Genotoxicity and oxidative stress among spray painter

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Abstract: Several organic solvents (OS_s) are potent carcinogens among population at risk. Their genotoxicity have important implications for cancer production. Genotoxicity could be related to lipid peroxidation with decrease of endogenous body antioxidants. This work included 27 car spray painters exposed to a mixture of mainly aromatic OS_s and a comparable group of 27 males. For both groups, chromosomal study and assay of serum glutathione peroxidase (GSH-P_x) were done. It was found that spray painters suffered a significantly higher percentage of chromosomal aberrations (CA_s%) and lower GSH-P_x activity specially among smoker ones. Positive correlations were found between CA_s% and duration of exposure and the lifetime hydrocarbon exposure score (HES). A significant negative correlation was found between GSH-P_x and duration of work and HES. Finally we recommend following up for workers who are at risk of genotoxicity by periodic examination and regular supplementation with antioxidants.

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Key words: GSH-Px; chromosomal aberrations; organic solvents; HES; lipid peroxidation; antioxidants.

1. Introduction:

OSs have a wide range of applications and exposure to them is an eminent risk factor in occupational and non-occupational environment Kim *et al*., (2011). Their inhalation can cause injury to several internal organs of human body and weather they are present alone or in a mixture, (Metwally and El-Shabrawy, 2000).

In fact, about 50% synthesized OSs are employed for the production of paints and thinners. Xylene, toleune, styrene , ethylbenzne , acetone and methyl ethylketone are some of most frequently and quantitatively represented solvents in the composition of paints, (Costa *et al*., 2005).

It is well documented that several OSs are potent carcinogens among population at risk. Their genetic effects have important implications for cancer induction , (Amal *et al*., 2011).

Genotoxicity could be proposed to be through excessive and persistent formation of reactive oxygen radical species (ROS) inducing lipid peroxidation and decrease endogenous antioxidants in the body such as reduced superoxide dismutase and (GSH-Px), (Coskum *et al*., 2005).There are, also, associated various gene expression changes, some of which may be responsible for oxidative stress (Kim *et al*., 2011).

The present cross sectional study aimed at assessing the genotoxic effects of chronic OSs inhalation among a group of spray painters. We, also, aimed at evaluating the oxidative stress, through GSH-Px assay as a potential indicator of DNA damage and carcinogenic mechanism.

2. Subjects and Methods:

2.1. Subjects:

This work included 29 male car spray painters exposed to a mixture of OSs mainly aromatic solvents as xylene, toluene, as well as, pigments and thinners.

They were randomly selected from 3 car painting workshops in Cairo. Spray painting was done either outdoor or in semi-open ill ventilated places for 5 hours/day and most of them used no personal protective devices. The control group consisted of 27 male. They have no past or present current history of exposure to OSs. Both groups were matched for age, sex, smoking habits and socioeconomic standard. Those who were taking regular medications or exposed to any sort of radiation during the last 12 months before sampling were excluded from both groups.

2.2. Methods:

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.

2.2.1. Study of chromosomal aberration*:*

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 hrs. metaphases were obtained by adding 0.2 ug/ml colchicines to the cultures 3 hrs 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. 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 Verma and Babu (1989).

2.2.2.Activity of GSH-Px*:*

The activity of GPx in serum was measured spectro-photometrically .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.

2.2.3. Assessment of exposure to OSs*:*

This was done by estimating the lifetime hydrocarbon exposure score (HES), which 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 32500/23501 taken as a cutoff point, (Yaqoob *et al.,* 1992).

2.2.4.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.

3. Results

Table (1) shows that there was no statistical significant difference between both groups concerning their age and smoking habits. The mean duration of exposure to spray painters for the exposed group was 15.2 ± 7.11 years.

As noticed in table (2) the mean levels of the different types of CA namely chromatid break, chromosome break, dicentric chromosome, chromosomal deletion and ring chromosome, were higher among exposed groups in comparison to the controls.

Table 1: characteristics of the studied population

Parameter	Exposed group N=29	Control N=27	*P* value
Age in years Mean± (SD)	43.8 (8.9)	41.86 (9.2)	> 0.05
Smokers No & % Non Smokers No & %	18 (62.07) 11 (37.93)	17 (62.92) 10 (37.08)	> 0.05
Duration of exposure to spray painters (years) Mean±(SD)	15.2 (7.11)		

The mean level of the percentage chromosomal aberrations in general was markedly higher among the exposed group compared to their controls; the differences were statistically highly significant $P < 0.01$.

