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Research Article

Lipid Peroxidation and Antioxidant Defense System among Workers of the Printing Industry

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ABSTRACT

Background: The printing industry is one of the largest and most geographically diverse manufacturing industries in the world. In the printing industry, volatile organic compounds main sources of chemical hazards associated with the using of different chemicals and solvents. As a result from environmental and workplace chemical exposures, the toxic chemicals are significant contributors to the human health effects.

Materials and methods: In this prospective study conducted in local printing company with two groups; the control group included (26) male healthy volunteer donors and the second is the workers group included (26) male volunteer workers, who have been exposed to chemicals for long time (10 years). Blood samples were obtained to evaluate oxidative stress biomarker and the antioxidant defense system. Statistical analysis was employed.

Results: When comparing between the two groups; The values of lipid peroxidation biomarker (MDA) and hydrogen peroxide were significantly high, while the levels of enzymatic and non-enzymatic antioxidants were detected with lower significant values (CAT, SOD, GST, GSH) ($P<0.05$).

Conclusion: Exposure of printing workers chemicals and solvents cause increased oxidative stress.

Keywords: Oxidative stress; Lipid peroxidation; VOCs; Antioxidants

INTRODUCTION

Printing industry, within various printing techniques, is abundant with a specific source and processes that commonly emit high levels of Volatile Organic Compounds (VOCs), such as carbonyls, alcohols, alkanes, alkenes, esters, aromatics, ethers and amides [1]. Although necessary and required as components of inks, alcohol, cleaners, solvents, emulsions, thinners, retardants and de-emulsifiers, the increased emissions of VOCs and their resulting impact on the air quality are now considered as a major environmental concern. VOCs are characterized by high vapor pressure, great flammability and low water solubility properties [2]. As chemical species with varying chemical reactivity, VOCs are very difficult to control. In the presence of oxygen, OH radicals, and UV radiation, VOCs can easily participate in the series photochemical reactions formatting smog as a final product. In the presence of Nitrogen Oxides (NOx), secondary contaminants, such as ozone, aldehydes and nitrates can also be easily generated [3-5].

MATERIALS AND METHODS

This prospective comparative study was conducted on 52 male volunteer of aged ≥ 25 worked in a local printing company in Alexandria, Egypt.

Study location: This was carried out in Institute of Graduate Studies and Research-Alexandria University, Egypt.

Sample size: 52 subjects.

Sample size calculation: We assumed that the Confidence Interval of 10% and Confidence Level of 95%. The sample size actually obtained for this study was 26 subjects for each group. We planned to include 52 volunteer (Control group-workers group).

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Subjects and selection method: The experimental design of this study contains two groups randomly selected at a local printing company at Alexandria city, Egypt: The first one is the control group included (26) male healthy volunteer donors of different age, sex, socio-economic level who were working in the other divisions of the same factory were classified into the control group and the second is the workers group included (26) male volunteer workers were also who have been exposed to chemicals for long time (10 years).

Procedure methodology

Prior to participation in this study all volunteers were advised about the procedure and signed the informed consent. The participants were told about the aim of the study, and they were informed that the data would be used for scientific purposes only. They were also given the right to refuse or participate in the study.

A Five milliliters venous blood sample were collected and aliquot was anticoagulant with Ethylene Diamine Tetra Aceticacid (EDTA). Plasma was obtained by centrifugation of the samples at 3000 rpm for 10 minutes. Malondialdehyde (MDA) was analyzed according to Satoh method [6]. Thiobarbituric Acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form Thiobarbituric Acid Reactive Product (TBARS). The absorbance of the resultant pink product can be measured at 534 nm using spectrophotometer and expressed as nmol/ml. Hydrogen peroxide was determined using commercial kits according to Aebi, et al. [7], method (Bio Diagnostic, Egypt). In the presence of Peroxidase (HRP), H_2O_2 reacts with 3,5-Dichloro-2-Hydroxy Benzene Sulfonicacid (DHBS) and 4-Aminophenazone (AAP) to form a chromophore. The color developed was measured at 510 nm. It was measured in UV-Double Beam Spectrophotometer. H_2O_2 was expressed by mM/L.

The activity of Superoxide Dismutase (SOD; EC 1.15.1.1) was expressed as the amount of enzyme that inhibits the oxidation of epinephrine to adrenochrome. The reaction was initiated by addition of epinephrine and the increase in absorbance at 480 nm was measured in UV-Double Beam Spectrophotometer by Misra, et al. [8], SOD was expressed by U/ml.

