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Biomonitoring Study on Workers Occupationally Exposed to Automobile Fuels

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ABSTRACT Petrol products remain unavoidable environmental pollutants as well as serious health hazards. Hence, the present study was undertaken amongst 70 petrol pump attendants and 70 Control subjects to evaluate the effects of exposure. The Exposed Group was further divided into two groups (Control Addiction and Control Non-addiction). Urinary phenol measurement, haematological analysis and Reactive Oxygen Species (ROS) parameters such as Super oxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Glutathione peroxidise (G-Px) were performed in serum. The haematological parameters were found to be within normal range. Urinary phenol levels and the ROS parameters were significantly increased in Exposed Group. Further, the ROS levels were significantly increased in Addicted group as compared to the Non-addicted group. The results showed a positive correlation between exposure and its effects on enzyme activity. Long term occupational exposure to automobile fuel may be linked to oxidative stress which can further alleviate due to confounding factors.

INTRODUCTION

Occupational biomonitoring in workplaces with exposure to hazardous chemicals and long work-shifts is of basic importance. Petrol pump attendants are individuals who get exposed to petrol and diesel derivatives primarily through inhalation of the volatile fraction of petrol. During refuelling at service stations, the toxic fumes are expelled out into the breathing zone of the attendants in that work area. This exposure process is repeated several times during a work shift and it is further intensified due to absence of personal protective measures. The components of petrol and their complex mixtures are readily absorbed and may cause a wide range of adverse health effects (Ekpenyaung and Asuko 2017). Occupational exposure to such derivatives may pose various health risks, depending on the route of exposure and quantity of petrol involved. The metabolic and xenobiotic pathways of various aliphatic and aromatic hydrocarbon chemicals present in automobile fuels may result into generation of free radical toxicity (Rezaeetalab et al. 2014). Petrol consists of many hazardous chemicals including Benzene, Toluene, Xylene, Volatile Organic Compounds etc., which are known to be potential neurotoxins, irritants as well as carcinogens (Edokpolo et al. 2015).

The volatile hydrocarbons present in petrol primarily get absorbed into blood via respiratory tract (Periago and Prado 2005), leading to toxic effect on various vital body organs such as liver (Friday et al. 2005). However, some of these substances formed in the liver do not leave the body as rapidly (ATSDR 1995). Phenol concentration in the urine of exposed workers represents 70-85 percent of urinary benzene metabolites at air concentrations of 0.1-10 ppm (Kim et al. 2006a). Hence, it can be used as a biomarker of external exposure. In studying the acute toxicological effects of diesel and crude oil in experimental animals (Rats), an increase in dose of the fuel administered into the animals caused a dose dependent decrease in haemoglobin (Hb) and packed cell volume (PCV) and White Blood

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Cells (WBC) (Dede and Kagbo 2002; Ovuru and Ekweozor 2004). A recent study found that exposure to gasoline vapor was associated with a time dependent increase in blood methaemoglobin (MetHb) concentration, due to this oxygen-carrying capacity of Hb was impaired. Further the severity of exposure can affect the individual with several clinical symptoms, including headache, cyanosis, fatigue, coma, and death (Udonwa et al. 2009). Exposure to petrol fumes has been reported to have toxic effects on haematological system of fuel pump attendants (Okoro et al. 2006).

Reactive oxygen species have a role in a number of cellular processes, including cell signalling, apoptosis, gene expression and the activation of cell signalling cascades (Ray et al. 2012). Considerable evidence has emerged in recent years implicating a central role for oxygen free radicals in the initiation of cellular injury that leads to the development of several diseases (Valko et al. 2007). Reactive oxygen species resulting from metabolism of toxic chemicals present in petrol and its fumes may damage biomolecules and also alter their function (O'Brien et al. 2005).

In India the petrol pump attendants are exposed to both petrol and diesel since both of them are being refilled at the petrol pump stations. There are few reports on petrol exposure in Indian population (Rekhadevi et al. 2010; Pandey et al. 2008; Gadhia et al. 2010). There is still limited data on Indian studies regarding information on the potential deleterious effect of exposure on oxidative damage and antioxidant systems in petrol exposed workers. Also, western part of India where the present study is conducted has hot climate during most part of the year; hence petrol and diesel which are volatile in nature readily disperse in the atmosphere especially at the petrol pumps resulting into recurrent exposure through inhalation.

