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Studying the effect of antioxidants on cytogenetic manifestations of solvent exposure in the paint industry

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Abstract

Objective: To investigate the antioxidant role in reversing cytogenetic changes caused by solvent exposure in paint industry. **Subjects and Methods:** A prospective controlled clinical trial was performed on 39 workers exposed to solvents and 39 workers not exposed to solvents by supplying a mixture of antioxidant vitamins (A, C, E and selenium) and the after effects of such regimen were analyzed. Environmental monitoring was carried out for air concentrations of different solvents at workplace. Exposed group was cytogenetically tested before and after giving the mixture of antioxidant vitamins for 1 month duration. **Results:** Frequency of chromosomal aberrations (CAs) and the mean of sister chromatid exchanges (SCEs) were statistically significantly higher among exposed workers than among controls. After the supplementation of antioxidants, there was a statistically significant decrease in the frequency of CAs, and 88% abnormal levels of SCEs were back to normal levels. **Conclusion:** Antioxidant supplementation decreases the frequency of CAs and SCEs among exposed workers.

Keywords

Organic solvents, paint industry, cytogenetic damage, antioxidants vitamins

Introduction

Paint industry is one of the most important industries all over the world. In Egypt, paint industry is one of the main industrial activities, which use solvents. According to the Egyptian Chemical Industries Chamber – Division of Paints, Inks & Resins (CCIE, 2008), there are 24 paint companies in Egypt, which produce 613 tons of paints/year and consume 40–50 tons of solvents/year in their industrial processes. Solvents used in paint industries represent about 45% of the total amount of solvents needed for the industry in Egypt. Thus, it is important to assess the health hazards on workers exposed to solvents in paint industry. One of the most important – yet not well studied – hazards are the cytogenetic hazards associated with occupational exposure to organic solvents. Different studies (Gajalakshmi et al., 2002; Gonzalez-Yebra et al., 2009 and Neto et al., 2009) found that chromosomal aberrations (CAs) were related to the duration of exposure among workers exposed to organic solvents. Epidemiological studies (Bonassi et al., 2000; Hagmar et al., 2004; Norppa, 2004) suggested that high frequency of CAs is predictive of an increased risk for cancers. Various studies on animals or in vitro have proven that antioxidants can provide protection against several forms of DNA damage (Fang et al., 2002; Turner et al., 2002 and Weiss and Landauer,

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2003); however, few experimental studies investigated the protective role of antioxidants in the field of occupational health (Elsayed and Gorbunov, 2003; Jacques-Silva et al., 2001; Qureshi and Mahmood, 2010; Ramanathan et al., 2003). The intake of antioxidants can neutralize reactive oxygen species, and it has been investigated in relation to DNA damage and cancer risk (Fang et al., 2002; Tapiero et al., 2004; Traber and Atkinson, 2007). Experimental studies (Aly and Donya, 2002; Siddique et al., 2005, 2007; Szeto et al., 2002) have shown that vitamins C and E are among the best known antioxidants used to inhibit CAs and sister chromatid exchanges (SCEs). The role of using antioxidants to ameliorate cytogenetic changes among workers exposed to solvents was not thoroughly studied before. The aim of this study is to investigate the genotoxic effects of exposure to solvents in paint industry and the potential ameliorating effects of antioxidants on these changes. To execute this task, we conducted a clinical trial by supplying a cocktail of antioxidants (vitamins A, C, E and selenium) known to prevent the chromosomal abnormality and analyzed the effect of such regimen.

Subjects and methods

Study population

Our target was a paint production factory that uses solvents in its industrial processes. This study has been conducted in a major paint production company in Cairo, Egypt, during the period from December 2009 to January 2010. The study was conducted on two groups: an exposed group and a control (nonexposed) group. The first group consisted of 39 male workers occupationally exposed to solvents. The control group included 39 male workers in other departments of the company, who have never been occupationally exposed to solvents. The control group was matched for age, sex, smoking habits and socioeconomic status. Our study is a prospective controlled clinical trial. The total number of working population exposed to solvents during the industrial processes of paint preparation was 263; however, only 190 workers from three shifts within the factory accepted to participate in this study. A simple randomization was carried out using software to enroll 40 workers into the study, and only one worker was dropped out from the study upon his request due to non-compliance with administering the antioxidant doses. The inclusion criteria were workers working in the company for at least 6 months, who were exposed to organic solvents; and the exclusion criteria were workers exposed to radiation – receiving radiotherapy, receiving treatment with chemotherapeutic drugs or had infectious diseases during the last 6 months. For the control group they had the same criteria above except for being not exposed to solvents.

