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RESEARCH ARTICLE

Carbon Disulfide (CS_2) Induced Chromosomal Alterations and Apoptosis in Circulated Blood Lymphocytes of Personnel Working in Viscose Industry

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Abstract

Background and objective: Carbon disulfide (CS_2) is a naturally occurring chemical substance in the environment and also an important endogenous substance in the human body. This study was done to collect data on the effects and to find a possible relationship between in-vivo CS_2 induced apoptosis and genotoxic effects. **Material and method:** Circulated blood lymphocytes (PBL) of 41 workers occupationally exposed to Carbon disulfide (CS_2) in viscose industry were investigated. The participants involved three groups. The first group included 41 participants exposed to CS_2 together with various confounding factors; the second group comprised of 41 participants who were inhabitant of viscose industry and were partially exposed to CS_2 in long periods; and third group consisting 41 participants as control group who were not exposed to any kind of chemicals and radiation hazards. Ambient air concentrations of CS_2 were measured in different workplaces. Measures of genotoxicity included the determination of the frequencies of chromosomal aberrations (CA), sister-chromatid exchange (SCE), HPRT mutations (variant frequency, VF), and measurement of UV-induced unscheduled DNA-repair synthesis (UDS). The percentages of premature centromere division (PCD) and of cells with a high frequency of SCE (HF/SCE) were also scored. Apoptosis and c ll proliferation were determined by flow cytometry. **Results:** In both CS_2 exposed groups, the apoptotic activity and the CA levels in PBLs were significantly higher than in controls. CA was mostly breaks of the chromatid type. In group II, CA was slightly lower in comparison with group I, which can be attributed to a different rate of elimination of damaged lymphocytes as a consequence of CS_2 induced apoptotic activity. **Conclusion:** The results demonstrate that exposure to CS_2 induced apoptosis and CA, indicating an excess cancer risk among participants occupationally exposed to CS_2. The results also emphasized the importance of the measurement of occupationally exposed pollutants such as CS_2 in order to avoid genotoxic effects in the workers.

Keywords: Carbon disulfide- viscose industry- blood lymphocytes

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Introduction

Carbon disulfide (CS_2) is a naturally occurring chemical substance in the environment and also an important endogenous substance in the human body. CS_2 is also an industrial toxicant and solvent mainly used in the viscose, rubber, and chemical manufacturing industries [1]. The largest use of CS_2 is in the viscose industry where it is used to obtain alkali cellulose. Exposure to high CS_2 concentrations in these industries may cause severe effects on human body [2-3]. Chronically revelation to CS_2 can cause eye, ear, cardiovascular, nervous, and reproductive system disturbances [4-11].

Currently, CS_2 exposure level is mostly below 31 mg/m^3. According to Guidotti et al (1999), Hoffman (1990), and Keil et al., (1996), short time exposure also caused deceleration of intra-cardiac impulse conduction and modified arrhythmia in the coronary occlusion. An extensive literature is available on the toxic effects and genetic and chromosomal damage of CS_2 on circulating

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blood lymphocytes and human sperm samples [12-14]. In addition, Bao et al., (1996) reported an increased incidence of chromosomal aberrations in the pronuclei of zygotes in adult female mice exposed to 10/100 mg/m^3 of CS_2 for 3 weeks and mated with unexposed male mice. In -vitro exposure of human sperm to CS_2 concentrations at 10 micromole/liter(µmol/L) resulted in increased occurrences of chromosomal aberrations [15]. Furthermore, the mutagenicity and genotoxicity potentials of CS_2 have been evaluated in vitro and in-vivo experiments using crucial confounding factors [14, 16-18]. Previously, CS_2 does not exhibit mutagenic activity in bacteria with or without the presence of activation system [19]. Additional in vitro tests, including host-mediated assay, unscheduled DNA synthesis in human fibroblasts and primary cultures of human leucocytes are unconvincing. However, the significance of these tests cannot be properly evaluated because of methodological problems such as the lack of proper positive controls [20].

