DOI: https://dx.doi.org/10.4314/gjpas.v29i1.8

GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 29, 2023: 63-71
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ENHANCEMENT OF OXIDATIVE STRESS, OXIDATIVE DNA DAMAGE AND LOWERED PEFR IN GASOLINE STATION ATTENDANTS IN CALABAR METROPOLIS

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(Received 22 June 2022; Revision Accepted 6 September 2022)

ABSTRACT

Exposure to gasoline fumes through inhalation or accidental ingestion has been associated with chronic inflammatory reactions leading to oxidative stress, oxidative DNA damage and increased risk of chronic lung conditions and cancer. This study assessed the peak expiratory flow rate (PEFR) and biomarkers of oxidative stress (total antioxidant capacity (TAC), total plasma peroxides (TPP), oxidative stress index (OSI)), oxidative DNA damage (8-hydroxy-2-deoxyguanosine (8-OHdG)), 1-hydroxypyrene and urine creatinine in Gasoline Station Attendants. A total of 100 consenting adults, aged 18-60 years, comprising 50 gasoline station attendants and 50 non-gasoline station attendants (controls) were enrolled into this comparative cross-sectional study. The PEFR was determined using the peak flow meter, TAC, TPP and creatinine by colorimetry, 8-OHdG by enzyme linked immunosorbent assay (ELISA), 1-hydroxypyrene (1-HOP) by high performance liquid chromatography and OSI by calculation. Data was analysed using unpaired Student t-test and Pearson's correlation analysis at $p<0.05$.

The body mass index, TPP, OSI and 8-OHdG levels were significantly higher and TAC and PEFR lower in gasoline pump attendants compared to the control groups ($p<0.05$). Positive correlations were observed between TPP and years at work ($r=0.638$, $p=0.000$), between TPP and OSI ($r=0.282$, $p=0.047$) and negative correlation between TAC and OSI ($r=-0.555$, $p=0.000$) only in gasoline station attendants. Exposure to gasoline is associated with increased lipid peroxidation and oxidative DNA damage, reduced lung function and depletion of antioxidants which may result in oxidative stress and increased risk for the development of chronic lung conditions in gasoline station attendants.

INTRODUCTION

Gasoline or petrol is ubiquitous environmental pollutants emanating from petrochemical industries, and are among the most hazardous compounds to human health (Satyanath, 2020). Gasoline contains a complex mixture of hydrocarbons and additives which are continuous source of pollution in homes and various occupational settings (Awasthi, *et al.,* 2016).

Gas station workers are exposed to gasoline because of their job environment which often increase their risks for acute and chronic health problems accruing from such exposures (Cezar-Vaz, *et al.,* 2012).The major sources of gasoline fumes exposure for petrol pump workers are exhaust emissions, evaporation losses from motor vehicles, and evaporation during the handling, distribution and storage of petrol, while the common routes of exposure are the respiratory, dermal and gastrointestinal tracts (Shaikh, *et al.,*2018).

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Health risks associated with exposure to gasoline has been attributed to the toxic components of gasoline including volatile organic compounds, polycyclic aromatic hydrocarbons (PAH) and heavy metals and their deleterious effects on multiple organs and systems (Abdel-Shafya, & Mansour, 2016).Exposure to gasoline vapors could be carcinogenic to humans, an observation made mainly on the basis of the well-established carcinogenicity of some components such as benzene and polycyclic aromatic hydrocarbons (benzo-a-pyrene) (Bukowska, *et al.,* 2022)

Accumulated evidence indicates that acute and long-term exposure to components of gasoline may be associated with several systemic health effects, including hematological, respiratory, reproductive, immunological, dermatological, renal, and central nervous system pathologies in humans. Other effects include hepatotoxicity, genotoxicity and carcinogenic potential. Cases of death following the inhalation of high concentrations of gasoline vapor have also been reported (Harrison, *et al.,* 2016). The degree of damage accruing from exposure to most chemical toxicants has been linked to the route of exposure, the dose and the duration of exposure. Biological monitoring and risk assessment associated with exposure to chemical toxicants at the work place can be evaluated by measuring indices of organ and systemic functions in the exposed populations. An inverse relationship has been reported between lung function indices and duration of exposure to gasoline fumes in occupationally exposed individuals (Moghadam, *et al.,* 2019). Higher levels of biomarker of oxidative DNA damage (8-OHdG) have been reported in gasoline station attendants compared to controls (Fenga, *et al.,* 2017), while elevation of lipid peroxidation products and oxidative stress have been demonstrated in petroleum attendants (Leong,2021).

