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BIOCHEMICAL MONITORING OF EXPOSURE TO BENZENE AMONG SMOKER AND NON-SMOKER PETROL PUMP WORKERS

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ABSTRACT

Background: Benzene contributes for much of the occupational exposure to toxic chemicals which is a major public health concern worldwide. Activation of benzene and its reactive metabolites leads to continuous production of reactive oxygen species (ROS), which damages DNA, RNA, and proteins by chemical reactions and consumption of antioxidants in the body. The toxicity of xenobiotics is usually determined biochemically by the monitoring of some plasma enzymes and lipids.

Aim: To estimate the extent of oxidative stress by measuring the Malondialdehyde (MDA) level in blood, assess the level of superoxide dismutase (SOD) in smoker and non-smoker petrol pump workers.

Material and Method: 100 workers at Petrol pump stations (50 smokers and 50 non-smokers) and 50 service workers not exposed to Benzene were selected for study. Study consisted of quessionaire, and blood investigations for MDA, SOD and liver enzymes AST, ALT and ALP. Statistical analysis done by student t test and ANOVA.

Result: MDA, SOD and all liver enzyme levels of exposed group were found to be significantly high as compared to non-exposed group.

Conclusion: This study has demonstrated a strong relationship between exposure to petrol fumes and increase in oxidative stress and liver toxicity.

KEYWORDS : Petrol pump workers, Oxidative stress, Antioxidants,Smokers,Non-smokers

Introduction:

Petrol is the generic term for petroleum fuel used for internal combustion of engines. It is complex, volatile, and inflammable and contains over 500 saturated and unsaturated hydrocarbons, containing 3 - 12 carbons[1]. Benzene is the major monocyclic aromatic hydrocarbon largely used in a variety of industrial and commercial purposes[2]. Also the daily use of petro outside the industrial settings is likely to have effect on users. It contributes much of the occupational exposure to toxic chemicals which is a major public health concern worldwide[3], the frequency is increasing due to rapid development in technology[4].Vehicle exhaust emissions and evaporation losses of petrol at petrol filling stations are the main environmental sources of benzene exposure. Activation of benzene and its reactive metabolites leads to continuous production of reactive oxygen species (ROS), which damages DNA, RNA, and proteins by chemical reactions and consumption of antioxidants in the body. There may occur chemical reactions such as oxidation, nitration, and halogenation leading to genetic modification and alteration in the functions of important lipids, enzymes and other proteins [5,6].

Our body has developed a robust mechanism using substances known as antioxidants to prevent this injury. Under normal conditions, antioxidants convert ROS to H2O to prevent overproduction of ROS. There are two types of antioxidants in the human body: enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants are composed of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, which also causes reduction of hydrogen peroxide to water and alcohol. Non-enzymatic antioxidants are synthetic antioxidants or dietary supplements[7].

The toxicity of xenobiotics is usually determined biochemically by the monitoring of some plasma enzymes and lipids. A rise in enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and cholesterol are indices for liver cells damage[8].

This study intends to investigate the effects of petrol fumes on the liver enzymes, total protein and albumin on petrol-pump workers in Aurangabad city. This is because of the increasing number of youth hawking petrol as business due to the immediate financial gain and also to create awareness of the danger involved in such business. This study also aim to estimate the extent of oxidative stress by measuring the Malondialdehyde (MDA) level in blood, assess the level of superoxide dismutase (SOD) enzyme and identify the personal and occupational factors that may be associated with level of Malondialdehyde (MDA) detected, activity of superoxide dismutase (SOD) enzyme in smoker and non-smoker petrol pump workers.

Materials and methods

The study was conducted in Department of Biochemistry during the period-Jan 2017 to June 2017. Approval from Institutional Ethical Committee (IAC) was taken before start of the study. The subjects selected for the study were the workers at various petrol pumps, in Aurangabad. The study comprised of two exposed groups and one control group:

Group I (Smokers): It included 50 workers at Petrol pump stations in Aurangabad City exposed to Benzene on performing their job and who were smokers,

Group II (Non-smokers): It included 50 workers at Petrol pump stations in Aurangabad City exposed to Benzene on performing their job and who were not smokers.

Group II (Control/Non-exposed): It included 50 service workers not exposed to Benzene at their current occupation nor even had a past occupational history of exposure to Benzene and were non-smokers. Also known as control group.

