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Occupational perchloroethylene exposure in dry-cleaning workers: Oxidative stress and hepatorenal biomarkers

Abbas Jafari[1,2], Soma Karimi[1], Zeinab Esmaeilzadeh[3], Mohammad Hajaghazadeh[1,4,]*

[1] Department of Occupational Health, School of Public Health, Urmia University of Medical Sciences, Urmia, Iran
[2] Cellular and Molecular Research Center, Cellular and Molecular Medicine Institute, Urmia University of Medical Sciences, Urmia, Iran
[3] Department of Nutrition, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[4] Social Determinants of Health Research Center, Clinical Research Institute, Urmia University of Medical Sciences, Urmia, Iran

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CORRESPONDING AUTHOR:
hajaghazadeh.m@umsu.ac.ir
Tel: (+98 44) 32752300
Fax: (+98 44) 32750048

ABSTRACT

Introduction: Perchloroethylene (PCE) is widely used in dry cleaning and has been linked to hepatorenal toxicity. We aimed to assess the relationship between occupational PCE exposure, oxidative stress, and biomarkers of liver and kidney function.

Materials and methods: We conducted a cross-sectional study of 30 male Iranian dry-cleaning workers and 30 frequency-matched controls. Personal full-shift air samples were collected for PCE. Serum biomarkers of oxidative stress ; Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (CAT); and organ function; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), bilirubin, creatinine, urea; were measured.

Results: Exposed workers had a mean Time-Weighted Average (TWA) of 29 ppm, exceeding the 25-ppm Occupational Exposure Limit (OEL). Compared with controls, Malondialdehyde (MDA), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST),

and creatinine were higher ($p<0.05$), while SOD and CAT were lower ($p<0.05$); ALP and bilirubin did not differ. Within the exposed group, longer employment was associated with worsening oxidative and hepatorenal markers. Multivariate regression analysis confirmed that PCE exposure remained a significant independent predictor of oxidative stress (MDA, SOD, CAT) even after adjusting for age and smoking.

Conclusion: Findings indicate oxidative stress accompanies subclinical liver injury and early renal impact at prevailing occupational exposures. Reducing PCE through engineering controls and safer technologies should be prioritized.

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Introduction

Perchloroethylene(PCE),ortetrachloroethylene, is a type of chlorinated solvent that has been employed for years worldwide, most notably in commercial dry-cleaning centers [1]. It is preferred in most dry-cleaning facilities because it works well as a degreasing agent and is nonflammable [2, 3]. As a result, a considerable number of employees are occupationally exposed to PCE, mainly because of inhaling solvent vapor exposure during machine operation and through handling processed clothing [4, 5].

PCE poses significant public health concerns because of its well-known toxicity [6]. PCE has been classified as a "probable human carcinogen" in Group 2A by the International Agency for Research on Cancer (IARC). Studies have shown that individuals who work dry are more likely to develop cancers such as bladder, kidney, and esophageal cancer and non-Hodgkin's lymphoma [6-9]. The carcinogenic effects of PCE are long known: chronic exposure to PCE is also known to cause noncancer health effects, especially to the central nervous system, liver, and kidneys, which are primary target organs [6, 10, 11]. There are links between occupational exposure to PCE and deficits in neurobehavioral functions. There are case reports that have shown the possibility of acute PCE exposure resulting in severe, fulminant liver failure [10, 12]. PCE has also been shown to cause genotoxicity through increases in DNA damage and chromosomal aberrations in exposed workers [13, 14].

Understanding PCE toxicity is essential for preventing its toxicity. Elevated oxidative stress may contribute to PCE-induced organ and cellular damage [15, 16]. As a result of oxidative stress, Reactive Oxygen Species (ROS) are produced in an imbalance to the body's ability to neutralize them through its antioxidant defense systems [16, 17]. As a result of PCE metabolism in the liver, reactive intermediates are produced that cause lipid peroxidation. Byproducts of this process, such as Malondialdehyde (MDA), damage cell membranes. [18, 19]. Owing to oxidative damage, antioxidants such as Superoxide Dismutase (SOD) and Catalase (CAT), as well as nonenzymatic molecules such as glutathione, may be depleted in the cell. Cellular injury is further aggravated by this depletion [2, 19, 20].

Although there is a link between PCE and organ damage, it is still necessary to evaluate exposure, oxidative stress markers, and markers of organ damage simultaneously. To resolve inconsistencies in prior research concerning antioxidant responses and strengthen mechanistic links, in vivo studies in humans are needed. Research has produced conflicting results concerning the antioxidant response [2] or has focused on different outcomes. In some studies, genotoxicity has been the focus [13, 21], whereas others have tried to validate various biomarkers of exposure, such as PCE levels in the blood or urine, rather than examining subsequent biomarkers of effects on the body [5, 22, 23]. Special attention must be given to this gap in small-scale dry-cleaning facilities, which often lack the resources to implement advanced engineering controls and continuous monitoring systems. Therefore, employees may be exposed to higher and more complex levels of VolatileO Compounds (VOCs) [24].