The exact mechanisms underlying gasoline toxicity is still uncertain, however, inflammatory, oxidative and apoptotic effects triggered by toxic components of gasoline especially benzene, toluene, ethylbenzene, and xylene (BTEX) and PAH have been implicated (Liao, *et al,* 2022).Reactive metabolites formed during the metabolism of these components has been shown to triggers the generation of free radicals and reactive oxygen species leading to inflammation, peroxidation and oxidative damage to cellular components such as cell membrane, nucleic acid, lipids and proteins and subsequent modifications of tissue and organ functions (Pizzino, et al., 2017).

Gasoline station workers spend 8-12 hours at their work place and are therefore exposed to higher concentrations of gasoline fumes more than individuals not working in gasoline stations. Most gasoline workers are ignorant of the health risks associated with their vocation and therefore work comfortably without the use of personal protective devices, many do not observe personal hygiene practice and often times, the regulatory bodies responsible for monitoring environmental levels and exposures to hazardous chemical in most developing countries are relatively inefficient. This places the gasoline station workers at risk of the various deleterious health effects accruing from long term exposure to hazardous components of gasoline. Despite the occupational health hazards associated with working in gasoline station, studies on the effects of these exposures on multiple organs and systems are not commensurate with the enormous health risk associated with such vocations. The indices of lung function, oxidative stress and oxidative DNA damage among gasoline station attendants and the effect of duration of exposure on the level of these indices were assessed in this study.

MATERIALS AND METHODS

Study Design

This case control study was carried out in Calabar Metropolis, Cross River State. Informed consent was obtained from these subjects through well-structured questionnaires before recruitment into the study. Test subjects were primarily adult active fuel attendants. Control subjects were apparently healthy individuals who have never been primary fuel attendant before the time of study.

Sample size

The sample size was obtained using the formula by Daniel and Cross (Daniel and Cross, 2013); $n = Z^2P(1 - P)/d^2$ (where Z = standard normal deviation at 95% confidence interval = 1.96; P = estimated prevalence of lung cancer; d = precision limit 5% = 0.05) with a global prevalence of lung cancer of 2.8% (Cheng, et al., 2016) and 10% attrition ratio giving a total of 100 participants (50 test participants and 20 controls).

Selection of Subjects

The study population comprised of 70 subjects (50 test and 20 controls) aged between 18-60 years who fulfilled the inclusion criteria were recruited for the study. The subjects were asked to complete a self-reported questionnaire indicating their socio-demographic information, medical history, occupation, alcohol consumption, smoking status, underlying health issues and dietary intake.

Data collection was done through administration of structured questionnaire after the subject consent has been sought for. Such data included names, age, sex, weight, BMI, years of exposure, disease and drug history, use of personal protective equipment. Data related to lifestyle, such as alcohol consumption and smoking were also taken.

Inclusion criteria: Male subjects who are apparently healthy, and are not fuel attendants were enrolled into this study.

Exclusion criteria: those who were former fuel attendants, or with any form of organ or systemic illness or prolonged medication such as those with asthma, tuberculosis, pneumonia, were excluded from this study.

Sample Collection

Venous blood samples were obtained from all the subjects. The samples were transported to the laboratory in plain sample containers. The blood samples were then centrifuged at 3000rpm for 5

minutes to segregate blood cells and obtain serum. The pre-tested samples were stored at -20^{o}c until analysis. The lung function test was carried out using a peak flow meter. The lung function parameter measured was peak expiratory flow rate, prior to the measurement, the subject were instructed and given a rapid and complete inhalation, the demonstration of the test, covering the correct posture for performance with the head slightly elevated, position of the mouth piece and exhalation with minimal force.

LABORATORY METHODS

Estimation of Peak Expiratory Flow Rate using Peak flow meter

Peak expiratory flow rate (PEFR) is the volume of air forcefully expelled from the lungs in one quick exhalation, and is a reliable indicator of ventilation adequacy as well as airflow obstruction. It is useful in monitoring the effects of air pollutants on lung function.

