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EVALUATION OF OXIDATIVE STRESS IN WORKERS EXPOSED TO DIFFERENT INDUSTRIAL ENVIRONMENTAL HAZARDS

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ABSTRACT

The aim of this study is to evaluate the antioxidant status and the end products of lipid peroxidation in blood of workers exposed to industrial environmental hazards. Sixty six workers are divided into 5 toxic vapor's exposure groups, phenol (group I), formaldehyde (group II), urea (group III), mixed vapors (group IV) and control group, (not exposed to any industrial hazards). Reduced glutathione (GSH), malondialdehyde (MDA) levels as well as superoxide dismutase (SOD) and catalase (CAT) activities were assayed in blood of workers. Moreover, protein profile of sera isolated from blood of workers and controls was analyzed by sodium dodecyl sulphate polyacrlamide gel electrophoresis (SDS-PAGE). Leukocyteic DNA isolated from blood samples of different groups was analyzed by agarose gel electrophoresis. A significant increase was observed in GSH content of blood workers of different groups compared to that in blood of control group. In addition, no significant difference was observed in the activities of antioxidant enzymes and MDA content when compared to the healthy control group. The resolved proteins showed no significant differences in protein pattern in comparison with those of control group. On the other hand, DNA breakage was observed in groups I, II and IV but no damage was observed in group III. In conclusion, the chronic exposure to formaldehyde, phenol and mixed vapor might induce DNA damage and can be used as a marker for the effect of these pollutants.

Key words: oxidative stress, industrial environmental hazards, and antioxidative enzymes

Abbreviations: CAT, catalase; FA, formaldehyde; GSH, reduced glutathione; Hb, hemoglobin; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase.

INTRODUCTION

Industrial environmental exposure for long period could cause oxidative damage to the biological systems **[US EPA (1999)]**. For example, formaldehyde (FA) is an environmental pollutant absorbed and oxidized in the liver after the inhalation, oral or dermal exposure **[Baj et al. (1994) and Pinkerton et al. (2004)]**. **El-Far et al. (2006)** demonstrated a novel finding about the effect of inhalation exposure to phenol, formaldehyde, urea and mixed vapors on level of serum carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), and prostate specific antigen (PSA). They showed that S-CEA can be used as an important prognostic screening marker for early prediction for malignancy, and for management of workers with lung cancer who exposed to the environmental hazards in industrial factories. Furthermore S-AFP can be used also as a biomarker if it is carried out and correlated with S-CEA.

Some studies have demonstrated that the toxicity of many organic compounds was mediated by reactive oxygen species (ROS) **[Datta & Namasivayam (2003) and IARC (1999)]** which induce or enhance the activities of antioxidant enzymes **[Holovska et al. (1998) and Meyer et al. (1998)]**. ROS are important mediators of cellular injury, and play a putative role in oxidative stress and can contribute to a variety of diseases, or be present in situations of toxicity **[Gurel et al. (2005)]**. ROS damage cellular macromolecules causing lipid peroxidation, nucleic acid and protein alterations **[Blokhina et al. (2002) and Yagi (1994)]**. ROS- initiated oxidative stress can be regulated by cell defense mechanisms, which include superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) **[Halliwell & Gutteridg (1999)]**. The aim of this study is to extend our previous work **[El-Far et al. (2006)]** and to evaluate antioxidant status and lipid peroxidation in the blood of workers after chronic inhalation of industrial environmental hazards (phenol, formaldehyde, urea and mixed vapors). Moreover, the study investigates and confirms the effect of these pollutants on causing leukocytes DNA damage.

MATERIALS AND METHODS

Chemicals:

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. (St.Louis, Mo, and USA).

Workers:

All workers of Mansoura for Resins and Chemical Industries Company exposed to industrial hazards were routinely examined in the factory by a specialist medical doctor. This study included fifty-one males workers with negative for both HCV and HBs-Ag. About 60% of workers were non smokers, others were not heavy smokers. The workers were divided into the following groups according to the type of industrial hazard:

Group I: included 12 workers exposed to phenol, with age range from 29 to 60 years. Average of exposure is 13 years (13 ± 11).

Group II: included 18 workers exposed to formalin, with age range from 26 to 46 years. Average of exposure is 9 years (9 ± 3.17).

Group III: included 8 workers exposed to urea, with age range from 35 to 55 years. Average of exposure is 8 years (8.1 ± 4.7).

