Short Communications

Editorial Process: Submission:10/01/2022 Acceptance:02/21/2023

DNA Damage on Buccal Epithelial Cells, Personal Working in the Rubber Industry Occupationally Exposed to Carbon Disulfide (CS_2)

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Abstract

Introduction: The most significant industrial utilization of carbon disulfide (CS_2) has been in the manufacture of cellulose rayon, cellophane, and rubber industry. CS_2 prompts expanded recurrence of chromosomal variations in laborers occupationally exposed to CS_2. **Materials and Methods:** In the current study, the DNA analysis was carried out from exfoliated buccal epithelial cells from rubber industry workers exposed to CS_2 and an equal number of healthy control subjects. Both the control and experimental subjects were categorized by their smoking habits such as smokers (S) and non-smokers (NS). Furthermore, experimental subjects were further separated based on their exposure period. Students t-test statistical tools were used to analyze the final results. **Results:** The present analysis identified a high frequency of DNA damage in rubber industry workers (16.55±0.43) than control subjects (9.8±0.21). Also, maximum number of DNA damage detected in smoking experimental group (18.27±0.02) than non-smoking experimental (15.02±0.01) and smoking control groups (10.25±0.04). **Conclusion:** Smoking habits synergistically increased the DNA damage in the rubber industry workers exposed to CS_2.

Keywords: Rubber Industry- DNA damage- Carbon di-sulfide

Asian Pac J Cancer Prev, **24 (2)**, 357-361

Introduction

Carbon disulfide (CS_2) is a vital industrial fluid natural dissolvable, which is basically utilized to treat soluble base cellulose within the viscose and rubber industries. In the past few decades CS_2 has remarkable cytotoxic effects on several mammals (NIOH, 2017). Intense and subacute harming effects are shown up due to exposure to CS_2 concentrations of 500-3000 mg/m3 and are predominantly considered by neurological effects, gastrointestinal disturbances, and homogenderual disorders (Liu et al., 2019; Sun et al., 2013; Wronska-Nofer et al., 2002; Wang et al., 2002; Krstev et al., 2003; Manikantan et al., 2009), while exposure to CS_2 concentrations over 5,000 mg/m3 may actuate coma or indeed death (Chalansonnet et al., 2018). More subtle neurological changes at lower CS_2 concentrations have been reported; the symptoms are a reduction of nerve conduction velocities and psychological disturbances (8-10). In workers exposed for 10-15 years to CS_2 in concentrations of around 10 mg/m^3, sensory polyneuritis and increased pain threshold were reported. These neurological disturbances were accompanied by psychological and neurobehavioural disorders. Occupational Health and Safety Administration, permissible exposure limits–time-weighted average. The current limit carbon disulfide concentration for daily exposures of 8 hours or fewer is 20 parts per million by volume, or 0.062 milligrams per liter at 25ºC and 760 mm Hg. The serious toxic effects of CS_2 on experimental animals (Guo et al., 2015) have been extensively identified and other epidemiological studies also demonstrated CS_2 exposure among viscose industry workers including heart related defects among viscose industry (Wang et al., 2015). Even though several studies fail to identify the mutagenic potential of CS_2. Studies on Salmonella typhimurium, Drosophila, human fibroblasts, human blood leucocytes, and rats have been indecisive. Recent study proved that DNA impairment and apoptosis of endometrial cells cause loss of the early undeveloped organism in mice to CS_2 exposure (Zhang et al., 2013). Various studies

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documented that the (SMR's) Standard Mortality Ratios for the persons who are exposed to CS_2 occupationally are precariously higher than other population. In any case, there are no reports available for CS_2 that give solid prove of genotoxic impacts on DNA and genetic materials (Stone et al., 1995). Since the buccal epithelium is a source of tissue for analyzing personals occupationally exposed to environmental genotoxins. The current analysis assesses the genetic toxicity impact of occupational exposure to CS_2 on buccal epithelial cell and the possible influence of alterations on personal occupationally exposed to CS_2 in the rubber industry.