Estimation of 1-hydroxy pyrene by HPLC

Urinary 1-hydroxypyrene was determined after enzymatic hydrolysis to release the conjugated 1-hydroxypyrene. After hydrolysis, the analyte was separated from the matrix and enriched by liquid/solid extraction in a reversed phase column. The components of the eluate obtained from the enrichment process were separated by means of high-performance liquid chromatography and 1-hydroxypyrene determined with a UV detector. A calibration curve was obtained using standards prepared with urine from unexposed persons. The urine was spiked with 1-hydroxypyrene, then processed and analyzed in the same way as the assay samples (Yosypchuk, et al., 2012).Concentration of 1-hydroxy creatinine per gram creatinine was obtained using the formula: Concentration of 1-hydroxy pyrene (ug/ L) / concentration of urine creatinine (ug/L)

Estimation of serum and urine Creatinine

In a protein free filtrate, creatinine reacts with picric acid under an alkaline medium to form a yellow complex chromogen whose degree of intensity is directly proportional to the concentration of creatinine in the sample and is read spectrophotometrically at 520nm (Jaffe Slot method, 1965).

Estimation of 8-hydroxy-2-deoxygnamosine (8-OHdG)

Biomarker of oxidative DNA damage; 8-hydroxy-2-deoxygnamosine (8-OHdG) was estimated using enzyme linked immunosorbent assay. The microelisa strip plate provided in the test kit has been pre-coated with an antibody specific to 8-OHdG. Standards or samples are added to the appropriate microelisa strip plate wells and combined to the specific antibody. Then a Horseradish peroxidase (HRP).Conjugated antibody specific for 8-OHdG is added to each microelisa strip plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain 8-OHdG and HRP conjugated 8-OHdG antibody will appear blue in color and then turns yellow after addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of 8-OHdG (Shekaftik, &Nasirzadeh, 2021).

Determination of Total Antioxidant Capacity (TAC)

TAC was determined based on the reaction of hydrogen peroxide (H_2O_2) with ferric ion-ethylene diamine tetraacetic (Fe-EDTA) complex to form hydroxyl radicals (OH•). Briefly, on addition of $H_2O_2$and Fe-EDTA to sample, the reactive oxygen species (ROS) (H_2O_2 and OH•) generated in the reaction mixture degrade benzoate leading to the release of thiobarbituric acid reactive substance (TBARS). The production of TBARS is suppressed by antioxidants present in the sample. This degree of inhibition of colour development is proportional to the concentration of the total antioxidant of the sample which was measured spectrophotometrically at 532nm (Koracevic, *et al.,* 2001).

Estimation of Total Plasma Peroxides

TPP was determined based on the ferrous-butylated hydroxytoluene-xylenol orange complex (FOX-2) test system. Briefly, the addition of serum sample to the test system leads to the oxidation of ferrous ions to ferric ions by various types of peroxides present in the serum sample, to produce a colored ferric-xylenol orange complex whose absorbance was measured spectrophotometrically at 560nm. The absorbance of the reaction mixture is proportional to the total plasma peroxides content of the sample (Harma, *et al.,* 2003).

Calculation of oxidative stress index (OSI)

The ratio of TPP to TAC was calculated as the OSI, an indicator of the degree of oxidative stress.

$$OSI\ (\%) = \frac{[TPP\ (\mu mol/l)]}{[TAC\ (\mu mol)]} \times 100$$

Statistical Analysis

Results were presented as mean ± standard deviation. Data analysis was done using SPSS version 20.0, IBM, USA. Analysis of variance (ANOVA) was used to test the variations within and among group means and Fisher's least significant difference (LSD) post-hoc test was used for the comparison of multiple groups means. Pearson's correlation was used to determine the associations between variables. A probability value $p<0.05$ was considered statistically significant.

RESULTS

Comparison of blood pressure, lung function, oxidative stress indices and PAH metabolite in gasoline pump attendants and controls

The comparison of age, Systolic blood pressure (SBP), Diastolic blood pressure (DBP), body mass index(BMI), peak expiratory flow rate (PEFA), total plasma Peroxides (TPP), Total antioxidant capacity (TAC), Oxidative stress Index (OSI), 8-hydroxy-2-deoxyguanosine (8-OHdG)), 1-hydroxypyrene and urine creatinine in gasoline pump attendants and control subjects were presented in table 1. The pump attendants had higher BMI, TPP, OSI and 8-OHdG (30.63±8.19 kg/m^2 vs 25.88±4.38 kg/m^2, 274.33±36.59 µmol/l vs 199.28±20.01 µmol/l, 23.54±15.96 % vs 9.15±1.56% and 1895.19±974.27 vs 1515.03±728.95 ng/ml respectively) levels and lower TAC and PEFR (1888.00±923.31 µmol/l 4288.00±2368.21 µmol/l and 245. 90±21.08 L/min 451.60±39.90 L/mins respectively) compared to the control groups ($p<0.05$).