Both exposed and control group were comparable as regards to sex (all were males) and age.

Inclusion criteria: All exposed were male workers in Aurangabad city, none of the workers included in this study had known exposure in any industry directly involved with benzene other than their current work. The subjects were apparently healthy between the age group of 25-55 years, with a minimum exposure of six months. The controls on the other hand were also males employed away from the petrol pumps and healthy. They were between 25 and 55 years with no exposure to petroleum fumes and matched for lifestyle with exposed workers.

A detail history was recorded which included questions regarding duration of work at petrol pumps, smoking habits, present respiratory symptoms if any, past history of respiratory/cardiac disease. The workers having past history of respiratory or cardiac disease were excluded from the study.

A structured questionnaire was used to collect information from all participants. Serum level of MDA was measured according to the method of Ohkawa et al. [9]. The reference range of MDA was 0.12 - 1.71 nmol/ml. The activity of SOD was assayed by the method developed by Kakkar [10]. The reference range of SOD was 0.16 – 0.24 u/l. Total Protein, Albumin and Liver enzymes ALT(Alanine Transaminase), AST (Aspartate Tranaminase) and ALP (Alkaline Phosphatase) were estimated using Vitros 5600 automated analyser. Hemoglobin was estimated using Siemens Advia 2120i. Statistical analysis was carried out after summarizing the data by computing mean and standard deviation (SD) of each study variable. Student t test and ANOVA was applied to compare the findings between exposed group (smokers and non-smokers) and non-exposed group.

A structured questionnaire was used to collect information from all participants about the following:

1. Socio demographic characteristics & Living conditions
• Age
• Gender
• Marital status
• Level of education
• Residence
• Current Smoking
• Duration of smoking
• Alcohol consumption
• Living in high polluted area
• Using sprays for pest control in your home
• Using household cleaning products in your home
2. Work environment
· Level of stress in your job
• Years of exposure to harmful chemical substances/ duration of employment
• Daily exposure in hours
3. Health conditions
• Having chronic emotional stress
• Having allergic tendencies
• Suffering from infections
• Using several rounds of antibiotics
• Suffering from aging symptoms

Result:

Results of this study showed that the exposed and control groups are comparable regarding age, marital status, the level of education and residence, living conditions and smoking, alcohol habits **(Table 1)**. Mean duration of smoking was 3 hours daily. The level of education was lower in smokers group. Even the habit of alcohol consumption was highly prominent in group I. Use of household cleaning products were high among the smokers group.

Table 2 shows the work environment and health status of all the groups. This table also shows that the level of stress was significantly moderate amongst both the groups. Almost 60 % of the exposed group-smokers were working for >10 years at the petrol pump station. The mean daily exposure of all exposed group workers was found to be 9.7 to 9.8 hours. There was no statistical difference between the duration of working. All the confounding factors in health status of smokers exposed group were high as compared to exposed non-smokers group and control.

Table 3 shows mean values of liver enzymes AST,ALT and ALP which are significantly raised in exposed group (smokers and non-smokers). The level of haemoglobin has decreased in exposed group but that is not statistically significant. The level of total protein and albumin decreased in exposed group which was found to be significant.

Figure 1 shows (mean) MDA levels of exposed-smokers group (6.49) which was found to be significantly high as compared to exposed non-smokers group (4.84) and non-exposed (3.04) group (p=0.0005). Similarly, figure 2 shows (mean) SOD levels of exposed-smokers group (0.72) which was found to be significantly low as compared to exposed non-smokers group (and non-exposed group (p=0.001).