Therefore, insufficient data are currently available to evaluate the mutagenic and genotoxic potentials of CS_2. A quantitative assessment of published data on workers heavily exposed to CS_2 can provide some evidence for/against the hypothesis regarding it association with numerous diseases. Current meta-analyses showed significant genetic changes induced by the potential confounding factors like smoking and alcohol. Today, chromosomal alterations in human peripheral lymphocytes (PBL) are recognized as a valuable biomarker of the effect of toxic chemicals, probably the only one which has been standardized and validated [21] method to detect the genetic level damage. SCE frequency in human PBL has been used as the best indicator of chromosome damage [22]. To assess early carcinogenic effects of various genotoxic markers involved, DNA alterations [23] have been used in molecular epidemiological studies [24-25].

Cytogenetic studies on occupational exposure have reported genetic damage among viscose industry workers [18-26] even though the results in this regard are rather contradictory. However, there is still no consistent evidence supporting that exposure to CS_2 causes chromosome damage. The inconsistent genotoxicity data could be due to differences in levels of direct and indirect exposures or in the utilized end-points. Hence, there is a need to evaluate different populations to analyze various genotoxic parameters of CS_2. Early identification of hazards is crucial to reduce the exposure and carcinogenic risk. A literature survey has revealed that no investigation has been conducted among these workers with different cytogenetic tools considering different levels of CS_2 exposure.

The focal aim of the present study was to identify genetic alterations of viscose factory workers exposed to CS_2 occupationally in Tamil Nadu, South India, by using the relevant cytogenetic tools such as apoptosis induced factors, chromosomal aberrations (CA), and Sister.

Chromatid exchange (SCE) assay and considering various confounding factors. To evaluate the possibility of toxic chemicals effect at the workplace, confounding factors and other influences associated with work duration must be taken into consideration.

Materials and Methods

Selection of subjects

Altogether 123 subjects from various viscose industries in Tamilnadu state especially Tiruppur and Erode districts were recruited for current study. Subjects were divided into three groups; namely group I consisting of 41 subjects directly involved and exposed to CS_2 in viscose industry, group II comprising 41 subjects who were not exposed to CS_2 but they were inhabitants near viscose industries, group III consisting of 41 subjects in the control group. Additionally, group I were divided based on their duration of exposure (0–3; 4–6; 7–9; 10–12; 13–15 years) and they were known to be exposed to CS_2 for a minimum of 8 hrs per day. Group II were those who were not directly exposed to CS_2, but they were inhabitants nearby viscose industry during past 2 decades and they were further categorized based on the year of residence (20–30; 31–40; 41–50; 51–60; and 61–70 years). Control participants were normal and healthy individuals who were not exposed to any kind of chemicals and radiation hazards, Control participants were matched to exposed groups with respect to age. Experimental groups were further categorized based on their age (Group I <40 years; Group II >40 years). A face to face questionnaire which recorded standard demographic profile as well as questions relating to lifestyle, consumption habits such as smoking, alcohol intake, medication, recent viral infections, vaccinations, diagnostic tests or previous occupational exposures to chemicals was completed by all the participants. It was also ensured that all the participants do not take any medicines nor been exposed to any kind of hazards for at least 12 months before sampling. Individuals (both experimental and controls) who smoked for at least 1 year (more than 15 cigarettes/day) were considered as smokers. Informed consent was obtained from all the participants.

CS_2 concentrations in ambient air were measured at fixed locations in the industries, and the concentrations were in the range of 0.13–1.20 mg corresponding to an 8-h time-weighted average exposure (TWA-8) of 0.9 mg/m^3, which exceeds the limits of both the short-term exposure (0.6 mg/m^3) and TWA-8 (0.6 mg/m3). All participants complained about acute toxic effects (eye irritation) and the presence of CS_2 was perceivable in each industry. Data concerning the ambient air concentration measurements for these chemicals were not available, but ambient air concentrations for each of these chemicals were much below both the 8-h time weighted average exposure (TWA-8) and short-term exposure limits in the selected region. Only active smokers were considered to be smokers.

