ORIGINAL ARTICLE

Chronic Exposure to Toluene and Heavy Metals and Changes in Indices of Liver Function, Inflammation and Oxidative DNA Damage among Automobile Workers

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Abstract

Background: Oxidative stress (OS), oxidative DNA damage and inflammatory response induced by chronic exposure to volatile organic compounds and heavy metals (HM) have been implicated in multiple organ dysfunction. The liver enzymes (alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT)), biomarkers of OS (nitric oxide (NO), glutathione (GSH), total antioxidant capacity (TAC), total plasma peroxides (TPP), malondialdehyde (MDA)) oxidative stress index (OSI)), oxidative DNA damage (8-hydroxy-2-deoxyguanosine (8-OHdG)), and inflammation marker (tumor necrosis factor alpha (TNF-α)); heavy metals (cadmium (Cd), lead (Pb)) and urine hippuric acid (uHA) levels were assessed in automobile workers.
Methods: Fifty automobile workers and 50 controls aged 18-60 years were enrolled into this study. The MDA, GSH, NO, TAC, TPP, ALT, ALP and GGT were estimated by colorimetry, 8-OHdG and TNF-α by enzyme linked immunosorbent assay, Cd, Pb by atomic absorption spectrophotometry and uHA by high performance liquid chromatography. Data were analyzed using t-test and correlation analysis at $p<0.05$.
Results: Automobile workers had significantly higher liver enzymes, lipid peroxidation, oxidative stress, oxidative DNA damage, nitric oxide, HM, uHA and lower total antioxidants relative to controls. Heavy metals were positively associated with MDA, TPP and OSI; TPP with duration of exposure; ALP with number of working hours; and liver enzymes with OSI only in automobile workers.
Conclusion: Association of exposure to toluene and heavy metals with increased liver enzymes activity, lipid peroxidation, oxidative stress, oxidative DNA damage, and depressed antioxidants in automobile workers suggest increased risk of hepatotoxicity and hepatocellular carcinogenesis.

Key words: antioxidants, heavy metals, liver enzymes, lipid peroxidation, toluene.

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INTRODUCTION

Occupational exposure to components of automobile exhaust gas and spray paints as seen in automobile workers have been associated with chronic illnesses. These health effects have been attributed to the mixed chemical components of exhaust gas and paints including carbon monoxide (CO), particulate organic matter (POM), volatile organic compounds (VOCs), and heavy metals and their degenerative effects on vital organs (1). Major toxic metals in the automobile workshop include cadmium, lead, and chromium and the major exposure routes are through dermal absorption, oral ingestion, and inhalation (2). The toxicity of these compounds is related to their lipophilic nature because of their ability to pass easily through cellular membranes and accumulate in fat-rich tissues (3). Volatile organic compound like toluene is used as solvents in paints; its metabolite hippuric acid has been used as a useful index in monitoring toluene levels in exposed workers (4). Cardio-pulmonary diseases, hepatocellular damage, immunologic, renal and heamatological disorders, and increased risk for cancer have been linked with exposure to lead, oxides of sulfur and nitrogen, acetaldehyde, heavy metals, and toluene in diesel and gasoline exhaust (5). The main pathologic mechanisms for functional and organic damage caused by these compounds are: inflammation, mitochondrial dysfunction, OS, and oxidative DNA damage (6). Perturbations in liver enzyme activities, albumin levels, and increased DNA damage have been associated with exposure to toluene. Likewise, environmental toluene levels have been correlated

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with biomarkers of inflammation, OS and immune response (7, 8). Exposures to Cd and Pb have been associated with decreased antioxidant system, increased lipid peroxidation, DNA damage, and disturbances in the ratio of activities of alanine transaminase and aspartate transaminases (9).

Safety in the work place and improvement in the evaluation of public health risk associated with exposure to chemical toxicants can be achieved by biological monitoring of the exposure levels of such chemicals in the work place (7). Poor adherence to safety precautions for occupational exposures to toxic environmental chemicals in most developing countries has made the automobile workers the victims of frequent exposures to these substances without appropriate maintenance of safety measures and hygiene of automobile workshop environment, which often results in adverse health outcome. The evaluation of multiple organ damage and alterations in the homeostasis of some biochemical indices may be necessary for detecting exposure level and predicting health risk and effects in occupationally exposed individuals. The levels of some indices of OS, inflammation, oxidative DNA damage, liver enzyme activity, and hippuric acid excretion in relation to duration of exposure to toluene and heavy metals components of automobile exhaust gas and spray paints were assessed in automobile workers.

