Research Article

Chromosomal aberrations and Oxidative stress induced by Occupational exposure to organic solvents: Role of antioxidant supplementation

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ABSTRACT

Previous studies have demonstrated that organic solvents could induce genotoxicity *in vivo* and *in vitro*, and oxidative stress may be an important factor involved. It was also suggested that antioxidant supplementation could ameliorate some of the physiological changes caused by DNA oxidation damage. In the current study, the effect of chronic exposure to organic solvent on chromosomes and enzymatic antioxidant system was assessed. Moreover, the present work was designed to elucidate the protective role offered by antioxidant supplementation to workers at risk. The results demonstrate that the percentage of chromosomal aberrations in general and mean level of MDA were significantly higher and those of GPx and SOD were significantly lower among the exposed groups in comparison to the controls. Chromosomal aberrations' percentage and MDA level showed significant positive correlations with the duration of work and hydrocarbon exposure score (HES), while significant negative correlations were found with GPx &SOD levels. After antioxidant administration, our results showed a significant decrease in CAs percentage &MDA level and increase in the activity of GPx & SOD in the exposed groups. The last results indicate that chronic exposure to OSs results in chromosomal damage and increased lipid peroxidation which might play an important role in malignancy. Also, we concluded that, antioxidants supplementation can ameliorate oxidative stress and genotoxicity in workers at risk.

Keywords: Antioxidants, Chromosomal aberration, Organic solvents, Oxidative stress.

INTRODUCTION

Organic solvents (OSs) have various effects on human health, whether the exposure is by vapour, mist or liquid form. In fact, about 50% of the synthesized organic solvents are employed for the production of paints and thinners. From the different groups of organic solvents, xylene, toluene, styrene, ethylbenzne, acetone and methyl ethylketone are considered to be some of the most frequently and quantitatively represented solvents in the composition of paints.[1] According to the Egyptian Chemical Industries Chamber Division of Paints, Inks & Resins, there are 24 paint companies in Egypt producing about 613 tons of paints/year and hence consuming nearly 40 – 50 tons of OSs/year in their industrial processes.[2] It is well documented that several OSs are potent carcinogens among population at risk and their genetic effects have important implications for cancer induction. Several studies have suggested that induction of chromosomal aberrations (CAs) may play a role in solvent's induced carcinogenesis, and considered the high frequency of CAs is a predictive of an increased risk of cancer.[3-5]

Genotoxicity could be proposed to be through excessive and persistent formation of reactive oxygen radical species (ROS) inducing lipid peroxidation and decreasing endogenous antioxidants in the body such as reduced superoxide dismutase and glutathione peroxidase (GPx), causing oxidative stress. Cytogenetic hazards associated with occupational exposure to OSs & their control is one of the most important, yet not well assessed hazards.[6-9] It was proven that antioxidants supplementation can provide protection against several forms of DNA damage.[10,11] However, few studies investigated the protective role of antioxidants in the field of occupational health.[12] The intake of antioxidants can neutralize ROS , preventing DNA damage and cancer risk.[13] Experimental studies have shown that vitamin C and E are among the best known antioxidants used to inhibit chromosomal aberrations and sister chromatid exchanges , however the role of using antioxidants to ameliorate cytogenetic changes among workers exposed to solvents was not thoroughly studied before.[14]

The aim of this study is to assess genotoxicity and oxidative stress resulting of chronic occupational OSs exposure in paint industry. Also, to investigate the ameliorating effect of a relatively long time intake of antioxidants for those changes in exposed workers.

MATERIALS AND METHODS

Subjects

This work was conducted in 2 major companies for paint production in Egypt. The study included 3 groups: the first included 28 males of a paint production company in Cairo (Group 1). The 2nd were 29 males of another one in Giza (Group 2). Workers exposed to OSs for at least 5 years were included**.** Work in both companies was proceeding for about 5 hours /day in semi closed big chambers with

no proper ventilation. Protective devices were worn occasionally during work.