Methods

The study protocol was first approved by the Ethics Committee for Research in the National Research Centre. The study has been performed in accordance with the ethical standards laid down in 1964 Declaration of Helsinki. All participants gave their written informed consent, and the ethical guidelines of good clinical practices were followed during the study.

Environmental monitoring

Environmental monitoring was carried out for air concentrations of different solvents (workplace air sampling). Measurements were done in the Reference Laboratory – Faculty of Science – Ain Shams University, Cairo, Egypt; the laboratory is certified by ISO 17025 (Table 1). The factory is composed of two compartments: administrative and production. The production compartment contains multiple production units as follows: (1) newspaper ink production unit, (2) paste production unit, (3) liquid ink production unit, (4) mixing and grinding unit and (5) finishing and packaging unit. Measurements were taken during shift time from different production units. Three measurements were taken from each unit and the mean values were calculated. Samples of indoor air were collected in the production units by active sampling on 8×110 mm^2 adsorbent tubes containing activated charcoal at a flow rate of 200 mL/min, using an air sampling pump with electronic flow control. The flow of the pump was calibrated using a mini-BUCK Calibrator M-30 Electronic Primary Gas Flow Standard, United States. After 4 h, the sampling was stopped by placing caps on both ends of the tubes. The tubes were covered with aluminium foil and stored at 4°C until analysis (Scheepers et al., 2010). The measurements from all production units were compared with the maximum allowable exposure limits according to the Egyptian Environmental Law 4 for the year 1994 (EEAA, 1994).

Questionnaire and clinical examination

Thorough literature review of both recent and old studies was carried out to determine the study questions

Table 1. Air levels of different solvents at work place.

Solvents vapour (mg/m^3)	Newspaper ink production unit	Paste production unit	Liquid ink production unit	Mixing and grinding unit	Finishing and packing unit	MAC[a]
Toluene	2.8	11.3	12.2	116	35	188
Xylene	12.4	31.4	122	79.5	50	434
Ethyl acetate	–	18.6	20.9	31.3	9.1	1440
Butanol	3.1	7.1	11.2	61.8	63.2	303
Isopropyl alcohol	3.3	19.1	40	142.5	50.9	983
Ethanol	14.6	16	11.1	–		1880
Acetone	–	0.7	4.2	–		1187
Total hydrocarbons	523	–	1300	1199	394	1800

[a]Maximum allowable concentrations according to Egyptian Environmental Law 4 (EEAA, 1994).

that would be comprehensive and objective at the same time; then a questionnaire was developed in English language, which was then translated into Arabic language. The questions were designed to elicit objective replies by the workers. Every question was assigned a code for statistical analysis. The questionnaire included questions about age, sex, smoking, education, marital status, duration of employment, job title, use of chemicals, use of personal protective equipment, engineering controls, workplace conditions and health status. The questionnaire was designed to evaluate the health status of the workers with regard to occupational exposure to solvents. The participants were interviewed in Arabic and clinically examined by a specialist in occupational medicine.

Cytogenetic studies on blood samples

After informed consent was obtained, peripheral blood samples from all the participants were collected at the end of the working shift, by the occupational medicine specialist, before and after antioxidant supplementation, in heparinized tubes. Using venepuncture, we collected 5 mL of blood in heparin tubes for the CA tests to analyze for deletion and breaks in DNA and SCEs. All blood sample tubes were coded and transported in dark in less than 3 h to the laboratory and processed for immediate analysis upon arrival.