Associations between oxidative stress indices, and years at work in gasoline station

The correlation of total plasma peroxides with duration of years at work as gas station pump attendant was depicted in figure 1. A significant positive correlation was observed between TPP and years at work (r=0.638, p=0.000).

The correlation of TPP with OSI was shown in figure 2. A positive correlation (r=0.282, p=0.047) was observed between TPP and OSI in gasoline station attendants.

Figure 3 presents the correlation plot between TAC and OSI in gas station pump attendants. Negative correlation was observed between TAC and OSI(r=-0.555, p=0.000).

Table 1: Comparison of age, BMI, SBP, DBP, PEFR, TPP, TAC, OSI, 8-OHDG, 1-HOP and urine creatinine in Gasoline Pump Attendants and Controls.

Parameter	Pump Attendants n=50	Control n=20	*P*-value
Age(years)	27.90±5.27	26.56±4.84	0.189
BMI (Kg/m^2)	30.63±8.19	25.88±4.38	<0.000*
DBP(mmHg)	80.26±12.11	80.18±15.60	0.977
SBP(mmHg)	127.26±10.98	129.18±14.69	0.461
PEFR(L/mins)	245.90±21.08	451.60±39.90	<0.000*
TPP(µmol/l)	274.33±36.59	199.28±20.01	<0.000*
TAC (µmol/l)	1888.00±923.31	4288.00±2368.21	<0.000*
OSI(%)	23.54±15.96	9.15±1.56	<0.000*
8-OHDG(ng/ml)	1895.19±974.27	1515.03±728.95	0.029*
1-HOP (µg/gCr)	2.51±1.56	2.24±1.05	0.305
uCreatinine (µmol/l)	811.77±85.02	790.26±85.57	0.859

Values are expressed as Mean ± SD; SBP= Systolic Blood pressure; DBP= Diastolic Blood Pressure; BMI= Body Mass Index; PEFR= Peak Expiratory Flow Rate; TPP= Total Plasma Peroxides; TAC=Total Antioxidant Capacity; 8-OHDG=8-hydroxylGuanosine; OSI= Oxidative Stress Index, *=Significant at *P*<0.05

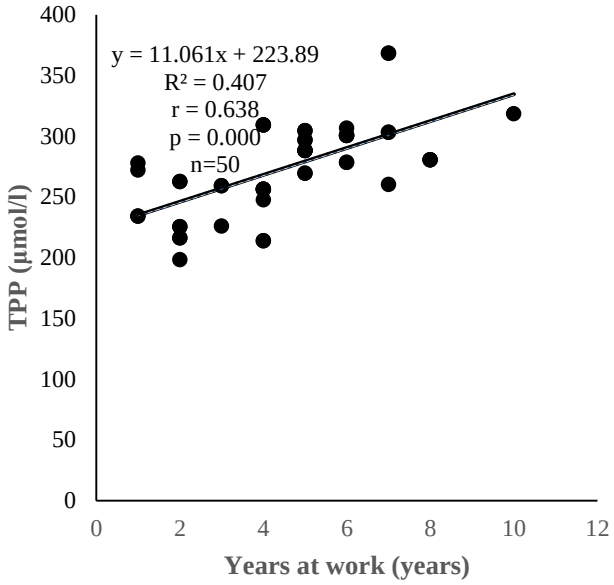

Figure 1: Correlation plot of TPP against years at work in Gasoline Pump Attendants.