Table 2: Chromosomal aberrations and serum GSH-Px among the studied groups.

Group / Chromosomal aberration	Exposed N=29 Mean ±SD	Control N=27 Mean ±SD	*P* value
Chromatid break	5.99 ±391	5.83 ±1.92	< 0.01
Chromosome break	6.89 ±3.54	4.01 ±1.33	< 0.01
Dicentric chromosome	5.82 ±2.91	1.99 ±0.99	< 0.01
Chromosomal deletion	4..89 ±2.01	1.91 ±0.98	< 0.01
Ring chromosome	1.52 ±1.33	0.29 ±0.38	< 0.01
Total chromosomal aberrations %	25.16 ±9.98	10.11 ±3.11	< 0.01
GSH-Px enzyme (U/mg protein)	15.91 ±5.67	27.19 ±4.99	< 0.01

This table shows that smokers exposed workers had higher frequency of CA and lower mean of GHS-Px compared to those of none exposed. The difference was statistically significant only for the frequency of CA ($P < 0.05$).

Table 3: Effect of smoking on CAs and GHS-Px among spray painters

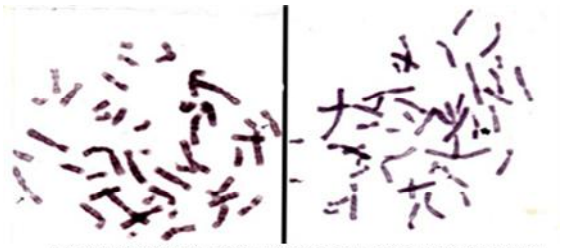
Parameter	Exposed group n = 29 (Mean ± SD)		*P* value
	Smokers N=18	Non smokers N=11	
CA %	27.66 ±10.99	22.11 ±9.19	< 0.05
GHS-P_x (U/mg protein)	14.89 ±5.18	16.99 ±5.32	> 0.05

HES: hydrocarbon exposure score which is calculated by arbitrary units.

Table 4: Frequency of CAs % and GHS-Px mean Concentration of exposure group in spray painters

Parameter	Higher exposure group N=13	Lower exposure group N=16	*P* value
	HES 32501 - 72500	HES 2500 - 32500	
	Mean ± SD	Mean ± SD	
CA %	27.88 ± 7.77	24.11 ± 7.53	< 0.05
GHS-Px	13.13 ± 4.17	19.11 ± 4.11	< 0.05

The mean percentage levels of CAs and GHS-Ps were higher for the first and lower for the letter among the high exposure group with HES (32501 – 72500) compared with those of lower exposure group with HES (2500 - 32500). The differences were statistically significant ($P < 0.05$).



Fig(1):G banding metaphases shows chromosomal breaks in ch.(3&5)(right) and ch.(13) (left)

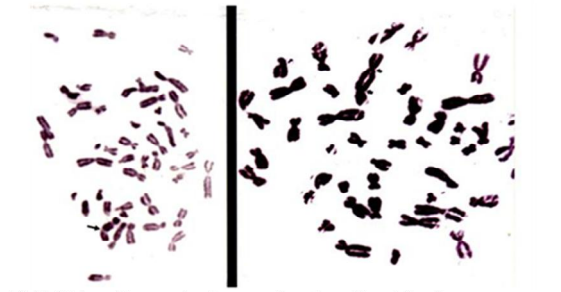


Fig2: G-banding metaphases showing dicentric chromosomes

CAs percentage showed a significant +ve correlation with the duration of work ($P < 0.05$) and highly significant with HES and GHS-Px ($P < 0.01$). GHS-Px showed a significant –ve correlation with the duration of work ($P < 0.5$) and highly significant with HES ($P < 0.01$).