Table 1: Demographic characteristics of petrol pump workers and controls

Socio demographic characteristics & Living conditions	Group I (Exposed-smokers) (n=50) (%)	Group II (Exposed-Non-smokers) (n=50) (%)	Group III (Non-exposed) (n=50) (%)
1. Age	41.38± 5.92	39.53 ± 6.37	36.82 ± 6.11
2. Gender Male	50 (100%)	50 (100%)	50 (100%)
3. Marital status Married Unmarried	40 (80%) 10 (20%)	37 (74 %) 13 (26%)	34 (68%) 16 (32%)
4. Level of education Illiterate Only read and write School education Higher education	15 (30%) 22 (44%) 10 (20%) 3 (6%)	10 (20%) 12 (24%) 24 (48%) 4 (8%)	5 (10%) 11 (22%) 21 (42%) 13 (26%)
5. Residence Urban Rural	17 (34%) 33 (66%)	27 (54%) 23 (46%)	29 (58%) 21 (42%)
6. Smoking Yes No Duration of smoking (hours)	50 (100%) 0 (0%) 3±0.86	0 (0%) 50 (100%) -	0 (0%) 50 (100%) -
7. Alcohol consumption Yes No	41 (82%) 9 (18%)	39 (78%) 11 (22%)	33(66%) 17(34%)
8. Living in high polluted area Yes No	39(78%) 11(22%)	34(68%) 16(32%)	29(58%) 21(42%)
9. Using sprays for pest control in your home Yes No	27 (54%) 23 (46%)	33(66%) 17(34%)	34(68%) 16(32%)
10. Using household cleaning products in your home Yes No	37 (74 %) 13 (26%)	27 (54%) 23 (46%)	17 (34%) 33 (66%)

Table 2: Work environment and health status of petrol pump workers and controls

Work environment and health status	Group I (Exposed-smokers) (n=50) (%)	Group II (Exposed-Non-smokers) (n=50) (%)	Group III (Non-exposed) (n=50) (%)
Work environment			
1. Level of stress in your job Mild Moderate	29(58%) 21(42%)	14(28%) 36(72%)	21(42%) 29(58%)
2. Years of exposure to harmful chemical substances/ duration of employment 5 or <5 years 5-10 years > 10 years	8 (16%) 12(24%) 30(60%)	10(20%) 16(38%) 24(48%)	-
3. Daily exposure in hours	9.8±0.9	9.7±1.2	-
Health conditions			
1. Having allergic tendencies Yes No	33(66%) 17(34%)	28(56%) 22(44%)	23(46%) 27(54%)
2. Suffering from infections Yes No	34(68%) 16(32%)	21(42%) 29(58%)	16(32%) 34(68%)

3. Using several rounds of antibiotics Yes No	39(78%) 11(22%)	34(68%) 16(32%)	29(58%) 21(42%)
4. Suffering from aging symptoms Yes No	34(68%) 16(32%)	33(66%) 17(34%)	21(42%) 29(58%)
5. Having chronic emotional stress Yes No	29(58%) 21(42%)	16(32%) 34(68%)	27 (54%) 23 (46%)

Table 3: Hemoglobin, liver enzymes and protein level in petrol pump workers and control group

Mean± SD	Group I and II (exposed) (n=100)	Group III (unexposed) (n=50)
Hb(%)	11.3±1.2	14.9±1.3
AST	52±6.8*	17±4.7
ALT	87±10.6**	29±8.1
Total Protein	4.82±1.40*	6.29±1.64
Albumin	2.75±0.72*	4.19±1.16
ALP	87.3± 22.6*	69.6± 16.5

*P<0.05 was considered as statistically significant
**P<0.001 was considered as highly statistically significant

Figure 1: Mean MDA levels in exposed smokers, exposed non-smokers and Non-exposed groups

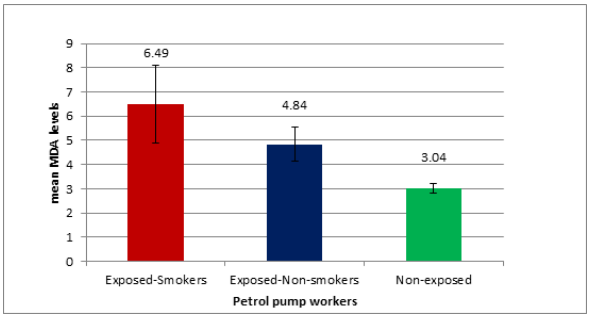
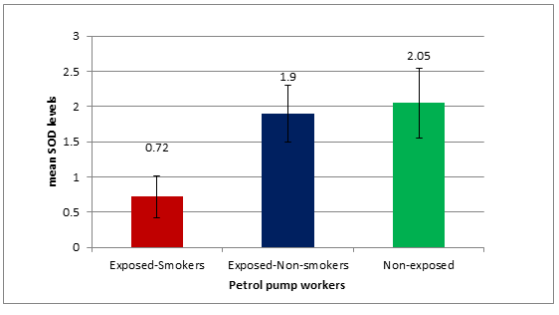