The aim of this study was to determine whether chronic exposure to PCE at work affects biomarkers of oxidative stress and organ dysfunction among Iranian dry-cleaners. As a result, we (a) determined the level of oxidative stress by measuring serum malondialdehyde (MDA) levels, Superoxide Dismutase (SOD) levels and Catalase (CAT) levels; (b) assessed subclinical liver and kidney injury by measuring the serum levels of Alanine aminotransferase

(ALT), Aspartate aminotransferase (AST), and creatinine; and (c) evaluated the relationships among these biological markers. Based on our a priori hypothesis, exposed workers would have higher MDA, ALT, and creatinine and lower SOD and CAT activities than matched controls. In addition, we expected that these biochemical changes would be positively related to the duration of occupational exposure, thus supporting the concept of cumulative damage.

Materials and methods

Study design and participants

This cross-sectional study was performed in the Alborz Province of Iran from September to December 2023. The study population consisted of an exposed group of 30 male employees from five dry-cleaning shops as well as a nonexposed control group of 30 males. The sample size was determined using the standard Eqation for comparing two independent means:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (SD_1{}^2 + SD_2{}^2)}{(\mu_1 - \mu_2)^2} \quad (1)$$

We utilized effect sizes from a comparable study by researchers to ensure sufficient power for both hepatorenal and oxidative biomarkers [20]. We based our calculation on the more conservative effect size observed for renal function (Creatinine: μ1±SD = 1.1 ± 0.2 mg/dL for dry cleaning workers vs μ2±SD = 0.92 ± 0.04 mg/dL for controls). Using standard parameters for 95% confidence Z(1-α/2)= 1.96) and 80% power (Z (1-β)= 0.84), the minimum sample size required to detect early renal dysfunction was calculated to be approximately 11 subjects per group. Therefore, our final sample of 30 subjects per group provides robust statistical power (>95%) to detect alterations across the full panel of measured biomarkers. Notably, this sample size aligns with that of the reference study by researchers which also utilized 30 participants per group to demonstrate significant PCE-induced toxicity [20].

The exposed participants were selected from three distinct job roles, with specific task definitions applied to ensure exposure homogeneity. Machine operators were exclusively responsible for the operation of dry-cleaning machines, including loading and unloading clothing, transferring solvent, and maintaining the machine, a role involving the highest proximity to the solvent source. Pressers were responsible for steam pressing and ironing cleaned garments in the finishing area, with exposure primarily derived from residual solvent off-gassing, while clerks were stationed at the front counter, responsible for reception, tagging, and bagging, with exposure limited to background ambient air and handling finished garments. Crucially, to prevent exposure misclassification, facilities were selected where these roles were distinct; during the full-shift sampling period, no task rotation was permitted, and workers remained in their assigned roles and locations throughout the shift. A primary inclusion criterion of a minimum of one year of work experience was required. For both groups, individuals with a self-reported history of liver disease, kidney disease, or regular alcohol consumption were excluded. The control group was frequency-matched to the exposed group to ensure comparable age distributions. To strictly control for confounding, all controls underwent a screening interview to exclude those with any history of occupational exposure to organic solvents. To rigorously control for any residual influence of age, multiple linear regression models were subsequently employed (see Statistical analysis) [4].

Occupational exposure assessment

To quantify worker exposure to PCE, personal breathing zone air samples (n=30) were collected and analyzed according to NIOSH Method 1003 for halogenated hydrocarbons [25]. Each sample was collected via a personal sampling pump (SKC pocket pump 210-1002, SKC Inc., USA) connected via flexible tubing to an activated carbon sorbent tube (Anasorb® CSC, 6×70 mm – 100/50 mg, SKC) clipped to the worker's collar.

Prior to sampling, each personal pump was calibrated to a flow rate of 0.1 L/min using a primary standard (soap bubble flowmeter). Post-sampling calibration was performed to verify flow stability; samples exhibiting a flow rate drift exceeding ±5% were excluded from the analysis. Sampling was conducted under typical environmental conditions (temperature: 25 ± 3°C; relative humidity: 40–60%). To minimize the Hawthorne effect, workers were monitored unobtrusively. Given the fixed-cycle, machine-paced nature of dry-cleaning tasks, significant behavioral modification during the sampling period was considered unlikely [26].

