ORIGINAL ARTICLE

Analysis of Oxidative Stress in Chronic Exposure to Petroleum Hydrocarbons in Karnataka, India

SUTTUR SRIKANTA NAIK MALINI [1,*], KATHIRESAN MAITHILY [1]

[1]Molecular Reproduction and Human Genetics Laboratory, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore, Karnataka, India

Abstract

Background: Several studies have reported the toxicological implications of inhalation of petroleum hydrocarbon fumes in animal models. But, there is certainly little or no documentation of the exposure to petroleum hydrocarbon fuel on oxidative stress levels in humans, unlike the pulmonary physiology. The present study was carried out to evaluate the effects of constituents of the hydrocarbon fuels on oxidative stress levels of the petrol fillers and tanker drivers.
Methods: The study involved 165 males divided into three groups were the petrol fillers, tanker drivers and the controls. Case control data set was established wherein the control subjects are not exposed to hydrocarbon fuels with similar age. Serum samples of the subjects were collected and subjected for various biochemical assays. The enzymatic antioxidants such as superoxide dismutase, malondialdehyde a byproduct of lipid peroxidation and total antioxidant capacity of the individuals along with non-enzymatic antioxidant Vitamin A was estimated.
Results: The results showed a no significant differences for age, body mass index, superoxide dismutase and levels of Malondialdehyde and total antioxidant capacity. But on the other hand, there is significant changes observed for total antioxidant capacity and vitamin A when exposed group is compared with control subject.
Conclusion: It is evidential from the present study that prolonged exposure to petroleum hydrocarbon fumes leads to an increase in their oxidative stress in turn resulting broad spectrum of diseases. Hence, there is a raised need for public awareness about the health hazards in order to enable petrol attendants.

Key words: Anti-Oxidants; India; Oxidative Stress; Petroleum Exposure

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INTRODUCTION

Total Petroleum Hydrocarbons (TPH) is a term used to describe a broad family of several hundred chemical compounds called hydrocarbons that originally come from crude oil (1). Crude oil varies in how much of each chemical they contain, and so can the petroleum products that are made from crude oil (2).

Petrol has mixture of volatile hydrocarbons which is readily available in the atmosphere dispenses easily at petrol filling stations and depots. Hence the inhalation is the most common form of exposure in those areas (3,4). Even though exposure to supra lethal concentration of petrol vapor is rare but possible in highly confined or poor ventilation areas (5). If the Petrol pump attendants are routinely exposed to hydrocarbon fuel vapor that has been reported to increase the risks for acute and chronic health problems (6). They are more prone to respiratory tract ailments due to the interruption by the particulate matter (PM) and fuel constituents (7). The intentional inhalation of vapor has been extensively documented resulting irritation of eyes, respiratory tract and skin at low doses (8). Exposure to higher concentrations of vapor may affect the central nervous system (CNS) resulting in staggered gait, slurred speech and confusion and very high concentrations may result in rapid unconsciousness and death due to respiratory failure (9). Chronic exposure leads to chronic inflammation of respiratory tract and lung parenchyma (10,11). The inflammatory response was also studied in mice, in which inhalation of 300 μm^3 of PM 2.5 caused increase in TNF-β, interferon- β, IL-6 and transforming growth factor- β resulting in ROS synthesis (12).

Benzene occupies the major composition of petroleum constituents and is a class I human carcinogen (13). Evaporation of petrol is common during its handling, distribution and storage which intern releases benzene along with vehicle exhaust (14). Activation of benzene and its reactive metabolites leads to continuous production of reactive oxygen species (ROS), which leads to lipid peroxidation and damages DNA, RNA, leading to genetic modification and alterations in the functions of important enzymes and proteins (15). Chronic benzene exposure leads to decrease in antioxidant enzymes activity and hematologic disorders. Benzene affects many enzyme activities in the liver,

