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Primary DNA Damage in Dry Cleaners with Perchloroethylene Exposure

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Abstract

Background: Perchloroethylene is a halogenated solvent widely used in dry cleaning. International agency of research on cancer classified this chemical as a probable human carcinogen.

Objective: To evaluate the extent of primary DNA damage in dry cleaner workers who were exposed to perchloroethylene as compared to non-exposed subjects. The effect of exposure modifying factors such as use of personal protective equipment, perceived risk, and reported safe behaviors on observed DNA damage were also studied.

Methods: 59 exposed and non-exposed workers were selected from Yazd, Iran. All the 33 exposed workers had work history at least 3 months in the dry cleaning shops. Peripheral blood sampling was performed. Microscope examination was performed under fluorescent microscope (400×). Open comet software was used for image analysis. All biological analysis was performed in one laboratory.

Results: Primary DNA damage to leukocytes in dry cleaners was relatively high. The median tail length, %DNA in tail, and tail moment in exposed group were significantly higher than those in non-exposed group. There was no significant difference between smokers and non-smokers in terms of tail length, tail moment, and %DNA in tail. There was no significant correlation between duration of employment in dry cleaning and observed DNA damage in terms of tail length, tail moment and %DNA in tail. Stratified analysis based on exposed and non-exposed category showed no significant relationship between age and observed DNA damage.

Conclusion: Occupationally exposure to perchloroethylene can cause early DNA damage in dry cleaners.

Keywords: DNA damage; Comet assay; Occupational exposure; Clothing

Introduction

Perchloroethylene ($\text{Cl}_2\text{C}=\text{CCl}_2$), also known as PERC, is a halogenated solvent widely used in metal cleaning, dry cleaning, textile industries, and chemical synthesis processes.¹ Moderate cost and lower toxicity in comparison with other cleaning chemicals made it a good

choice in most of dry cleaning shops.² Occupational and environmental inhalational exposure to PERC varies considerably from place to place and in various occupations.¹ International agency of research on cancer (IARC) classified PERC as a probable human carcinogen (B2).³ The DNA-damaging effect of PERC observed in the liver of mice after oral administration of

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PERC, suggesting a genotoxic property of this chemical.⁴ However, another study found no significant difference in chromosome translocation frequencies between the PERC-exposed dry cleaners and the laundry workers.⁵

The comet assay, also known as single-cell gel electrophoresis, is a well-known DNA damage test developed by Singh nearly 30 years ago.⁶ Simplicity and availability of comet assay test make it a good choice for screening in occupational and environmental genotoxicity studies. Applicability of the comet assay in occupational biomonitoring has been reviewed by Valverde and Rojas.⁷ Available literature about DNA damage in dry cleaners exposed to PERC is limited and to the best of our knowledge, there is only another study based on comet assay on dry cleaners exposed to the PERC.⁸ The objective of this study was to evaluate the extent of primary DNA damage in dry cleaners who were exposed to PERC in their work environment. The effect of exposure modifying factors such as use of personal protective equipment, perceived risk, and reported safe behaviors on observed DNA damage were also studied.

Materials and Methods

Study Groups and Questionnaires

The study was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences. Informed verbal and written consents were obtained from all participants. Sample size calculation was conducted for tail length effect size based on what reported in Everatt study,⁸ where the mean of tail length for exposed and non-exposed groups was 10.45 (SD 6.52) and 5.77 (2.31), respectively. The minimum sample size in each group for a power of 80% and a two-sided significance level of 0.05 was 31. Finally, 33 dry cleaners with

at least three months of work history and one week of exposure to dry cleaning process during last month were enrolled into the study as the exposed group. We could also find 26 non-exposed subjects selected from healthy general population with no exposure to known carcinogens during the same period in Yazd city. Subjects with history of therapeutic or diagnostic radiation exposure during the last six months were excluded from the study. The study groups were matched for smoking, age, and sex. Demographic characteristics, exposure history, level of personal protection during working hours and safety behaviors were asked by researcher-made questionnaires.

