa (p=0.001). We did not find significant improvement of liver steatosis after chelation therapy (mean pre-and post-chelation CAP were 208.6 ± 31.7 and 207.0 ± 45.0 dB/m, p=0.738, respectively). (Table 3)

Table 3 Comparison of lead-related parameters between pre- and post-chelation therapy

Parameters	Pre-chelation (mean ± SD)	Post-chelation (mean ± SD)	Mean difference between post- and pre-chelation (95% Confidence Interval)	p-value
Blood lead level (µg/dL)	81.8 ± 9.9	56.6 ± 16.8	-25.2 ± 13.8 (21.9 – 28.5)	<0.001
Liver stiffness (kPa)	5.3 ± 0.9	4.8 ± 1.4	- 0.5 ± 1.2 (0.2-0.8)	0.001
Steatosis (dB/m)	208.6 ± 31.7	207.0 ± 45.0	-1.6 ± 41.0 (-8.1-11.4)	0.738
TNF-α (pg/mL)	371.6 ± 211.3	215.8 ± 142.7	-155.8 ± 137.4 (120.9-190.7)	<0.001
Interleukin-1β (pg/mL)	29.8 ± 1.7	25.9 ± 4.3	-3.8 ± 3.7 (2.9-4.8)	<0.001
Interleukin-6 (pg/mL)	46.8 ± 10.2	35.0 ± 11.9	-11.8 ± 10.6 (9.1-14.5)	<0.001
Glutathione (µg/mL)	3.3 ± 3.3	13.1 ± 3.7	9.8 ± 3.7 (10.8 - 8.9)	<0.001

TNF, tumor necrosis factor

Effects of chelation therapy on oxidative stress and inflammatory markers

The mean level of inflammatory biomarkers for TNF-α, IL-1β and IL-6, were significantly reduced after chelation therapy by 41.93% (371.6 ± 211.3 to 215.8 ± 142.7 pg/mL), 13.09% (29.8 ± 1.7 to 25.9 ± 4.3 pg/mL), and 25.21% (46.8 ± 10.2 to 35.0 ± 11.9 pg/mL), respectively. On the other hand, the mean GSH level significantly increased after chelation therapy from 3.3 ± 3.3 to 13.1 ± 3.7 µg/mL (297.0%). (Table 3) However, the correlation between

the degree of change in BLL and the reduction in TNF-α and IL-6 levels were not significant. The elevated level of GSH was also not significant. (Table 4)

Table 4 Degree of correlation between reduced BLL, LS, and CAP for each inflammatory markers studied

Inflammatory markers affected by chelation treatment	**Reduced BLL**		**Reduced LS**		**Reduced CAP**	
	Pearson's Correlation	p-value	Pearson's Correlation	p-value	Pearson's Correlation	p-value
TNF-α	0.212	0.919	-0.014	0.909	-0.237	0.048
Interleukin-1β	0.034	0.778	0.045	0.714	0.008	0.945
Interleukin-6	0.118	0.332	0.020	0.872	0.055	0.652
Glutathione	-0.100	0.410	-0.030	0.802	-0.079	0.517

BLL; blood lead level, LS; liver stiffness, CAP; controlled attenuation parameters, TNF; tumor necrosis factor

Discussion

Each day, about 0.1-2 mg of lead enters the human body through ingestion (75%), inhalation and skin contact (25%). Once the lead is absorbed and enters the blood stream, it is distributed and deposited in various types of soft tissue in the human body. The lead accumulates in the bone, followed by the liver kidney, neuron, and spleen[8].

In the blood, 95% of lead binds to the erythrocytes and has a mean half-life of 35 days. There are various ranges of normal BLL depending on the age and environmental exposure to lead. The BLL of 25-40 μg/dl in adults and 5-10 μg/dL in children are considered to be normal reference levels in non-lead exposure population while BLL of 40-60 μg/dl is an acceptable normal value among occupational lead-exposure workers[9]. The diagnosis of chronic lead poisoning is based on the BLL regardless of the presence of signs or symptoms.

