SERUM CYTOKERATIN 18 AND CYTOKINE ELEVATIONS SUGGEST A HIGH PREVALENCE OF OCCUPATIONAL LIVER DISEASE IN HIGHLY EXPOSED ELASTOMER/POLYMER WORKERS

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Abstract

Objective—Cytokeratin 18 (CK18) is a novel serologic biomarker for occupational liver disease. The purpose of this study is to determine the prevalence of CK18 elevation in elastomer/polymer workers exposed to acrylonitrile, 1,3 butadiene, and styrene.

Methods—82 chemical workers were evaluated. CK18 was determined by ELISA and pro-inflammatory cytokines were measured by multi-analyte chemiluminescent detection.

Results—39% (32 of 82) had elevated CK18 levels which were not explained by alcohol or obesity, except in potentially 4 cases. The pattern of CK18 elevation was consistent with toxicant-associated steatohepatitis (TASH) in the majority of cases (78%). TNFα, IL-6, IL-8, MCP-1, and PAI-1 were increased in these workers compared to those with normal CK18 levels.

Conclusions—These results suggest a high prevalence of occupational liver disease and TASH in elastomer/polymer workers with elevated pro-inflammatory cytokines.

Keywords

1,3 butadiene; acrylonitrile; chemical workers; cytokeratin 18; elastomer workers; occupational liver disease; polymer workers; TASH; toxicant-associated steatohepatitis; styrene

Introduction

Occupational exposures to industrial chemicals may result in chronic occupational liver disease, a potentially serious health problem. While initially asymptomatic, chronic occupational liver disease may progress to cirrhosis, liver cancer, and death (1–5). Some industrial chemicals have consistently been associated with occupational liver disease, such as vinyl chloride and its association with hepatic hemangiosarcoma (4, 6, 7). In fact, for some chemicals, the association with occupational liver disease is so strong, that exposure limits and medical surveillance programs are mandated by the Occupational Safety and

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Health Administration (OSHA). For example OSHA's Vinyl Chloride Standard (29 CFR 1910.1017), mandates semi-annual measurements of liver chemistries (bilirubin, alkaline phosphatase, aspartate aminotransferase or AST, alanine aminotransferase or ALT, and gamma-glutamyl transferase) in vinyl chloride or polyvinyl chloride production workers exposed for over 10 years. Unfortunately, these liver tests are frequently normal, even in advanced forms of chronic occupational liver disease (1, 8). Therefore, occupational hepatotoxicity is likely to be under-recognized, even in workers enrolled in medical surveillance programs specifically targeting liver disease.

Toxicant-associated steatohepatitis (TASH) is a recently named form of occupational liver disease (1). Pathologically, TASH is characterized by hepatic steatosis, inflammation, and fibrosis. Although TASH histologically resembles alcoholic steatohepatitis (ASH) and nonalcoholic steatohepatitis (NASH), it occurs in non-drinking, non-obese, subjects with occupational exposures to some industrial chemicals (1, 9, 10). However, like ASH and NASH, TASH may be associated with elevated serum pro-inflammatory cytokines (1, 11). However, unlike ASH and NASH, TASH is frequently characterized by normal liver enzymes making its detection problematic (1, 8).

Cytokeratin 18 (CK18) is a cytoskeletal protein found in hepatocytes and other epithelial cells. Dying cells release CK18 into the extracellular compartment where they can be measured in serum. Both the whole CK18 protein (CK18 M65) and the caspase 3-cleaved fragment (CK18 M30) may be measured. While CK18 M65 measures total cell death, CK18 M30 specifically measures caspase-3 dependent (apoptotic) cell death. While NASH and ASH are characterized by hepatocellular apoptosis, non-apoptotic death (presumably oncotic necrosis) occurs in many forms of chemical hepatotoxicity (1, 12, 13). Indeed, elevated CK18 M30 is a clinically useful biomarker for NASH, while CK18 M65 is an emerging biomarker for TASH (particularly when it occurs in the setting of normal ALT, AST, and CK18 M30) (1, 14).