*Correspondence to: Suttur Srikanta Naik Malini; MSc, PhD. Assistant professor, DOS in Zoology, University of Mysore, Manasagangothri,Mysuru-06, India. Email: ssmalinisri@yahoo.co.in, drssmalini@gmail.com
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tissues, and peripheral blood and this can lead to a decrease in the activity of antioxidants enzymes and may result in oxidative stress (16). Oxidative stress occurs as a consequence of the imbalance between pro-oxidants and antioxidants. This imbalance is due to excessive accumulation of reactive oxygen species or antioxidant depletion or both together resulting in cellular damages (17-21). Increased levels of hydrogen peroxide, hypochlorous acid (HClO) and free radicals including hydroxyl radical (OH) and superoxide anion (O^{2-}) enhances the production of ROS in those organisms which are exposed to petroleum compounds. Animals will maintain a balance between generation and neutralization of ROS under normal physiological conditions. If otherwise the inflammation of the alveolar surfaces due to inhalation of the petrol fumes is quite common

Antioxidants such as glutathione (GSH), uric acid, ascorbate and α-tocopherol present in epithelial lining fluid (ELF) may protect the airways from oxidant injury induced by exposure to air pollutants (22).Antioxidants prevents the oxidation of macromolecules such as lipids, proteins and carbohydrates by scavenging oxidant pollutants from the air ways. But insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Till date analysis of the effect of exposure to hydrocarbon in humans is not documented vastly. The related studies were limited to fly or murine model even though few researchers made an attempt, still the sample size was comparatively small to get conclusive message. Hence, in view of this the present investigation was carried out to analyze the oxidative stress level due to hydrocarbon fuel fumes on fuel station attendants.

METHODS

A cross-sectional toxic-biochemical study with prior Ethical Clearance (IHEC –UOM No.49/MSc /2015-16) and consent from the subjects, was conducted among 165 apparently normal males within the age group of 20-50 years with a minimum exposure of 8hrs per day (as the work schedule has fixed to 8hrs) for 8 yrs. (Workers had various years of experience. Since majority was in the group of 7-8years we considered 8y of exposure).The subjects were classified into three groups and were included in the study. The first group was the fuel fillers (n-55) and the second group was the fuel tanker drivers (n-55) who were frequently exposed to hydrocarbon fuel fumes during loading, unloading and filling petrol. The third group constitutes healthy males (n-55) of similar age group without frequent exposure to hydrocarbon fumes. Exclusion criteria being female workers, subjects with medication and nutrient supplements, known cases of cardiac disorders, dyslipidemias, pulmonary disorders and metabolic errors and family history of above said illness. The information about personal data like eating habits, smoking, alcoholism and any other addictions were enquired for and noted. A note on the duration of smoking and alcoholism, duration and experience of work, marital status, domicile, literacy level, occupation, socio-economic status, was done. Cases with smokers and alcoholics were excluded from the study and finally three groups with 55 individuals in each group was maintained to have case-control data set. The respective bio assays were standardized, tabulated and analysed using SPSS software version 21. Analyses of variance (ANOVA) and t-test (independent test) was employed to find out any significance difference existing between different groups, $p<0.05$ was considered as significant

Sample collection and analysis: 5ml of venous blood collected under strict aseptic precaution and allowed to clot at room temperature. The serum was separated by centrifugation and stored at -20^0c for biochemical assay. The assays were done for the markers like superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity (TAC) and vitamin A.

Biochemical Assays

Superoxide dismutase (SOD): The concentration of SOD which was consumed to nullify superoxide radical in order to reduce oxidative stress at cellular level was estimated by Das *et al.,* (23). Preparation of reaction mixture was done using appropriate volume of the following reagents such as phosphate buffer, α methionine, triton X, hydroxylamine hydrochloride (HAC) and Ethylenediamine Tetra Acetic acid (EDTA). 139µl of the reaction mixture was added to 10µl of sample and incubated for 5 min at room temperature. Simultaneously a blank was also run which constituted distilled water instead of sample. 8µl of phosphate buffer and riboflavin was then added to the blank and samples respectively. 100µl of Griess reagent was added to them after 10 minutes of incubation at room temperature. Optical Density was measured at 543nm. Protein estimation of the respective subject was done by Lowry's method in order to express the SOD level.