Methodology for cytogenetic analysis

A total of 39 subjects were cytogenetically tested before and after giving antioxidants. Conventional structural CA analysis was carried out according to the method followed by Verma and Babu (1995), a 48-h lymphocyte cell culture was initiated using F-10 medium (HAM's medium) obtained from GIBCO, enriched with 20% calf serum. Phytohaemagglutinin (FITO KRKA, Cairo, Egypt) served as a mitotic stimulator and 5 ug/mL of bromodeoxyuridine (BrdU) (SIGMA, Cairo, Egypt) was added into each culture in order to enable the detection of first division cells. At the final preparation stage, the slides were stained by 5% Giemsa solution and then analyzed microscopically. An experienced microscopist scored 100 metaphases/person for CAs from the slides. Metaphase spreads (25–30 cells) were analyzed and any structural or numerical anomalies were recorded. For SCE detection, 72-h lymphocyte cell culture was carried out according to the standard procedure (Verma and Babu, 1995). In order to enable exact SCE visualization, 10 μg/mL of BrdU were added into each culture. After the appropriate Giemsa staining, SCEs were counted either under the microscope or from the photographed cells. SCEs were scored only in cells that had 46 chromosomes. Usually 25–30 complete cells were analyzed from each case and SCEs/metaphase were scored (Kato, 1974).

Antioxidant supplementation

The exposed subjects were instructed to take a daily dose of antioxidants: vitamin A (500 IU), vitamin C (60 mg), vitamin E (30 IU), selenium (100 μg), folic Acid, zinc and iron (Antox – Mepaco Company, Egypt), which is the most preferable prophylactic drug prescribed by physicians, at the present time. The supplementation was taken on a daily basis in the form of capsules for 30 days' duration under the supervision of the health supervisor of the factory. This regimen is corresponding to the one studied by Gaziev et al. (1996) who gave a mixture of antioxidant supplementation to volunteer donors. After 1 month of antioxidants administration, the blood

Table 2. Frequency of Chromosomal Aberrations (CAs) manifested among both exposed workers and controls.

		Group					
		Control *N*(39)		Exposed *N* (39)			
		N	%	*N*	%	χ^2	*p* Value
CAs	Normal	35	89.7	30	76.9	0.26	0.046
	Abnormal	4	10.3	9	23.1		

Table 3. Mean ± SD of Sister Chromatid Exchanges (SCEs) among studied groups and SCE before and after administration of antioxidants.

	Group	*N*	Mean ± SD	*t* Test	*p* Value
SCE	Control	39	7.16 ± 1.50	−2.47	0.01
	Exposed	39	8.51 ± 3.05	7.26	<0.001
Exposed	SCE (before)	39	8.51 ± 3.05		
Workers	SCE (after)	39	5.96 ± 1.68		

samples were collected for the follow-up of cytogenetic studies.

Data entry

The results of baseline and follow-up cytogenetic studies were tabulated in Microsoft Excel 2007. Data were verified and then validated twice to ensure accuracy.

Statistical analysis

Data obtained from the study were coded and entered using the statistical package SPSS version 16. The mean values, standard deviations and ranges were then estimated for quantitative variables; as for the qualitative variables, the frequency distribution was presented. Comparisons between exposed and control groups were carried out using the independent simple *t* test. The correlations between individual variables were calculated using Pearson correlation coefficient. p Values < 0.05 was considered statistically significant.

Results

The exposed group consisted of 39 male workers occupationally exposed to solvents; their mean age was 50.7 ± 4.6 years, with an employment duration of 25.9 ± 6 years. The control group (nonexposed) included 39 male workers from other departments of the company. Their mean age was 49.5 ± 4.9 years. There was no statistically significant difference between exposed and control (nonexposed) group as regards the age ($p = 0.27$). The frequency of smokers among exposed workers and control group was 23 (59%) and 25 (64.1%), respectively, and there was no statistically significant difference. Table 1 shows the measured levels of different solvents in the working atmosphere. The total hydrocarbon levels were higher in the liquid ink production unit (1300 mg/m^3) and in mixing and grinding unit (1199 mg/m^3) than the rest of the units involved in the production line. All air concentrations were below maximum allowable concentrations according to the Egyptian Law and International Regulations. Among exposed workers, majority of them (76.9%) used personal protective equipment (PPE; gloves, masks and aprons). The frequency of CAs was statistically significantly higher among exposed workers (before taking antioxidant supplementation) than that in controls (Table 2). There was a statistically significant increase in the mean SCEs among the exposed workers (before taking antioxidant supplementation) compared to controls (Table 3). Also, there was a positive correlation between age and duration of exposure with both CAs and SCEs before supplementation with antioxidants, but it did not reach the statistically significant values (Table 4). A statistically significant decrease was observed in the frequency of CAs among exposed workers by 11% after supplementation of antioxidants, that is, one out of nine workers exposed to solvents showed decrease in the CA number, meaning that there is amelioration in the frequency of CAs after supplementation of antioxidants. Also, after

Table 4. Correlations between age and duration of exposure with chromosomal Aberrations (CAs) and sister chromatids exchange (SCEs) before supplementation of antioxidants among exposed workers.