A 13-item questionnaire was developed to elucidate protective behaviors of dry cleaners in working with PERC (Cronbach's α 0.74). Protective behaviors such as routine medical checkup, use of appropriate protective equipment, use of local ventilation in the dry cleaning shop, use of natural ventilation in working environment, accumulation of cloths in the shop, proper disposal of waste chemicals and storage of dry cleaning solvents were asked. A 5-point Likert scale ('1' completely disagree/false to '5' completely agree/true) was used for scoring the questions. A grand score of behaviors was then calculated based on summation of all questions.

Blood Sampling and Comet Assay

Peripheral blood sample was collected in morning from each participant. The sample was poured into a heparinized tube and transported to laboratory in cold box in less than two hours. Whole blood was diluted (1:1) by phosphate buffer saline (PBS). Ficoll density gradient solution (Baharafshan, Iran) was added and centrifuged for 20 min at 114 g. Lymphocytes were isolated at the end of another 15 min spin down at 114 g, and diluted in 900 μ L of PBS. Viability was checked regularly by Trypan blue and was kept above 90% for

For more information on DNA damage in workers of a copper processing industry see <http://www.theijoem.com/ijoem/index.php/ijoem/article/view/612>



TAKE-HOME MESSAGE

- Perchloroethylene (PERC) is widely used in dry cleaning.
- PERC is classified as a probable human carcinogen.
- Early DNA damage increases in dry cleaners occupationally exposed to PERC compared with a group of non-exposed people.
- In contrast to other studies, there is no significant correlation between DNA damage and each of the level of safety behavior, use of personal protection equipment, and ventilation of dry cleaning shops.

all samples.

The comet assay was performed according to the protocol developed by Singh, *et al*,⁶ with slight modifications.⁸ Briefly, a frosted microscopic slide was cleaned with ethanol and then dipped into 1% normal melting point agarose (NMA). Twenty μL of blood cell suspensions were mixed with 80 μL of 0.7% (w/v) low-melt agarose (LMA) at 37 °C. Thirty μL of LMA/cell suspension mixture was placed on NMA and covered with a cover slip. The slides leaved for 10 min on ice cooled metal surface, then 100 μL of NMA was dropped on the prior layer of LMA and kept at 4 °C for 5 min. Slides were dipped into an alkaline lysis solution (2.5 M NaCl, 100 mM Na₂-EDTA, 10 mM tris-HCl, pH 10; and Triton™ X-100 1%, Dms010% solution added freshly to the solution just before use) for 1 hr. and then washed gently with deionized water. Slides were placed horizontally in an electrophoresis tank (Padideh Nojen Pars, HU150, Iran; connected to a Padideh Nojen Pars, PNP1000D DC power supply) filled with 4 °C electrolysis buffer (0.3 M NaOH, 1 mM Na₂-EDTA; pH >13) and kept for 30 min at 4 °C. Electrophoresis was performed for 30 min at 300 mA and 0.8 V/cm based on electrophoresis tank dimensions.⁹ After completion of electrophore-

sis, slides dipped in neutralization buffer (0.4 M Trisbase, pH 7.5) for 5 min and then washed with deionized water. Slides were stained with EdBr solution for 5 min. All slides were prepared in triplicate. Microscopic analyses were performed under fluorescent microscopes (400 \times , Nikon Eclipse E200, Nikon, Japan). Open comet software was used for image analysis.¹⁰

Statistical Analysis

Fifty cells were counted in each comet slide. Tail length (length of comet tail from right border of the head to the end of tail), the percent of DNA in the comet tail (%DNA in tail), tail moment (the %DNA in tail multiplied by the tail length), and olive tail moment (the %DNA in tail multiplied by [tail center of gravity – head center of gravity]) were measured for each single cells.¹¹ To overcome inter-observer and inter-laboratory variability, all biological analyses on blood samples were performed in a same laboratory by the one trained researcher. Image analysis was performed by a blind observer. For each study participant the mean value and SD of the tail length, %DNA in tail, tail moment, olive tail moment, and the number of comets scored was calculated according to Hartman. There is no consensus on a unified statistical method for the analysis of comet assay data given the complexity of the distribution of the values.¹¹ In this study, two-sided statistical test of mean difference was used for hypothesis testing. Normality of comet assay values was tested with the Shapiro-Wilk's test. For data not normally distributed, difference between the exposed and non-exposed subjects was tested with Mann-Whitney U-test. Correlation analysis was performed to examine the possible correlation between levels of protective behaviors, demographic characteristics of subjects and observed DNA damage. All statistical analyses were performed using the SPSS® for Windows® ver 20 (SPSS Inc,