The determination of HPRT gene mutations and UV-induced DNA synthesis (UDS) in PBL

To determine HPRT gene mutations, PBLs were separated from the blood samples on Histopaque

1,077 gradients and cultured in RPMI-1640 medium supplemented with 20% fetal calf serum and 0.5% phytohemagglutinin -P (PHA) without antibiotics in a standard thermostat at 37oC in a humidified atmosphere containing 5% CO_2. The determination of the frequency of gene mutations at the HPRT locus (variant frequency, VF) was performed by autoradiography of the cells cultured in the presence of 3H-thymidine reagent (3H-TdR) and a selective agent (10−4 M 6-thioguanine) applying the modified method of Strauss and Albertini (1979). The lectin labeling index (LI) and the HPRT mutation frequency were determined from the same samples, as previously described [27, 28]. Finally, the VF was calculated according to the equation of Strauss and Albertini (2000).

The measurement of UDS was carried out according to Bianchi et al. (1981), as previously described briefly. PBLs were separated on Histopaque 1,077 gradients by density centrifugation. After separation, PBLs were irradiated in open petri-dishes by UV light (24 J/m^2) and then incubated for 3 hrs with 10 pC i/ml3H-TdR in the presence of 2.5 m Mhydroxy urea. The degree of 'de novo' UDS was measured by scintillometry based on 3H-TdR incorporation in separated lymphocytes. UDS was calculated as the difference between radioactivities of the incorporated 3H-TdR in UV-irradiated and control participants.

Determination of CA and SCE frequencies

Whole blood samples were processed for studying CA and SCE. The cell culture methods were identical in both protocols: samples of 0.8 ml heparinized blood were cultured in duplicate at 37 oC, in 5% CO_2 atmosphere, in 10 ml RPMI-1640 supplemented with 20% fetal calf serum and 0.5% PHA without antibiotics. For CA and SCE analyses, the cultures were incubated for 50 and 72 hrs, respectively. 5- Bromo-2µ- deoxyuridine (BrdU), used in SCE analysis to identify the first and subsequent metaphases, was added at 5 µg/ml concentration for 22 hrs. Culture harvest, slide preparation, and staining were conducted following the standard methods using 5% Giemsa stain for CA (Moorhead et al, 1960) and according to the Fluorescence-plus-Giemsa method of Perry and Wolff (1974) for SCE. CA characterization was carried out in 100 metaphases with 46±1 chromosomes per subject according to Carrano and Natarajan (1988). Mitoses with unmatched (45 or 47) chromosomes were considered as aneuploidy cells. Mitoses containing only achromatic lesions (gaps) and/or aneuploidy were not considered aberrant. Premature centromere division (PCD) was scored according to Mehes and Bajnoczky (1981). Total PCD and mitoses with more than three chromosomes with PCD (PCD/CSG, centromere separation general) were also scored. Cells with a higher number of SCE per cell than the 95 percentile of the control (≥10) were considered as high- frequency SCE cells (HFC/SCE) according to Tates et al., (1991) and Major et al., (1996).

Flow-cytometric analysis of apoptosis and cell proliferation in PBL

For the measurement of the percentage apoptosis and the S-phase, PBL were separated from the blood samples on Histopaque 1,077 gradients and cultured in RPMI-1640 medium supplemented with 20% fetal calf serum and 0.5% phytohemagglutinin-P (PHA) for 50 hrs without antibiotics in a standard thermostat at 37 °C in a humidified atmosphere containing 5% CO_2. One hour prior to termination of the incubations, 5 µg/ml BrdU was added to the cultures. Cells were washed twice with PBS, fixed in 1ml ice-cold 70% ethanol, and stored at -20 °C until further processing. DNA denaturation prior to propidium iodide (PI) and fluorescein isothiocyanate (FITC) labeled monoclonal anti-BrdU staining was performed at room temperature with 2MHCl containing 0.2 mg/ml pepsin according to the method proposed by van Erp et al. (1988). DNA was stained with PI and the incorporated BrdU was detected by use of immunocytochemistry with FITC labeled monoclonal antibody.