The control group consisted of 25 males, who have no past or present history for exposure to OSs. All groups were matched for age, sex, smoking habits and socioeconomic status. The total number of exposed working force was 263 in the first company and 270 in the second; however, only 191 and 198 workers of both factories respectively accepted to participate in this study. A randomization stage then followed; simple randomization was done using software to enroll the 2 exposed workers groups in the study. Those who were taking regular medications, had infectious diseases or exposed to any sort of radiation during the last year before sampling were excluded from the 3 groups.

Industrial processes

Both exposed groups are exposed to a mixture of OSs, mainly aromatics like xylene, toluene, and pigments during different steps of industrial processes of paint production which could be summarized in the following:

Mixing process

In which the worker is exposed to solvents while filling the mixing containers with the raw materials, where he is standing near the open mixer machine to monitor and adjust the process of mixing.

Grinding Process

Where the workers can be exposed to significant solvents' vapors as the entire process is open and the used roll mill machine produces some heat during work, permitting evaporation of a significant amount of solvents during that process.

Finishing Process

The worker is exposed to solvents as in mixing process, because most of the solvent content of paints is added during this step.

Packaging Process

While the worker pours the paint into packages, he is exposed to solvents, as the whole process of packaging is manual and open.

Cleaning Processes

Workers are using solvents to clean machines on a regular basis.

Methods

The study protocol was first approved by the Ethical Committee of National Research Centre and all participants gave their written informed consent. All Participants were interviewed and subjected to a detailed questionnaire including detailed medical and occupational histories. A through clinical examination was done for each one. For the three studied groups, the following was done at the start of the study and repeated again for groups 1&2 after antioxidants intake by group 2:

Study of chromosomal aberrations

The CA analysis was conducted following a standard protocol. A total of 1 ml aliquot of whole blood was cultured in F-10 medium supplemented with 20% fetal bovine serum, 0.5 ml PHA, 5000 IU/ml penicillin and 1000 IU/ml streptomycin. Each culture was incubated at 37°C for 27 h. metaphases were obtained by adding 0.2 ug/ml colchicines to the cultures 3 h before harvesting, cells were collected by centrifugation, re-suspended in a pre-warmed hypotonic solution (0.075 M KCL) for 15 min at 37°C and fixed in acetic acid : methanol (1:3 v/v). Chromosome preparations were stained with 3.3% Giemsa stain. The slides were analyzed at 1000 magnification using a light microscope. One hundred metaphases cells were screened per each individual. Cells with 46 chromosomes were scored for CA. The analysis of CA included chromatid and chromosome breaks, chromatid deletions, chromatid rings and di-centric chromosomes according to the method of Verma and Babu.[15]

Activity of Glutathione peroxidase (GPx)

The activity of GPx in serum was measured spectrophotometrically. The enzyme reaction was initiated by the addition of H_2O_2 to the reaction medium and the rate of NADPH oxidation was followed at 340 nm. The amount of enzyme that oxidizes 1 mol NADPH per minute was considered to be one unit.

Activity of Superoxide dismutase (SOD)

This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.[16] We used 0.5 ml of heparinized EDTA whole blood then centrifuge for 10 minutes at 4000 rpm and the plasma was removed, we determined the activity of the enzyme in the erythrocyte which was washed four times with 0.9% NaCl. Then a 25 fold dilution of lysate was used in the assay.

Malondialdehyde (MDA)

MDA was measured in the serum by the method of Ohkawa.[17] The principle of the method is that thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product, the absorbance of the resultant pink product can be measured at 534 nm. The concentrations of MDA were expressed in nmol/ml.

Assessment of exposure to Organic solvents

The lifetime hydrocarbon exposure score (HES), was estimated. It equals the product of intensity of exposure (coded 2, 1 and 0.5) and lifetime hours of exposure. The units were, then, arbitrary ones which represent hours weighted by the exposure intensity factor. As a general rule, exposure to hydrocarbons while working indoors without protection was given an intensity factor 2, while

working indoors with protection or outdoors without protection was allocated an intensity factor1. Exposure to outdoor activities with protection was given an intensity factor 0.5.Therefore, the solvent –exposed workers were classified into low exposure group (LEG), and high exposure group (HEG) with the score value of 32500/32501 taken as a cutoff point.[18]

Antioxidant supplementation

The exposed subjects 1n the 2nd, factory (group 2) 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 & Iron (a constituents of an Egyptian preferable prophylactic drug described by physicians). The supplementation was taken on daily basis in the form of capsules over 6 month's duration under the supervision of the health supervisor of the factory. That regimen is corresponding to Gaziev et al., *1996*[19], who gave a mixture of antioxidants supplementation to volunteers. After 6 months of administering antioxidants to group 2, blood samples were collected again for the follow-up of oxidative stress and cytogenetic changes in both group 1 and 2.