METHODS

Design of the Study

This comparative cross-sectional study which involved automobile workers as test subjects and non-automobile workers as controls was conducted in Calabar, southern Nigeria. The participants of the study were apparently healthy automobile workers aged between 18–60 years and age matched controls. They were spray painters, automobile mechanics, welders, panel beaters, radiator repairers, and battery recyclers who were routinely engaged in auto activities in automobile workshop for the past one year and above. The control group were individuals who had never worked in an automobile workshop or resided in the vicinity of an automobile workshop or exposed to paint in their environment for the past one year. The aims and scope of the study were explained to participants, after which written consent was obtained before enrollment in the study. The study protocol was approved by the Cross River State Ministry of Health research ethic committee (REC No. CRSMOH/RP/REC/2017/833). Moreover, the study was conducted in compliance with Helsinki declaration of 1975 on ethical principles guiding medical research involving humans.

Selection of Subjects

A total number of 50 professional automobile workers who had been actively working in an automobile workshop without personal protective device for the past one year and above and a control group of 50 apparently healthy non-automobile workers (not exposed to automobile exhaust gas) who were residents of Calabar metropolis were recruited into the study.

A demographic information questionnaire was employed for the collection of information on socio-demographics (age, marital status, education) work experience in the automobile work shop (number of working hours, years at work), safety precautionary measures (use of personal protective equipment as hand gloves and face masks), family and medical history of past illness, and social lifestyle (smoking habit, alcohol use, drug addiction and substance abuse) of the target participants. All the subjects who were not literate were assisted in filling the questionnaire to minimize errors. Individuals with chronic illness, on long-term medication, and with a history of cigarette smoking, alcoholic or drug addiction were excluded from the study. The blood pressure of all participants was obtained while anthropometric indices as weight and height were measured and body mass index (BMI) calculated as body weight divided by height (10).

Collection of Samples

Whole venous blood samples (5ml) were collected into plain anticoagulant free sample containers (3ml) and sera obtained for the estimation of MDA, GSH, NO, TAC, TPP, GGT, ALP and ALT. The remaining 2ml was dispensed into K_2EDTA tubes for the estimation of cadmium (Cd) and lead (Pb). Spot urine samples were collected for the estimation of creatinine and hippuric acid. All samples were collected after at least 5 hours at the work place.

Analytical Methods

Analysis of TAC

The estimation of TAC was done following the reaction of a standard solution of Fe-EDTA with H_2O_2 to form hydroxyl radicals which degrades benzoate to liberate thiobarbituric acid substance (TBARS). Antioxidants present in the sample inhibits the production of TBARS and colour development. This inhibition of color development at 532nm is proportional to TAC in the sample (11).

Analysis of TPP

The analysis of TPP was based on the reaction of serum peroxides with ferrous-butylated hydroxytoluene-xylenol orange complex (FOX-2 reagent) to yield a colored ferric xylenol orange complex that was measured at 560 nm (12).

Calculation of OSI

The OSI was calculated as the ratio of TPP to TAC and is a measure of the degree of OS; OSI (%) = [TPP (μM H_2O_2) × 100] ÷ [TAC μM] (12).

Analysis of NO

The NO (total nitrate and nitrite level) in the sample was estimated based on the Griess test. The α-naphthylamine reagent reacts with NO containing compounds to yield a pink colored dye whose intensity was read at 540nm (13).

Analysis of GSH

The modified Ellman's reaction was employed in the estimation of GSH. The GSH in the sample reacts with Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) to give a colored complex which was read at 412nm (14).

Analysis of MDA

The analysis of malondialdehyde (a break down product of polyunsaturated acid) was done by its reaction with

thiobarbituric acid to give a red colored complex absorbing at 532 nm (15).

Analysis of Alanine Amino Transaminase (ALT) Activity

The enzyme activity of alanine aminotransaminase was determined using colorimetric method with test kit procured from Elabscience (USA)

Alanine aminotransaminase catalyses the following reaction:

α -Keto glutarate + L-alanine $\xrightarrow{ALT}$ L -glutamate + pyruvate

The product pyruvate is complexed with 2, 4-dinitrphenol hydrazine (2,4-DNPH) to give a brown colored hydrazine in an alkaline medium at 505 nm (16).

Analysis of Alkaline Phosphatase (ALP) Activity

The enzyme activity of alkaline phosphatase was determined using colorimetric method with test kit procured from Elabscience (USA).