In the laboratory, the front and back sections of each activated carbon tube were transferred to separate vials and desorbed with 1 mL of carbon disulfide. The resulting solutions were analyzed for PCE concentration via a DANI model GC 1000 instrument equipped with a split/splitless injector, a Flame Ionization Detector (FID), and a capillary column (30 m × 0.32 mm ID × 0.25 µm df). A separate analysis of the back section was used to check for sample breakthrough, which was confirmed to be negligible if the PCE mass on the back section was less than 10% of the mass on the front section.

Biological sampling and processing

Following the completion of air sampling, venous blood samples (5 mL) were collected from each participant (n=30 per group) by a certified medical technician into gel-clot vacuum tubes. To obtain serum, the samples were allowed to clot at room temperature before being centrifuged at 2000 rpm for 15 min. Crucially, centrifugation was performed within 1 hour of sample collection to verify sample integrity. The resulting serum was immediately separated and divided into two distinct aliquots before being stored at -80°C. All biochemical analyses were completed within 1 week of sampling. To prevent the degradation of sensitive oxidative stress markers caused by repeated freeze-thaw cycles, the first aliquot was utilized specifically for oxidative stress assays (MDA, SOD, CAT), while the second aliquot was used for hepatorenal biomarkers [19].

Biochemical analyses

All biochemical parameters were measured via commercially available colorimetric assay kits and a spectrophotometer following the manufacturer's protocols.

Liver and kidney function: Serum biomarkers were analyzed to assess liver and kidney function. Liver function was evaluated by measuring the enzymatic activities of alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), and alkaline phosphatase (ALP; EC 3.1.3.1) via methods recommended by the International Federation of Clinical Chemistry (IFCC). For kidney function, serum creatinine was quantified via the Jaffe method, and urea was determined via a Urease-GLDH kinetic assay [11]. All parameters were measured via commercial kits (Pars Piunvand, Tehran, Iran).

Oxidative stress markers

Lipid peroxidation: Malondialdehyde (MDA)

levels were quantified via a Thiobarbituric Acid Reactive Substances (TBARS) assay kit (Zell bio, Germany), which measures the colorimetric product of the MDA-TBA reaction at 532 nm.

Enzymatic and nonenzymatic Antioxidants: Superoxide Dismutase (SOD) and Catalase (CAT) activities were determined via commercial assay kits (Zell bio, Germany) according to the manufacturer's protocol. Reduced Glutathione (GSH) levels were measured via Ellman's reagent (DTNB), which reacts with thiol groups to produce a yellow-colored compound, as measured at 412 nm.

Statistical analysis

The data were analyzed via SPSS (version 26). The distribution of all continuous variables was first assessed for normality via the Kolmogorov–Smirnov test. Descriptive statistics are presented as the mean ± Standard Deviation (SD) for normally distributed variables and as the median (Interquartile Range [IQR]) for non-normally distributed variables.

Comparisons between the exposed and control groups were conducted as follows: an independent samples t-test was used for age (to verify matching efficacy), and the chi-square (χ^2) test was used for categorical variables (smoking, marital status). For biomarker comparisons, which were not normally distributed, the nonparametric Mann–Whitney U test was employed. To evaluate dose–response relationships, the association between work experience and biomarkers was analyzed using the Kruskal–Wallis test.

To assess the independent effect of occupational exposure on oxidative stress and organ function while controlling for potential confounders, multiple linear regression models were constructed. In these models, biomarkers served as dependent variables, with study group (exposure status), age, and smoking entered as predictors. To assess monotonic relationships between biomarkers, Spearman's rank correlation coefficient (rho) was calculated. A p-value < 0.05 was considered statistically significant

Results and discussion

Participant characteristics

The study included a total of 60 male participants, comprising 30 dry-cleaning workers in the exposed group and 30 individuals in a frequency-matched control group. The exposed workers were distributed across three job roles: machine operator, presser, and clerk. The mean ages of the exposed and control groups were 39.73 ± 9.3 and 35.73 ± 8.97 years, respectively. An independent samples t-test indicated no statistically significant difference in age between the two groups ($t(58) = 1.69$, $p = 0.096$), confirming that the groups were adequately matched. The mean work experience of the exposed workers was 9.16 ± 6.99 years, ranging from 3 to 28 years.

Among the exposed workers, 36.7% (n=11) were smokers and 83.3% (n=25) were married, whereas 26.66% (n=8) were smokers and 76.66% (n=23) were married in the control group. Chi-square tests revealed no statistically significant differences between the exposed and control groups for smoking status ($\chi^2(1) = 0.69$, $p = 0.40$) or marital status ($\chi^2(1) = 0.42$, $p = 0.51$). The detailed demographic data, including educational levels, are presented in Table 1.