Statistical analysis

Data were collected and statistically analyzed. Quantitative data were compared using t-test and for the qualitative data chi-square test x2 was used. Statistical difference p< 0.05 was considered a significant difference and the p< 0.01 was highly statistically significant. Those with p > 0.05 were not significant.

RESULTS

Table 1 shows that there was no statistical significant difference between both exposed groups when each compared to the control concerning their ages and smoking habits. Also, both exposed groups showed no statistically significant difference compared to each other as regards their ages smoking habits and, mean duration of work.

As seen in table 2, the percentage chromosomal aberrations in general and the mean levels of the different types of CAs namely chromatid break, chromosome break, dicentric chromosome, chromosomal deletion and ring chromosome, were higher among both exposed groups in comparison to the controls. The differences were statistically highly significant P < 0.01. Also, it is apparent of the same table that although the percentage chromosomal aberrations and the mean levels of the different types of CAs were slightly higher in group 2 compared to group 1; yet the differences were not statistically significant P > 0.05. The mean level of GPx and SOD was significantly lower while that of MDA level was significantly higher in the exposed groups when each compared to controls. In the meanwhile they showed no significant difference when both exposed groups compared with each other P > 0.05.

The solvent exposed workers (Gr.1&2) were classified according to HES to LEG, and HEG (Table 3). The mean levels of CAs % and MDA were higher and those of GPx and SOD were lower in HEG with HES (32501 –72500) compared with those of LEG with HES (2500 - 32500). The differences were statistically significant (P < 0.05).

Chromosomal aberrations percentage showed a significant +ve correlation with the duration of work (P < 0.05) and highly significant one with HES and MDA level. At the same time it showed a highly significant –ve correlation and GPx and SOD (P < 0.01). A significant –ve correlation was found between GPx & SOD and the duration of work (P < 0.5). A highly significant –ve correlation was found between GPx and SOD on one side and HES & MDA on the other (P < 0.01), Table 4.

It is quite evident from table 5 that after antioxidant administration by group 2, the percentage of chromosomal aberrations in general and the mean levels of the different types of CAs & MDA were much lowered in that group while those of Group 1 who are still exposed with no antioxidant intake were much increased "compared to the values found at the start of the study seen in table2". On comparing both groups 1&2, the differences were statistically significant P < 0.05.

Moreover, the mean levels of GPx & SOD were much increased in Group 2 and lowered in Group 1. The differences were statistically significant P < 0.05 for SOD and highly statistically significant GPx for (P < 0.01) when both exposed groups compared to each other.

Table 1: Characteristics of the studied population

Parameter	Group 1 (n=28)	Group 2 (n=29)	Control (n=25)
Age in years (mean ±SD)	43.33±7.3	43.52±6.8	42.11±8.01
Smoking Habits Smoker (no & %) Non Smoker (no & %)	17 (60.71%)11 (39.29%)11	17 (58.62%) 12 (41.38%)	15(60%) 10(40%)
Duration of Exposure to OSs in years (mean ±SD)	19.5±2.8	19.8±2.91	----

DISCUSSION

Chromosomal aberrations (CAs) in lymphocytes are considered as an end point in carcinogenic progress and are the best validated cytogenetic biomarkers to predict cancer risk. The physical discontinuity of the chromosome may cause loss of genetic information and even cell death if a housekeeping gene is involved [1, 20]. It was reported that the high frequency of CAs is a predictive of an increased risk of cancer, and it plays an important role in solvent's induced carcinogenesis (4&5). In our work not only the percentage total chromosomal aberrations but also, the mean levels of the different types of CAs namely chromatid break, chromosome break, dicentric chromosome, chromosomal deletion and ring

chromosome, were statistically significantly higher (P < 0.01) in both exposed groups when each was compared to the controls (table 2).