P-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ Phosphate + P-nitrophenol

Phenyl phosphate is broken down to inorganic phosphate and phenol by alkaline phosphatase in the serum at pH 10.0. The product phenol reacts with 4-amino antipyrine in the presence of the oxidizing agent potassium ferricyanide in an alkaline medium to form an orange complex measured at 510 nm (17).

Analysis of Gamma Glutamyl Transferase (GGT) Activity

The enzyme activity of gamma glutamyl transferase was determined using colorimetric method with test kit procured from Elabscience (USA).

L-gamma-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{GGT}$ L-gamma-glutamyl–glycylglycine + 5-amino-2-nitrobenzoate

The gamma glutamyl transferase enzyme in the serum sample catalyzes the reaction of glycylglycine with glutamyl-3-4-nitroanilide to form L-gamma-glutamyl-glycylglycine and 5-amino-2-nitrobenzoate. The rate of reaction was measured at 405nm (18).

Analysis of Cadmium and Lead.

The analysis of Cd and Pb was done by atomic absorption spectrophotometry (AAS) (model 2380 Perkin Elmer Inc. Norwalk, CT. USA). In AAS, when a beam of electromagnetic radiation emitted from a light source passes through a vaporised sample, the atoms in the sample absorbs some of the radiation and the amount of light absorbed is proportional to the concentration of the targeted element in the sample (19). The reference blood Cd and Pb levels in adult humans is 0.03-0.12µg/dl and <10 µg/dl respectively (20).

Analysis of Urine Hippuric Acid

The hippuric acid was determined using high performance liquid chromatography (Agilent Technologies, Nova-Pak C18 (3.9 x 150 mm) column brand and head space hamilton syringe 50 µl) which is based on the interaction of the analyte with the mobile phase and the stationary phase to effect a separation (21). The standard biological exposure index (BEI) of hippuric acid in the urine is 1.6 g/g creatinine (22).

Analysis of Urine Creatinine

The absorbance of the yellow complex formed when creatinine reacts with picric acid in an alkaline pH is proportional to the concentration of creatinine in the sample (23).

Data Analysis

Results were presented as Mean±SD and data was analyzed using the statistical package for social sciences (SPSS version 20.0, IBM, USA). The t-test analysis and Pearson's correlation were used to determine mean differences and associations among variables respectively at $p<0.05$.

RESULTS

Anthropometric indices, heavy metals, indices of oxidative stress, oxidative DNA damage, inflammation, liver functions and toluene exposure in automobile and non-automobile workers.

The comparison of age, WC, BMI, biomarkers of OS (MDA, TPP, TAC, OSI, NO, GSH), oxidative DNA damage (8-OHdG), inflammation (TNF-α), liver enzymes (ALT, ALP, GGT), heavy metals (Pb, Cd,) uHA and uCr in automobile workers and non-automobile workers were depicted in table 1. Automobile workers had higher MDA, TPP, OSI, NO, 8-OHdG, ALT, ALP, GGT, Pb, Cd, uHA and lower TAC and uCr compared to non-automobile workers ($p<0.05$). The levels of other indices were not significantly different in the two groups ($p>0.05$).

Table 1. Comparison of age, WC, BMI, indices of OS, oxidative DNA damage and inflammation, liver enzymes, heavy metals and uHA in automobile workers and non-automobile workers.

Parameter	Automobile workers n=50	Non-automobile wokkers n=50	*P*-value
Age (years)	34.76±4.78	34.22±7.04	0.655
WC (cm)	32.02±2.95	32.45±2.96	0.469
BMI (kg/m^2)	24.22±4.49	23.28±2.33	0.192
MDA (mmol/L)	9.11±2.64	2.41±2.08	0.000*
TPP (mmol/L)	48.83±18.02	33.85±9.96	0.000*
TAC (mmol/L)	99.04±15.19	127.52±4.36	0.000*
OSI (%)	50.48±20.04	26.52±7.64	0.000*

Table 1. Continued.

