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Chromosomal alterations in circulated blood lymphocytes of personnel exposed with Carbon di-sulfide (CS_2)

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ABSTRACT

Circulated blood lymphocytes (PBL) of 41 workers occupationally exposed to Carbon di-sulfide (CS_2) from viscose industry were investigated to find a possible relationship between CS_2 and genotoxic effects. The subjects were divided into three groups: group I consisted of 41 subjects occupationally exposed to CS_2, another 41subjects who were nearby residents of viscose industry indirectly exposed to CS_2. About 41 subjects who were directly or indirectly exposed to viscose served as controls. Ambient air concentrations of CS_2 were measured in different work places. Measures of genotoxicity including the frequencies of chromosomal aberrations (CA) and sister chromatid exchanges were analyzed in group I, II and III subjects. The subjects occupationally exposed to viscose (group I) showed higher chromosome aberrations and sister chromatid exchanges, whereas in group II CA were slightly lower in comparison with group I which may be attributed to a different rate of elimination of damaged lymphocytes as a consequence of CS_2 induced apoptotic activity. In conclusion, the results demonstrate that exposure to CS2 induces apoptosis and CA, indicating an excess cancer risk among subjects occupationally exposed to CS_2. The results also emphasize the importance of the measurement of pollutants in the work place in order to avoid genotoxic effects to the workers.

Keywords: Carbon di-sulfide, sister chromatid exchange, chromosome aberration, occupational exposure, genotoxocity

INTRODUCTION

Carbon di-sulfide (CS_2) is naturally occurring chemical substances in the environment and also an important endogenous substance in the human body. CS_2 also an industrial toxicant and solvent mainly used in the viscose, rubber and chemical manufacturing industries (Fielder and Shillaker 1981). The largest use of CS_2 is in the viscose industry where it

is used to yield from alkali cellulose. Exposure to high CS_2 concentrations in these industries may cause severe effect to human body (Ruijten et al., 1990, 1993). Chronic exposure to CS_2 can cause eye, ear, cardiovascular, nervous and reproductive system disturbances (Vanhoorne et al., 1994; Huang, 2002; Sulsky et al., 2002; Tsai et al., 2002; Tan et al., 2004; Sills et al., 2005; Nishiwaki et al., 2004; Korinth et al., 2003).

Currently, exposure level of CS_2 is mostly below 31 mg/m^3, according to Guidotti et al (1999), Hoffman (1990) and Keil et al., (1996) short time exposure also cause 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 blood lymphocytes and human sperm samples (Liss and Finkelstein, 1996; Swaen et al., 1994; Tang and Xuan, 2003). 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. *Invitro* exposure of human sperm to CS_2 concentrations of 10 µmol/L resulted in increased occurrences of chromosomal aberrations (Le and Fu 1996). Furthermore the mutagenicity and genotoxicity potential of CS_2 has been evaluated *in vitro* and *in-vivo* experiments with crucial confounding factors (Wang et al., 1999; Wabg et al., 2000; Tang and Xuan, 2003; Manikantan et al., 2010). Previously CS_2 does not exhibit mutagenic activity in bacteria with or without the presence of activation system (Beauchamp, 1983). 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 including the lack of proper positive controls (Izmerov, 1983; Struwe, 1984).

Today, chromosomal alterations in human peripheral lymphocytes (PBL) are recognized as a valuable biomarker of effect of toxic chemicals, probably the only one which has been standardized and validated (Albertini et al., 2000) method to detect the genetic level damage.

Cytogenetic studies in occupationally exposed viscose industry workers resulted in genetic damage (Medeiros et al., 2003; Manikantan et al., 2010). Early identification of hazards is crucial to reduce the exposure and carcinogenic risk. A literature survey has revealed that no investigation has been conducted on this region of workers with different cytogenetic tools based on the different levels of CS_2 exposure.

The focal aim of present study was to identify genetic alterations of viscose factory workers occupationally exposed to CS_2, Tamil Nadu, South India by analyzing the structural chromosomal aberrations (CA).

MATERIALS AND METHODS

Selection of subjects

A total of 123 subjects from various viscose industries in Tamilnadu state especially Tiruppur and Erode districts were recruited. Subjects were divided into three groups, group I consisted of 41 subjects occupationally exposed to CS_2 in viscose industry, group II comprising 41 subjects occupationally not exposed to CS_2 but they are inhabitants near viscose industries, group III consisted of 41 subjects served as controls. In addition, subjects of group I further divided based on their duration of exposure (0–3; 4–6; 7–9; 10–12; 13–15 years) to CS_2. Group I subjects were exposed to viscose minimum 8 hrs per day. Group II subjects indirectly exposed to viscose because of their two decades of residence in and around viscose industries were further sub grouped based on the number of years of residence (20–30; 31–40; 41–50; 51–60 and 61–70 years). Control subjects were normal and healthy individuals who have not been exposed with any kind of chemicals and radiation hazards. Control subjects were matched to the age of exposed groups.