Table 1. Demographic and lifestyle characteristics of the study participants

Characteristic	Category	Exposure Group (N=30)	Control Group (N=30)
Age (Years)	Mean ± SD	39.73 ± 9.3	35.73 ± 8.97
	< 35 Years	9 (30.0%)	16 (53.3%)
	35-45 Years	13 (43.3%)	10 (33.3%)
	> 45 Years	8 (26.7%)	4 (13.3%)
Work Experience (Years)	Mean ± SD	9.16 ± 6.99	N/A*
	< 10 Years	6 (20.0%)	N/A*
	10-18 Years	19 (63.3%)	N/A*
	> 18 Years	5 (16.7%)	N/A*
Smoking Status	Smoker	11 (36.7%)	8 (26.7%)
	Nonsmoker	19 (63.3%)	22 (73.3%)
Marital Status	Married	25 (83.3%)	23 (76.7%)
	Single	5 (16.7%)	7 (23.3%)
Education Level	Elementary	6 (20.0%)	N/A*
	Middle School	17 (56.7%)	N/A*
	High School Diploma	3 (10.0%)	N/A*
	Associate's Degree	2 (6.7%)	N/A*
	Bachelor's Degree	2 (6.7%)	N/A*

*Data not applicable to the control group.

Occupational exposure to PCE

The overall mean full-shift TWA exposure for all workers was 29.006±5.52 ppm, a level that consistently exceeds the recommended 8-h Time-Weighted Average (TWA) Occupational Exposure Limit (OEL) of 25 ppm. As detailed in Table 2, a significant variation in PCE exposure was observed, with concentrations highly dependent on specific job tasks and individual facility characteristics.

An initial comparison of Arithmetic Means (AM) revealed an exposure hierarchy: machine operators (31.96 ppm) experienced the highest average exposure, followed by pressers (28.05 ppm) and then clerks (23.70 ppm). While the AM for clerks was just below the OEL of 25 ppm, the geometric mean (GM)—a more suitable indicator for exposure data due to its reflection of log-normal distributions—showed a different pattern. The GM was above the OEL (25 ppm) for all three job roles. Specifically, clerks presented the highest GM (30.813 ppm), followed by machine operators (29.128 ppm) and pressers (26.851 ppm). The divergence between AM and GM for clerks suggests a highly skewed exposure distribution within this job role.

Similar variations in exposure were observed across facilities. Analysis by facility revealed that four of the five businesses had arithmetic mean concentrations above the 25 ppm OEL, with only Facility G (23.40 ppm) remaining below the limit. When considering the geometric means for facilities, three businesses (T, P, S) had concentrations above the OEL, whereas Facilities M (20.02 ppm) and G (23.60 ppm) remained below the 25 ppm OEL.

Table 2. Occupational exposure to PCE by job role and facility

Exposure Group	Category	N	Arithmetic Mean (AM) ± SD (ppm)	Geometric Mean (GM) (ppm)	Geometric Standard Deviation (GSD)	Range (Min–Max) (ppm)
Overall	All Workers	30	29.006 ± 5.52	28.51	1.20	19.20 - 42.20
By Job Role	Machine Operator	12	31.96 ± 5.34	29.12	1.20	23.90 - 42.20
	Presser	12	28.05 ± 4.39	26.85	1.19	19.20 - 36.90
	Clerk	6	23.70 ± 2.86	30.81	1.24	19.20 - 37.20
By Facility	T	6	31.83 ± 6.45	31.32	1.21	25.70 - 42.20
	M	9	28.44 ± 5.50	20.02	1.19	24.10 - 38.40
	P	8	28.98 ± 4.59	28.65	1.17	21.50 - 35.70
	S	4	30.27 ± 5.36	29.90	1.20	23.90 - 35.60
	G	3	23.40 ± 4.97	23.60	1.23	19.20 - 28.90

Biomarker analysis: Oxidative stress and organ function

To evaluate the impact of occupational exposure, key biochemical and oxidative stress parameters were compared between the exposed and control groups. Biomarker data were not normally distributed; therefore, all comparisons between the exposed and control groups were conducted via the nonparametric Mann–Whitney U test.

Markers of oxidative stress

This study revealed significant evidence of increased oxidative stress in the exposed group compared with the control group. This is characterized by both an increase in a key marker of cellular damage and reduced activity of the body's antioxidant defense system. Specifically, the median level of MDA—an indicator of lipid peroxidation—was significantly greater in the exposed workers (0.350 nmol/mg protein; IQR: 0.150) than in the controls (0.285 nmol/mg protein; IQR: 0.080) ($p = 0.001$).