Malondialdehyde (MDA):MDA being a byproduct of lipid peroxidation at cellular level was estimated by the method of Mossa *et al.,* (24). Three reagents namely I, II, III were prepared using trichloro acetic acid 17.5% (TCA), TCA 70% and thiobarbituric acid 0.6% (TBA) respectively. 1ml each of these reagents were mixed with 1ml of the sample and incubated in boiling water bath for 15min. It is left undisturbed for 20min and then centrifuged at 2000rpm for 15min. Optical Density of the supernatant was observed at 534nm. A blank was also run simultaneously.

Total Antioxidant Capacity (TAC): Estimation of TAC was done by the phosphomolybdenum method. To 100µl of serum 100µl of 5% TCA was added and left undisturbed for precipitation and then centrifuged at 3000 rpm for 5min. 1ml of TAC reagent was added to 100µl of this supernatant to and incubated for 90min at 90ºC. Optical Density was checked at 695nm. Simultaneously a blank was also run which constituted distilled water instead of sample.

Vitamin A: Vitamin A levels of the study subjects were estimated by Rutkowski *et al.* (25) method as they are the premier indictors of stress produced by chemical or physical carcinogens and mutagens. To 1ml of the serum 1ml of KOH was added and incubated for 20min at 60ºC after shaking vigorously. To this 1ml of xylene was added and shook vigorously for 1min. It was then centrifuged at 1500 rpm and OD of the separated extract was observed at 335nm.

Table 1. Comparison of oxidative stress parameters of exposed and non-exposed group for petroleum fumes (BMI- Body Mass Index, TAC-Total Antioxidant Capacity, SOD- Super oxide Dismutase, MDA- Malondialdehyde).

Parameter	Study group	Mean ±SE	F	p< 0.05
Age (Years)	Fillers Drivers Controls	34.63±1.46 34.68±1.29 35.40±1.30	0.828	0.682
BMI (Kg/m^2)	Fillers Drivers Controls	22.16±0.63 22.66±0.63 22.89±0.52	0.741	0.462
TAC (µg/ml)	Fillers Drivers Controls	25.73±1.42 25.12±0.85 28.19±0.87	0.123	0.781
SOD (Units/mg of protein)	Fillers Drivers Controls	0.33±0.02 0.18±0.00 0.45±0.02	20.6	0.106
MDA (µMol/ml)	Fillers Drivers Controls	1.27±0.02 1.59±0.09 1.39±0.03	0.559	0.472
VIT A (µM)	Fillers Drivers Controls	18.61±0.92 28.42±1.34 4.90±0.40	29.701	0.01*

*P value is significant at the 0.05 level

RESULTS

Comparison of age and BMI was done along with the oxidative stress markers such as SOD, TAC, MDA and vitamin A in three groups such as drivers, petroleum product fillers and control subjects (table 1). No significant difference exist for SOD, TAC and MDA but vitamin A shows fairly significant difference ($p<0.05$) between three groups. Similarly SOD, MDA levels remain same for exposed (drivers) and non-exposed group but differences was significant for TAC and Vitamin A between drivers and control group (Figure1).

Comparison of oxidative stress parameters between petroleum product fillers and control subject was depicted in the figure 2. Results were similar as that of the previous figures wherein no difference for SOD and MDA but for TAC and vitamin A significant difference were evident among controls and fillers. Variation in the levels of TAC and increased levels of vitamin A indicating that body homeostasis is maintained by scavenging free radicals which were produced due to oxidative stress as a result of exposure to petroleum products.

Figure 3 shows the comparison among exposed group like drivers and fillers wherein the levels of vitamin A was preferably higher among drivers compared to fillers indicating drivers were exposed to long duration for petroleum fumes compared to fillers who have brief exposures to petroleum fumes require more attention.