Elastomer/polymer workers are exposed to a multitude of potentially toxic chemicals including acrylonitrile, 1,3 butadiene, and styrene (ABS). While acrylonitrile is metabolized into cyanide, a potent inhibitor of cytochrome c oxidase; 1,3 butadiene and styrene are metabolized into reactive intermediates including crotonaldehyde and styrene 7,8-oxide (15–17). Regardless of its etiology, steatohepatits is invariably associated with mitochondrial dysfunction, oxidative stress and elevated pro-inflammatory cytokines (1, 11, 18, 19). On the basis of these observations, we hypothesize that ABS-exposed elastomer/polymer workers may be at risk for TASH owing to the toxicity of the metabolites of the parent compounds. The purpose of this study is to i) determine the prevalence and pattern of serum CK18 abnormalities in ABS-exposed elastomer/polymer workers, and ii) determine if workers with abnormal CK18 levels also have elevated levels of pro-inflammatory cytokines previously implicated in occupational liver disease (1).

Materials and Methods

Subjects

Clinical data and biological specimens (serum archived at −80°C) were obtained through a bio-repository which was instituted as described previously in response to a cluster of hepatic hemangiosarcomas occurring at a chemical manufacturing complex (4, 20). For the purposes of this study, 82 male ABS-exposed chemical workers were selected from 2 facilities. The analyzed serum samples were collected between the mid-1970's and late 1990's. All research subjects gave informed consent and institutional IRB approval was obtained prior to the initiation of this project in accordance with the 1975 Declaration of Helsinki.

Chemical Exposure Indices

Chemical exposures were characterized based on employment records. Although workers were exposed to multiple industrial chemicals, exposures were higher for acrylonitrile, 1,3 butadiene and styrene. An index of cumulative exposure, the cumulative exposure rank month (CERM), was determined for each employee for each of these chemicals as described previously (21). Briefly, all jobs in the plant were assigned a rank from 0–6 based on level of exposure. Workers who were absent from the plant for a month were assigned a score of zero for that month, while workers with direct skin contact or high inhalational exposures were assigned a score of six. Ranks were summed yearly (maximum yearly score 72), and the CERMS were calculated through the date of specimen collection.

Serum Analysis

Routine serum liver enzymes, glucose, and lipids had previously been measured by an automated clinical chemistry analyzer and these data were obtained from the occupational toxicology database for analysis. Serum cytokeratin 18 whole protein (M65®) and caspase-cleaved fragments (M30 Apoptosense®) were measured by ELISA (diaPharma, Columbus, OH) as previously described (1). Abnormal CK18 was defined as either an M65 ≥ 300 U/L or an M30 ≥ 200 U/L based on prior studies (1, 14, 22). The prevalence of CK18 elevation in this cohort was determined based on these parameters. The CK18 M30:M65 ratio was determined by dividing serum CK18 M30 by M65. Serum cytokines were determined by multi-analyte chemiluminescent detection using Milliplex kits (Millipore, Billerica, MA) on the Luminex® (Austin, TX) platform as previously described (1, 18). Specifically, interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), and tumor necrosis factor α (TNFα) were measured. Results are reported as mean ± standard deviation.

Statistical Analysis

Subjects were stratified into three subgroups based on CK18 levels: normal CK18 M30 and M65 (healthy workers without liver injury, group 1), elevated CK18 M65 with normal CK18 M30 (suspected TASH, group 2), and elevated CK18 M30 also with elevated CK18 M65 (other suspected liver disease, group 3). Demographic, chemical exposure, and laboratory variables were then compared between these three subgroups. Intergroup comparisons were performed by one-way ANOVA with Tukey's post-test using GraphPad Prism version 5.00 for Windows (San Diego, California). In some cases of non-parametric data, the Kruskal-Wallis test with Dunn's post-test was utilized to determine statistical significance. Likewise, linear regression analysis was performed between serum CK18 M65 and serum aminotransferases and pro-inflammatory cytokines with all subjects grouped together also using GraphPad Prism version 5.00. A p value less than 0.05 was considered statistically significant.

