Research Article
Effect of Exposure to Petroleum Fumes on Plasma Antioxidant Defense System in Petrol Attendants

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Abstract: Several studies have reported toxicological implications of inhalational exposure to petrol fumes in animal models; however, there is little or no documentation on the probable effect of exposure in human subjects. This study investigated the relationship between exposure to petrol fumes and lipid peroxidation and antioxidant levels among petrol station attendants in Ibadan, South-West Nigeria A total of 150 subjects consisting of 100 petrol attendants and 50 control subjects were recruited. Ten mL of blood was collected from ante-cubital vein of subjects for analysis. Results reveal that exposure to petrol fumes is associated with oxidative stress. Significant ($p<0.001$) elevation of malondialdehyde was associated with marked decreases in superoxide dismutase ($p<0.01$), catalase ($p<0.001$) and glutathione ($p<0.05$) when compared with the control. Chain breaking antioxidant vitamins results include significant ($p<0.05$) decreases in vitamin E and no significant difference in vitamin C ($p>0.05$) when compared with control. Also there was a significant decrease in total protein ($p<0.05$) but no significant difference in albumin ($p>0.05$) in petrol attendants compared with the control. Our findings imply that exposure to petroleum fumes is a risk factor and is associated with oxidative stress which raises the need for public awareness about the health hazards in order to enable petrol attendants to take necessary precautionary measures.

Keywords: Antioxidants, health effect, lipid peroxidation, occupational exposure, petrol attendants

INTRODUCTION

Petrol is distilled from crude petroleum and vapour obtained as a result of its evaporation may be considered as petrol fumes. The volatile nature of petrol makes it readily available in the atmosphere any time it is dispensed, especially at petrol filling stations and depots. Petrol contains mixture of volatile hydrocarbons and so inhalation is the most common form of exposure (Cecil *et al*., 1997). Petrol vapour can reach supra-lethal concentrations in confined or poorly ventilated areas, although such exposures are rare (Takamiya *et al*., 2003).

The intentional inhalation of vapour ('sniffing' or 'huffing') has been extensively documented (Cairney *et al*., 2002). At low doses, petrol vapour is irritating to the eyes, respiratory tract and skin. Exposure to higher concentrations of vapour may produce CNS effects such as staggered gait, slurred speech and confusion. Very high concentrations may result in rapid unconsciousness and death due to respiratory failure (Chilcott, 2007). Motorists are exposed to gasoline fumes during fuelling at gas stations, but the gas station attendants are more at risk by virtue of their occupational exposure (Micyus *et al*., 2005).

Gasoline vapour is not safe when inhaled even for a brief period of time (seconds). Vapour concentrations, expressed in parts per million (ppm) or mass of total hydrocarbons per unit volume (mg/m³) of air above open barrel in unventilated out-house on 'hot' day is 25,000, air around tanker during bulk-loading is between 50 and 320 while air around petrol pump in service station during fuelling of vehicles is between 20 and 200 ppm (Micyus *et al*., 2005; Lewne *et al*., 2006).

Gasoline fumes contains aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons which are a continued source of pollution in various occupational setting It has been demonstrated that after inhalation of petroleum vapour through chronic exposure, lower concentrations of saturated hydrocarbons are detected in human and animal blood than that of the unsaturated aromatic hydrocarbons (Yamamoto and Wilson, 1987). Both diesel and gasoline engine exhausts are known to contain, in either the particulate or the vapour phase, a variety of mutagenic and carcinogenic agents (Yamamoto and Wilson, 1987). Benzene and toluene are major monocyclic hydrocarbons present in the refined petrol. Biological monitoring of exposure to bitumen fumes during road-paving operations indicated urinary excretion of 1-hydroxypyrene and thioethers in the exposed workers (Burgaz *et al*., 1992).

Ueng *et al*. (1998) reported that exposure of rats to motorcycle exhaust and organic extracts of the exhaust

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particulate caused a dose- and time-dependent increase in cytochrome P_{450} dependent monoxygenases and glutathione-S-transferase in the liver, kidney and lung microsomes. Since petrol contains some of these constituents, chronic or frequent exposure to their fumes may affect the oxidant/pro-oxidant balance in exposed individuals. Data on the potential deleterious effect of exposure on oxidative damage and antioxidant systems are still very scanty. So far, the biochemical mechanisms involved in xenobiotic biotransformation in petrol exposed individuals have not been clearly elucidated in Nigeria. In this present study, therefore, we assessed the levels of biomarkers of oxidative stress in petrol attendants in Ibadan, South-West of Nigeria.