DISCUSSION

In the recent past, attempts have been made regarding the evaluation of health impact and toxicological implications of inhalational exposure to petrol fumes (26, 27) The biomarkers evaluated in this work may provide early signals

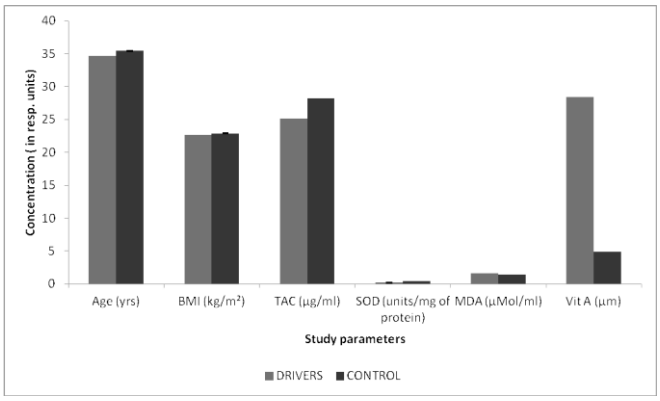

Figure 1. Comparison of oxidative stress parameters between drivers and control group (BMI- Body Mass Index, TAC-Total Antioxidant Capacity, SOD- Super oxide Dismutase, MDA- Malondialdehyde)

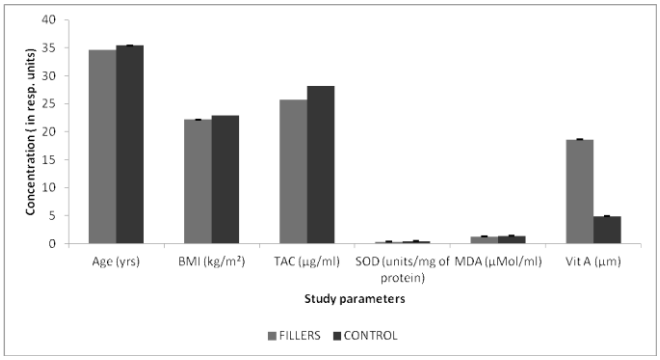

Figure 2. Comparison of oxidative stress parameters between fillers of petroleum products and control group (BMI- Body Mass Index, TAC-Total Antioxidant Capacity, SOD- Super oxide Dismutase, MDA- Malondialdehyde)

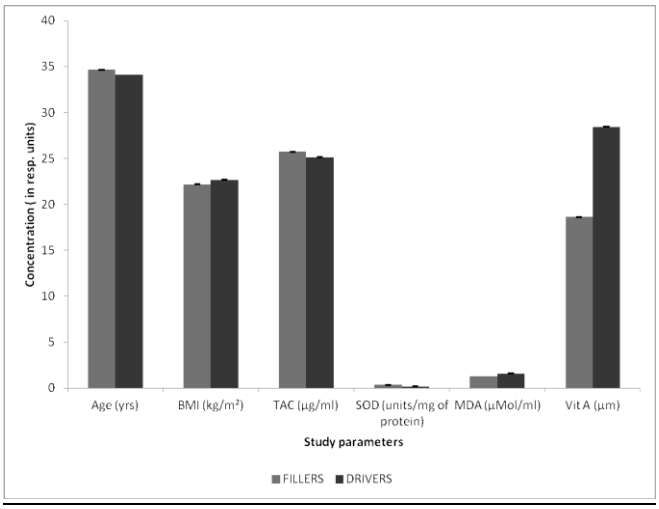

Figure 3. Comparison of oxidative stress parameters between fillers of petroleum products and drivers (BMI- Body Mass Index, TAC- Total Antioxidant Capacity, SOD- Super oxide Dismutase, MDA- Malondialdehyde)

of damage in subjects occupationally exposed to benzene, class I human carcinogen which may have genotoxic and carcinogenic effects.

About 95% of compounds in petrol vapors are aliphatic compounds and less than 2% are aromatics. Hence it readily available in the atmosphere at any time especially at petrol filling stations (28). Many studies have linked excess generation of ROS with cellular damage. Oxidative stress caused by the unbalance between the generations of ROS and the rate of their consumption by antioxidants (29).Generally, ROS is generated in two different ways. Firstly, ROS is generated by intrinsic properties of particles and iron content of the dust particles. Secondly, the intensive formation of ROS occurs by the oxidative burst of macrophages and neutrophils activated during phagocytosis and persistent inflammation (30).

Free radicals are produced due to oxidation process and these radicals intern causes cellular damages due to their chain reactions. Antioxidants terminate these chain reactions by inhibiting the oxidation of these molecules. They do this by being oxidizing themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid (vitamin C), or polyphenols. Glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases were complex system of multiple types of antioxidants which are predominant in both plants and animals.