Catalase (CAT; EC 1.11.1.6) reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase (HRP), remaining H_2O_2 reacts with 3,5-Dichloro-2-Hydroxybenzene Sulfonicacid (DHBS) and 4-Aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample. The color developed was measured at 510 nm. Catalase activity was measured in UV-Double Beam Spectrophotometer by using commercial kits according to Aebi, et al. [7], (Bio Diagnostic, Egypt). CAT was expressed by U/ml.

Glutathione S-Transferase (GST; EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. According to the method of Habig the measurement of the activity of GST is determined by using para-nitrobenzylchloride as a substrate [9]. The absorbance was measured spectrophotometrically at 310 nm using UV double beam spectrophotometer. GST was expressed by µmol/hr/ml.

Determination of Reduced Glutathione (GSH) by the method of Beutler which based on the reduction of 5,5` Dithiobis (2-Nitro Benzoicacid) (DTNB) with Glutathione (GSH) to produce a yellow compound [10]. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. GSH was expressed by mmol/l.

Statistical analysis

All measurements were triplicately performed in independent experiments for all treatments. The results were expressed as mean ± Standard Error (SE). Statistical analyses were made with One-Way Analysis of Variance (ANOVA) when differences were found multiple comparisons by Tukey's post-hoc test using the SPSS version 21. The criterion for statistical significance was $P<0.05$.

RESULTS

Table 1 Shows MDA, the lipid peroxidation biomarker was significantly higher in workers (2.66 ± 0.14 nmol/ml) in comparison to controls (4.58 ± 0.10 nmol/ml) ($P<0.05$) whereas, Workers showed increased H_2O_2 levels when compared to the control group (0.07 ± 0.00 mM/L) *vs.* (0.15 ± 0.01 mM/L) ($P<0.05$) as represented in Table 2.

Table 1: Shows distribution of mean values of biomarker of free radicals generation control and worker groups.

Parameter	Control N (26)	Workers N (26)	P value
MDA	2.66 ± 0.14	4.58 ± 0.10*	P<0.05

Note: Data presented as mean ± SE. *Significant higher than control (P<0.05).

Table 2: Shows distribution of mean values of hydrogen peroxide (H_2O_2) control and worker groups.

Parameter	Control N (26)	Workers N (26)	P value
H_2O_2	0.07 ± 0.00	0.15 ± 0.01*	P<0.05

Note: Data presented as mean ± SE. *Significant higher than (P<0.05).

Table 3 shows comparison of antioxidants parameters in which the activity of CAT was significantly lower in workers (481.55 ± 6.13 U/ml) in comparison to controls (656.06 ± 10.42 U/ml) ($P<0.05$); the activity of SOD was significantly lower in workers (158.63 ± 4.02 U/ml) in comparison to controls (285.94 ± 10.15U/ml) ($P<0.05$); the activity of GST was significantly lower in workers (0.74 ± 0.03 µmol/hr/ml) in comparison to controls (1.30 ± 0.03 µmol/hr/ml) ($P<0.05$) and the content of GSH was significantly lower in workers (0.29 ± 0.01 mmol/l) in comparison to controls (0.43 ± 0.01 mmol/l) ($P<0.05$).

Table 3: Shows distribution of mean values of antioxidants control and worker groups.

Parameter	Control N (26)	Workers N (26)	P value
CAT	656.06 ± 10.42	481.55 ± 6.13**	P<0.05
SOD	285.94 ± 10.15	158.63 ± 4.02**	P<0.05
GST	1.30 ± 0.03	0.74 ± 0.03 **	P<0.05
GSH	0.43 ± 0.01	0.29 ± 0.01**	P<0.05

Note: Data presented as mean ± SE.
** Significant lower than control (P<0.05).

DISCUSSION

Printing is one of the businesses that have traditionally had a high occupational exposure to health hazards [11]. The work environment usually contains a large number of chemicals, which may be inhaled and absorbed by the body [12]. Solvents used in the printing industries are usually mixtures of chemicals rather than a single substance [13]. During printing, the major exposure is to ink in addition to fountain solution hydrocarbon-based cleaning solvents and isopropanol from damping solutions [1,14]. All types of organic solvents are lipophilic volatile liquids at room temperature [15,16]. Adverse health effects related to exposure to organic solvents through inhalation and skin contact in the workplace. Exposure of solvent vapours affects not only the lungs but the whole circulatory system and hence the whole body. Solvents accumulate principally in lipid and fat-rich cells in the nervous system, brain, bone marrow, liver, and body fat. Long-term health effects may be damage to internal organs after absorption into the body [17-20]. Lipids such as free and ester forms of polyunsaturated fatty acids and cholesterol are vulnerable target to oxidation by both enzymes and nonenzymatic oxidants [21].