Objectives

The objectives of the present study were, to assess the level of exposure through urinary phenol and to check the toxic effects of exposure on haematological parameters and antioxidant enzyme activity in the occupationally Exposed Group at petrol filling stations.

MATERIAL AND METHODS

Sample Collection

The study was approved by Institutional Ethical Committee and work was carried out according to the provided ethical guidelines. The samples were collected during the year 2014 to 2015, with prior consent of the occupational workers. The study was conducted amongst 70 petrol pump attendants working at various petrol pumps located in Ahmedabad city. The Control Group consisted of 70 healthy, age matched individuals working in offices, without indication of excess exposure to petrol derivates or other potential genotoxic substances. The Exposed Group was further divided into two groups based on their addiction habits (Addiction and Non-addiction). Participants were informed about the study and a standard questionnaire was filled to obtain necessary data on lifestyles and personal factors (age, work experience, health, medications, other symptoms etc.).

Total Blood Count

Collected blood samples were transferred into EDTA vaccutainer and the levels of blood count parameters like Hb, RBC, WBC, PCV, MCV, MCH, MCHC, Polymorphs, eosinophils, basophils, lymphocytes, monocytes, and Platelets were determined.

Urinary Phenol Measurement

Samples were obtained from workers during their work shift. Collected urine samples were treated with 6N HCl and stored in refrigerator. Urinary phenol measurements were performed following the method of Yamaguchi and Hayashi (1977).

Antioxidant Enzyme Levels

For oxidative stress parameters, serum was separated and used to perform following parameters:- (1) Superoxide dismutase activity (SOD): Kakkar et al. 1984; (2) Glutathione activity (GSH): Ellman 1959; (3) Catalase (CAT): Sinha 1972; (4) Glutathione peroxidase activity (G-Px): Rotruck et al. 1973.

Statistical Analysis of all the data was done using student's t-test and multiple comparisons amongst the Addiction groups were done by Tukey's Multiple Comparison Test. All the values were expressed as Mean ± Standard Error.

RESULTS

The demographic details of Exposed Group are given in Table 1. It was observed that the exposed individuals were having long term exposure of 12 to 25 years and 8-12 hours of daily work-shift. Age range of the subjects was between 30 to 40 years. Amongst the Exposed Group 64 percent were vegetarians and remaining 36 percent were consuming mixed (vegetarian and non-vegetarian) diet. The Control Group was strictly non-addicted, while 74 percent of the Exposed Group were having addiction such as *Gutkha/Pan masala*, smoking, alcohol. Most of the exposed individuals reported of headache (15%), bodyache (15%) and allergy (20%) and breathing problem (12%).

Table 1: Demographic details of study subjects

	Control	*Exposed*
No. of Samples	70	70
Age Range	30-45 years	30-46 years
Work Experience	-	12-25 years
Duration of Work-shift	-	8-12 hours
Diet	Veg – 64% Mixed-36%	Veg - 35% Only non-veg - 5% Mixed – 60%
Addiction	No-addiction	26% no-addiction 74 % addicted *Pan masala/Gutkha* – (52%) Smoking – (14%) Alchohol – (8%)
Allergy/ Symptoms	No complaints	Headache - 15% Body ache - 15% Allergy - 20% Breathing problem - 11.7%

The mean frequencies of all the parameters of the Total blood count such as Hb, RBC, WBC, PCV, Polymorphs Lymphocytes, Eosinophils, Monocytes, Basophils, MCV, MCHC, MCH and Platelets were observed to be within normal limits (Table 2) showing minimum or no effect of petrol exposure on haematological system of the Exposed Group. The mean frequencies of urinary phenol in Exposed Group (0.289 ± 0.0101) was found to be significantly higher ($p<0.01$) as compared to the Control (0.240 ± 0.01) subjects as shown in Table 3, which suggests that the level of petrol constituents in Exposed Group is much higher than in Control subjects.