IL, USA). A two-sided p value <0.05 was considered statistically significant.

Results

From 65 participants approached, 59 agreed to participate. Participants aged between 18 and 62 years. The exposed subjects had a median employment duration of 8 (IQR 1 to 13.5) years. Most of participants were males (94% of exposed, and 92% of non-exposed). There was no significant difference between the two groups in terms of age, sex, and smoking habits (Table 1).

None of the measured comet assay parameters had a normal distribution (p<0.001). All comet assay parameters in the exposed group were significantly higher than those in the non-exposed group. The tail length in the exposed group ranged from 6.63 to 67.2 (median 25.85), significantly (p<0.001) longer than that in the non-exposed group (median 5.61, range 2.65 to 18.53). The %DNA in tail in exposed group ranged from 5.73 to 48.85 (median 23.03) and was significantly (p<0.001) higher than that in the non-

Table 1: Descriptive characteristics of the exposed (dry-cleaner) and non-exposed (general population) groups. Figures are either mean (SD) or n (%).

	Exposed (n=33)	Non-exposed (n=26)
Age	32.9 (10.1)	32.1 (9.1)
<40 yrs	27 (82%)	23 (89%)
≥40 yrs	6 (18%)	3 (12%)
Sex		
Male	31 (94%)	24 (92%)
Female	2 (6%)	2 (8%)
Smoking		
Smoker	12 (36%)	10 (39%)
Non-smoker	21 (64%)	16 (62%)

exposed group (median 8.77, range 3.05 to 21.03). The tail moment in the exposed group (median 7.07, range 0.42 to 44.29) was also significantly (p<0.001) higher than that in the non-exposed group (median 1.03, range 0.14 to 5.12) (Tables 2).

To assess the modifying effect of smoking on the observed results, smokers and non-smokers were analyzed separately.

Table 2: The comet assay parameters stratified by sex and smoking status in the exposed (n=33) and non-exposed (n=26) groups. Figures are mean (SD; range).

Sub-group	Tail length	%DNA in tail	Tail moment	Olive tail moment
Exposed group				
Female	26.03 (0.99; 25.33 to 26.73)	20.7 (4.89; 17.25 to 24.16)	7.41 (2.94; 5.33 to 9.49)	6.25 (1.06;) 5.5 to 7
Male	26.32 (14.03; 6.63 to 67.2)	22.52 (10.78; 5.73 to 48.85)	9.84 (9.23; 0.42 to 44.29)	7.76 (4.93; 1.39 to 23.45)
Non-smoker	26.08 (15.29; 6.63 to 67.2)	22.03 (10.88; 5.73 to 48.85)	10.02 (10.11; 0.42 to 44.29)	7.66 (5.27; 1.39 to 23.45)
Smoker	26.69 (10.58; 8.4 to 41.33)	23.08 (10.17; 10.13 to 38.5)	9.13 (6.93; 2.12 to 22.51)	7.69 (4.01; 2.17 to 14.61)
Non-exposed group				
Female	6.79 (4.32; 2.65 to 18.53)	8.84 (4.5; 3.05 to 21.03)	1.33 (1.29; 0.14 to 5.12)	1.96 (1.15; 0.6 to 5.68)
Male	6.17 (1.88; 4.84 to 7.5)	10.43 (2.15; 8.9 to 11.95)	1.29 (0.25; 1.11 to 1.47)	2.04 (0.02; 2.02 to 2.05)
Non-smoker	6.73 (4.23; 2.65 to 18.53)	8.65 (4.19; 3.05 to 20.75)	1.26 (1.12; 0.14 to 4.94)	1.94 (1.18; 0.6 to 5.68)
Smoker	6.75 (4.28; 3.09 to 16.08)	9.46 (4.8; 4.05 to 21.03)	1.44 (1.46; 0.33 to 5.12)	2 (1.04; 0.88 to 4.24)