To date, there were few literatures about hepatotoxicity from lead poisoning. Most were case reports of participants with acute lead poisoning symptoms and abnormal liver chemistry tests; the ranges of the liver enzymes for SGOT and SGPT were 63-66 mg/dL and 75-256 mg/dL, respectively[4-6, 10-15]. No liver failure was reported. There were only four analytical studies that focused on hepatotoxicity among occupational lead-exposed workers[4-6, 17]. Every study found that there was a mild elevation of the liver enzymes. Two of the studies showed significant differences in the level of the liver enzyme between occupational lead-exposed workers and healthy control participants.[5, 6] However, in the other two studies, there were no differences in the level of liver enzymes in the people who were exposed to lead versus the control group[4, 16]. In our study, majority of participants have normal levels of liver enzymes. However, approximately 20% of the participants had elevated SGOT and/or SGPT levels without any explainable causes. Bilirubin and ALP levels were also normal. These findings were in concordance with the previous reports[4-6,17]. Lead is accumulated in the liver among workers who

are constantly exposed to lead. Therefore, we recommend that further investigations of the chronic toxicities from chronic lead poisoning be conducted.

Our study found GSH level was markedly elevated and that BLL dropped after chelation therapy. This indicated that lead depleted antioxidants which was consistent with findings from animal studies[10,18]. Lead-induced oxidative stresses were the main mechanisms of lead poisoning according to the animal models; there was a decrease in GSH reserve and an increase in reactive oxygen species (ROS). Lead inactivates GSH by binding to the sulfhydryl groups and inhibits GSH synthesis[8, 17]. Besides this, lead destabilizes the cell membrane by inducing lipid peroxidation and changes the membrane's biophysical properties and causes cell damage[17]. Our study was the first human study that confirmed findings from animal studies that GSH depletion contributed to liver injury in individuals with chronic lead poisoning.

Results from our study supported the systemic inflammation theory. We showed that after chelation therapy, BLL and the levels of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 were reduced. Lead exposures could enhance production of various pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IFN-γ and TNF-α. Overall, lead causes tissue damage by inducing inflammation and inhibits anti-inflammatory mechanisms[18-22].

Liver is one of the major organs that collects lead. We hypothesized that sustained lead exposure contributed to chronic inflammation which predisposed hepatic fibrosis. Histopathological findings from animal experiments[23, 24] and two human case reports of acute lead poisoning with unexplained hepatitis demonstrated extensive microvesicular and macrovesicular steatosis, portal and intralobular lymphocytic infiltrate, disrupt liver parenchymal architecture and pericellular fibrosis[11, 23]. Our study used LS as a non-invasive parameter that represented the degree of liver fibrosis. Although the mean LS in our study was within normal range, 26.7% of the participants had LS values above the significant fibrosis cut-off level. As of note, 82.6% of the participants with significant fibrosis had non-severe steatosis, thus the significant liver fibrosis might be the consequences from lead poisoning. Our study found that duration of lead exposure was the major factor that contributed to the development of liver fibrosis. This finding supported our hypothesis that liver injury came from chronic lead poisoning.

After chelation therapy, we found that the degree of LS and levels of inflammatory cytokines were significantly reduced and that there was an elevation of GSH. These findings suggested that liver fibrosis was associated with lead poisoning. Although the change in degree of LS did not significantly alter in proportion to the change in any single biomarker, we postulated that each cytokine exerted small effects in concert, rather than a single cytokine that contributed to hepatic fibrosis.

Evidently, chronic liver inflammation from chronic lead poisoning does not only lead to hepatic fibrosis, but it also induces various pathways that contribute to the development of hepatic steatosis. Animal studies found that lead-intoxicated rats had altered-gene expressions of hepatic enzymes in cholesterol and triglyceride homeostasis[7,25-27]. Few literatures observed significant hypertriglyceridemia and hypercholesterolemia among lead-exposed workers[28, 29] with scant histological reports of macrovesicular steatosis[11]. The mean CAP in our study was 225.1 ± 49.3 dB/m which was considered to be mild steatosis (S1). Noticeably, 54.7% of our participants had significant steatosis. However, we did not find a significant correlation between degree of steatosis and lead-associated parameters such as duration of exposure and BLL. Obesity might overcome the

effect of chronic lead poisoning. We saw that there was a strong significant correlation between degree of steatosis and BMI as well as WC.