Flow-cytometric analysis was performed with a FACS Calibur flow cytometer. Data for at least 10,000 lymphocytes per sample were acquired; CellQuestPro Software was used for the analysis.

Cell proliferation was also characterized by flow-cytometric measurement of the expression of the cell-activation marker CD71 on T- lymphocytes (Biro et al., 2002). Briefly, heparinized whole blood was mixed and incubated at room temperature for 20 min with the appropriate amount of fluorescence-labeled monoclonal antibodies against surface antigens. The following monoclonal antibodies were used: peridinin–chlorophyll–protein complex (PerCP)-labelled anti-CD3 (T-cell marker), FITC -labelled anti-CD71 (transferrin receptor), and allophycocyanin (APC)-labelled anti-CD45. The erythrocytes were removed through lysis by addition of FACS Lysing solution. After washing with phosphate-buffered saline (PBS), samples were analyzed within 4 hrs after labeling or fixed with 2% paraformaldehyde. Flow-cytometric analysis was performed on a Becton Dickinson FACS Calibur flow cytometer. Standard forward and side scatter gating combined with CD45 was used to set the lymphocyte gate. Data for at least 10,000 leukocytes per samples were acquired; CellQuest Pro Software (Becton Dickinson) was used for analysis. Phenotypes are expressed as a percentage of positive cells of the given lymphocyte subpopulation.

Statistical analysis

Statistical analysis was made using GraphPad Prism 3.02 software. Differences between the experimental groups and the control group were tested using Student's t-test. $P< 0.05$ was considered as statistically significant. A standard linear correlation analysis was used to determine the relationship between apoptosis, CA, and PCD.

Results

Main demographic data of the experimental and control groups are summarized in Table 1. The participants aged 22 to 60 years. The time of occupational exposure as viscose industry workers were 21.8 years (ranging from 7 to 34 years) and 17.7 years (ranging from 4 to 34 years) in experimental and control group, respectively. The number of current smokers was slightly elevated among the exposed donors. The present study described the lifestyle characteristics such as gender, smoking status. Considering smoking status, 43.96% were smokers (and 48.78% were nonsmokers.

The participants in the experimental group and control group were matched for age and gender and no difference was observed in terms of gender. Totally, 26 males (63.41%) and 15 females (36.58%) participated in the present study. Data on chromosomal aberrations and SCE are depicted in Table 2. Circulated lymphocytes of Group I and II were similar to those in the control group. The comparative data on general characters, chromosomal alterations, and SCE/1,000 cells are presented in Table 2. In addition, CA and SCE frequency are also depicted in the same Table based on the CS_2 exposure in air and in urine.

In total, Group I CS_2 exposure was higher (1.42±0.004 (mg/m^3)), compared to that in Group II (0.054±0.002 mg/m^3) and group control (0.021±0.004 mg/m^3). With respect to mean ± SD values, it was found that the total CA (5.41±2.48) and SCE (13.62±3.46) for Group I was found to be higher compared to group II (9.31±2.73) and control group (3.62±1.18). The values obtained in Group I were found to be statistically significant when compared to their respective controls. Tables 3 and 4 depict the mean ± SD values of CA and SCE frequencies for group I and