	Age		Duration of exposure	
	r	*p* Value	*r*	*p* Value
CAs (before) $N = 39$	0.106	0.523	0.166	0.313
SCE (before) $N = 39$	0.175	0.286	0.141	0.392

Table 5. Frequency of chromosomal aberrations (CAs) and sister chromatids changes (SCE) among exposed workers before and after supplementation of antioxidants.

		Before antioxidants $N = 39$	After antioxidants $N = 39$
CAs	Normal	30	31
	Abnormal	9	8
SCEs	Normal	14	36
	Abnormal	25	3

supplementation of antioxidants, 22 out of 25 abnormal cases of SCEs, that is, 88% returned back to normal levels Table 5).

Discussion

As the main interest of this work is to study the effect of antioxidants on cytogenetic changes caused by solvent exposure, the studied groups were compared for exclusion of confounding factors as age, sex and smoking habits. Statistical analysis revealed that no statistically significant difference as regards the age and smoking status between exposed workers and controls. Exposure to solvents was considered to be the only factor that is supposed to contribute to our findings in the cytogenetic testing. In the current study, although the air concentrations of different solvents in the working atmosphere are within maximum allowable concentrations according to the Egyptian law, we evaluated the genotoxic effects of actual exposure conditions in paint industry, where workers were directly exposed to a complex mixture of organic solvents. In the present study, the frequency of CAs were statistically significantly higher; also, there was a statistically significant increase in the mean of SCEs among the exposed workers compared with the controls. Our results agreed with Pinto et al. (2000) who found that CAs and SCEs were significantly elevated in 25 public building male painters exposed to organic solvents when compared to a similar number of age- and gender-matched controls. Also Kim et al. (2004 and 2008) reported that the frequencies of CAs and micronuclei (MN) were significantly higher in workers exposed to benzene at a petroleum refinery than unexposed controls after adjusting for age, sex, smoking status and alcohol intake. Recently, Hoyos-Giraldo et al. (2009) investigated car painters exposed to thinners where organic solvents were used as diluents. They detected a significant increase in the frequency of CAs among exposed workers, representing a higher risk, in relation to the matched referent. Our findings of positive correlation between the prevalence of CAs with the duration of solvent exposure did not reach the level of statistical significance. However, Gonzalez-Yebra et al. (2009) and Burgaz et al. (2002) detected cytogenetic damage as frequency of MN in buccal cells among shoe workers exposed to mixture of organic solvents, such as *n*-hexane, toluene and methyl ethyl ketone. In these studies, cytogenetic damages were statistically significant associated with the duration of exposure. On the contrary, Pitarque et al. (2002) found different results when studying 52 Spanish female workers exposed to organic solvents such as toluene, gasoline and acetone, in two shoe factories. They compared them with 36 unexposed age- and sex-matched referents. They found no statistically significant difference between the groups as regards the SCE analysis. These contradictory results, to our findings, may be attributed to the difference in sex and the duration of exposure. The levels of air concentrations of solvents in the paint factory did not exceed the regulated maximal allowable concentrations according to the Egyptian Law 4 for the year 1994 for the environment and according to international regulation. However, in our study, the frequencies of CAs and SCEs in the exposed workers compared with the controls were significantly higher. Also, assessment of the frequency of using PPE among exposed workers within the production units revealed that the majority of workers (76.9%) were using PPE. This can be attributed to the understanding among workers that exposure to a mixture of organic solvents for long durations can have cytogenetic impacts. Many