Our results were greatly supported by those of others, on their studies on paint formulating workers and public building painters[21] and in workers exposed to organic solvents,[22] who found a significant elevation of chromosomal aberrations (CAs) and sister chromatids exchanges when compared to a matched controls.

Moreover, similar results were reported by Gonzalez-yebraet al[8], who found a significant increase in the frequency of different CAs among shoe workers exposed to OSs. Our findings are, also, in accordance with the findings of Hoyos-Giraldo et al[23] in workers exposed to OSs in petroleum refinery compared to their controls. Furthermore, the same results were detected in car painters exposed to thinners where organic solvents were used as diluents.[5]

Table 2: Chromosomal aberrations% "CA %", GPx, SOD and MDA in the studied groups

Parameter	Group 1(n=28) Mean ± SD	Group 2 (n=29) Mean ± SD	Control (n=25) Mean ± SD
Chromatid break	5.89±3.91**	6.56±3.32**	4.11±1.23
Chromosome break	6.97±3.31**	7.11±3.34**	3.89±1.55
Chromosomal deletion	5.53±3.11**	5.98±3.19**	1.41±0.71
Dicentric chromosome	4.11±1.55**	4.83±1.71**	1.35±0.99
Ring chromosome	1.28±1.12**	1.33±1.11**	0.31±0.21
Total chromosomal aberrations %	18.35±5.12**	19.23±5.32**	10.01±3.03
GSH-Px enzyme (U/mg protein)	15.35±5.61**	15.93±5.99**	17.19±4.81
SOD(U/g Hb)	78.71±14.21**	76.14±15.67**	98.55 ±12.15
MDA(nmol/ml)	10.99±2.55**	10.01±2.11**	6.04 ± 2.33

** Values differed significantly from control (P < 0.01)

Table 3: Chromosomal aberrations %, GPx, SOD and MDA mean values in the exposed workers by exposure group

Parameter	High exposure group n=32 HES 32501 - 72500	Low exposure group n=25 HES 2500 -32500	P value
	Mean ± SD	Mean ± SD	
CA %	27.81 ± 9.11	24.08 ± 7.99	< 0.05
GPx (U/mg protein)	12.98 ± 5.01	18.01 ± 4.88	< 0.05
SOD (U/g Hb)	79.94 ± 10.67	73.24 ± 11.07	< 0.05
MDA (nmol/ml)	13.88 ± 3.31	9.11 ± 1.01	< 0.05

Table 4: Correlation coefficient between exposure indices and effect indices

Parameter	Age (year)	Duration of exposure in years	HES	GPx	SOD	MDA
CAs%	0.35	0.58*	0.72**	-0.75**	-0.74**	0.76**
GPx	-0.41	-0.59*	0.75**-	--	0.53*	-0.73**
SOD	-0.38	-0.55*	-0.69**	0.53*	--	-0.72**
MDA	-0.44	0.62**	0.78**	-0.73*	0.72**	--

* = P < 0.05; ** = P < 0.01

Table 5: Chromosomal aberrations CA % and (GPx, SOD & MDA) mean levels in the exposed groups after antioxidants intake by Gr. 2

Parameter	Group 1 (n=28) Mean ± SD	Group 2 (n=29) Mean ± SD	P value
Chromatid break	7. 91±3.31	4. 65±2.34	< 0.05
Chromosome break	7.99±4.98	5.08±2.02	< 0.05
Chromosomal deletion	6.12±3.99	3.99±2.57	< 0.05
Dicentric chromosome	5.75±1.99	2.91±0.99	< 0.05
Ring chromosome	2.98±1.51	0.85±0.52	< 0.05
Total chromosomal aberrations %	22.65±6.57	13.88±4.55	< 0.05
GSH-Px enzyme (U/mg protein)	12.86±5.01	23.85±7.59	< 0.01
SOD(U/g Hb)	69.51±10.21	88.74±18.65	< 0.05
MDA(nmol/ml)	13.01±1.05	7.28±2.55	< 0.05

Several studies have implicated oxidative stress as one of the important mechanisms of toxic effects of Oss.[5,24] They also confirmed that occupational exposure to high concentration of solvents induced lipid peroxidation and decreased endogenous antioxidants in the body such as and superoxide dismutase and GPx which are a seleno enzyme responsible for elimination of reactive oxygen species (ROS).