Moreover, the body's primary enzymatic antioxidant defenses were significantly diminished in the exposed group. SOD activity was significantly lower in exposed workers (189.81 U/ml; IQR: 46.40) compared to the control group (262.60 U/ml; IQR: 102.57) ($p = 0.001$). Similarly, CAT activity was significantly lower in the exposed group (14.70 U/ml; IQR: 7.69) versus the controls (29.67 U/ml; IQR: 7.32) ($p = 0.001$). The median levels of nonenzymatic GSH were also lower in the exposed group (3.22 µmol/L) compared to controls (3.39 µmol/L), although this difference did not reach statistical significance ($p = 0.151$). These results are summarized in Table 3.

Table 3. Comparison of oxidative stress markers between the exposed and control groups

Parameter	Unit	Exposed Group (N=30) (Median(IQR))	Control Group (N=30) (Median(IQR))	P-value
Damage Marker				
MDA	nmol/mg protein	0.350 (0.150)	0.285 (0.080)	0.001
Antioxidant Defenses				
GSH	µmol/L	3.22 (0.58)	3.39 (1.37)	0.151
SOD	U/ml	189.81 (46.40)	262.60 (102.57)	0.001
CAT	U/ml	14.70 (7.69)	29.67 (7.32)	0.001

Table 4. Comparison of liver and kidney function markers between the exposed and control groups

Parameter	Unit	Exposed Group (N=30) (Median(IQR))	Control Group (N=30) (Median(IQR))	P-value
Liver Function Markers				
ALT	U/L	23.40 (12.90)	17.91 (9.86)	0.009
AST	U/L	22.50 (6.25)	19.40 (6.27)	0.003
ALP	U/L	185.50 (80.25)	183.70 (45.97)	0.684
Total Bilirubin	mg/dl	0.78 (0.25)	0.83 (0.25)	0.529
Kidney Function Markers				
Creatinine	mg/dl	1.20 (0.21)	1.10 (0.21)	0.004
BUN	mg/dl	15.68 (4.63)	16.52 (3.56)	0.295
Urea	mg/dl	33.55 (9.90)	35.35 (7.63)	0.296

Markers of liver and kidney function

The present study revealed statistically significant alterations in markers of both liver and kidney function in the exposed group. Evidence for hepatocellular effects was observed, with the median activities of ALT and AST being significantly greater in exposed workers than in controls. Specifically, the median ALT was 23.40 U/L in the exposed group versus 17.91 U/L in controls (p=0.009), and the median AST was 22.50 U/L versus 19.40 U/L (p=0.003). While ALP levels were not significantly different between the groups (p=0.684), notably, the median activity for both groups (185.50 U/L and 183.70 U/L) was above the normal clinical range. Total bilirubin levels remained unaffected (p=0.529).

In terms of kidney function, the serum creatinine level was significantly elevated in the exposed group (median: 1.20 mg/dl) compared to the control group (median: 1.10 mg/dl) (p=0.004). In contrast, the levels of urea (p=0.296) and blood urea nitrogen (BUN) (p=0.295) were not significantly different between the two groups. A complete summary of these findings is presented in Table 4.

Impact of employment duration

To investigate the presence of a dose–response relationship, we analyzed the association between the duration of employment (work experience) and biomarker levels within the exposed group. A clear pattern of cumulative toxicity was observed. Greater work experience was significantly associated with worsening markers of liver injury, including increased activity of ALP ($p = 0.002$), ALT ($p = 0.001$), and AST ($p = 0.015$). Similarly, indicators of oxidative stress worsened with increasing years of employment; MDA levels increased significantly ($p = 0.03$), whereas the activities of antioxidant enzymes decreased significantly (SOD: $p = 0.016$; CAT: $p = 0.001$). These findings suggest that PCE-induced organ damage and oxidative depletion accumulate over time.

Multivariate analysis of confounders

To evaluate the independent impact of PCE exposure while controlling for potential confounders, multiple linear regression analyses were performed for MDA, SOD, and CAT. The models included Study Group (Exposed vs. Control), Age, and Smoking as predictors. The full results of these adjusted models are presented in Table 5.

Lipid Peroxidation (MDA): The regression model explained 36.5% of the variance in MDA levels ($R^2 = 0.365$). While age was identified as a significant positive predictor of lipid peroxidation ($p < 0.001$), occupational exposure remained a statistically significant predictor after adjustment ($p = 0.004$). Specifically, the control group exhibited significantly lower MDA levels compared to the exposed group, independent of age.