Malondialdehyde (MDA) is one of the most studied carbonyl products of oxidative stress. Reactive oxygen species react with multiple, long-chain, un-saturated fatty acids in cell membranes to generate lipid peroxidation products, particularly MDA. Leading to protein crosslinking MDA alters the membrane structure and transmembrane transport [22].

Although the mechanisms by which VOCs induce these adverse health effects are not well understood, but it is believed that oxidative stress and DNA damage have a basic role in the induced adverse health effects due to the occupational exposure to VOCs. The possible damage mechanism of these compounds are related to their lipophilic structure, they pass easily from cellular membranes and accumulate in fatty-rich tissue causes induced ROS production and increased ROS is related with DNA damage [23,24].

As illustrated in Table 1, MDA, the lipid peroxidation biomarker, was significantly higher (P<0.05) in workers in comparison to controls in compliance with Hussein, et al. [24], elevated MDA levels observed in workers could be linked to toxic effects of organic solvents by the formation of free radicals and reactive oxygen species [25-28].

Hydrogen peroxide is a ubiquitous oxidant present in all aerobic organisms. It is a particularly interesting oxidant because of its stability; its longer life time allows it to participate either as a secondary messenger molecule or a lethal oxidant [29]. Since its first identification in a living cell, H_2O_2 was considered a toxic byproduct of aerobic metabolism, it was discovered that neutrophils use H_2O_2 toxicity and produce massive amounts of H_2O_2 during the oxidative burst to kill invading pathogens [30]. The main pathways to decompose hydrogen peroxide involve catalase, cytoplasmic Glutathione Peroxidase (GPx) and Peroxiredoxin (Prx). Decomposition of H_2O_2 by CAT is greatly dependent on H_2O_2 concentration; this enzyme presents lower affinity for H_2O_2 than GPx and Prx [31].

As represented in Table 2, elevation of hydrogen peroxide content in workers that exposed to organic solvents showed statistically significant difference (P<0.05) with their matched controls could indicate the high rate of production of H_2O_2 induced by organic solvents exceeds the system's ability to neutralize and eliminate them and the lower rate of H_2O_2 consumption by antioxidant systems.

A condition occurring when the generation of reactive species in a system exceeds the system's ability to neutralize and eliminate them, described as oxidative stress [32]. Antioxidant defense enzymes, dietary and endogenous antioxidants (Free-radical scavengers) are important to counteract the damages induced by oxidative stress [33]. VOCs exposure cause oxidative stress by producing highly reactive radicals that might cause progression of numerous pathological conditions in the process of these VOCs metabolism [23]. Among various antioxidant mechanisms in the body a mitochondrial enzyme, SOD an important factor in limiting oxygen toxicity, it represents the primary defense against oxidative stress as superoxide is such a strong initiator of chain reactions. SOD dismutated superoxide radical by into O_2 and hydrogen peroxide [34]. Another important part of the enzymatic defense system is Catalase (CAT) which is one of the most active catalysts produced by nature and it is a tetrameric heme-containing enzyme found in all aerobic organisms. Once hydrogen peroxide formed must be reduced by CAT to water to prevent it forming the hydroxyl radical [34]. Reduced glutathione protects hemoglobin from oxidation by agents that may be present in the cell [35].

Findings of Hussein, et al. [25], concur with our work, where it was observed a decrease in SOD and CAT activities in the exposed workers in relation to the control. It is known that both solvents and metals can generate oxidative stress [17,22,26,27] which could be partially reduced by antioxidant enzymes. The decrease in SOD activity observed in this work suggests the formation of superoxide radical by volatile organic compounds and metals, which possibly depleted the enzyme. CAT activity reduction due to consumption in hydrogen peroxide reduction. Activities of GST of worker group were found to be significantly decreased (P<0.05) when, compared to control group as shown in Table 3.

As clarified in Table 3 the mean of the content of GSH was statistically significant lower (P<0.05), among the exposed workers as compared with control, is probably caused by its depletion and direct binding to the cysteine thiol groups. Our findings confirmed with those of Habib, et al. [27].

Solvents involved in the generation of reactive oxygen species that were found to induce cell damage through the increase in the level of lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals [28,36].

Tharshanapriya, et al. [37], Study demonstrated a significant decrease in the activities of SOD and GST while the activities of CAT and GPX were found to be significantly increased in the exposed painting group participants when compared to those of control group participants.

CONCLUSION

Organic solvent which can be enzymatically bioactivated to reactive intermediates that can lead to increased formation of ROS key participants in cell damage. Workers in printing industry exposed to VOCs initiate a state of oxidative stress. This resulted from an increase in the production of active oxygen radicals or from inhibiting the ability of the antioxidants. Excess amount of ROS leads to the depletion of the protective endogenous antioxidant defenses.

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