Materials and Methods

Subject recruitment

The experimental subjects were 40 rubber industry workers and 40 non exposed people as controls who were chosen from different cities of southern India between August 2018 and January 2019. Earlier to enrolment within the ponder, all subjects gave composed ethical assent. A questionnaire was utilized to gather data on sexual orientation, age, exposed duration, protective covers, common wellbeing status, smoking propensities, and introduction to drugs for each experimental and control subject. The purpose of the questionnaire is to collect the participants' daily routines in order to incorporate specific routine metrics pertinent to the study's aims . There were 18 smokers and 22 non-smokers in both control and experimental subjects. The normal cigarette consumption of smokers in both bunches was about 14.2 ± 2.0 (mean ± standard deviation) cigarettes/day. Ethical approval for the present study was granted by the World Medical Association (WMA) make at Helsinki (2008) regarding the ethical principles for medical research.

Sample collection

Buccal epithelial cells were collected by oral brushing or by swabbing method. After collecting saliva, buccal swabs were taken with two Copan FLOQ swabs by pressing them on the inside of each participant's cheek twenty times, then in the maxillary and mandibular buccal sulcus (the upper and lower furrows between the gingiva and the inner cheek) for 10 seconds each side. Each buccal swab was wiped along the length of a standard-sized microscope slide and fixed as described for saliva before. Before this, subjects washed their mouth with typical saline to evade the obstruction of bodily fluid. Added cold phosphate buffer saline (PBS) solution for collections of pellets. Then cells were resuspended in 300 µl PBS and 50 µl of suspension buffer.

Comet assay

DNA examination was performed under acidic conditions followed by the previous method (Stone et al., 1995) with some minor changes (Maluf and Erdtmann, 2000). Cells were fixed in low softening point agarose on a glass slide precoated with 1% ordinary agarose. After cementing of gel, the slide was lowered into cool lysis solution [2.5M NaCl, 100 mM EDTA, 10 mM Tris (pH 10.0), 1% LSS lauryl sarcosine sodium salt to which 10% DMSO, 1% Triton X- 100 were newly added] and kept for the time being at 4°C. The slides were at that point set on the level electrophoresis unit loaded up with newly arranged antacid electrophoresis buffer solution (300 mM NaOH, 1 mM EDTA, pH 13) for 30 min and afterward exposed to electrophoresis at 25V/ 300mA for 40 min. After electrophoresis, the slides were counteracted for ~60 min in 0.4 M Tris/HCl, pH 7.5 on-ice, trailed by staining in ethidium bromide (stock fixation 25 mg/mL in distilled water) furthermore, mounting on glycerol. All these processes were performed on ice to forestall the evacuation of a thin agarose gel layer from the slide. The stained slides were analyzed under Nikon fluorescent magnifying instrument with a 580nm emission filter.

Statistical analysis

Results are correlated as mean ± standard deviation. The understudy's t-test was performed to look at the DNA levels between the test and controls. Significant levels were considered at $p < 0.05$.

Results

The subjects were chosen from the rubber industry laborers who are occupationally exposed to CS_2. The tail development of comets observed in the buccal cells of exploratory and controls are given in Table 1. Among the control group, the level of DNA damage noted among smokers was higher than that seen among non-smokers, however, without measurably critical information was seen aside from the subjects matured 26-35 years. An age-related increment in DNA damage as seen in both

Table 1. Percentage of DNA Damage among the Control and Experimental Non-Smoking Subjects

Subjects	Smoking Habits	Groups	Number of subjects	Percentage of DNA damage
Control	Non-smokers	<25	7	6.1± 0.5
		26-35 years	5	7.5 ± 0.3*
		36-45 years	6	10.7 ± 1.2
		46-55 years	4	13.2 ± 0.5
Experimental	Non-smokers	<25	7	10.3 ± 0.7*
		26-35 years	5	13.3 ± 0.4
		36-45 years	6	17.4 ± 1.2*
		46-55 years	4	19.1 ± 0.3

Table 2. Percentage of DNA Damage among the Control and Experimental Smoking Subjects

Subjects	Smoking Habits	Groups	Number of subjects	Percentage of DNA damage
Control	Smokers	<25	6	5.2 ± 0.1
		26-35 years	4	7.2 ± 0.32
		36-45 years	5	13.1 ± 0.3*
		46-55 years	3	15.5 ± 0.5
Experimental	Smokers	<25	6	13.2 ± 0.6
		26-35 years	4	15.0 ± 0.4**
		36-45 years	5	20.1 ± 0.1
		46-55 years	3	24.2 ± 0.4*