Regarding the change in degree of liver steatosis, we did not find a significant reduction in the level of CAP after treatment. In terms of inflammatory marker analysis, only TNF-α level changed which was significantly negatively correlated with CAP change. It should be noted that the mean pre-chelation CAP level was rather low and within normal reference range. It is possible that the sample size was small so that we could not detect a change in CAP after therapy. Thus we cannot confidently conclude that there was no relationship between lead toxicity and steatosis.

Our study has some limitations. We did not use the gold standard of histopathology in detecting liver fibrosis and steatosis. Due to ethical and safety concerns, we opted to use a non-invasive technique, the FibroScan®, which has been validated by other investigators. Not only that, but it has shown good accuracy in evaluating the degree of fibrosis. It can even replace liver biopsy[30]. Another limitation was the chelation regimen that might be insufficient because the mean post-treatment BLL was still above the normal level and the follow-up time might have been too short to detect any change after treatment.

Conclusion

Continuous exposure to lead has an adverse effect on the liver. Duration of lead exposure was significantly associated with degree of fibrosis. Lead depletes antioxidants and increases systemic inflammatory response. Treatment by chelation increases the level of GSH and reduces levels of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6. Hence, chelation therapy can reduce the degree of LS.

Abbreviations

GSH, glutathione; LS, liver stiffness; TNF, tumor necrotic factor; IL, interleukin; BLL, blood lead level; US, United States; OSHA, Occupational Safety and Health Administration; HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; WC, waist circumference; CAP, controlled attenuation parameters; NCEP ATP, National Cholesterol Education Program Adult Treatment Panel; TE, transient elastography; ULN, upper limit normal; SGPT, serum glutamate-pyruvate transaminase, ALP, alkaline phosphatase; BMI, body mass index; SGOT, serum glutamic-oxaloacetic transaminase

Declarations

Ethics approval and consent to participate:

This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB. Number 297/61). All participants provided written consent prior to study enrollment.

Consent for publication:

Not applicable

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests

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Author's contributions:

TT designed the study, performed the research, analyzed data, and wrote the full manuscript. RC designed the study and revised the manuscript for final submission. PP designed the study and wrote the manuscript and revised for final submission. DW collected specimens, advised for laboratory interpretation, and revised for final manuscript submission.

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References

1. Kianoush S, Sadeghi M, Balali-Mood M. Recent Advances in the Clinical Management of Lead Poisoning. Acta Med Iran. 2015;53:327-36.
2. Ericson BLP, Taylor MP, Frostad J, Caravanos J, Keith J, Fuller R. The Global Burden of Lead Toxicity Attributable to Informal Used Lead-Acid Battery Sites. Ann Glob Health. 2016;82:686-99.
3. Latif Wani AA Ab, Jawed Ahmad Usmani. Lead toxicity: a review. Interdiscip Toxicol. 2015;8:55-64.
4. Bhagwat VR, Patil AJ, Patil J, et al. Occupational lead exposure and liver functions in battery manufacture workers around Kolhapur (Maharashtra). Al Ameen J Med Sci. 2008;1:2-9.
5. Dioka CE, Orisakwe OE, Adeniyi FA, et al. Liver and renal function tests in artisans occupationally exposed to lead in mechanic village in Nnewi, Nigeria. Int J Environ Res Public Health. 2004;1:21-5.
6. Dongre NN, Suryakar AN, et al. Occupational Lead Exposure In Automobile Workers In North Karnataka (India): Effect On Liver And Kidney Functions. Al Ameen J Med Sci. 2010;3(4):284-92.
7. Vanduangden K, Thanapirom K, Sonsiri K, Treeprasertsuk S. Prevalence of nonalcoholic fatty liver disease (NAFLD) diagnosed by controlled attenuation parameter with transient elastography in subjects with and without metabolic syndrome. Chula Med J. 2017;61:483-95.
8. Kim HC, Jang TW, Chae HJ, et al. Evaluation and management of lead exposure. Ann Occup Environ Med. 2015;27:30.