Antioxidant Enzyme Levels

GSH – Glutathione-S-Transferase: The increase in mean frequencies of GSH in Exposed Group (3.884 ± 0.11) was found to be highly significant ($p<0.001$) when compared with the Control Group (2.698 ± 0.13) as shown in Table 4. It was observed that the addicted group (3.865 ± 0.034) showed highly significant increased ($p< 0.001$) levels of GSH as compared to the Control (2.698 ± 0.13) Group and significant increased levels ($p<0.01$) as compared to the non-addicted groups (3.20 ± 0.05) as shown in Table 5.

G-Px – Glutathione Peroxidise: The increase in mean frequencies of GPx in Exposed

Table 2: Total blood count in exposed individuals

Parameters	*Exposed*	*Normal range*
Haemoglobin	14.4 ± 0.274	13.8-17.2 gm/dl
RBC	4.36 ± 0.317	4.7 - 6.1 million cells/mcL
WBC	8392.3 ± 547.52	4500-10,000 cells/ mcL
PCV	0.42 ± 0.608	0.40-0.52
Polymorphs	0.61 ± 1.9286	(2-7.5) x 10^9/L
Lymphocytes	0.28 ± 1.8902	(1.3-3.5)x10^9 /L
Eosinophils	0.58 ± 0.396	0-0.20x10^9 /L
Monocytes	0.4 ± 0.189	0-0-0.01x10^9 /L
Basophils	0 ± 0	0.01x10^9 /L
M.C.V	85.515 ± 0.9501	80-95 femtolitre
M.C.H	29.323 ± 0.5144	27-31 pg/cell
M.C.H.C	34.215 ± 0.3303	32-36 gm/dL
Platelets	24007.7 ± 2010.1	1,50,000-4,50,000 /dL

Table 3: Urinary phenol measurements in control and exposed individuals

	Control	*Exposed*
Mean ± S.E	0.24 ± 0.01	0.289 ± 0.010 ***

***= (p<0.001) highly significant

Group (2.824 ± 2.38) was found to be highly significant (p<0.001) when compared with the Control Group (2.187 ± 0.1901) (Table 4). It was noted that the addicted group (2.6125 ± 0.008) showed significantly (p<0.01) increased levels of G-Px as compared to the Control (2.187 ± 0.1901) and the non-addicted Groups (2.45 ± 0.035) as shown in Table 5.

SOD – Superoxide Dismutase: The increase in mean frequencies of SOD in Exposed Group (6.590 ± 0.2289) was found to be highly significant (p<0.001) when compared with the Control Group (4.694 ± 0.247) as shown in Table 4. Also, the addicted group (6.385 ± 0.027) showed highly significant (p<0.001) increased levels of SOD as compared to both the Control (4.694 ± 0.247) and non-addicted group (5.815 ± 0.075) as shown in Table 5.

CAT - Catalase: The increase in mean frequencies of CAT in Exposed Group (49.97 ± 0.9812) was found to be highly significant (p<0.001) when compared with the Control Group (37.94 ± 0.9708) (Table 4). The addicted group (46.36 ± 0.067) showed highly significant (p<0.001) increased levels of CAT as compared to both the Control (37.94 ± 0.9708) and non-addicted group (43.52 ± 0.137) as shown in Table 5.

DISCUSSION

Urinary phenol measurement are considered to be a strong biomarker to analyse the level of exposure. It is an important means to determine various metabolites of hazardous chemicals such as benzene and other aromatic compounds present in petrol (Khoschsorur and Petek 2000). The researchers analysed urinary phenol in petrol pump attendants and found that the Exposed Group showed highly significant levels of urinary phenol when compared with the Control Group. Previously, biological monitoring of petrol pump attendants showed substantially higher levels of urinary phenol when workers were compared with subjects with no known exposure to either petrol or benzene (Verma and Rana 2001; Gadhia et al. 2010; Kim et al. 2006b).The interactive and synergistic mechanisms of various chemical toxicity and carcinogenicity present in petrol, can lead to the different disease endpoints such as aplastic anaemia, leukaemia, and multiple myeloma as suggested by Yardley-Jones et al. (1991). Moreover, the urinary phenol level can be affected by other confounding factors, such as diet, smoking and ingestion of medicine. Therefore, analysis of other urinary biomarkers such as *trans*, *trans* muconic acid and *S* phenylmercapturic acid should