MATERIALS AND METHODS

Subjects and study design: The study comprised of a total of 150 subjects (aged between 21 and 37 years with a median of 24 years), consisting of 100 petrol attendants and 50 healthy control matched with respect to age, sex and smoking habit and with no known chemical exposure at work in Ibadan metropolis, Nigeria. Only those individuals who had not been on antioxidant supplements or had conditions (such as diabetes, asthma, hypertension, malaria) with underlying inflammatory or immune responses and the use of drugs which interfere with oxidative metabolism were recruited. Participants gave informed written consent in accordance with Helsinki Declaration of 1964 as amended in 1983 (World Medical Organization, 1996). Ten (10) ml of blood sample were collected from the ante-cubital vein of subjects for analysis.

Determination of malondialdehyde and reduced glutathione: Lipid peroxidation was estimated spectrophotometrically by the thiobarbituric acid reactive substance (TBARS) method as described by Varshney and Kale (1990) and malondialdehyde (MDA) was quantified using $\Sigma = 1.56 \times 10^5$/M/cm (Buege and Aust, 1978). Reduced glutathione (GSH) level was estimated at 412 nm according to the method of Beutler *et al.* (1963).

Assay of antioxidant enzymes: Catalase (CAT) activity was determined according to the spectrophotometric method described by Clairborne (1995). The assay was based on the ability of CAT to induce the disappearance of H_2O_2. Superoxide dismutase (SOD) activity was determined based on the ability of SOD to inhibit the spontaneous oxidation of adrenaline to adrenochrome as described by Magwere *et al.* (1997).

Determination of plasma level of vitamins C and E: The level of vitamin E was determined in the plasma level by using the method of Baker (1968) which is based on the principle that vitamin E extracted in

xylene is made to react with alpha, alpha-dipyridyl. The product produces a reddish color with ferric chloride, which was read at 520 nm while vitamin C was determined colorimetrically according to the method of Omaye *et al.* (1979) in which vitamin C reacts with acidic 2, 4-dinitrophenylhydrazine to form a red bis-hydrazone which was measured at 520 nm.

Statistical analysis: Results are presented as mean±standard deviation (S.D.). Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 16.0. Comparison between Petrol attendants and control was performed using Student's *t*-test for unpaired data. The statistical significance was set at p<0.05.

RESULTS

A total of 150 subjects comprising of 40 females and 110 males were recruited for the study, 100 subjects (25 females and 75 males) were petrol attendants and the remaining 50 subjects (15 females and 35 males) were control.

Antioxidant parameters: Table 1 depicts blood levels of MDA, GSH, as well as the activities of SOD and CAT in petrol attendants and control groups. Petrol attendants exhibited significant (p<0.001) increases in MDA with marked decreases in SOD (p<0.001) and CAT (p<0.001) activities as well as GSH (p<0.05) levels when compared with control.

Plasma proteins and antioxidant vitamins: Table 2 shows plasma proteins and antioxidant vitamins of petrol attendants and control groups. Vitamin E and total protein decreased significantly (p<0.05) while total leukocytes count was significantly increased (p<0.01) in the MSW workers when compared with control. There were no significant (p>0.05) changes between albumin and vitamin C of petrol attendants and control.

Table 1: Markers of oxidative stress/antioxidant status of petrol attendants

Parameters	P.A (n = 100)	Control (n = 50)	t-value	p-value
MDA (nmol/mL)	4.61±0.27*	2.57±0.31	10.715	0.000
GSH (mg/dL)	0.77±0.26*	1.67±0.30	-18.695	0.000
SOD[†]	2.17±0.14*	4.35±0.41	-6.975	0.000
CAT[††]	41.67±8.54*	43.33±8.99	3.635	0.000

Values are expressed as mean ± standard deviation (S.D.); n = number of subjects; P.A: petrol attendants; [†]: Activity expressed as units of enzymes required to inhibit auto-oxidation of adrenaline to adrenochrome; [††]: Activity expressed as µmol H_2O_2 consumed/min/mg Hb. MDA: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase; CAT: catalase, *: Significantly different from control

Table 2: Levels of plasma proteins and antioxidant vitamins of petrol attendants

Parameters	P.A (n = 100)	Control (n = 50)	t-value	p-value
TP (g/dL)	6.70±0.70*	7.05±0.75	-2.821	0.005
ALB (g/dL)	3.76±0.38	3.60±0.62	1.895	0.060
Vit. C (mg/dL)	14.26±8.69	16.73±5.77	-1.813	0.072
Vit. E (mg/dL)	0.16±0.14*	0.68±0.78	12.419	0.000

Values are expressed as mean±standard deviation (S.D.); n = number of subjects; P.A: petrol attendants; TP: total protein; ALB: albumin; Vit C: vitamin E; Vit. E: vitamin E; *: Significantly different from control