Results

Demographic and Cumulative Exposure Data

Demographic and exposure data for the entire cohort of 82 subjects are given in Table 1. All workers were males. The mean age (±s.d.) was 47.43±7.46 years old. The mean BMI was 25.08±3.31 kg/m². Only two workers were obese (BMI 31 and 33). The mean alcohol consumption was 2±5.34 drinks per week. 27 of the 82 workers reported drinking ethanol. Of these, only 3 workers consumed, on average, greater than 2 alcohol containing beverages per day. These 3 workers each reported drinking 24 such beverages weekly. Concerning the workers' chemical exposure history, the mean duration of employment was 21.57±9.17

years. The mean cumulative exposures (CERMs) for acrylonitrile, butadiene, and styrene were 1123.58±425.26, 1055.39±449.26, and 944.00±352.68 respectively.

Liver Tests

39% (32 of 82) of workers had abnormally high serum CK18 levels. Workers were stratified into groups 1–3 based on CK18 levels (Table 2). The mean serum CK18 M65 and M30 levels for group 1 (normal CK18 M65 and M30, healthy chemical workers), group 2 (elevated M65 with normal M30, suspected TASH), and group 3 (elevated CK M65 and M30, other suspected liver disease) were respectively 107.57±63.36 and 32.80±38.63 U/L; 444.43 ±87.98 and 103.19±38.51 U/L; and 541.88±175.32 and 317.3±93.31 U/L. The mean CK18 M65 levels were significantly higher for both groups 2 and 3 *vs.* group 1 (p<0.001); and for group 3 *vs.* group 2 (p<0.05). The mean CK18 M30 levels were significantly higher for both groups 2 and 3 *vs.* group 1; and for group 3 *vs.* group 2 (p<0.001). The CK18 M30:M65 ratio was significantly lower in group 1 (33.43±37.34%, p<0.01) and group 2 (23.06±7.64%, p<0.05) *vs.* group 3 (61.90±21.82%), but not *vs.* each other.

Mean routine liver chemistries (AST, ALT, total bilirubin, albumin, and alkaline phosphatase) were in the normal range for all groups (Table 2). In fact, only 3 of 82 total subjects (1 subject from each group) had any lab abnormality on routine liver tests. One subject from group 1 had mildly elevated ALT (94 U/L, reference range 0–55) and AST (53 U/L, reference range 0–50). A single subject from group 2 and a mild alkaline phosphatase elevation to 157 U/L (reference range 25–140); while one subject from group 3 had an isolated ALT elevation to 64 U/L. Although mean ALT activity was significantly higher in group 2 (23.67±11.98 U/L) compared to group 1 (14.82±14.1 U/L) (p<0.05), all workers in group 2 had ALT levels within the normal reference range. 30% of workers (25 of 82 – group 2) had a serum biochemical signature consistent with TASH (elevated CK18 M65 with normal CK18 M30, AST, and ALT). Using pooled data from all subjects, linear regression analysis found a significant positive correlation between CK18 M65 and ALT (r^2=0.1732, p=0.0001) but not AST (Table 3).

Concerning the 3 workers who drank greater than 2 alcoholic beverages daily, 2 of these were in group 2, while the other was in group 1. AST and ALT activities were normal in all three cases with ALT ranging from 10–12 U/L and AST ranging from 13–17 U/L. Concerning the two workers who were obese, one worker was in group 1 while the other was in group 3. Both of these workers had normal liver enzymes with ALT activities ranging from 21–29 U/L and AST activities ranging from 20–24 U/L.

Demographic and exposure data were then determined for groups 1–3. There were no significant differences for either age, BMI, or duration of employment between groups (Table 1). Unexpectedly, acrylonitrile cumulative exposures (CERM) were significantly higher in group 1 (1316.11±252.46) than in either group 2 (805.86±476.58) (p<0.001) or 3 (591.96±282.28) (p<0.001). Cumulative butadiene exposures were also significantly higher in group 1 (1240.69±327.97) than in either group 2 (740.19±473.25) (p<0.001) or 3 (597.98±284.53) (p<0.01). Likewise, cumulative styrene exposures were also significantly higher in group 1 (1097.73±214.42) than in either group 2 (681.20±406.18) (p<0.001) or 3 (571.98±252.68) (p<0.01). Cumulative acrylonitrile, butadiene, or styrene exposures were not significantly different between groups 2 and 3. Thus the incidence of suspected TASH or other liver disease did not appear to increase with ABS exposures.