Antioxidant Enzymes (SOD and CAT): The models for antioxidant enzymes demonstrated strong predictive power ($R^2 = 0.512$ for SOD; $R^2 = 0.698$ for CAT). Although age was a significant negative predictor for both enzymes ($p < 0.001$)—indicating that antioxidant activity naturally declines with age—the effect of occupational exposure remained highly significant. Even after adjusting for age, the control group showed significantly higher enzymatic activity for both SOD ($p < 0.001$) and CAT ($p < 0.001$) compared to the exposed workers.

Impact of Smoking: Smoking status was not a statistically significant predictor in any of the multivariate models ($p > 0.05$). These findings confirm that the observed oxidative stress in dry-cleaning workers is primarily attributable to PCE exposure and is not an artifact of demographic factors such as aging or smoking habits.

Table 5. Multiple linear regression analysis estimating the association between occupational PCE exposure status and oxidative stress biomarkers, adjusted for age and smoking (N=60)

Biomarker	B (Unstandardized)	SE	β (Standardized)	t	p-value
MDA(nmol/ml)					
(Constant)	0.248	0.08	—	3.1	0.003
Study Group (Control)	-0.085	0.028	-0.331	-3.03	0.004*
Age (Years)	0.006	0.002	0.443	4.04	< 0.001*
Current Smoker	-0.009	0.03	-0.031	-0.29	0.774
SOD (U/ml)					
(Constant)	291	35.07	—	8.3	< 0.001
Study Group (Control)	53.33	12.39	0.413	4.3	< 0.001*
Age (Years)	-3.5	0.67	-0.499	-5.19	< 0.001*
Current Smoker	-2.18	13.11	-0.016	-0.17	0.868
CAT (U/ml)					
(Constant)	27.83	4.05	—	6.88	< 0.001
Study Group (Control)	10.36	1.43	0.547	7.24	< 0.001*
Age (Years)	-0.55	0.08	-0.532	-7.03	< 0.001*
Current Smoker	0.95	1.51	0.046	0.63	0.535

*Statistically significant at $p < 0.05$. Model fit (R-square): MDA = 0.365; SOD = 0.512; CAT = 0.698.

Correlation analyses

To explore potential mechanisms of toxicity, a series of nonparametric correlation analyses using Spearman's rank correlation coefficient were performed to assess the relationships between exposure, biomarkers of effect, and other key variables (Table 6).

Correlation with PCE exposure

The direct relationships between individual PCE exposure concentrations and the measured biomarkers were examined. This analysis did not reveal any statistically significant correlations between PCE exposure levels and any of the oxidative stress, liver, or kidney function parameters within the exposed group ($P > 0.05$ for all).

Interparameter correlations and mechanistic links

The relationships between the health markers themselves revealed strong mechanistic links. Specifically, the Markers of oxidative Damage (MDA) were positively correlated with the markers of liver injury (ALT and AST). Conversely, the body's primary antioxidant defenses (SOD, CAT, and GSH) were negatively correlated with these factors. These findings suggest that as antioxidant systems are depleted, liver injury becomes more pronounced. The key correlation coefficients are presented in Table 6.

Table 6. Mechanistic correlations between oxidative stress and liver function markers

Biomarker	ALT	AST
Damage Marker		
MDA	0.458*	0.588*
Antioxidant Defenses		
GSH	−0.550*	−0.543*
SOD	−0.430*	−0.470*
CAT	−0.706*	−0.571*

All the coefficients are Spearman's rank correlation coefficients (rho); *$P < 0.01$.

In this study, we found compelling evidence that chronic occupational exposure to PCE creates a significant biological burden for dry-cleaning workers. This burden is characterized by a combination of effects: oxidative stress, subclinical liver damage, and early signs of kidney impairment. Our results demonstrate not only a significant increase in the lipid peroxidation marker MDA and a corresponding depletion of the antioxidant enzymes SOD and CAT in exposed workers but also a strong mechanistic correlation between this oxidative imbalance and the elevation of the liver enzymes ALT and AST. This relationship is further supported by the dose-dependent nature of these effects, which worsened with increased duration of employment. These findings collectively suggest that oxidative stress is a key pathway through which chronic PCE exposure, at levels found in these occupational settings, contributes to organ pathophysiology.

The mean TWA exposure to PCE in this study was 29.01 ppm, a level that notably exceeds the recommended 8-hour OEL of 25 ppm. This finding indicates a significant ongoing occupational risk for the workers in the investigated facilities. This exposure level is remarkably consistent with findings from another recent study on Iranian dry-cleaning workers, which reported a mean personal exposure of 27.49 ppm [20]. The fact that two independent studies conducted in Iran reported similarly high exposure levels suggests that this may be a systemic issue within the industry in this region, emphasizing the need for targeted interventions.