Table 1. Demographic Data of Experimental Subjects

Particulars		No of Samples	Percentage (%)
Group I	0-3	8	19.5
(Work Duration in Years)	4-6	13	31.7
	7-9	11	26.82
	10-12	7	17.07
	13-15	2	4.87
Group II	20-30	11	26.82
(Year of Residence)	31-40	13	31.7
	41-50	6	14.63
	51-60	5	12.19
	61-70	6	14.63
Controls		41	100
Gender	Male	26	63.41
	Female	15	36.58
Smoking Status	Smokers	18	43.96
	Non-Smokers	23	48.78
Age (mean±SD)	Group I	31.05 ±2.14	
	Group II	42 ± 2.47	

Table 2. Comparative Analysis Of Chromosome Aberrations And SCE Based On The CS2 Level In Workplace Environment And Urine Samples

Groups	No. of Subjects	CS2 amount in workplace mean±SD	CS2 content in urine mean±SD	Total CA (mean±SD)	Total SCE (mean±SD
I	41	1.42±0.004	1.82±0.84	5.41±2.48	13.62±3.46
II	41	0.054±0.002	1.01±0.46	4.08±3.16	9.31±2.73
III	41	0.021±0.004	0.54±0.39	1.41±2.04	3.62±1.18

Table 3. Chromosome Aberration Frequency and SCE in Group I Subjects Based on Their Work Duration

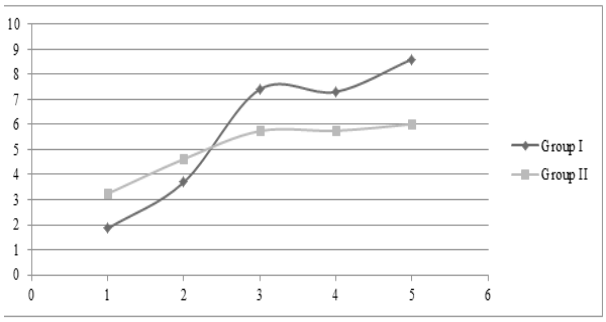
Particulars	0-3	4-6	7-9	10-12	13-15
Age	34.42±5.64	35.41±3.72	38.22±4.82	41.16±3.74	38.3±6.57
Work Duration	3.18±0.49	5.46±1.02	8.67±0.09	11.36±0.28	14.56±8.46
CTAs	1.46±0.87	2.57±1.03	3.67±0.05	5.62±1.18	6.08±1.40
CSAs	0.43±0.68	1.12±0.9	4.42±1.03	9.41±1.12	7.93±0.76
Total	1.86±0.62	3.70±0.84	7.40±1.14	7.32±0.81	8.62±2.36
SCE/1,000 Cells	13.04±3.46	16.80±3.39	18.06±1.86	21.06±3.42	19.32±3.82

Table 4. Chromosome Aberration Frequency and SCE in Group II Subjects Based on Their Year of Residence

Group II	20-30	31-40	41-50	51-60	61-70
Age	36.46±3.28	41.1±2.86	38.1±1.47	39.62±4.68	38.21±6.16
Year of Residence	23.74±1.82	33.80±2.62	46.28±2.30	52.63±3.47	63.62±4.62
CTAs	3.51±1.68	3.01±1.46	3.87±1.02	6.28±0.56	6.02±0.83
CSAs	1.02±0.48	0.96±1.04	1.87±1.06	0.89±1.01	2.46±0.96
Total	3.24±0.92	4.62±1.53	5.74±1.67	5.75±1.07	6.01±2.10
SCE/1000 Cells	4.96±2.15	6.46±1.03	13.02±3.71	16.28±2.52	18.26±1.86

Table 5. Mean Value of Cell Induction (%) Cell Proliferation, HPRT Mutation Frequency and DNA Repair Capacity in Cultured Peripheral Lymphocytes among Viscose Factory Workers Occupationally Exposed to CS2

Groups	N	Apoptosis %	S-Phase	CD cells %	LI (PHA) %	VFX10-6
I	41	8.57±1.10	22.13±1.38	1.86±0.31	12.12±1.08	1.86±0.07
II	41	5.04±0.48	17.13±1.56	1.52±0.58	9.60±0.51	2.32±1.62
III	41	3.91±0.27	13.86±1.03	1.08±0.27	8.14±1.16	4.26±1.03



Flow Chart 1. Graphical Representation of CA Value in Group I and Group II Subjects

II, respectively.