Table 5 : correlation coefficient of exposure indices and effect indices

Parameter	Age	Duration of exposure	HES	GHS-P_x
CA_s%	0.44	0.58^{x}	0.75^{xx}	-0.71^{xx}
GHS-P_x	- 0.31	$- 0.56^{x}$	$- 0.69^{xx}$	---------

x = P < 0.05 *x x = P < 0.01*

4. Discussion:

Several studies have suggested that induction of CAs may play a role in solvent's induced carcinogenesis, (Catalan *et al*., 2009) . Many epidemiological studies reported that the high frequency of CAs is a predictive of an increased risk of cancer, (Bonassi *et al*. 2005) .The genotoxicity resulting from occupational exposure can be evaluated using different genetic endpoints, e.g. DNA damage, chromosomal aberrations and micronuclei, (Celik and AKbas 2005). In this work CAs analysis was utilized to evaluate the extent of genome damage in spray painters. CAs are particularly dangerous to the cell ,as well as, the physical discontinuity of the chromosome may cause loss of genetic information and even cell death if a housekeeping gene is involved, (Pasguini *et al*. 2001). In our Work , the mean values of the different types of CAs, namely; chromatid break, chromosome break, dicentric chromosomes, chromosomal deletion, and ring chromosome, as well as, the total chromosomal aberrations percentages were significantly higher ($P< 0.01$) among spray painters compared to their controls (Table2). Similar results were reported by Gonzalez-yebra *et al.* (2009) who found a significant increase in the frequency of different CAs among shoe workers exposed to Oss. These data are, also, in accordance with the findings of kim *et al.* (2008) in workers exposed to OSs in petroleum refinery compared to their controls. Moreover, our results were greatly supported by those of Ihsan *et al.*(2000);Rueff *et al*.(2009) ; Amal *et al.* (2011) on their studies on painters, Our results also, revealed a significant decrease of GHS-Px concentration in spray painters compared to their controls (Table2). GHS-Px is a seleno enzyme responsible for elimination of reactive oxygen species (ROS).

Several studies have implicated oxidative stress as one of the important mechanisms of toxic effects of Oss. They also confirmed that exposure to high concentration of solvents induced lipid peroxidation and decreased endogenous antioxidants in the body such as GHS-Px and superoxide dismutase Farahat and Kamel, 2010).

Cigarette smoking was found to have an additional genotoxic effect (Table3). We found that smokers spray painters reported high frequencies of CAs and lower levels of GHS-Px compared to their controls.

Some studies pointed out the very complex interaction between smoking and occupational exposure to genotoxic agents. Cigarette smoking itself is a well known risk factor for several types of cancer and it is a well known confounding factor influencing the frequency of cytogenetic damage and lipid per-oxidation, (Palus *et al*., 1998; Rekhadevi *et al*., 2009) .This might explain the higher frequency of CAs and lower levels of GHS-Px in smoker spray painters,

A dose response relationship was found between the prevalence of CAs , decreased concentration of

GHS-Px and exposure level to spray painting (Table 4). Workers belonging to higher exposure group reported statistically significant higher frequencies of CAs and lower concentration of GHS-Px ($P < 0.01$). Moreover, in the current study, spray painters showed a dose response relationship between exposure indices (age, duration of work and HES) and effect indices (CAs% and GHS-Px) (Table 5).

A significant +ve correlation was found between CAs% and both duration of work and HES, being stronger with HES. On the other hand, significant -ve correlations were found between CAs and levels of GHS-Px which in turn showed significant –ve correlation of duration of work and HES. Our results, therefore, are in accordance with those of other workers, (Amal *et al*., 2011; Ihsan *et al*., 2000; Kim *et al*., 2011; Rueff *et al.,* 2009) who confirmed that heavily exposed workers to OSs and those with longer duration of work reported a significant increased frequency of CAs and decrease levels of GHS-Px supporting the claims of induction of ROS by OSs exposure with depletion of substrate molecules.

Our results were in agreement with those reported by others like catalan *et al*.(2009), who found that workers handling complex chemical mixture and painters whaling paint thinners. Ihsan *et al*., 2000); Angela *et al*., 2010) showed higher frequency of CAs and decreased concentration of GHS-Px.

The intimate relation between ROS and DNA damage is well documented in table (5) where a –ve correlation between CAs frequency and GHS-Px (r = - o.71 & $P < 0.01$). Our results were greatly supported by those recorded by Rekhadevi *et al.*(2009) who detected a similar association between decreased superoxide dismutase and GHS-Px on one side and increased frequency of CAs , micronuclei and abnormal comet assay on the other one.

Finally, we conclude that chronic exposure to OSs results in chromosomal damage and lipid peroxidiation which might play an important role in activating proto-oncogenes predisposing to transformation to malignancy. Hence, educational programs to educate workers about the potential health hazards of exposure to OSs with stressing on importance of using protective measures should be implemented. We stress on the importance of regular bio-monitoring of genotoxic effects for workers at risk with regular supplementation with antioxidants.

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