Table 3. Percentage of DNA Damage According to Duration of Exposure in Experimental Subjects

Exposure period	Number of subjects	Percentage of DNA damage
<10 years	18	11.4±2.5
10-29 years	10	11.8±2.6
>20 years	12	12.32±3.6

control and exploratory subjects. Experimental subjects more than 46 years old demonstrated the greatest DNA damage (24.2 ± 0.4). A significant increase ($p < 0.05$) of the DNA damage was identified in many people of the experimental group when compared with the control subjects. A high degree of DNA damage was seen in the rubber industry workers with smoking propensities when compared with smoking controls and non-smoking rubber industry workers. To decide the impact of duration of exposure to CS_2 on DNA damage, the laborers were separated into 2 groups relying upon whether they had under 10 years of exposures or over 10 years of exposure (Table 2). No significant huge contrast in DNA damage was seen with a higher degree of workers occupationally exposed to CS_2 (Table 3).

Discussion

Mutagenesis has a role in the pathophysiology of several cancers. In many instances, genotoxic aberrations are implicated in occupational exposure processes that may lead to the onset of pernicious illnesses. Continual efforts have been made to define the conditions of hazardous exposure, identify genotoxic chemicals, and monitor populations overexposed to these conditions (Kuang et al., 2022). The current study was intended to evaluate the DNA damage among rubber industry workers who are occupationally exposed to CS_2. Eukaryotic cell DNA strand breakage may be quickly calculated using the comet test, which is also known as single-cell gel electrophoresis. To produce nucleoids, cells that are embedded in agarose on a microscope slide are lysed with detergent and a high salt concentration. Nucleoids are composed of supercoiled DNA loops that are attached to the nuclear matrix. The number of DNA breaks may be determined by comparing the intensity of the comet tail to the intensity of the comet head using fluorescence microscopy. These comet-like structures are shown when electrophoresis is performed at high pH. The test is useful for assessing the genotoxicity of novel substances, monitoring the genotoxicity of the environment, doing molecular epidemiology research, performing human biomonitoring, and conducting fundamental research on DNA damage and repair. The fact that the experiment can be performed with any eukaryotic cell that can be obtained as a single cell suspension, including cells isolated from blood, cells from tissue biopsies that can be homogenized, buccal cells, whole blood, and cultured cells, is a major factor in the experiment's widespread acceptance. This factor also plays a significant role in the experiment's notoriety. The comet assay has been widely used as a hazard evaluation and characterization tool (Velverde et al., 1998), and it has also been shown to be useful for detecting the negative effects of air pollution (Rojas et al., 1996), cigarette smoking (Bajpayee et al., 2005), and other exposures in in vitro and in vivo studies (Szeto et al., 2005). In the current investigation a striking DNA damage was observed among the experimental and control subjects. It is because of the test being generally utilized in contemplating DNA damage in solid individuals (Szeto et al., 2005) and everyday variety in buccal epithelial cell strand breaks (Daemen et al., 1999). There was a noteworthy distinction among exploratory and control subjects who are occupationally exposed to CS_2. In the past few years, CS_2 fixations in the rubber industry found the value of around 250 mg/m3; they were accordingly decreased to 50-150 mg/m3 and all the more as of late presentation levels of CS_2 are generally under 31 mg/m3,33. A report on hypospermia, asthenospermia, and teratospermia in young workers exposed to 40-80 mg/m3 of CS_2 affirmed gonadal injury (Lancranjan et al., 1969). Le and Fu (1996) demonstrated that the CS_2 prompt chromosome distortion in human sperm. Various epidemiological reports inferred that the CS_2 is poison to occupational workers (Guidotti and Hoffman, 1999). In this examination, experimental subjects with smoking propensities appeared greatest degrees of DNA harm when contrasted with particular controls, which shows that the CS_2 exposure with cigarette smoking synergistically affects the DNA damage. Chromosomal variations were demonstrated to be acceptable markers of the future danger of cancer (Hagmar et al., 1994). Similarly, DNA harms are a definitive reason for disease in light of the fact that DNA base changes can be mutagenic (Poirier, 1997). The recent invention noted the significance of

examining the genotoxicity of CS_2 on rubber industry laborers occupationally presented to this compound when the smoking propensity is related since this data gives an expanded level of CS_2 for the positive reaction.