Table 3: Spearman ρ between age, employment duration, and protective behaviors and comet assay parameters in the exposed subjects (n=33)

Parameters	Age	Background history	Use of PPE	Local ventilation	Clothing	Storage of clothes	Natural ventilation
Tail length	-0.136	0.020	0.168	0.009	-0.038	-0.175	-0.226
%DNA in tail	-0.254	-0.102	0.156	-0.009	0.090	-0.135	-0.089
Tail moment	-0.218	-0.061	0.134	0.059	-0.018	-0.104	-0.179

There was no significant difference in tail length, tail moment, and %DNA in tail between smokers and non-smokers. However, the difference in comet assay parameters between exposed and non-exposed group remained significant ($p < 0.001$) after subgroup analysis based on smoking status. Similar finding was observed when analysis was performed for male subjects. It was not possible to conduct the tests on females because of small number of cases (n=4).

There was no significant correlation between each of the comet assay parameters and duration of employment in the dry cleaning shop (Table 3). The results were the same after adjustment for age. Analysis on results based on age on all participants (n=59) showed no significant increase in the observed DNA damage adjusted for participant's age. Stratified analysis on cases and controls also showed no significant correlation between each of the comet assay parameters and age. Use of specific safety measures such as ventilation and personal protective equipment, and storage of cloths in a shop were also explored in participants. No significant correlation was found between safety measures and early DNA damage measures.

Discussion

We found a significant increase in early DNA damage in dry cleaners occupationally exposed to PERC in comparison with a group non-exposed people. This could

be considered evidence for potential genotoxic and carcinogenic effects of occupational exposure to PERC. Several studies reported an increase in cancer risk in those occupationally exposed to PERC. Recent findings suggest a close relationship between comet assay results and cancer risk in human. While the genotoxic effects of exposure to PERC have been investigated previously based on chromosomal aberration and micronucleus test,¹² to the best of our knowledge, there is only another human study that used comet assay to evaluate genotoxicity in dry cleaners and found significant early DNA damage in dry cleaners.⁸ Despite several animal and *in vitro* studies on genotoxicity of PERC based on comet assay, there are no conclusive remarks about this issue based on cytogenetic tests. Cederberg, *et al*, found a weak but significant increase in early DNA damage in rats exposed to PERC.⁴ However, their results are criticized because of the statistical methods they used and the observed effect size.¹³

Alkaline comet assay is capable of determining the level of single-strand and double-strand lesions as well as alkaline-labile site lesions, simultaneously. Comet assay on peripheral blood lymphocytes reflects the level of DNA damage resulting from recent exposures, which generally are easily repairable.¹⁴ This could be an explanation for no observed correlation between duration of occupational exposure to PERC and level of DNA damage in our study. Everatt, *et al*, also found no significant correla-

tion between duration of employment and level of DNA damage by the comet assay.⁸ Singh, *et al*, in a study on workers exposed to petroleum fumes,¹⁵ and Costa, *et al*, in another study on a group of formaldehyde-exposed workers,¹⁶ found similar results. The effect of age, as a comorbidity in comet assay results, is questionable. Two studies found that age has no effect on endogenous single-strand break levels and repair capacity,^{17,18} whereas other studies reported an increase in DNA damage in comet assay with advancement of age.¹⁹⁻²¹ However, other studies found no significant association between duration of employment or age with the observed level of DNA damage measured by comet assay.^{22,23}