9. Administration OOSaH. Lead Exposure In Construction; Interim Final Rule–Inspection and Compliance Procedures. In: Occupational Safety and Health Administration OoHCA, ed. OSHA Instruction CPL 2-2.58: Occupational Safety and Health Administration, Office of Health Compliance Assistance, 1993.
10. Marginean CO, Melit LE, Moldovan H, et al. Lead poisoning in a 16-year-old girl: a case report and a review of the literature (CARE compliant). Medicine (Baltimore). 2016;95:e4916.
11. Verheij J, Voortman J, van Nieuwkerk CM, et al. Hepatic morphopathologic findings of lead poisoning in a drug addict: a case report. J Gastrointestin Liver Dis. 2009;18:225-7.
12. Menezes G, D'Souza H S, Venkatesh T. Chronic lead poisoning in an adult battery worker. Occup Med (Lond). 2003;53:476-8.
13. Grasso IA, Blattner CMR, Short ET, et al. Severe Systemic Lead Toxicity Resulting From Extra-Articular Retained Shrapnel Presenting as Jaundice and Hepatitis: A Case Report and Review of the Literature. Mil Med. 2017;182:e1843-e1848.
14. Pagliuca A, Mufti GJ, Baldwin D, et al. Lead poisoning: clinical, biochemical, and haematological aspects of a recent outbreak. J Clin Pathol. 1990;43:277-81.
15. Cullen MR, Robins JM, Eskenazi B. Adult inorganic lead intoxication: presentation of 31 new cases and a review of recent advances in the literature. Medicine (Baltimore). 1983;62:221-47.
16. Onyeneke EC and Omokaro EU. Effect of occupational exposure to lead on liver function parameters. Int J Pharm Med Sci. 2016;6:15-9.
17. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. Interdiscip Toxicol. 2012;5:47-58.
18. Dietert RR, Lee JE, Hussain I, et al. Developmental immunotoxicology of lead. Toxicol Appl Pharmacol. 2004;198:86-94.
19. Hsiao CL, Wu KH, Wan KS. Effects of environmental lead exposure on T-helper cell-specific cytokines in children. J Immunotoxicol. 2011;8:284-7.
20. Garcia-Leston J, Roma-Torres J, Mayan O, et al. Assessment of immunotoxicity parameters in individuals occupationally exposed to lead. J Toxicol Environ Health A. 2012;75:807-18.
21. Dobrakowski M, Kasperczyk A, Pawlas N, et al. Association between subchronic and chronic lead exposure and levels of antioxidants and chemokines. Int Arch Occup Environ Health. 2016;89:1077-85.
22. Chang WJ, Joe KT, Park HY, et al. The relationship of liver function tests to mixed exposure to lead and organic solvents. Ann Occup Environ Med. 2013;25:5.
23. Jarrar BM, Taib NT. Histological and histochemical alterations in the liver induced by lead chronic toxicity. Saudi J Biol Sci. 2012;19:203-10.
24. Hegazy AMS, Fouad UA. Evaluation of Lead Hepatotoxicity; Histological, Histochemical and Ultrastructural Study. Forensic Med Anat Res. 2014;2:70-79.
25. Mudipalli A. Lead hepatotoxicity & potential health effects. Indian J Med Res. 2007;126:518-27.
26. Kojima M, Masui T, Nemoto K, et al. Lead nitrate-induced development of hypercholesterolemia in rats: sterol-independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis. Toxicol Lett. 2004;154:35-44.
27. Wahlang B, Beier JI, Clair HB, Bellis-Jones HJ, Falkner KC, McClain KJ, Cave MC. Toxicant-associated Steatohepatitis. Toxicol Pathol. 2013;41:343-60.