Blood Glucose and Lipids

Overall, 20% of subjects had impaired fasting glucose as defined by the World Health Organization as fasting glucose values between 110 and 126 mg/dL, and one had diabetes.

In group 1, 26% (13/50) subjects had elevated fasting blood glucose, but only one had a blood sugar in the diabetic range (>126 mg/dL). The remaining 12 subjects had impaired fasting glucose. 12% (3/25) of subjects in group 2 and 14% (1/7) subjects in group 3 had impaired fasting glucose and none had diabetes. The mean glucose values were 108.70±33.04, 99.28±10.33, 101.20±6.76 mg/dL for groups 1–3 respectively and were not different between groups (Table 2).

Overall, 20% (16/82) of subjects had hypertriglyceridemia defined by the American Heart Association as values > 199 mg/dL. 18% (9/50), 20% (5/25), and 28% (2/7) of subjects in groups 1–3 respectively had hypertriglyceridemia. The mean triglycerides were 167.73±153.99, 163.29±67.25, 167.17±130.36 mg/dL for groups 1–3 respectively and were not different between groups (Table 2).

Pro-inflammatory Cytokines

Mean pro-inflammatory cytokines were higher in workers with suspected liver disease than those with normal CK18 (Table 4). Mean TNFα was higher in both groups 2 (9.76±5.02 pg/ml, p<0.001) and 3 (5.72±4.78 pg/ml, p<0.05) *vs.* group 1 (0.73±2.35 pg/ml). Group 2 had a higher mean IL-6 (7.45±4.92 pg/ml) than both group 1 (1.62±2.85 pg/ml, p<0.001) and group 3 (4.98±4.01 pg/ml, p<0.05). IL-8 was higher in group 2 (5.67±5.78 pg/ml) than in group 1 (1.79±2.92 pg/ml, p<0.01). However, IL-8 was no different in group 3 (3.01±2.64 pg/ml) than in either groups 1 or 2. Likewise, serum MCP-1 was higher in group 2 (283.14±122.63 pg/ml) than in group 1 (179.48±151.21 pg/ml, p<0.05); but it was no different for group 3 (243.76±101.71 pg/ml) *vs.* either groups 1 or 2. Similarly, mean PAI-1 was higher in group 2 (42.57±50.94 ng/ml) than in group 1 (15.40±15.65 ng/ml, p<0.05). PAI-1 was not different in group 3 (17.09±7.11 ng/ml) *vs.* either groups 1 or 2. Using pooled data from all subjects, linear regression analyses found positive correlations between CK18 M65 and TNFα (r^2=0.5596, p< 0.0001); IL-6 (r^2=0.4219, p< 0.0001); IL-8 (r^2=0.1307, p=0.0009); MCP-1 (r^2=0.1625, p=0.0002); but not PAI-1 (Table 3).

Discussion

The main finding in this study is the unexpectedly high prevalence of CK18 elevation (39%) in this cohort of ABS-exposed chemical workers with normal liver enzymes. The observed CK18 elevations were not explained by obesity or ethanol, except possibly in 4 cases. However, in each of these cases, AST and ALT activities where well within the normal range, arguing against NASH which is typically associated with ALT elevation and ASH which is typically associated with AST elevation. This suggests that effected workers had occupational liver disease. In the majority of cases (25 of 32 or 78%), the pattern of liver test abnormalities (increased CK18 M65, normal M30, normal AST/ALT) was consistent with toxicant-associated steatohepatitis. Indeed, we recently reported on a cohort of highly-exposed vinyl chloride workers with biopsy-proven steatohepatitis featuring this same serologic pattern of liver test abnormalities (1). The ABS workers with suspected TASH (group 2) also had elevated pro-inflammatory cytokines including TNFα, IL-6, IL-8, MCP-1, and PAI-1. Importantly pro-inflammatory cytokine elevation was previously reported in TASH and may be an important disease mechanism (1). We believe that these findings support our hypothesis that subjects in group 2 have TASH attributable to ABS exposures. While we believe that subjects in group 3 also have liver disease, we have not previously encountered this pattern of liver test abnormalities (increased CK18 M30 with normal AST/ALT) before in chemical workers. Therefore, we cannot make any supposition regarding the histopathologic lesion in these workers. However, we believe that these workers are truly different from those in group 2 because there were also differences in cytokine levels between these two groups. The positive correlation between CK18 M65 and ALT supports the hypothesis that the CK18 elevations observed in these chemical workers