Author Contribution Statement

MP contributed to design, methodology, manuscript writing and critical review AM, and AC contributed to data collection and manuscript writing KK contributed to design and critical review of the manuscript.

Acknowledgements

The study was approved by the ethics committee of Sakra World hospital and Institutional ethical committee with approval No ECR/793/Inst/KA/2015/RR-18. This particular study is part of a student thesis. Name of the Student: Aditi from M.Sc Zoology, Reg.No: 2047802.

Conflict of interests

Present study does not contain the any conflict of interest.

References

Bajpayee M, Pandey AK, Parmar D, et al (2005). Comet assay responses in human lymphocytes are not influenced by the menstrual cycle: a study in healthy Indian females. *Mutat Res*, **565**, 163–72.

Beauchamp RO, Jr Bus JS, Popp JA, Boreiko CJ, Goldberg L (1983). A critical review of the literature on carbon disulfide toxicity. *Crit Rev Toxicol*, **11**, 169–278.

Carbon disulfide. Geneva: World Health Organization (2018). Environmental Health Criteria, 10.

Chalansonnet M, Carreres-Pons M, Venet T, et al (2018). Combined exposure to carbon disulfide and low-frequency noise reversibly affects vestibular function. *Neurotoxicology*, **67**, 270–8.

Commission of the European Communities (CEC) (1988). Solvents in Common Use.

Daemen E, van Risseghem M, de Bacquer D, et al (1999). Preliminary external quality assessment for the biological monitoring of carbon disulfide with urinary 2-thiothiazolidine-4-carboxylic acid. *Annals Occup Hyg*, **43**, 125–30.

Guidotti TL, Hoffman H (1999). Indicators of cardiovascular risk among workers exposed to high intermittent levels of carbon disulphide. *Occup Med (Lond)*, **49**, 507–15.

Guo L, Luo C, Fan J, et al (2015). Serum miRNA profiling identifies miR-150/30a as potential biomarker for workers with damaged nerve fibers from carbon disulfide. *Ind Health*, **53**, 38–47.

Hagmar L, Brøgger A, Hansteen IL, et al (1994). Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. *Cancer Res*, **54**, 2919–22.

Hoffmann P, Müller S (1990). Subacute carbon disulfide exposure modifies adrenergic cardiovascular actions in rats. *Biomed Biochim Acta*, **49**, 115–20.

Krstev S, Perunicić B, Farkić B, Banićević R (2003). Neuropsychiatric effects in workers with occupational exposure to carbon disulfide. *J Occup Health*, **45**, 81–7.

Kuang HX, Li MY, Li LZ, et al (2022). Co-exposure levels of volatile organic compounds and metals/metalloids in children: Implications for E-waste recycling activity prediction. *Sci Total Environ*, **863**, 160911. Advance online publication.

Lancranjan I, Popescu HI, Klepsch I (1969). Changes of the gonadic function in chronic carbon disulphide poisoning. *Med Lav*, **60**, 566–71.

Le JY, Fu XM (1996). Human sperm chromosome analysis--study on human sperm chromosome mutagenesis induced by carbon disulfide. *Biomed Environ Sci*, **9**, 37–40.

Liu X, Wang S, Sun Y, Zhang T, Wang Z (2019). The suppressed autophagy induced by carbon disulfide could be rescued by N-carbamoyl glutamate during the window of embryo implantation in mice. *Chem Biol Interact*, **312**, 108751.

Maluf SW, Erdtmann B (2000). Evaluation of occupational genotoxic risk in a Brazilian hospital. *Genet Mol Biol*, **23**, 485-8.

Maluf SW, Erdtmann B (2000). Follow-up study of the genetic damage in lymphocytes of pharmacists and nurses handling antineoplastic drugs evaluated by cytokinesis-block micronuclei analysis and single cell gel electrophoresis assay. *Mutat Res*, **471**, 21–7.