workers can work for extra hours/day. There was no regular maintenance of PPE, which causes low or no functionality of PPE and increases the exposure to solvents for these workers. CAs in lymphocytes are considered as an end point in carcinogenic progress and are the best validated cytogenetic biomarkers to predict cancer risk (Albertini et al., 2000; Bonassi et al., 2000; Hagmar et al., 2004 and Norppa, 2004). Therefore, the current study gives a better cancer risk insight in relation to exposure to organic solvents, which may help to avoid future cancers. This study is an attempt to provide a mean for medical intervention in preventing the risk of genetic damage among workers exposed to organic solvents. We investigated the role of antioxidant supplementation in reversing cytogenetic changes caused by solvent exposure in paint industry. In the current study, there is a statistically significant decrease in the frequency of CAs among exposed workers by 11% after administration of antioxidants, that is, one out of nine workers exposed to solvents showed decrease in the frequency of CAs. After antioxidant supplementation, in 22 (i.e. 88%) out of 25 abnormal cases the levels of SCE were back to normal.

Our results are in accordance with those of Gaziev et al. (1996) who investigated the effect of consumption of a vitamin–antioxidant mixture (VAM) on the frequency of spontaneous and in vitro γ-radiation-induced MN in peripheral blood lymphocytes in the donors of various ages. The donors of groups took VAM containing the vitamins A, C and E as well as β-carotene, folic acid, and rutin daily for 4 months. They suggested that consumption of VAM favours a decrease in the chromosome damage produced by endogenous and exogenous factors in human lymphocytes; however, in our study VAM were given daily for 1 month. Also, Velanganni et al. (2007) and Velanganni and Balasundran (2010) investigated the preventive effects of antioxidant vitamins A, C, E and their analogues against DNA damage induced by a hepatocarcinogen *p*-dimethylaminoazobenzene (DAB) as assessed using comet assay on groups of rats. They concluded that administration of high doses of vitamin A, L-ascorbic acid and vitamin E succinate individually prevented the DNA damage. However, administration of a mixture of these vitamins at low doses prevented the DAB-induced DNA damage, which may be due to their synergistic effect. The results indicate that there is a significant advantage in mixed vitamins therapy at low dose compared to the treatment with individual vitamin. In the occupational field, there is limited human data supporting these associations; however, recently, Horska et al. (2011) investigated the possible interactions between polymorphisms in glutathione S-transferase (*GST*) genes and plasma vitamin C, tocopherols and carotenoids in 149 reference subjects and 239 subjects occupationally exposed to mineral fibres that induce oxidative stress. They concluded that the correspondence of lower vitamin C levels with non-functional GST isoenzymes may indicate a causal connection between antioxidant defence pathways, and they concluded that the underlying mechanism is not yet clear. Also Wilhelm et al. (2010) measured several enzymatic biomarkers (such as glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD)) and non-enzymatic (such as protein carbonyls (PC), protein thiols (PT), α-tocopherol (AT), reduced glutathione (GSH)) of oxidative stress in the blood of coal mining and incineration of solid residues of health services (SRHS), which generate several contaminants that are delivered into the environment. They gave vitamin E (800 mg/day) and vitamin C (500 mg/day) supplementation to the study participants for 6 months, and the results were compared with the situation before antioxidant intervention. Plasma lipid peroxidation thiobarbituric acid reactive substance and protein carbonyls concentrations that were elevated before antioxidant intervention decreased after the antioxidant supplementation. Similarly, the contents of α-tocopherol and GSH, which were low before antioxidant intervention, reached values nearer to those found in the controls. The results showed that the oxidative stress condition detected prior to antioxidant supplementation in both directly and indirectly exposed subjects to the airborne contamination from coal dusts and SRHS incineration was attenuated after antioxidant intervention.

From our study, we concluded that antioxidant supplementation ameliorates the frequency of CAs and SCEs in exposed workers. This study indicates the importance of antioxidant supplementation on a regular basis to counteract the cytogenetic impacts of solvents on workers in the paint industry and its possible protective role against cancer vulnerability in those workers. So, our results could help in establishing surveillance strategies and prevention programmes that will be of utmost importance for more precise regulations for exposure to organic solvents and perhaps decreasing the threshold limits stated in the Egyptian legislations. We recommend further studies with longer follow-up of larger cohorts of workers

with a wide range of exposure levels to support our findings and provide a better understanding of genotoxicity due to solvent exposure.

Conflict of interest

The authors declared no conflicts of interest.

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