The increased production of ROS also enhances lipid peroxidation. The major aldehyde product of lipid peroxidation is MDA, a widely used index to estimate oxidative stress.[25] Organic solvents exposure has been associated with increase in the overall formation of MDA.[26,27] In our study we illustrated a significant increase in the level of MDA in the workers than in referents. Karagozler et al.[28] reported that MDA concentration was significantly elevated in house painters occupationally exposed to organic solvents. Halifeoglu et al. [29] also found that inhalation of organic solvent increases lipid peroxidation. In accordance with the results of previous studies, MDA in the current study was found to be higher in the exposed groups especially HEG and positively correlated with increasing years of exposure confirming the pathogenic role of oxidative stress.

Our results also, revealed a significant decrease of GPx and SOD activities in exposed groups [1 & 2] compared to controls (Table 2). The same finding was found by others.[4,5]

A dose response relationship was detected between the exposure level in workers exposed to OSs and the prevalence of CAs oxidative stress.[5,23] Their findings supports those found in Table 3 & 4 in this work as we found that workers belonging to the higher exposure group reported statistically significant higher frequencies of CAs and lower concentration of GPx & SOD. Also, a significant +ve correlation was found between CAs% & MDA and both duration of work and HES, "being stronger with HES".

The intimate relation between ROS and DNA damage is well documented in table (4) where a–ve correlation between CAs% and GPx & SOD ($r = -0.75$ & -0.74 respectively $P < 0.01$).

Our results are in accordance with those recorded by Kim et al.,[1] & Ehab et al.[5] who detected a similar association between decreased superoxide dismutase and GPx on one side and increased frequency of CAs, micronuclei and abnormal comet assay on the other one.

Our results, also, are also, greatly supported by those reported by other researchers,[30,1,4] who confirmed that heavily exposed workers to OSs like those handling complex chemical mixture and painters or those with longer duration of work reported a significant increased frequency of CAs and decreased levels of GPx & SOD supporting the claims of induction of ROS by OSs exposure with depletion of substrate molecules.

To get a better insight of antioxidants supplementation's role on oxidative stress and cytogenetic changes caused by solvent exposure, two similar groups of exposure conditions were investigated twice, firstly , at the start of the study and secondly after 6 months of antioxidants administration only by one group (group 2).

We found (table 5) that the percentage chromosomal aberrations in general ,the mean levels of the different types of CAs and MDA were much lowered in that group "group 2" while those of Group 1 who are still exposed with no antioxidant intake were much increased. Moreover, the mean level of GPx & SOD were increased in Group 2and lowered in Group 1comared to values found previously in "table2". The differences were statistically significant when both exposed groups compared to each other. Our results are in accordance with those of Gaziev et al.[19] Velanganni et al.[31] and Amal et al.,[4] They insured the preventive effect of antioxidant vitamins A,C,E supplementation against DNA damage is especially when taken at low doses in combination due their synergistic effect .

Also, Wilhelm et al.[32], measured several enzymatic and non-enzymatic biomarkers of oxidative stress in the blood of coal mining and incineration of solid residues of health services after supplementing the study groups with vitamin E (800 mg/day) and vitamin C (500 mg/day) for 6 months, and comparing the situation before antioxidant intervention, they found that biomarkers of oxidative stress markedly decreased after the antioxidant supplementation.

CONCLUSION

Finally, we can conclude that chronic exposure to OSs results in chromosomal damage and lipid peroxidation which might play an important role in activating proto-oncogenes predisposing to transformation to malignancy. Also, we concluded that, antioxidants' supplementation can ameliorate oxidative stress and genotoxicity in exposed workers. So, our results could help in establishing surveillance strategies and prevention programs that will be of utmost importance for more precise exposure regulations to organic solvents, Further studies with longer follow-up of larger cohorts of workers with a wide range of exposure levels are recommended.

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