The frequency of total CA found in group I was higher [8.62±2.36 (13-15 yrs)] compared to other age groups [7.32±0.81 (10-12 yrs), 9.46 ± 4.04 (7–9 yrs), 3.70±0.84 (4-6 yrs); 1.86±0.62 (0–3)] (Table. 4). In group I, the total SCA was higher in 10-12 years exposure (21.06±3.42) compared to other age groups who were exposed to CS_2 [19.32±3.82 (13-15 yrs), 18.06±1.86 (7-9 yrs), 16.80±3.39 (4-6 yrs) and 13.04±3.46 (1-3 yrs)] which were found to be statistically significant when compared to other age groups and their respective controls. For group II, the total CA (6.01±2.10) and SCA (18.26±1.86) frequencies were higher in age group of 61–70 years. The present study showed levels of CA in Group I, II, and controls (Table 4). Furthermore, statistical significance for the frequency of CA expressed in mean ± SD was established using the analysis of variance (ANOVA). Cell cultures were effectively established from the blood samples collected from all the participants. The overall CA and SCA frequencies for CS_2 exposures were significantly different (Tables 2, 3, 4) from those of the controls for both chromatid and chromosome type aberrations (P value is 0.03 by ANOVA).

The results of the flow-cytometric measurements on the percentage of apoptotic cells, cells in S-phase, and CD71 expression, together with the mean values of LI, VF, and UDS are summarized in Table 5. The mean percentage of apoptotic cells was significantly higher in groups I and II (8.57±1.10, 5.04±0.48) in comparison with controls (3.91±0.27). Fascinatingly, the percentage of apoptotic cells was even higher in group I exposed to CS_2 only, in comparison with the groups II and III exposed to CS_2; however, this difference was not statistically significant (p = 0.33).

The mean percentage of cells in S-phase, measured by flow cytometry, as well as the mean values of LI slightly increased in both groups I and II in comparison with the control participants. There was no significant difference between experimental groups and control group in terms of SCE. The mean percentage of HF/SCE showed a non-significant (p = 0.16) increase among participants in group II, exposed mainly to CS_2. The percentages of PCDs were significantly higher in both experimental (Groups I and II) groups in comparison with the controls (Group III).

Table 6.X Axis Value

X Axis Value	1	2	3	4	5
Group I	0-3	4-6	7-9	10-12	13-15
Group II	20-30	31-40	41-50	51-60	61-70

Discussion

The results of the present study revealed cytogenetic alterations and induction of apoptosis among viscose factory workers occupationally exposed to CS_2. Moreover, the considerable CS_2 concentration was always present in the working environments, and the presence of CS_2 in the air was always observable by olfactory perception. Safety measures and devices have been introduced in the last few years in these industries, and the employees have used these protective devices during their work. However, none of the workers have worn masks or similar personal safety devices equipped with specific filters for CS_2. Therefore, to assess the excess risk among these workers in the second group, we should consider the effects of low dose exposure to organic solvents as a confounding factor and as a cause of bias on the effects of CS_2. On the other hand, the first group was almost exclusively exposed to CS_2, as they situated nearby these industries.

Furthermore, major effluent water mostly contaminated with CS_2. Moreover, some of the industries in our study area release their effluents either on the open land or in surrounding surface water bodies contaminating the soil and surface water ultimately turned into groundwater. Much of the groundwater is unsuitable for irrigation, and hundreds of wells in the region can no longer be used. Based on these earlier reports, we recruited experimental subjects who were known to live for the past 3 decades in and around our study area and were occupationally exposed to CS_2 by mode of air and water (drinking).