However, the concentrations observed in our cohort and in the other Iranian study [20] stand in stark contrast to the substantially lower exposures typically reported in recent European studies. For example, a study in Lithuania reported a mean TWA of 31.40 mg/m³ (~4.6 ppm), which, while below their national OEL of 70 mg/m³, still resulted in overexposures for some individual workers [13]. Other studies have reported even lower mean or median values, such as 52.32 mg/m³ (~7.7 ppm) in Italy [23] and 7 ppm in France [4]. A large multicountry study of Nordic countries documented a clear decreasing trend over time, with median exposure levels falling to just 3 ppm by the year 2000 [3]. More recently, another Italian study reported a median PCE concentration of only ~1.6 ppm, attributing the low levels to the implementation of new technologies and improved preventive strategies [5].

The higher concentrations observed in our

study may be attributable to several factors, including the age of the dry-cleaning machines, the effectiveness of engineering controls, and specific work practices. Studies have shown that modern closed-loop machines equipped with controls such as refrigerated condensers and carbon adsorbers can dramatically reduce PCE emissions [27, 28]. The absence or poor maintenance of such technologies in the studied facilities could explain the elevated airborne concentrations. Furthermore, our finding that machine operators had the highest mean exposure (31.96 ppm) is consistent with the exposure hierarchy reported in other studies, which consistently identify tasks involving the opening and closing of the machine door as the primary sources of worker exposure[5, 23, 29]. This is broadly supported by the Lithuanian study, which also revealed that administrative staff had the lowest exposures (mean 6.52 mg/m³), whereas operational staff, such as pressers (35.76 mg/m³), machine operators (38.12 mg/m³), and counter clerks (44.42 mg/m³), experienced similarly elevated exposure levels [13]. The significant exposure levels found in our cohort, in contrast with the general downward trend reported in Europe and the US, emphasize that a substantial health risk persists in dry-cleaning facilities that have not fully adopted modern emission control technologies and safe work practices.

Our observation of increased oxidative stress is a central finding and aligns with a significant body of research. The increase in serum MDA, indicating elevated lipid peroxidation, is consistent with in vitro work by Zhu et al., who demonstrated that PCE directly caused an increase in MDA in human keratinocytes[18], and with animal studies by Wang et al., who reported increased MDA-protein adducts in PCE-treated mice[19]. The increase in MDA levels has also been reported in other studies on dry cleaning workers. For example, increased plasma levels of MDA were reported in young Chinese female dry-cleaning workers exposed to PCE, suggesting increased oxidative stress [24]. Similarly, elevated levels of Lipid Peroxidation (LPO) in B lymphocytes of Iranian dry-cleaning workers have been reported [20]. These findings emphasize that PCE exposure, even at varying concentrations, can induce oxidative damage to cellular components.

Furthermore, the corresponding decrease in the activity of the antioxidant enzymes SOD and CAT in our exposed cohort supports the hypothesis that the body's defense mechanisms are depleted or overwhelmed. This finding is also in agreement with the in vitro results from Zhu et al., which showed an inhibition of SOD activity by PCE [18]. In contrast to the significant decrease in SOD and CAT activities observed in our study, some studies [2, 24] reported increased plasma or blood activities of these enzymes in PCE-exposed dry cleaning workers. These increases are often interpreted as a compensatory response by the body's antioxidant defense system to counteract the increased production of reactive oxygen species induced by PCE exposure. The discrepancy may reflect different stages or severities of the biological response; an initial compensatory increase in antioxidant enzymes may, under conditions of prolonged or higher-level exposure, as observed in our cohort, progress to a state of depletion and system exhaustion. This interpretation is supported by studies on other occupational exposures, where a decrease in SOD activity was observed in workers exposed to carbon disulfide [30], and a marginal decrease in both SOD and CAT activities was reported in highly exposed pesticide workers [31]. Such reductions indicate a compromised ability to neutralize free radicals, increasing the vulnerability of cells to oxidative damage.

The evidence of subclinical hepatotoxicity in our study, demonstrated by significantly higher ALT and AST levels, aligns with the established role of PCE as a potent hepatotoxin and confirms that the liver is a primary target organ for this solvent. Researchers similarly reported that AST levels

were significantly higher in female dry-cleaning workers than in controls, although they did not observe a significant difference in ALT [11]. Evidence for hepatocellular effects was observed, with the median activities of ALT and AST being significantly greater in exposed workers than in controls. While the elevations in our study are subclinical, they are toxicologically significant, as a case report by other researchers documents that acute, high-level PCE exposure can lead to fulminant acute liver failure with massively elevated transaminases [10]. Because ALP median levels were elevated in both groups and we lacked GGT or ALP isoenzymes, we interpret ALP cautiously and do not ascribe cholestasis. Our findings therefore represent an early stage on a continuum of potential liver injury.