were in fact due to liver injury. The lack of correlation between CK18 M65 and AST in this cohort was not surprising because these subjects did not drink significant amounts of alcohol. Furthermore, the pattern of serum transaminases (ALT>AST) observed in this study was similar to what we previously noted in a different cohort of chemical wokers (1).

Importantly, the 30% prevalence of suspected TASH in elastomer/polymer workers is consistent with that noted for other etiologies of steatohepatitis. Only 20–35% of alcoholics develop alcoholic steatohepatitis while only 25–55% of obese subjects develop nonalcoholic steatohepatitis (19, 23). Therefore, it is hardly surprising that not all exposed chemical workers developed suspected TASH in the present study. This moderate prevalence (30%) of suspected TASH in ABS workers is in contrast to the extremely high prevalence (80%) of biopsy-proven TASH in our previous study of highly-exposed vinyl chloride workers. These findings are not unexpected because acrylonitrile, 1,3 butadiene, and styrene are certainly recognized to have less hepatotoxic potential than vinyl chloride. Although there is little experimental data to support the hepatotoxicity of 1,3 butadiene, the hepatotoxic potential of styrene (centrilobular hepatic necrosis) and, to a lesser degree, acrylonitrile have been documented (24–26). To our knowledge, experimental studies on ABS mixtures have not been performed.

Although not specifically addressed in this study, we believe that the CK18 M30:M65 ratio, may be able to differentiate chemical liver injury (TASH) from obesity-related liver injury (NASH), as TASH seems to be characterized by a lower ratio reflective of caspase-3 independent (presumably necrotic) cell death. In this study, the CK18 M30:M65 ratio in ABS workers suspected of having TASH was 33%. This relatively low ratio was consistent with that observed in our cohort of biopsy-proven vinyl chloride-related TASH (26%), and is much lower than the ratios historically reported in adult (55%) and pediatric (59%) obesity-related NASH studies (1, 22, 27), as well as alcoholic hepatitis (28). Interestingly, although the absolute levels of both CK18 M30 and M65 were normal in group 1, the CK18 M30:M65 ratio was lower than healthy controls from our previous study who did not work in the chemical industry and thus did not have high-level industrial chemical exposures (33% *vs.* 76%) (1). These findings suggest that chemical exposures could affect the mode of hepatocyte death even when the level of hepatocyte death is in the normal range. Although much work needs to be done, we believe that CK18 ratios could potentially differentiate TASH from NASH, and this may be reflective of differences in cell death mechanisms in these pathologically similar forms of steatohepatitis. The precise cellular mechanisms behind these observed differences are, as yet, unknown.

Interestingly, subjects with suspected TASH in this study had significantly lower cumulative ABS exposure than subjects with normal CK18 levels. This suggests that TASH may not necessarily be a dose-dependent exposure phenomenon. We hypothesize that susceptibility factors which were not evaluated in this study could be critically important in TASH. Specifically, genetic and nutritional susceptibility factors could be relevant for TASH just as they are in ASH and NASH (11, 19). Genetic polymorphisms in enzymes implicated in the metabolism of industrial chemicals (cytochrome P450 2E1, or Cyp2E1, and glutathione S-transferases, or GST) influenced chemical toxicity in previous studies (29, 30). Importantly, the 30% prevalence of suspected TASH in our study is similar to the previously reported prevalence of GST polymorphisms in white populations (20–45%) (31). We hypothesize that Cyp2E1 and GST genotype may have influenced the development of suspected TASH in our cohort, and future studies should examine this possibility. Limited data suggest that diets high in antioxidants are protective against NASH, and could likewise be protective against TASH, although this has not been studied (32, 33). However, fatty meals have been shown to increase absorption of inhaled 1,3 butadiene by changing the partition coefficient (solubility) of this lipid-soluble chemical in blood (34). Therefore, differences in diet could