Mutagenesis is involved in the pathogenesis of many neoplasias. Occupational exposure may contribute to the development of pernicious illnesses through mechanisms that involve genotoxic changes. Continuous efforts have been made to identify genotoxic agents, determine conditions of harmful exposure, and monitor populations that are excessively exposed to these conditions [29]. The present study was designed to assess the genetic damage among viscose plant workers who are occupationally

exposed to CS_2. CA and SCE is a valuable method for detection of occupational and environmental exposures to genotoxicants, and it can be used as a tool in risk assessment for hazard characterization [29-30] of various in vitro and in vivo studies [31-33].

An epidemiological study showed a strong correlation between CS_2 concentrations from workers in viscose rayon factory and CS_2 factory indoor air concentrations up to 64 mg/m3 (20.5 ppm). Ghittori et al., (1998) used personal passive samplers installed in the breathing zones to measure the airborne CS_2 levels for 4 hours. Most of the indoor air samples revealed CS_2 at levels below the TLV of 31 mg/m^3.

Previous studies concluded that urinary CS_2 may be a good indicator to estimate the levels of exposure to CS_2 in the workplace. Investigating the correlation between urinary concentrations and indoor air levels indicated that a mean level of 15.5 µg/ CS_2/L (95% CI 13.8–17.1 µg) was excreted following exposure to CS_2 at 31 mg/m3 current occupational exposure limit in viscose industry [34]. Cox et al., (1998) investigated 2-thiothiazolidine-4-carboxylic acid (TTCA) concentrations as a biomarker of CS_2 exposure. They concluded that more emphasis should be placed on workplace protection factors rather than just addressing the indoor air CS_2 concentrations. Present findings also suggested that urinary concentrations can be a determinant of the workplace protection factor among workers using respirators and even among those who did not wear respirators and adhere to safety precautions in the workplace [35]. Moreover, the present finding depicted that CS_2 level was more in the first group of subjects in comparison with subjects of the second and controlgroups.

On the other hand, Jian and Hu (2000) investigated the mechanism of CS_2 action on the antioxidative stress mechanisms and measured enzymes linked to oxidative stress, including Cuprozinc-superoxide dismutase (CuZnSOD) and malonyl dialdehyde (MDA) levels in the serum of viscose rayon factory workers and in control subjects. The workers aged between 20 to 41 years and had been exposed to CS_2 from 2 to 14 years, with an average exposure of 8 years. They found a dose-response relationship. Serum MDA levels also increased in CS_2 exposed workers in both a concentration- and an exposure duration-dependent manner. The enzymes (i.e., CuZnSOD and MDA) may serve as biomarkers for worker's health surveillance to determine early stages of impairment of the anti-oxidative stress response resulting from CS_2 exposure (Jian and Hu, 2000).

Flow-cytometric measurements detected an induction of apoptosis among both experimental and control groups who occupationally exposed to CS_2 from viscose factory. Our results are in agreement with the findings of the in vitro experiments by Bao et al., (1996) demonstrating that higher doses of CS_2 induce apoptosis. Cell proliferation, as determined by flow-cytometric measurements of the S-phase and expression of CD71 and LI measurement, was not decreased in our study. However, in vitro experiments have shown a decrease in the viability and mitotic activity of cells at CS_2 doses inducing apoptosis.

These differences between the findings of the in vitro studies and our results may be attributed, among others, to differences in doses, duration of exposure, and differences in cell types. The slight decrease in apoptosis among workers who aged more than the mean age in the second group and exposed mainly to CS_2 may be a consequence of adaptation to CS_2 exposure. Mean of CA frequencies were significantly elevated in both groups of pathology personnel, in comparison with the controls. This increase is indicative of exposure to clastogenic agents among viscose industry workers. The mean of CA frequencies also significantly elevated in viscose factory workers in the second group who were partially exposed to CS_2. The CA-inducing effect found in our study can be attributed to CS_2 as a clastogenic agent. Our findings on the elevated CA frequencies in both groups of workers exposed to a mean ambient air concentration of 0.9 mg/m^3 CS_2 are in good agreement with most of the literature dealing with CS_2 induced CA (and/or SCE) among viscose industry workers [7, 9, 10] Cho et al., 2002; [6, 8, 11] Takebayashi et al., 2004; [5].