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 0.9mg/m^3, which exceeds the limits of both the short-term exposure (0.6mg/m3) and TWA-8 (0.6mg/m^3). All subjects complained about acute toxic effects (eye irritation) and the presence of CS_2 was perceivable in each industry.

Analysis of Chromosome aberrations

Whole blood samples were processed to study chromosome aberrations. In brief: 0.8 ml heparinized blood were cultured in duplicate at 37^{o}C, in 5% CO_2 atmosphere, in 10 ml RPMI-1640 supplemented with 20% fetal calf serum and 0.5% PHA without antibiotics.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 3.02 software. Differences between the study groups and the control group were tested with Student's t-test, $p < 0.05$ was considered as statistically significant.

RESULTS

The demographics pertaining to different groups of exposed and controls subjects are presented in Table 1. The mean age of occupationally exposed subjects was 31.05±2.14 (group I) whereas 42 ± 2.47 for the group II (residents in and around viscose industry). The average duration of occupational exposure to viscose range from 21.8 years (group I) and 17.7 years (group II).

The amount of CS_2 in the premises of industry was found to be higher than the amount found in and around the industry (Table 2). The workers occupationally exposed to viscose had higher concentrations of CS_2 in their urine than nearby residents and controls (Table 2). Further, CA analysis revealed that, the mean amount of aberrations was higher in occupationally exposed (group I) followed by nearby residents (group II) in comparison with controls (group III) (Table 3). It was

Table 1: Demographics of exposed (Occupationally and as nearby residents)

Particulars		No of Samples	Percentage (%)
Group I (Work Duration in Years)	0-3	8	19.5
	4-6	13	31.70
	7-9	11	26.82
	10-12	7	17.07
	13-15	2	4.87
Group II (Year of Residence)	20-30	11	26.82
	31-40	13	31.70
	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
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 CS_2 level in workplace environment and urine samples

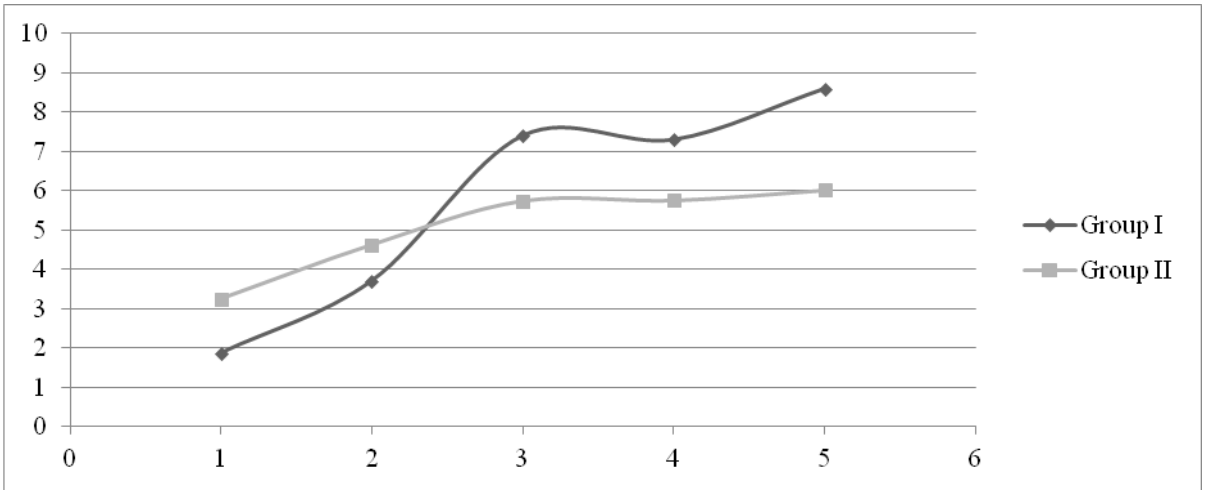
Groups	No. of Subjects	CS_2 amount in workplace mean±SD	CS2 content in urine mean±SD	Total CA (mean±SD)
I	41	1.42±0.004	1.82±0.84	5.41±2.48
II	41	0.054±0.002	1.01±0.46	4.08±3.16
III	41	0.021±0.004	0.54±0.39	1.41±2.04

Table 3: Chromosome aberration frequency and SCE in Group I subjects based on their duration of work

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

Table 4: Chromosome aberration frequency and SCE in Group II subjects based on their years 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



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

Figure 1: Graphical representation of CA value in Group I and Group II subjects

also observed that, with an increase of age and years of exposure the chromosome aberration and the mean frequency of sister chromatid exchange increases in group I subjects (Table 3).