alter systemic exposure to at least some of the hepatotoxicants in this study. Therefore, we believe that genetic and nutritional susceptibility factors may have influenced the development of suspected TASH in this cohort which did not appear to be entirely explained by cumulative exposure.

Workers with suspected TASH had higher mean levels of multiple pro-inflammatory cytokines including IL-6, IL-8, MCP-1, PAI-1, and TNFα, than workers with normal CK18 levels. We have previously reported abnormally high IL-6, IL-8, and TNFα levels in chemical workers with TASH (1). Although PAI-1 and MCP-1 elevations have not to our knowledge been reported in chemical workers with TASH, they certainly have been implicated in other etiologies of steatohepatitis (35, 36). The cytokine elevations observed in this study provide further evidence that TASH is a pro-inflammatory lesion. Furthermore, we believe that these pro-inflammatory cytokines may play a role in the genesis and perhaps progression of TASH. The observed positive correlations between serum levels of CK18 M65 and TNFα, IL-6, IL-8, and MCP-1 support this hypothesis.

A major limitation of our study is that neither imaging nor liver biopsies were performed. However, the finding of CK18 elevation supports future ultrasound and/or liver biopsy studies for the confirmation of liver disease in ABS-exposed elastomer/polymer workers. CK18 is expressed in all cells of epithelial origin, and it is highly expressed in hepatocytes (37, 38). Thus serum CK-18 may be increased by a wide variety of diseases affecting epithelial cells such as malignancies (39) or inflammatory conditions leading to epithelial cell necrosis or apoptosis. In addition to chronic hepatitis, non-malignant diseases associated with serum CK18 elevation include chronic lung diseases and chronic inflammatory kidney diseases (40, 41). Serum CK18 concentration is a function of the amount of epithelial cell death, the concentration of CK18 within these dying cells, the size of effected organ, and the probability that the contents of the dying cell will enter the general circulation. The liver is the largest solid abdominal organ with a dense population of epithelioid derived hepatocytes, which express large quantities of CK18, and the liver is centrally located so that the cellular contents enter the systemic circulation.

Therefore, the liver is a major contributor to the serum CK18 level. Malignancy and chronic lung disease rapidly become symptomatic, while chronic liver and kidney diseases may be asymptomatic for long periods of time. We have over 10 years of clinical follow up data on all subjects, which by in large were healthy workers. During the follow up interval, only 1 subject was diagnosed with cancer, and this diagnosis was made four years after the date of serum sample collection. Thus the possibility that the elevated CK18 levels observed in this study were due to unidentified malignancies is remote. Furthermore, none of the workers with elevated CK-18 levels in group 2 had active pulmonary or renal diseases (by history, physical exam or labs – data not shown), although three workers reported having pneumonia within 3 months of the serum sample collection date. Thus, the absence of malignancy and chronic lung/kidney disease suggests that the observed serum CK18 elevations were due to chronic liver disease. In addition to specificity, a second potential problem with using CK18 as a biomarker for chronic liver disease is that the selected cutoffs have never been rigorously validated in a liver biopsy study of TASH, but rather were based on the previously published NASH literature and our own experience with vinyl chloride-related TASH studies (1, 22, 27). Anecdotally, however, liver-related mortality has not historically been greater than expected in this cohort of ABS workers, and modern-day exposures may be less than those experienced by this cohort. The findings of our study coupled with these historical observations call for future liver biopsy studies to rigorously validate CK18 as a biomarker for TASH and for the precise determination of its sensitivity and specificity at various cutoffs in present-day ABS workers. Of minor concern, viral hepatitis serologies were not determined in this study. Furthermore, a non-exposed healthy control group was