Table 4: Reactive oxygen species parameters in control and exposed individuals

	GSH	*G-Px*	*SOD*	*CAT*
Exposed	3.884 ± 0.11***	2.824 ± 0.238***	6.590 ± 0.2289***	49.97 ± 0.9812***
Control	2.698 ± 0.13	2.187 ± 0.1901	4.694 ± 0.247	37.94 ± 0.9708

***= (p<0.001) highly significant

Table 5: Frequencies of antioxidant enzyme activity parameters in Control and Exposed-groups as per addiction

Parameters	*Control*	*Non-addiction*	*Addiction*
GSH[a]	2.698 ± 0.13	3.20 ± 0.05**	3.865 ± 0.034***,++
G-Px[b]	2.187 ± 0.1901	2.45 ± 0.035***	2.6125 ± 0.008**,++
SOD[c]	4.694 ± 0.247	5.815 ± 0.075***	6.385 ± 0.027***,+++
CAT[d]	37.94 ± 0.9708	43.52 ± 0.137**	46.36± 0.067***,+++

*Control Vs Non-addiction and Addiction: ** = significant (p<0.01), *** = highly significant (p<0.001),
+Addiction Vs Non-addiction: ++ = significant (p<0.01), +++ = highly significant (p<0.001).
a = units/mg protein, b = mM GSH consumed/mg/protein, c = units/mg protein,
d = μ moles (H_2O_2) consumed /min/mg protein

also be carried out to check the level of exposure to gasoline constituents (Qu et al. 2000).

Complete blood count (CBC) has been considered as an easy and quickly available screen for haematoxicity following occupational exposure. There have been few reports of haematological study in fuel pump attendants, which showed a significant decrease in total blood count (RBC, WBC, Hb, PCV and MCV) of subjects exposed to petrol fumes (Tanasorn et al. 2013). Another study on benzene exposure in petrol pump attendants showed significant decrease in Haemoglobin concentrations at various exposure levels (Ragia and Hala 2014). An Indian study on occupational exposure of benzene from vehicular exhaust has reported decrease in Haemoglobin levels as well as other haematological parameters such as erythrocyte, lymphocyte and platelet levels (Ray et al. 2007). However, in the present study all the parameters of total blood count and haemoglobin levels in exposed population were within normal range (Table 2), which suggests least toxic effects of exposure on haematological system of the subjects. Larger number of subjects can be studied in future including various confounding factors to reach a better understanding of the toxic effects on haematological system.

A small percentage of gasoline constituents undergoes metabolism in the liver and is eradicated either unchanged or as inactive metabolites in the urine. The active metabolites undergo further toxicokinetic processes, such as generation of reactive oxygen species, oxidative tissue damage, leading to altered structure and functions, and multi-system toxicity (Ekpenyong and Asuquo 2017). A study by Takano et al. (2002) suggested that the lung expression of CYP1A1 can be used as a biomarker of acute inhalation exposure to fuel exhaust particles and may be implicated in an accelerated production of ROS and the subsequent aggravation of lung injury. Various petrol constituents such as BTEX (Bezene, Toluene, Ethylene and Xylene) were observed to induce single and double-strand breaks in DNA, and the oxidative DNA bases modification, in Lymphocyte cultures treated with benzene, m-xylene, o-xylene, or p-xylene-treated with lymphocytes (Chen et al. 2008).