Figure 2: Mean SOD levels in exposed smokers, exposed non-smokers and Non-exposed groups



Discussion-

The risk of exposure to petrol fumes is even greater in the developing countries, compared to the developed countries. Limited facilities for safe handling of such toxic substances and lack of knowledge of reducing the over exposure and the toxic effects of such chemicals contribute to the increase in the exposure in the developing nations. Poverty has also made people to engage in such occupations without consideration to the health effects of such occupations. Results of this study showed no statistical significant difference (p-value > 0.05) regarding suffering from chronic emotional stress, allergic tendencies, infections and aging symptoms, also using of several rounds of antibiotics among exposed and control groups.

Results of this study showed that there is a higher statistical significant difference (p-value < 0.05) concerning the level of malondialdehyde (MDA) being higher among exposed smokers compared to exposed non-smokers, also there is lower statistical significant difference (p-value <0.05) regarding the level of superoxide dismutase (SOD) as it was lower in exposed smokers than exposed non-smokers. Tobacco smoke is a rich source of oxidants. Increased production of reactive oxygen species has been associated with smoking , which may exceed the capacity of oxidant defense system, resulting in oxidative damage[11]. This study showed that there was a higher statistical significant difference among smokers concerning the level of Malondialdehyde (MDA) compared to non-smokers. These findings were in agreement with other studies which detected that Malondialdehyde levels were significantly increased in cigarette smokers.[12,13,14,15]

Also there was lower statistical significant difference regarding the level of superoxide dismutase (SOD) among smokers compared to non-smokers. This was consistent with other studies that found decreased activity of SOD among smokers.[13,14]

This study showed high statistical significant difference among exposed group compared to control group regarding the level Malondialdehyde (MDA) which was higher among exposed**.** MDA increased with increase work duration while the level of SOD decreased with increased work duration. This is in agreement with other studies that showed workers had significant uptake of benzene due to prolonged work hours and increased years of exposure which will also leads to the formation of ROS, decreases antioxidant activity and hence increases oxidative stress.[16,17]

Benzene is often found in automobiles and solvent gasoline[18]. However the most affected are those who occupationally exposed to the fumes[19,20,21]. A high level of benzene in the breathing zone of fuel service station during refueling of automobiles has been reported[22]. Benzene and related hydrocarbons are metabolized through an intermediate epoxide, which is highly reactive and possibly binds to hepatic microsomal proteins and nucleic acids leading to cytotoxic effects[23]. Petroleum constituents can cause liver damage and may disturb the normal biochemical process in the hepatobilliary system, this ranges from increased enzymes to hepatic failure, again adults can be affected and have neurological damage, anaemia, hypertension, impotence, sterility and miscarriage[24].

Concerning the health conditions study results showed that most workers complaining of chronic emotional stress has significantly higher levels of Malondialdehyde (MDA) compared with those that don't complain of chronic emotional stress. This is in agreement with other studies which indicated that in stress condition oxidative damage to DNA and sensitivity to lipid oxidation were significantly increased when compared with the same parameters in “non-stress” conditions[25,26]. Sivonová et al. [26] found a significant decrease in plasma antioxidant activity (SOD) in students who were under stress.

The statistical results of the current study showed that the hemoglobin of the refuel station workers were lower in the petrol exposed group compared to the control but were not statistically significant. The liver enzymes were significantly raised in exposed group while the total proteins and albumin levels significantly decreased. An increase levels in these enzymes activities in the plasma are linked to hepatocellular damage caused by either toxins, toxins in drugs or herbs[27]. Nasterlack et al, 1994 had found that ALT and AST are used as sensitive biomarkers for possible hepatocellular damage due to exposure to organic solvents[28].

Conclusion:

This study has demonstrated a strong relationship between exposure to petrol fumes and increase in oxidative stress. The decreased levels of antioxidants resulting from working at petroleum fuel stations for long period could be causes for serious health problems, liver toxicity and other organs and tissue damage. Also this study supports the hypothesis that Benzene and its metabolites induce oxidative stress which was noticed by the results of the study that showed increased level of MDA and decreased antioxidants activity among the petrol station workers who were smokers and this plays a role in benzene-initiated toxicity. Regular examination of liver function tests and attention to use safety gloves and face mask is recommended for petroleum fuel stations workers.

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