not included for comparison of CK18 and cytokine levels. However, excluding subjects with viral hepatitis is not likely to influence our results because chronic hepatitis B is extremely rare in Caucasians in this region and the prevalence of chronic hepatitis C is expected to be less than or equal to the overall US national average which is only 2.2 to 2.4% in adults (42). Our lab has determined mean CK18 and cytokine levels for a healthy unexposed control group and these results have been previously published (1). Anecdotally, workers in the present study had higher mean CK18, IL-6, IL-8, and TNFα levels than the unexposed healthy controls from that study.

In conclusion, 39% of acrylonitrile, 1,3 butadiene, and styrene-exposed elastomer/polymer workers had elevated serum cytokeratin 18 levels suggestive of liver disease. Pro-inflammatory cytokines previously implicated in toxicant-associated steatohepatitis were elevated in effected workers. Future ultrasound and liver biopsy studies should be performed in chemical workers with biochemical signatures consistent with occupational liver disease and suspected toxicant-associated steatohepatitis.

Acknowledgments

Abbreviations

ABS	acrylonitrile, butadiene, and styrene
ALT	alanine aminotransferase
ASH	alcoholic hepatitis
AST	aspartate aminotransferase
CK18	cytokeratin 18
CK18 M30	cytokeratin 18 caspase-cleaved fragment or M30 antigen
CK18 M65	cytokeratin 18 whole protein or M65 antigen
CERM	cumulative exposure rank month
Cyp2E1	cytochrome P450 2E1
IL-6	interleukin-6
GST	glutathione S-transferase
IL-8	interleukin-8
MCP-1	monocyte chemotactic protein-1
NASH	nonalcoholic steatohepatitis
OSHA	Occupational Safety and Health Administration
PAI-1	plasminogen activator inhibitor-1
TASH	toxicant-associated steatohepatitis
TNFα	tumor necrosis factor α

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Table 1

Demographic Information and Cumulative Exposure Data

	All Workers	Group 1 Healthy Workers	Group 2 Suspected TASH	Group 3 Other Suspected Liver Disease
Number	82	50	25	7
Age (yr)	47.43±7.46	45.50±5.81	49.38±3.87	51.83±12.41
BMI	25.08±3.31	25.09±3.02	25.09±3.87	25.00±3.67
CERM-ACRYL	1123.58±425.26	1316.11±252.46	805.86±476.58[1]	591.96±282.28[1]
CERM-BUT	1055.39±449.26	1240.69±327.97	740.19±473.25[1]	597.98±284.53[2]
CERM-STYR	944.00±352.68	1097.73±214.42	681.20±406.18[1]	571.98±252.68[2]
Duration of Employment (yr)	21.57±9.17	21.01±6.91	21.58±10.39	24.89±15.98

[1] p < 0.001 vs. group 1

[2] p < 0.01 vs. group 1

Note: Workers were sub-grouped by serum CK18 levels as follows: Group 1, normal CK18 M65 and M30; Group 2, Elevated M65 and normal M30; Group 3 Elevated M65 and M30. Serum CK-18 M65 was measured by ELISA and elevated levels were defined as values ≥300 U/L, while serum CK-18 M30 was measured by ELISA and elevated levels were defined as values ≥200 U/L.

Abbreviations: BMI, body mass index; CK18 M30, cytokeratin 18 caspase 3-cleaved fragment; CK18 M65, soluble cytokeratin 18; CERM-ACRYL, cumulative exposure rank month for acrylonitrile; CERM-BUT, cumulative exposure rank month for 1,3-butadiene; CERM-STYR, cumulative exposure rank month for styrene; TASH, toxicant-associated steatohepatitis.