Group IV: included 13 workers exposed to mixed vapors (phenol, formaldehyde and urea) with age range from 31 to 51 years .Average of exposure is 13.5 years. (13.5 ± 5.4). All workers in each department worked for 8 hours every day. Fifteen healthy subjects not exposed to any industrial pollutants served as a control group with age range from 30 to 55 years. In fact the amount of pollutant vapors in each department was not exactly quantatively available as it varies from day to another pending on the maintenance of different equipments of the factory as explained to us during the time of this study, although the vapors are easily detected and recognized by their specific distinguish odors.

All approvals for this human subject protocol and for blood sample collection were given from the director of the factory as well as the protocol of the research accepted from our Mansoura University Council.

Blood samples and hemolysate

3 ml of blood samples were collected from workers of all groups as well as controls in EDTA-coated tubes and the usual precautions for venipuncture were taken. They were centrifuged at 3000 g for 15 min and plasma was removed, then erythrocytes were washed with saline solution three times, and hemolysed by diluting with deionized water.

Hemoglobin (Hb) contents of the samples were measured as described by **[Wintrobe et al., (1965)]**. The hemolysate was kept in –70°C.

Biochemical measurements

Reduced glutathione (GSH) content was estimated as described by **[Beutler (1982)]** Thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation (malondialdehyde(MDA), was determined by the method mentioned by **[Ozcankaya & Delibas (2002)]**. SOD activity was assayed by the method of **[Nishikimi et al. (1972)**. The activity of catalase was assayed as reported by **[Abei (1984)]**.

Leukocytes separation and DNA extraction:

Leukocytes were prepared by dextran sedimentation **[Paya et al. (1994)]**. Whole blood samples from selected workers and controls were collected in EDTA-coated tubes. They were centrifuged for 15 min at room temperature. The upper layer was removed, and the residual blood was combined with an equal volume of dextran. The blood was left at room temperature for 45-60 min to permit erythrocytes to sediment. The upper polymorphonuclear leukocytes phase was collected and concentrated by centrifugation 2000 g for 15 min at room temperature. DNA was extracted from leukocytes as described by **[Miller et al. (1988)]**.

SDS-polyacrylamide gel electrophoresis.

The protein profile of serum samples was analyzed using SDS-PAGE according to the method of **[Laemmli (1970)]**.

Agarose gel electrophoresis.

DNA isolated from leukocytes was analyzed by agarose gel electrophoresis as described by **[Mannaiates et al. (1982)**.

Statistical Analysis.

Program package. (Graphed version 2.3) was used for statistical analysis. $P< 0.05$. $P<0.01$ and $P<0.001$ were considered to indicate significant differences, highly significant differences and extremely significant differences respectively. The unpaired alternate t test and Separman's rank correlation tests were used.

RESULTS

Non significant change was observed in SOD, CAT activities and MDA levels in erythrocytes of the studied industrial hazards vapor exposed workers compared to that of control group. However, a significant increase in erythrocytes GSH, and plasma GSH, was obtained in the four different vapor exposed worker groups when compared to control group. In addition, erythrocytes SOD activity was positively correlated with erythrocytes GSH level and MDA level ($r = +0.59$, $P<0.01$) and ($r = +0.57$, $P<0.01$) respectively (Table 1). However, no significant correlation was found between the other pairs as indicated in Table 2. The sera isolated from selected exposed workers and controls were resolved by 10 % SDS-PAGE under reducing conditions and the electrophoresed polypeptides were stained by coomasie brilliant blue R-250. As indicated in electropherogram (Fig 1) no significant different was observed in the protein profile of sera of all workers when compared to controls. Figure 2 shows the pattern of DNA extracted from blood leukocytes of control (Lane A), workers exposed to phenol (lane B), workers exposed to formaldehyde (Lanes C and D), workers exposed to urea (Lanes E and F) and workers exposed to mixed vapors (Lanes G and H). It was found that exposure to formaldehyde, phenol and mixed vapors induce damage of DNA. In contrast no significant change was observed in workers exposed to urea.

Table (1): Mean and standard deviation of erythrocytes SOD and CAT activities as well as MDA and GSH contents in erythrocyte and plasma of different groups of workers and healthy control group.

Group Parameter	Control (n=15)	Group I (n=12)	Group II (n=18)	Group III (n=8)	Group IV (n=13)
SOD(U/gHb)	2.66 ±0.77	3.05±0.90	3.50±0.91	3.70±0.90	3.35 ±1.30
CAT(KU/gHb)	0.50 ±.0 16	0.90±0.50	0.70 ±0.31	0.70 ±0.10	0.70 ±0.20
GSH(µmol/gHb)	0.23±0.05	0.33±0.09[a]	0.29 ±0.05[a]	0.32 ±0.07[a]	0.38±0.15[a]
GSH(µmol/ml)	10.20 ±2.40	14.80±1.90[b]	15.34 ±3.90[a]	15.40 ± 3.70[a]	17.60 ± 2.70[b]
MDA(nmol/gHb)	10.50 ±3.25	11.13 ±3.40	12.50 ±1.60	11.65 ±3.30	11.74±2.10

a= $P<0.05$, b= $P<0.01$

Fig. (1): Coomassie blue- stained 10% polyacrylamide gel under reducing conditions in presence of SDS showing the polypeptide pattern of:

Lane (A) : Serum from worker exposed to formalin (group II).