A similar trend was also observed with group II subjects, where the chromosome aberrations and sister chromatid exchange increases with a corresponding increase in age and years of residence in group II subjects (Table 4, Fig. 1).

DISCUSSION

The results of the present study revealed cytogenetic alterations among viscose factory workers occupationally exposed to CS_2. Our study also observed considerable amount of CS_2 always present in the work place and the presence of CS_2 in the air was always observable by olfactory perception. Safety measures and devices were introduced in the last few years in these industries, and the employees are using these protective devices during their work. But 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 have considered 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 persons in the first group were almost exclusively exposed to CS_2, as they were situated near by these industries.

Furthermore, majority of effluent water released by the industries in the study area was mostly contaminated with CS_2 and some of the industries 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 are no longer in use. Based on the

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).

Occupational exposure may contribute to the development of pernicious illnesses, many times through mechanisms that involve genotoxic changes. Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposure and to monitor populations that are excessively exposed (Moller, 2006, Collins et al., 1997). The present study was designed to assess the genetic damage among viscose plant workers who are occupationally exposed to CS_2. CA 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 (Dusinska et al., 2008; Moller, 2006) of various in vitro and in vivo studies (Valverde, et al., 1998; Rojas et al., 1996; Fairbairn et al., 1995).

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/m^3 (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. A positive correlation or 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/m^3 current occupational exposure limit in viscose industry (Ghittori et al., 1998). Cox et al., (1998) investigated 2-thiothiazolidine-4-carboxylic acid (TTCA) concentrations as a biomarker of CS_2 exposure. The observation of CS_2 in the urine samples of workers suggests that more emphasis should be placed on workplace protection factors rather than just addressing the indoor air CS_2 concentrations. Present findings suggest us that, workers should be insisted to wear protective equipment's like respirators and follow safety precautions recommended by relevant authorities.

Certain *in vitro* experiments (Le and Fu, 1996) clearly showed that CS_2 induces CA in PBL among viscose factory workers. An increased frequency of CA in PBL was observed in the study of Le and Fu, 1996 which is in line with the results of our study. However, in group II subjects, an increase in SCE frequency was observed. A possible explanation for this may be due to smoking habits of the subjects. Because a study (Bao et al., 1996) reported no increase in SCE frequency among subjects exposed to CS_2.

The observation of higher frequencies of CA in the lymphocytes of exposed individuals agrees with the earlier reports Le and Fu 1996 regarding viscose rayon workers. These are considered S-dependent alterations, frequently observed due to human chronic exposure to chemical mutagens. In the present study, CA was observed to increase with age and exposure period in the exposed groups and based on age in the controls, though it was very low in the latter. The results of the present study also indicate a role played by age in the development of CA observed in PBL of controls.

A report on hypospermia, asthenospermia and teratospermia in young workers exposed to 40-80 mg/m^3 of CS_2 confirmed gonadal injury (Lancranjan et al., 1969). Le and Fu (1996) showed that the CS_2 induce chromosome aberration in human sperm. Numerous epidemiological reports concluded that the CS_2 is toxicant to viscose industry workers (Guidotti and Hoffman, 1999; Wang et al., 1999, Wang and Shiu, 2000). In this study, experimental subjects with smoking habits showed maximum levels of chromosome and SCE alterations when compared to respective controls, which shows that the CS_2 exposure with cigarette smoking has synergistic effect on inducing genetic damage. Chromosomal aberrations were shown to be good indicators of future risk of cancer (Hagmar et al.,1994). On the other hand genetic damages are the ultimate causes of cancer because DNA base changes can be mutagenic (Poirier, 1997). The present findings highlight the importance of investigating the genotoxicity of CS_2 on viscose plant workers occupationally exposed to this organic solvent when the smoking habit is associated, since this information provides an increased degree of identification for the positive response.

The relation between exposure to CS_2 and the induction of CA 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 maximum men only, 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..

Conflicts of interest: The authors stated that no conflicts of interest.

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