DISCUSSION

In the last few years, attempts have been made regarding the evaluation of health impact of petrol fumes in human and laboratory animals in Nigeria (Uboh *et al*., 2005 and Akinosun *et al*., 2006). Several studies have reported toxicological implications of inhalational exposure to petrol fumes in human and animal models (Smith *et al*., 1993; Pranjic *et al*., 2002; Lewne *et al*., 2006; Azeez *et al*., 2012). Xenobiotics within the organism undergo a series of reactions and biotransformation to facilitate their excretion. Oxyradicals are continually produced in eukaryotes as unwanted byproducts of normal oxidative metabolism and their production can be increased by conditions such as hypoxia/hyperoxia, redox cycling xenobiotics like metals, quinones, nitroaromatic compounds and induction of enzymes, such as cytochrome P_{450} and P_{450} reductase (Premereur *et al*., 1986). Consequently, aerobic organisms have developed defence systems against oxidative damage (Di Giulio *et al*., 1989), consisting of antioxidant scavengers (glutathione, vitamin C, vitamin E, carotenoid pigments) and specific antioxidant enzymes: catalase, superoxide dismutase and glutathione peroxidase. These enzymes participate in the removal of reactive oxygen species.

Our results indicate significant elevation of lipid peroxidation in these subjects when compared with the control. Environmental pollutants and petrol fumes have been reported to enhance peroxidative processes and oxidative stress within cells (Wright and Welbourne, 2002). Elevated levels of the thiobarbituric acid reactive substance, malondialdehyde, reveal peroxidative damage to cell membranes and other lipid-derived macromolecules. Lipid peroxidation results from release of free radicals that can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes to form malondialdehyde (MDA). This oxidative stress may have resulted from the build-up of ROS such as O_2^- and H_2O_2 following the decrease in the activities of the enzymatic antioxidants. The increased generation of O_2^- and H_2O_2 leads to increase production of the more reactive hydroxyl (OH$\cdot$) radicals via Fenton and Haber-Weiss reactions (Stadman, 1990). OH$\cdot$ Radicals react at nearly diffusion-limited rates with any component of the cell including lipids, DNA and proteins. The net result of this non-specific free radical attack is a loss of cell integrity, enzyme function and genomic stability (Gille *et al*., 1994). The enhanced lipid peroxidation observed in the petrol attendants when compared with control subjects correlates with decrease in the antioxidant defense system in their blood. This is evident in the significant decrease in the enzymatic antioxidants- superoxide dismutase (SOD) and catalase (CAT)- as well as the non-enzymatic redox sensitive thiol compound, reduced glutathione (GSH). SOD and CAT enzymes are major primary antioxidant defense components that primarily catalyze the dimutation of superoxide radical (O_2^-) to H_2O_2 and decomposition of H_2O_2 to H_2O, respectively (McCord and Fridovich, 1969; Cheng *et al*., 1981). The decreased SOD and CAT activities induced by exposure to petroleum fumes probably results in accumulation of O_2^- and H_2O_2 which react with metal ions to promote additional radical generation, with release of the particularly reactive hydroxyl radicals (OH$^-$) (Stadman, 1990). OH$^-$ reacts at nearly diffusion-limited rates with any component of the cell including lipids, DNA and proteins. The net result of this non-specific free radical attack is a loss of cell integrity, enzyme function and genomic stability (Huhhes *et al*., 1996). The involvement of these Reactive Oxygen Species (ROS) in inflammatory responses has been reported (Khansari *et al*., 2009).

A significant decrease in the level of glutathione was accompanied by a significant increase in MDA level in this study. This observation is in agreement with the reports that inverse relationship exists between lipid peroxidation and glutathione status (Hill and Singal, 1996; Singal *et al*., 1993). Glutathione depletion impairs the cell defense against the toxic action of xenobiotic which could lead to cell injury or death. Under acute oxidative stress, the toxic effects of the pollutants may overwhelm the antioxidant defenses (McCord, 1996). Furthermore, the apparent decrease in glutathione detoxification system as a result of environmental xenobiotics indicates that this system is a sensitive biochemical indicator of environmental pollution (Kono and Fridovich, 1982).

Vitamin E is a powerful chain-breaking antioxidant, primarily preventing lipid peroxidation by breaking the chain of events leading to the formation of hydroperoxides. This action should also lead to a reduction in DNA damage since the intermediate products of lipid peroxidation include lipid peroxides, which can cause strand breaks in DNA (Cheeseman, 1993). Significant decrease of vitamin E observed in petrol attendant may be attributable to its role in preventing lipid peroxidation which may cause its depletion. No significant difference was observed in vitamin C levels in petrol attendants when compared with the control. Albumin level in petrol attendants did not change significantly when compares with the controls. Our results agreed with the study of Akinosun *et al*. (2006). Albumin transports free fatty acids in the plasma and possesses cysteine residue which enhances its capacity to neutralize peroxyl radicals (Young and Woodside, 2001). Data from this study seem to suggest that oxidative stress is associated with occupational exposure to petrol fume in these individuals. Petroleum attendants therefore should take necessary precautionary measures and have regular medical check-up to ascertain their health condition.

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