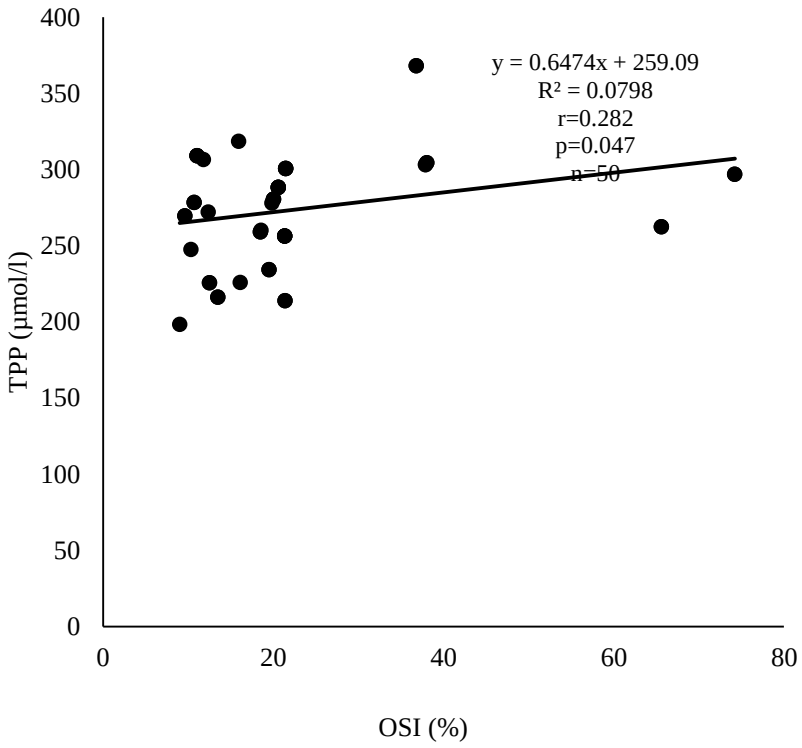

Figure 2: Correlation plot of TPP against OSI in Gasoline Pump Attendants.

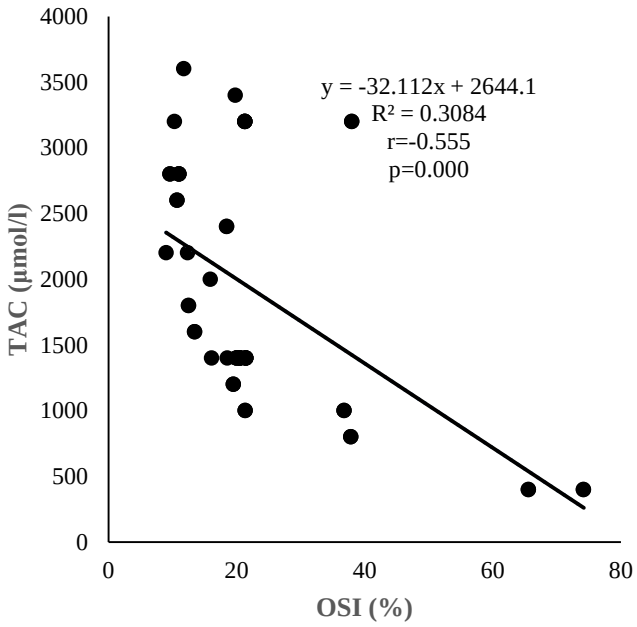

Figure 3: Correlation plot of TAC against OSI in Gasoline Pump Attendants.

DISCUSSION

In this study, gasoline pump attendants had higher BMI compared to non-gasoline pump attendants. Our observation is in line with reports from a previous study which demonstrated that exposure to gasoline was associated with increased BMI, cardiovascular dysfunction and impairment of respiratory function (Moghadam, *et al.,* 2019; Dixon, & Peters, 2018).Exposure to gasoline has been shown to induce weight and fat mass gain in mice. The relationship between gasoline exposure and BMI has been attributed to PAHs present in gasoline (Scinicariello, & Buser, 2014; Wang, et al., 2022). A positive dose dependent association between obesity and 2-phenanthrene has been reported. PAH is related to obesity and the expression of a number of obesity-related cardiometabolic health risk factors (Ranjbar, *et al.,* 2015).