Manikantan P, Balachandar V, Sasikala K, Mohanadevi S (2009). DNA damage in viscose factory workers occupationally exposed to carbon di-sulfide using buccal cell comet assay. *Braz J Oral Sci*, **8**, 197-200.

Moorman MP, Sills RC, Collins BJ, Morgan DL (1998). Carbon disulfide neurotoxicity in rats: II. Toxicokinetics. *Neurotoxicology*, **19**, 89–97.

National Institute for Occupational Safety and Health. Criteria for a recommended standard occupational exposure to carbon disulfide. Washington, DC: US Department of Health Education and Welfare; 2017.

Poirier MC (1997). DNA adducts as exposure biomarkers and indicators of cancer risk. *Environ Health Perspect*, **105**, 907–12.

Rojas E, Valverde M, Sordo M, Ostrosky-Wegman P (1996). DNA damage in exfoliated buccal cells of smokers assessed by the single cell gel electrophoresis assay. *Mutat Res*, **370**, 115–20.

Sills RC, Harry GJ, Valentine WM, Morgan DL (2005). Interdisciplinary neurotoxicity inhalation studies: carbon disulfide and carbonyl sulfide research in F344 rats. *Toxicol Appl Pharmacol*, **207**, 245–50.

Stone JG, Jones NJ, McGregor AD, Waters R (1995). Development of a human biomonitoring assay using buccal mucosa: comparison of smoking-related DNA adducts in mucosa versus biopsies. *Cancer Res*, **55**, 1267–70.

Sulsky SI, Hooven FH, Burch MT, Mundt KA (2002). Critical review of the epidemiological literature on the potential cardiovascular effects of occupational carbon disulfide exposure. *Int Arc Occup Environ Health*, **75**, 365–80.

Sun Y, Dai B, Wu Y, et al (2013). Carbon disulfide exposure at peri-implantation disrupts embryo implantation by decreasing integrin β3 expression in the uterine tissue of pregnant mice. *Chem Biol Interact*, **206**, 126–33.

Swaen GM, Braun C, Slangen JJ (1994). Mortality of Dutch workers exposed to carbon disulfide. *Int Arc Occup Environ Health*, **66**, 103–10.

Szeto YT, Benzie IF, Collins AR, et al (2005). A buccal cell model comet assay: development and evaluation for human biomonitoring and nutritional studies. *Muta Res*, **578**, 371–81.

Tang GH, Xuan DF (2003). Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = *Chin J Industrial Hygiene Occupational Dis*, **21**, 440–3.

Valverde M, del Carmen López M, López I, et al (1997). DNA

damage in leukocytes and buccal and nasal epithelial cells of individuals exposed to air pollution in Mexico City. *Environ Mol Mutagen*, **30**, 147–52.

Wang Q, Fu K, Wu Q (1999). Effects on fertility and menstrual cycle of female workers exposed to carbon disulfide. *Chin Publ Health*, **15**, 215-7.

Wang YF, Shiu YF (2015). Investigation on eye injury of workers exposed to CS_2. *J Lab Med*, **17**, 89.

Wang C, Tan X, Bi Y, et al (2002). Cross-sectional study of the ophthalmological effects of carbon disulfide in Chinese viscose workers. *Int J Hyg Environ Health*, **205**, 367–72.

Wang S, Sun Y, Wu Y, et al (2015). Down-regulation of uterine LIF expression induced by the hormonal level disorder causes embryo implantation loss after mice exposed to carbon disulfide at peri-implantation. *Biochem Biophys Res Commun*, **467**, 7–13.

Wronska-Nofer T, Chojnowska-Jezierska J, Nofer JR, Halatek T, Wisniewska-Knypl J (2002). Increased oxidative stress in subjects exposed to carbon disulfide (CS_2)--an occupational coronary risk factor. *Arch Toxicol*, **76**, 152–7.

Zhang B, Shen C, Yang L, et al (2013). DNA damage and apoptosis of endometrial cells cause loss of the early embryo in mice exposed to carbon disulfide. *Toxicol Appl Pharmacol*, **273**, 381–9.