Lane (B): Serum from worker exposed to urea (group III).

Lane (C): Serum from worker exposed to mixed vapors (group IV).

Lane (D) to (F): Sera from worker exposed to phenol (group I).

Lane (G) to (I): Sera from control.

Lane St: Molecular weight markers.

Table (2): Correlation between SOD, GSH, CAT and MDA levels in erythrocytes of workers exposed to different industrial hazards.

	r	P
SOD& GSH	+0.59	P<0.01
SOD & CAT	+0.17	P>0.05
SOD & MDA	+0.57	P<0. 01
GSH & CAT	-0.03	P>0.05
GSH &MDA	+0.33	P>0.05
CAT &MDA	-0.03	P>0.05

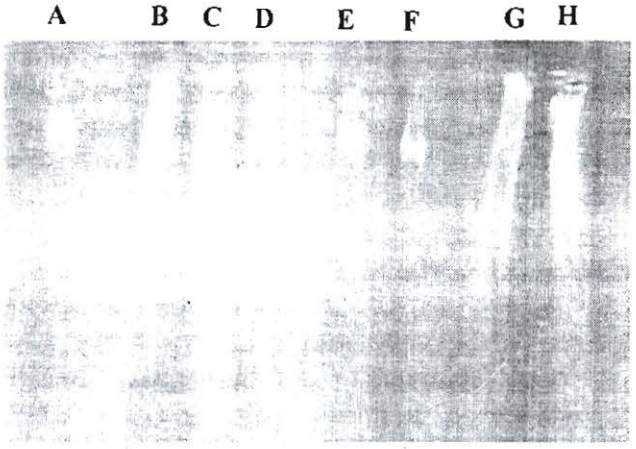

Fig. (2): Agarose gel electrophoresis of DNA samples isolated from leukocytes

A: DNA isolated from control sample

B: DNA isolated from worker exposed to phenol (group I)

C, D: DNA isolated from workers exposed to formaldehyde (group II)

E, F: DNA isolated from workers exposed to urea (group III)

G, H: DNA isolated from workers exposed to mixed vapors (group IV)

DISCUSSION

A lot of chemical compounds such as pesticides, herbicides, metals. many organic compounds can generate extremely ROS leading to damage of cellular macromolecules **[Winzer et al. (2002)]**. Their formation is considered as a pathochemical mechanism involved in the initiation of progression phase or various diseases such as atherosclerosis. ischemic heart diseases, diabetes, initiation of carcinogenesis or liver disease **[Holovska et al. (2005)]**. In the present study. results revealed changes in activity of SOD, CAT and also the MDA as an index of lipid peroxidation and GSH content in workers erythrocytes and plasma after chronic inhalation of the industrial environmental hazards. The results also showed no significant differences in the activity of antioxidant enzymes under investigation and erythrocytes MDA content between the control and industrial hazards exposed worker groups, except GSH. Although, it is known that oxidative stress in many causes induces or enhances the activities of antioxidant enzymes, numerous studies have shown important individual differences in response of these enzymes to oxidative attack **[Holovska et al. (1996)]**. Our findings, however, are in contrast with these previous indicated reports and in agreement with the results reported by **[Holovska et al. (2005) and Sogut et al. (2004)]**. Our results in case of workers exposed to phenol are in agreement with **[Bukowska & Kowalska (2003)]** were reported that phenol has not shown a negative effect on SOD and with **[Bukowska and Kowalska (2004)]** were reported that lipid peroxidation in erythrocytes incubated with phenol was not observed and did not significantly change catalase activity. Moreover. our results showed that the contents of plasma and erythrocytes GSH were significantly increased in workers after chronic inhalation of industrial environmental hazard. For example formaldehyde can induce toxic effects in distant organs such as liver, kidney, testes, etc **[Barber & Donohue (1998)]**. When FA was ingested and/or inhaled excessively, glutathione-dependent formaldehyde dehyrdogenase (GSH-FDA) will detoxify this compound more than normal metabolic situation and GSH will run out in the liver **[Barber & Donohue (1998)]**. We suggest that the increasing in GSH content in plasma and erythrocytes may be due to increased de novo synthesis of glutathione. MDA released by the lipid peroxidation in human erythrocytes might be metabolized immediately by a mitochondrial MDA-metabolizing enzyme and increase