Phenolic compounds present in the petrol enter in the body through different route of exposures, and after penetrating into the cell they may undergo active transformations, and participate in metabolic reactions. Such transformation processes sometimes lead to increase of toxicity of individual compounds by the formation of electrophilic metabolites that may bind and damage DNA or enzymes (Michalowichz and Duda 2007). The antioxidant enzymes SOD, CAT, GPx and GSH serve as a primary line of defence in destroying the free radicals produced by oxidative stress. SOD reduces the radical superoxide ($O^{\cdot}_2$) to form hydrogen peroxide (H_2O_2) and oxygen (O_2). CAT is an ion containing hemoprotein, which converts hydrogen peroxide to water (Vlastis et al. 2010). The researchers observed a significant increase in SOD and CAT in the Exposed Group as compared to the Controls. GPx is an enzyme containing a selenium ion as a co-factor and for catalysing reaction it requires glutathione (GSH). GPx reduces the peroxide and hence it is helpful for preventing cellular damage (Lu 2013). The results of this study have showed significant increase in activities of GPx and GSH in Exposed Group. The increased protective free radical scavenging activity of the antioxidant enzymes suggests that exposure to petrol has caused oxidative stress. These altered enzyme activities could lead to an imbalance in structure and function of cells, tissues and organs which may result into some malignant diseases, diabetes, atherosclerosis, chronic inflammation, infection etc. (Behrend et al. 2003; Bergamini 2004). Similar results were also recorded by previous studies (Odewabi et al. 2014; Bayraktar et al. 2006). ROS can also cause oxidative DNA, protein damage, changes in tumour suppressor genes and enhanced expression of protooncogenes (Waris and Ahsan 2006). In a study on benzene pollution in urban areas, it was reported that the enzyme activity and LDH levels were altered by the exposure of benzene and heavy metals (Carletti and Romano 2002). The results of present study also support the results found in various other occupational (bus drivers and painters) exposure studies (Karagozler et al. 2002; Rossner et al. 2007), which shows that occupational exposure is related to increased levels of oxidative stress biomarkers.

Within the exposed individuals 8 percent reported of having habit of consuming alcohol, 14 percent had smoking habit, while many of them were having habit of chewing *gutkha/pan masala* (52%) and the remaining (26%) were non-addicted. Hence, the Exposed Group was further divided into two sub-groups namely Addiction

and Non-addiction group. It was noted that the activity of all the antioxidant enzymes (SOD, CAT, GSH and G-Px) were significantly elevated in the addicted group when compared with non-addicted group. Reports of several studies on addiction (*Gutkha/pan masala* and smoking) have shown occurrence of cytogenetic toxicity through production of ROS (Smita et al. 2013; Nair et al. 2004). In addition health risk assessment study indicates that occupational workers exposed to BTEX (Benzene, Toluene, Ethylene, Xylene) are prone to have carcinogenic effects and this risk also exceeds the US-EPA cancer limits (Raeesa et al. 2015). Hence, it is suggested that workers exposed to petrol should be given proper counselling about their addiction habits, diet and lifestyle factors, since these can further increase the health consequences related with petrol exposure. In this study, more than 70 percent of the petrol pump attendants complained of various symptoms including headache, body ache, allergy and breathing problem. These complaints by petrol pump workers could be exposure related consequences. Petrol pump attendants therefore, should take necessary precautionary measures and have regular medical check-up to ascertain their health condition.

CONCLUSION

The increased urinary phenol measurement in the petrol pump attendants is indicative of the petrol exposure and can be used as a potential biomarker to determine the exposure level in occupational workers exposed to petrol and its derivatives. Increased oxidative stress observed in petrol pump workers is also associated with occupational exposure to petrol in these individuals, which was further increased due to certain confounding factors such as addiction of *gutkha/pan masala*, alcohol, and smoking. Thus, occupational workers are at a high risk of developing petrol exposure related health hazards. It is emphasized that there is a need of effective assistance between the health services, occupational workers and their employers in order to minimize the risk of work related exposure and its effects.

RECOMMENDATIONS

The occupational workers should be monitored regularly and provided with safety and precautionary measures such as safe breathing masks and their working hours should be managed in a manner that they are not exposed to the petrol fumes continuously. They should also be counselled regarding their addiction habits which further alleviates their susceptibility to health consequences due to long term occupational exposure.

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