We also observed a statistically significant increase in the level of serum creatinine, a marker of renal dysfunction. This finding is particularly noteworthy because previous studies on workers with low-level PCE exposure have not always detected changes in creatinine. Other researchers, for example, reported no difference in creatinine but did find a significant dose-related increase in more sensitive, specific urinary markers of tubular damage, such as glutamine synthesis [11]. The fact that our study detected an elevation in the less sensitive marker creatinine suggests a tangible impact on renal function in our cohort. This finding is further supported by epidemiological evidence from a large mortality study by researchers, which revealed a significant exposure–response relationship between solvent exposure in dry cleaners and death from kidney cancer [7].

One of our key findings was a clear dose–response relationship: the longer a worker was employed, the more severe their liver injury and oxidative stress became. These findings strongly suggest that the damage caused by chronic PCE exposure is cumulative. Furthermore, our multivariate regression analysis confirmed that these effects were independent of potential confounders, distinguishing the impact of occupational exposure from age-related oxidative changes. This aligns with a genotoxicity study, which revealed that the frequency of chromosome aberrations in dry-cleaning workers was significantly associated with the duration of employment [13]. In contrast, a study did not find a correlation between employment duration and DNA damage as measured by the comet assay, suggesting that some biomarkers may reflect recent exposure, whereas others, such as the liver enzymes in our study, reflect the cumulative burden of chronic exposure [14].

The strong correlation we observed between markers of oxidative stress (e.g., high MDA and low SOD/CAT) and markers of liver injury (high ALT/AST) provides compelling support for our central hypothesis that oxidative stress is a primary mechanism driving PCE-induced hepatotoxicity. This is strongly supported by the experimental work of a study, which was demonstrated that lipid peroxidation-derived aldehydes from PCE exposure could trigger an autoimmune response, establishing oxidative stress as a critical immunologic trigger [19]. Interestingly, we did not find a significant correlation between individual workers' single-day air PCE concentrations and their biomarker levels. The absence of same-day exposure-biomarker correlations is plausible given PCE's toxicokinetics and day-to-day variability [22]; a single air measurement may not accurately reflect the long-term internal dose that leads to the observed chronic effects. Accordingly, we view these correlation analyses as exploratory descriptors rather than definitive dose-response tests within a cross-sectional design. Indeed, studies such as that of a study in 2019, have shown that while urinary PCE correlates well with airborne PCE, metabolites such as trichloroacetic acid do not, reflecting interindividual differences in metabolism [5].

The strengths of this study include the use of a frequency-matched control group and a

comprehensive panel of biomarkers for assessing both organ function and a plausible underlying mechanism. However, several limitations must be acknowledged. First, the cross-sectional design prevents us from establishing causality, although the dose–response findings with work duration provide suggestive evidence. Second, while our sample size was sufficient for statistical comparisons, it was relatively small, limiting the generalizability of the findings. Third, personal air sampling was conducted over a single work shift. While we selected representative days with typical workloads, this snapshot may not fully capture day-to-day fluctuations or seasonal variability in PCE concentrations. Finally, although we statistically adjusted for age and smoking, data on lifestyle habits were self-reported, and other potential confounders, such as Body Mass Index (BMI) and dietary patterns, were not quantitatively assessed.

Conclusion

This study provides robust evidence that occupational exposure to Perchloroethylene (PCE) among dry-cleaning workers—at levels frequently exceeding the recommended occupational exposure limit (mean 29.01 ppm)—is associated with significant oxidative stress, subclinical liver injury, and early signs of renal dysfunction. Specifically, exposed workers exhibited elevated lipid peroxidation (MDA) and a concurrent depletion of antioxidant defenses (SOD, CAT). Crucially, multivariate regression analysis confirmed that this oxidative imbalance and organ dysfunction are primarily driven by PCE exposure and are independent of potential confounders such as age and smoking habits. The observed dose–response relationship with employment duration further supports a cumulative mechanism of toxicity. These results emphasize the importance of continued efforts to reduce PCE exposure in this industry through the implementation of engineering controls [27, 28] and the adoption of safer alternatives [32, 33], as recommended by health agencies [6]. Future longitudinal studies are warranted to confirm these findings and to further elucidate the long-term health consequences of chronic PCE exposure.

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Competing interests

The authors report there are no competing interests to declare.

Authors' contributions

MH and AJ designed the study. SK and ZE collected the data. MH and AJ analyzed the data and wrote the initial draft. All of the authors revised and approved the paper.

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Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Urmia University of Medical Sciences (UMSU) (approval number: IR.UMSU.REC.1400.179). All workers provided their written informed consent to participate in the current study.

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