MDA level is not seen **[Sogut et al. (2004)]**. The results were also confirmed by SDS-PAGE observations that showed no significant difference in the protein profile of different worker sera. However, these observations are in contrast with **[ATSDR (1998)]** who reported that acute exposure to phenol causes protein denaturations. **[Thrasher & Kilburn (2001)]** reported that formaldehyde reacts chemically with proteins by addition and condensation reactions. Our results showed damage in leukocytes DNA of the workers exposed to formaldehyde, phenol and mixed vapors but no significant damage in DNA of workers exposed to urea. Formaldehyde exposed groups does not reveal an increase in blood. This may be mainly due to its rapid metabolism and succeed in reacting with DNA **[Shaham et al. (2003)]**. Our findings are consistent with **[Bukowska & Kowalska (2003) and Do Ceu Silva et al. (2003)]** who reported that phenol and formaldehyde had mutagenic effect by inducing cell transformation, gene mutations, unscheduled DNA synthesis, chromosomal aberrations and sister chromatid exchanges. In the present study, there is a positive correlation between SOD and MDA. Also, there is a positive correlation between SOD and GSH. However, there is no significant correlation between other pairs of antioxidants. One can use these correlations to calculate roughly, SOD if MDA and/or GSH are practically determined. These positive correlations between SOD and GSH, MDA in the studied groups have not previously reported.

Therefore, inhalation of industrial environmental hazards under investigation induces the antioxidant system in the blood of exposed workers, and consequently stimulates biosynthesis of both erythrocyte levels of SOD and GSH as a defense mechanism. Also, this inhalation would lead to increase ROS, which in turn increases lipid peroxidation. MDA is measured as indicator for lipid peroxidation, consequently when ROS increases then MDA increases with concomitant increase of SOD, as the last can diminish the ROS effect.

CONCLUSION

DNA damage according to our technique, we reported here for first time confirm the dramatic harmful effect on the workers under exposure to phenol, formaldehyde and mixed vapors and this effect confirmed with the our previous work by **El-Far et al. (2006)** done on the same workers under same environments in the same factory where these pollutants enhanced the levels of serum CEA and AFP of workers.

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تقييم بعض مضادات الأكسدة لدى العمال المعرضين لمخاطر البيئة الصناعية

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يهدف هذا البحث الى تقييم بعض مضادات الأكسدة في دماء العمال المعرضين لمخاطر البيئة الصناعية.

تم اجراء هذه الدراسة على ٦٦ عاملا تم تقسيمهم الى أربع مجموعات طبقا للغازات التى تم التعرض لها وهى على التوالى الفينول (المجموعة الأولى)، الفورمالين (المجموعة الثانية)، اليوريا (المجموعة الثالثة)، الأبخرة الثلاث مجتمعة (المجموعة الرابعة) بالاضافة الى المجموعة الضابطة وتشمل ١٥ رجل من الأصحاء الذين لم يتعرضوا لأية مخاطر بيئية.

تم قياس مستوى MDA. GSH وكذلك نشاط أنزيمات CAT. SOD في دماء العمال، وفصل بروتينات مصل الدم باستخدام الهجرة الكهربية على هلام عديد الأكريلاميد (SDS-PAGE)، وفصل الـ DNA من كريات الدم الحمراء من عينات الدم المجمعة وتم تحليلها باستخدام الهجرة الكهربية على هلام الأجاروز، وقد أسفرت الدراسة عن النتائج التالية:

١- زيادة ملحوظة في مستوى الجلوتاثيون في كل من كرات الدم الحمراء وبلازما الدم لدى جميع العمال مقارنة بالمجموعة الضابطة.

٢- عدم وجود زيادة ملحوظة في نشاط أنزيمات CAT. SOD ومستوى MDA في كريات الدم الحمراء لجميع العمال مقارنة بالمجموعة الضابطة.

٣- عدم وجود فرق واضح بين مجموعات الدراسة بالنسبة لبروتينات مصل الدم التي تم فصلها.

٤- وجود تكسير في الـ DNA في جميع الحالات الخاضعة للدراسة فيما عدا العمال المعرضين لليوريا.

ويمكن أن نستخلص من هذه النتائج أن التعرض المستمر لأبخرة الفورمالين والفينول يمكن أن يؤدي الى تكسير الـ DNA عن طريق الأكسدة وزيادة نسبة الجلوتاثيون في كرات الدم الحمراء والبلازما.