Our study observed lower PEFR in gasoline station attendants compared to their corresponding controls. Similar reductions in FVC, FEV1, FEV1%, FEF25-75%, PEFR and all pulmonary parameters has been described in petrol fill workers compared to the controls(Mandal, & Mukherjee, 2020; Aprajita, & Sharma, 2011). Higher ambient air concentrations of solvents and pollutants has been associated with marked systemic pulmonary inflammatory response with decreased forced vital capacity(FVC), forced expiratory volume in the first second (FEV1), inspiratory and expiratory flow rates (Awadallah, *et al.,* 2020). Decreased lung function indices observed in gasoline station attendants may be attributed to constant exposure to gasoline vapor whose inhalation is reported to cause widespread peroxidative processes, decreases antioxidant defense system and induces oxidative stress which results to non-specific free radical attacks and inflammatory responses (Maksoud, *et al.,* 2019). These effects of oxidative stress and chronic inflammation may also alter the concentration and properties of pulmonary surfactant as well as the fibromuscular layers of the airways which may contribute to early closure of smaller airways, reduction in mid-expiratory flow rate and other characteristics of peripheral airway obstruction (Santus, *et al.,* 2014; Domej, *et al.,* 2014). Consequently, decreased static and dynamic lung volumes occur, causing reduced pulmonary ventilation and poor blood oxygenation.

Lower TAC and higher levels of TPP and OSI were recorded in gasoline station attendants compared to the controls. Previous studies have also demonstrated significant reductions in TAC and elevation of MDA and lipid peroxidation in petroleum attendants compared to controls (Abdel Maksoud, et al., 2019; Azeez,, *et al.,* 2012. Significant decrease in enzymatic and non-enzymatic antioxidants has also been reported with exposure to gasoline (Owagboriaye, *et al.,* 2018). Gasoline metabolites has been shown to be potent inducers of reactive oxygen species (ROS) which enhances lipid peroxidation via its reactions with biomolecules (membrane lipids, proteins and nucleic acids) and oxidative stress through exhaustion of antioxidants (Juan, *et al.,* 2021). Enhanced lipid peroxidation and consequent OS induced by gasoline metabolites may be responsible for the higher TPP, OSI and lower TAC observed in this study. This observation may also explain the positive association between OSI and TPP and negative associations between OSI and TAC also observed in this study. The observed positive association between TPP and number of years at work may be attributed to continuous and cumulative peroxidation of cellular molecules

associated with persistent and chronic exposure to toxic components of gasoline. Oxidative stress, inflammation and apoptotic effects accruing from exposure to toxic components of gasoline has been implicated in the development of acute and chronic health issues associated with gasoline exposure (Abubakar, *et al.*, 20015; Nour-Eldine, *et al.,* 2022)

Gasoline station attendants recorded higher levels of 8-OHdG compared to their control counterparts. Workers exposed to components of gasoline (PAHs, BTEX and heavy metals) have been shown to have increased urinary 8-OHdG levels compared to unexposed individuals (Louro, *et al.,* 2022). OS and oxidative DNA damage accruing from exposure to gasoline may be responsible for higher urine levels of 8-OHdG observed in gasoline attendant workers in this study. 8-OHdG; a product of oxidized nucleoside emanating from oxidative DNA damage has been conveniently used in many studies not only as a biomarker for measurement of endogenous oxidative DNA damage but also as a risk factor for many chronic illnesses including cancer (Lee, *et al.*, 2017).

Our study is limited by small sample size and single spot sampling method. Because of the short half-life of PAH metabolites, urinary concentrations of PAH metabolites are more reflective of immediate or short-term PAH exposure and do not account for the PAH levels stored in fatty tissue. Therefore, we still require studies to investigate PAH levels in fat tissue as it relates to health risk over an extended period of time. This study employs a cross-sectional design and therefore does not allow for the extrapolation of causal or temporal relationships between PAH and health risk. The strength of our study lies its ability to establish an association between exposure to gasoline and redox imbalance, oxidative DNA, reduction in lung function indices; which is the first to establish such relationship in the area of study.

CONCLUSION: Exposure to gasoline is associated with increased lipid peroxidation and oxidative DNA damage, reduced lung function and depletion of antioxidants which may result in oxidative stress and increased risk for the development of chronic lung conditions in gasoline station attendants. The use of personal protective devices and antioxidant rich diet may reduce the risk of development of chronic lung conditions in exposed individuals.

Author's Contribution

All authors contributed to the conception and design of the work, data acquisition, analysis and interpretation of the results, revision, and final approval and are accountable for the originality of the work.

Conflict of interest

Authors declare no conflict of interest with regards to publication of this work.

Funding

There was no funding for this research work

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