Free radicals like OH^- radical, H_2O_2, HOCl or O^{2-} radical attempts to destroy the cellular integrity by acting on the bilipid layer of the plasma membrane. At this position the enzymatic antioxidant SOD catalyzes the destruction of superoxide radical; $2O^{2-} + 2H^+ \rightarrow O_2 + H_2O_2$. This enzyme protects the oxygen metabolizing cells against harmful effects of superoxide free radicals defined as lipid peroxidation (31). MDA is one of the byproducts of this process, widely used index to estimate oxidative stress (32). Benzene exposure has been associated with increases in the overall formation of MDA. Hence, it is clear that increased oxidative stress causes frequent lipid peroxidation there by leading to decrease in the concentration of SOD and, eventually that causes increase in the concentration of MDA in the blood plasma. Hence it is understood that if there is higher concentration of superoxide free radicals there would be more consumption of SOD enzyme.

In the present study the association between oxidative stress and exposure to hydrocarbon between control subjects and exposed individuals was not evident. The major reason behind this could be the purity of the fuel. Studies carried out in other parts of the globe reported that 30% of the fuel petroleum constitutes benzene. But, this concentration of benzene is 3% in Indian petroleum fuels (33, 34). This concentration of benzene determines the purity level of the hydrocarbon fuel and the dietary practices, hygienic and daily activities followed by the study subjects are healthy as observed from the questionnaire. Probably due to these reason there are no remarkably changes in exposed group for SOD and MDA levels when compared control group.

Total antioxidant capacity considers the cumulative effect of all antioxidants present in blood and body fluids (35).In the present study it is observed that there is slight variation exist for TAC level of the study subjects and control. This observation accords the previous documentations (6).A significant increase in the concentration of Vitamin A in the petrol pumps workers than the controls was evident in the present study. Several researchers made an attempt to emphasize the importance of dietary vitamins such as A,C and E in protecting several types of cancers and cardiovascular diseases (36). It is usually suggested that the activity of these compound is related to their ability to scavenge toxic forms of oxygen and other free radicals in living systems (37). Vitamin A has remarkable response when exposed to mutagens. High concentration of Vitamin A is consumed during cellular metabolism when the cells are exposed to any of the chemical or physical mutagens.

Developmental process, cell growth, and differentiation of the animals are regulated by key regulators which are mainly derived by Vitamin A (all-trans retinol) and its derivatives the retinoids. Retinoid action is mediated by specific nuclear retinoic acid receptors (RARs) and retinoid receptors (RXRs) belonging to the steroid/thyroid superfamily of transcription factors (38). Many researchers have proved that retinol may also act directly on the modulation of different PKC isoforms, serine/threonine kinases and cRaf activities (38). Retinol supplementation changes the phosphorylation pattern of histones and the high mobility group (HMG) of proteins, altering the organization and function of chromatin, which in turn modulates the switching on and off of transcriptionally active regions of DNA in cultured rat sertoli cells (39). Retinol treatment being proved to be highly effective also on chromatin structure and DNA repair processes, as well as on reactive oxygen species metabolism (40).

In the present study increased concentration of vitamin A in the petro pump workers than the controls at the particular instant could be a counter acting mechanism against oxidative stress which has been produced by the mutagens in petroleum constituents. As such the vitamin A is one of the important

antioxidants which in turn ameliorating the toxic effects in exposed workers due to the production of free radicals which are highly reactive causes adverse health issues. Hence, vitamin A level was significantly increased both in drivers and fillers of petroleum products.

CONCLUSION

Disease manifestation of various organs in petrol bunk attendants could be due toxic effect of petrol fumes which might act as genotoxic, agent. Chronic petroleum exposure is predictable cause of cellular toxicity. By developing appropriate biochemical markers in disease diagnosis will help the needy to for appropriate health management. The observed decrease in TAC and increased vitamin A levels attributes to increased oxidative stress. The subjects who are exposed should be given full attention in medical surveillance. There is a strong need to advocate stringent policies to safe guard the health of the neglected petrol station workers and they should be ensured safety and healthy working atmosphere to alleviate the health hazards that they may encounter.

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