Table 2

Serum Liver Tests, Glucose, and Triglycerides

	All Workers	Group 1 Healthy Workers	Group 2 Suspected TASH	Group 3 Other Suspected Liver Disease
Number	82	50	25	7
ALT (0–55 U/L)	18.36±14.67	14.82±14.1	23.67±11.98[1]	25.43 ± 20.53
AST (0–50 U/L)	19.32±6.87	19.88±7.54	17.63±5.44	21.14 ±5.81
ALK PHOS (25–140 U/L)	73.32±21.05	69.94±17.69	78.13±26.71	84.00 ± 16.67
Bilirubin (0.2–1.2 mg/dL)	0.61±0.27	0.57±0.27	0.64±0.28	0.74 ± 0.26
Albumin (3.5–5.5 mg/dL)	4.26±0.26	4.22±0.26	4.3±0.28	4.4 ±0.16
Glucose (70–110 mg/dL)	105.21±27.05	108.70±33.04	99.28±10.33	101.20±6.76
Triglycerides (<200 mg/dL)	166.39±131.03	167.73±153.99	163.29±67.25	167.17±130.36
CK-18 M65 (<300 U/L)	247.34±196.33	107.57±63.36	444.43±87.98[2]	541.88 ±175.32[2,3]
CK-18 M30 (<200 U/L)	78.55±91.61	32.80±38.62	103.19±38.51[1]	317.30 ±93.31[1,4]
CK-18 M30/M65 ratio (%)	32.70±31.60	33.43±37.34	23.06±7.64	61.90±21.82[3,5]

[1] $p < 0.05$ *vs.* group 1

[2] $p < 0.001$ *vs.* group 1

[3] $p < 0.05$ *vs.* group 2

[4] $p < 0.001$ *vs.* group 2

[5] $p < 0.01$ *vs.* group 1.

Note: Workers were sub-grouped by serum CK18 levels as follows: Group 1, normal CK18 M65 and M30; Group 2, Elevated M65 and normal M30; Group 3 Elevated M65 and M30. The provided normal reference ranges were determined by the hospital lab except for fasting glucose where upper limit of normal was defined by World Health Organization, and cytokeratin 18 which was defined by the investigators as CK18 M65 <300 U/L, CK18 M30 <200 U/L. Serum CK18 M65 and M30 were measured by ELISA.

Abbreviations: ALT, alanine aminotransferase activity; AST, aspartate aminotransferase activity; ALK PHOS, alkaline phosphatase; CK18 M30, cytokeratin 18 caspase 3-cleaved fragment; CK18 M65, soluble cytokeratin 18 whole protein.

Table 3

Linear Regression Analysis Between Serum Cytokeratin 18 M65 and Serum Aminotransferases and Pro-inflammatory Cytokines

	r^2	p-value
AST	0.0012	0.7608
ALT	0.1732	0.0001
TNFα	0.5596	< 0.0001
IL-6	0.4219	< 0.0001
IL-8	0.1307	0.0009
MCP-1	0.1625	0.0002
PAI-1	0.05157	0.0626

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemotactic protein-1; PAI-1, plasminogen activator inhibitor-1; TNFα, tumor necrosis factor α.

Table 4

Serum Pro-inflammatory Cytokines

	All Workers	Group 1 Healthy Workers	Group 2 Suspected TASH	Group 3 Other Suspected Liver Disease
Number	82	50	25	7
TNFα (pg/ml)	3.83±5.41	0.73±2.35	9.76±5.02[1]	5.72±4.78[2]
IL-6 (pg/ml)	3.64±4.50	1.62±2.85	7.45±4.92[1]	4.98±4.01[4]
IL-8 (pg/ml)	3.05±4.29	1.79±2.92	5.67±5.78[2]	3.01±2.64
MCP-1 (pg/ml)	218.13±145.48	179.48±151.21	283.14±122.63[3]	243.76±101.71
PAI-1 (ng/ml)	24.60±33.88	15.40±15.65	42.57±50.94[3]	17.09±7.11

[1] p<0.001 *vs.* group 1

[2] p<0.01 *vs.* group 1

[3] p<0.05 *vs.* group 1

[4] p<0.05 *vs.* group 2

Abbreviations: IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemotactic protein-1; PAI-1, plasminogen activator inhibitor-1; TNFα, tumor necrosis factor α.