Moreover in vitro experiments [15] showed that CS_2 induces CA in PBL among viscose factory workers. Increased frequencies of CA in PBL were observed in a study by Le and Fu (1996), which is line with our findings, revealing elevated CA level in the PBL of the exposed workers. However, in the second group of the exposed participants aged more than the mean age, a significantly increased SCE was observed, but this increase could probably be due to the effect of smoking. In a study by Bao et al., (1996), no increase in the SCE frequencies in lymphocytes from CS_2 exposed viscose factory workers was observed. PCD and PCD/CSG, a disturbance in the process of mitotic division of chromosomes, were significantly increased in both groups, indicating an effect of genotoxic exposure inducing PCDs as suggested by previous studies.

The observation of higher frequencies of CA in the lymphocytes of exposed individuals agrees with the earlier reports by Le and Fu (1996) among viscose rayon workers. These are considered S-dependent alterations, frequently observed due to human chronic exposure to chemical mutagens. In the present study, CA increased with the increase of age and exposure period in the exposed groups. The results of the present study also indicated the role of age in the development of CA observed in PBL of controls.

In the present investigation, a notable CA was observed among the healthy controls. There was a significant difference between experimental and control participants who were occupationally exposed to CS_2. In past years, CS_2 concentrations in viscose rayon plants averaged about 250 mg/m^3; they were subsequently reduced to 50-150 mg/m^3 and more recently to below 31 mg/m^3 [36].

A report on hypospermia, asthenospermia, and teratospermia in young workers exposed to 40-80 mg/m^3 of CS_2 confirmed gonadal injury [37]. Le and Fu, (1996) showed that CS_2 induced chromosome aberration in human sperm. Numerous epidemiological reports have concluded that the CS_2 is toxicant to viscose industry workers

[16, 17, 38]. In this study, experimental participants with smoking habits experienced maximum levels of chromosome and SCE alterations when compared to respective controls, demonstrating that CS_2 exposure with cigarette smoking has a synergistic effect on inducing genetic damage. Chromosomal aberrations were shown to be good indicators of future risk of cancer [24]. On the other hand, genetic damages are the ultimate causes of cancer because DNA base changes can be mutagenic [25]. The present findings highlighted the importance of investigating the genotoxicity of CS_2 among viscose plant workers occupationally exposed to this organic solvent and also revealed that the smoking habit was associated with maximum levels of chromosome and SCE alterations.

The relation between exposure to CS_2 and the induction of apoptosis and changes in CA, PCDs and PCD/CSG needs further investigation, and more data from larger groups of CS_2 exposed individuals from different industries are also needed to analyze the confounding effects of smoking. Similarly, as in the present study, the investigated viscose factory workers were mostly men, further investigations are needed to collect data from women workers in order to study the possible effect of gender on CS_2 induced apoptosis and changes in the cytogenetic end-points.

In conclusion, the results of this study demonstrated that occupational exposure to CS_2 can induce apoptosis, CA and SCE on circulating fluids, indicating a possible excess cancer risk among exposed subjects. The multiple end-point genotoxicological monitors, developed and run in our laboratory, is able to detect changes in cytogenetic and cell proliferation biomarkers. The results emphasized the importance of personal safeguard at workplaces, with possible occupational exposure. Therefore, the aim of our study was to investigate the genotoxic effects associated with occupational exposure to CS_2 by analyzing apoptosis induction, CS, and SCE in circulated blood lymphocytes of workers associated with viscose industry and subjects living in the region surrounding these industries. However, the magnitude of this risk depends on the extent of exposure. Therefore, studies employing indicators for assessing the exposure and biological effects are strongly recommended.

Conflict of intersert

None declared

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