28. Kristal-Boneh E, Coller D, Froom P, Harari G, Ribak J. The association between occupational lead exposure and serum cholesterol and lipoprotein levels. Am J Public Health. 1999;89:1083-7.
29. Shyam VS, Kumar P, Virendra A, Anoop V, Murthy R. Lipid profiles with increase blood lead level: Risk of cardiovascular disease in battery workers of Lucknow city. J Indian Acad Forensic. 2012;34:971-3.
30. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015;63:237-64.

Figures

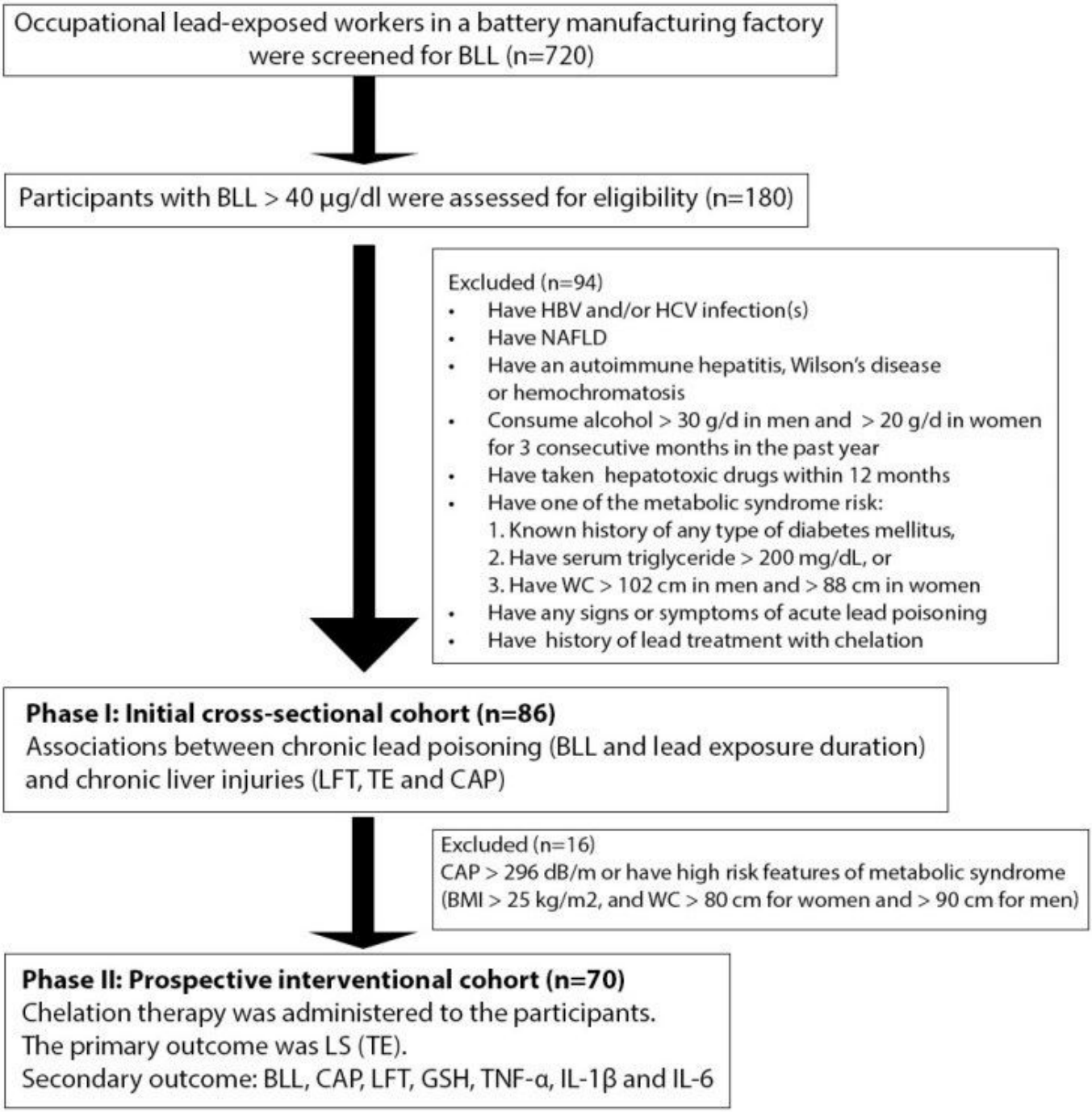

Figure 1

Study flow

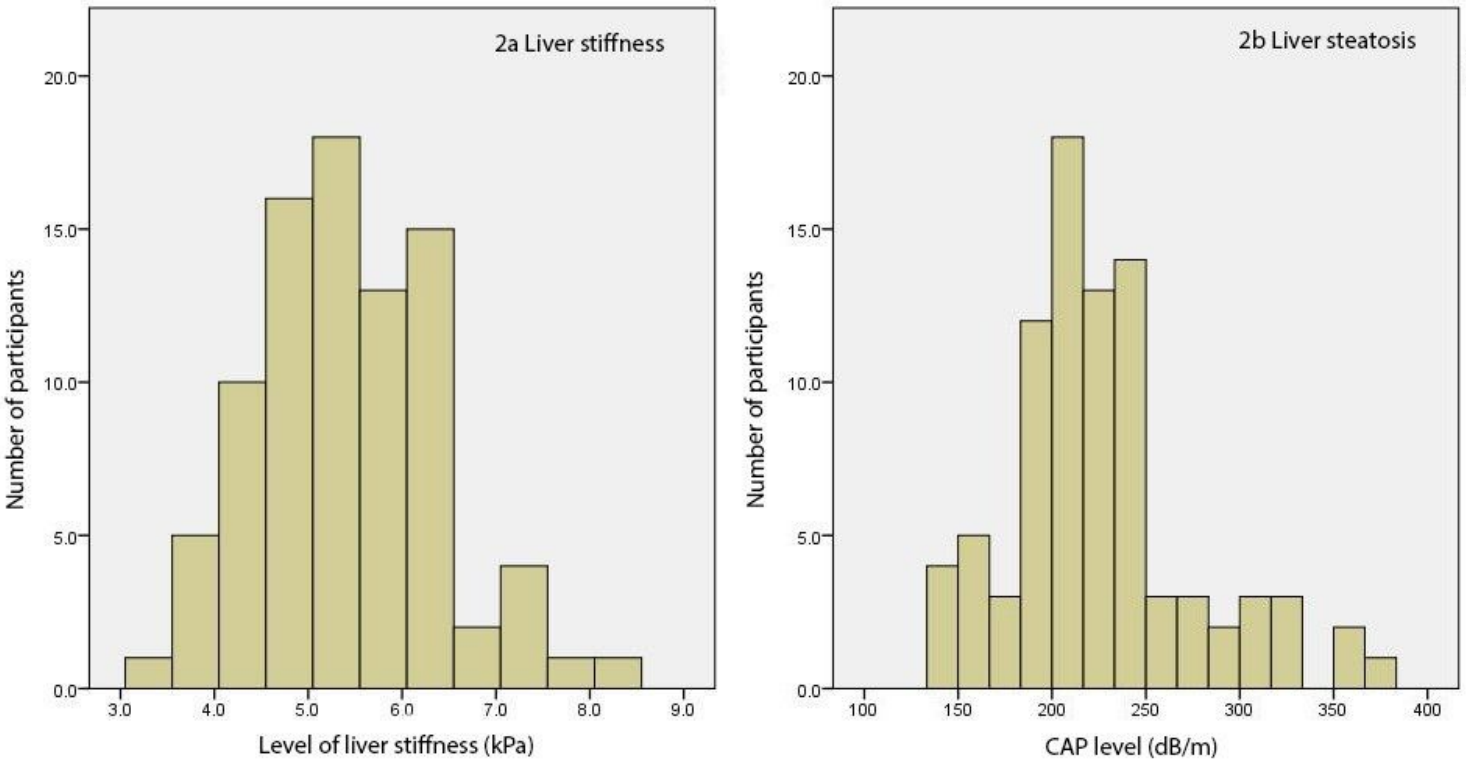

Figure 2

a Proportion of study participants and their level of liver stiffness (LS) b Proportion of study participants and their level of steatosis (CAP)

Supplementary Files

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- SupplementaryTable1.docx