In our study, there was no significant association between DNA damage and smoking status. There is no conclusive result on the effect of smoking on DNA damage in comet assay test.²⁴ Classification of subjects based on dichotomous measure of smoking status did not definitely mean the same level of DNA damage in all group members, and thus the level of DNA damage could be related to severity and frequency of smoking, number of cigarettes smoked per day, tobacco type, smoking style, and exposure to second-hand smoke. Use of dichotomous variable instead of continuous or ordinal variables can also reduce the power of analysis and underestimate possible difference between groups of interest. Another explanation for this finding would be misclassification of exposure because of false response of subjects about their smoking status. Most of people tend to hide their smoking behavior in countries such as Iran. Use of more objective measures such as urinary cotinine in future studies could eliminate this source of error in the results. Another explanation could be attributed to the exact degree of exposure to genotoxic chemicals in smoking products in comparison with other sources of exposure to this class of

compounds such as occupational exposure. Occupational exposure to chemicals is usually several times higher than those values occur in environmental cases. Therefore, the intensity of effect of exposure to occupational pollutants can mask the possible effect of smoking. A study on a group of fuel station workers exposed to petrol vapors showed that exposure to fuel vapors can increase %DNA in tail by 16.5% in comparison with controls, whereas smoking was only responsible for 35% increase in %DNA in tail.²⁵

Effect of sex on DNA damage is an ongoing research topic and the results are inconclusive. We were unable to test the difference between males and females results because of small number of females. In terms of occupational factors, women generally have lower workload duties and are thus less exposed to chemical substances. In our study, the women were shopkeepers and apparently had lower exposure to PERC in comparison with men who worked in machine delivery section. There is significant difference in the level of exposure depending on the task and thus the possible difference observed between men and women could be due to lower exposure for women. The role of body composition (more fat in females),²⁶ lifestyle between men and women could also be responsible for the observed difference.

We found no significant correlation between DNA damage and each of the level of safety behavior, use of personal protection equipment, and ventilation of dry cleaning shops. There are several explanations for this finding. Most of dry cleaners in this study stated different levels of use of personal protective equipment. However, it did not mean "good level of protection," as the use of personal protective equipment is generally considered the last resort in control of occupational inhalation exposures. Improving knowledge of personnel can enhance the perception and

finally the behavior of workers in exposure to carcinogens.

Regular and strict procedure for blood sampling in this study was its strength in comparison with Everatt, *et al.*⁸ This study has several limitations. First of all, we did not perform exposure assessment in our study. However, previous results from Zare Sakhvidi, *et al.*, showed relatively high exposure intensity with PERC in Iranian dry cleaners.²⁷ Due to similar working procedures and similar dry cleaning machines across workshops, comparison of results seems reasonable. This was a preliminary study evaluating potential genotoxic effects in an occupationally exposed group; however, it would have been stronger if more than one test was used to evaluate these effects. Use of multi-biomarker approach based on cytogenetic tests such as micronucleus assay, chromosome aberration, and sister chromatid exchange can enhance our understanding about possible genotoxic mechanisms of PERC. Our findings have external validity for some other industrial such as metal cleaning and chemical industries, where workers are exposed to PERC. Effect of exposure window on observed effects should be accounted in future studies. Adding a third exposure group, one where previous exposure to PERC is known to have occurred, but has not occurred in the preceding 3–6 months, to investigate whether or not the effects are transient or not. We did not perform exposure assessment in this study to evaluate a possible dose-response relationship. Similar to other comet assay application, standardization of protocol to reduce inter-laboratory variations was performed. Use of positive control samples such as irradiated blood cells in combination with exposure assessment can enhance the quality of findings.

In conclusion, exposure to PERC can induce DNA damage in dry cleaners. Application of strategies in lowering exposure

intensity such as use of protective equipment can prevent this effect. Our results indicated that use of personal protective equipment by itself, has no significant correlation with the observed level of DNA damage. However, proper use of personal protective equipment should be emphasized. Healthier lifestyle such as avoiding smoking is also advisable for prevention of undesired damages. Development of safety culture in dry cleaners based on proper working with solvents and sanitation of the workplace can also prevent future toxic effects from exposure to PERC.

Conflicts of Interest: None declared.

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