Parameter	Automobile workers n=50	Non-automobile wokkers n=50	*P*-value
NO (nmol/L)	24.90±6.73	10.70±3.57	0.000*
GSH (mmol/L)	62.76±18.54	64.76±12.20	0.524
8-OHdG (ng/ml)	15.74±22.61	8.73±1.10	0.031*
TNF-α (pg/mL)	1.04±0.28	0.98±0.23	0.227
ALT (IU/L)	39.54±22.43	13.60±15.53	0.000*
ALP (IU/L)	258.62±121.97	130.82±69.17	0.000*
GGT (IU/L)	56.44±9.06	28.82±16.45	0.000*
Pb (µg/dl)	9.66±4.23	4.89±2.22	0.000*
Cd (µg/l)	1.76 ± 0.65	0.85±0.32	0.000*
uHA (g/gCr)	1.52±0.57	0.73±0.27	0.000*
uCr (mg/L)	252.28±26.94	268.49 ± 24.86	0.002*

Results presented as mean±SD, *=significant at p<0.05, WC=waist circumference, BMI=body mass index, MDA=malondialdehyde, TPP=total plasma peroxides, TAC=total antioxidant capacity, OSI=oxidative stress index, NO=nitric oxide, GSH=reduced glutathione, 8-OHdG=8-hydroxy-2-deoxyguanosine, TNF-α=tumour necrosis factor alpha, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma glutamyl transferase, uHA=urine hippuric acid, uCr=urine creatinine

Association of heavy metals, liver enzymes and oxidative stress indices in automobile workers.

Table 2 shows the correlation between heavy metals, liver enzymes and oxidative stress indices in automobile workers. Cadmium and lead correlated positively with MDA (r=0.855, p=0.000); (r=0.805, p=0.000), and TPP (r=0.824, p=0.000); (r=0.815, p=0.000) respectively. Significant positive correlations were also observed between OSI and Cd (r=0.757, p=0.000), Pb (r=0.777, p=0.000), ALT (r=0.310, p=0.029) and ALP (r=0.311, p=0.028) respectively in automobile workers.

Association of number of working hours and number of years at work with liver enzyme and lipid peroxidation in automobile workers

The correlation plot of ALP with number of daily working hours in automobile workers was depicted in figure 1.

Table 2. Correlation between heavy metals (Cd, Pb), liver enzymes (ALT, ALP), oxidative stress biomarkers (MDA, TPP) and oxidative stress index in Automobile Workers (n=50).

Biochemical	Indices	R	*P*-value
Cd versus	MDA	0.855	<0.001*
	TPP	0.824	<0.001*
Pb versus	MDA	0.805	<0.001*
	TPP	0.815	<0.001*
OSI versus	Cd	0.757	<0.001*
	Pb	0.777	<0.001*
OSI versus	ALT	0.310	0.029*
	ALP	0.311	0.028*

*=significant at p<0.05, MDA=malondialdehyde, TPP=total plasma peroxides, TAC=total antioxidant capacity, OSI=oxidative stress index, ALT=alanine aminotransferase, ALP=alkaline phosphatase.

Positive correlations between ALP and number of working hours (r=0.344, p=0.014) were observed in automobile workers. Figure 2 shows the correlation plot of TPP with number of years at work in automobile workers. Significant positive correlations between TPP and number of years at work (r=0.286, p=0.044) were observed in automobile workers.

DISCUSSION

Chronic exposure to automobile exhaust gas, heavy metals, and volatile organic compounds present in automobile workshop has been associated with undesirable health events. The effect of exposure and duration of exposure to toluene and HM on liver enzyme activities,

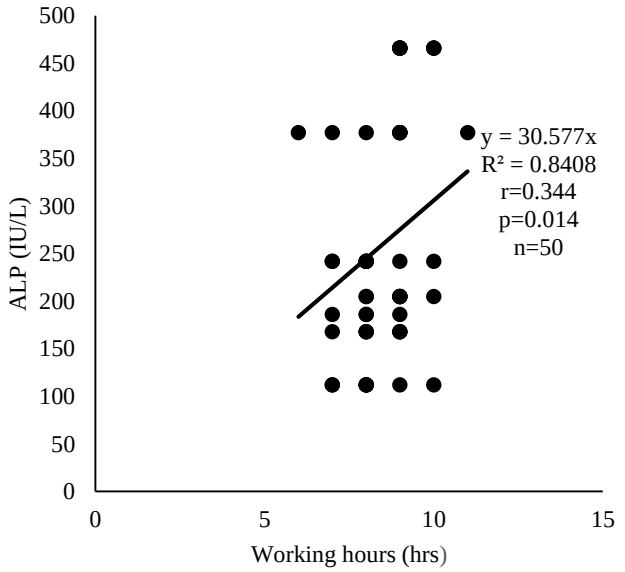

Figure 1. Correlation plot of ALP against numbers of daily working hours in automobile workers.

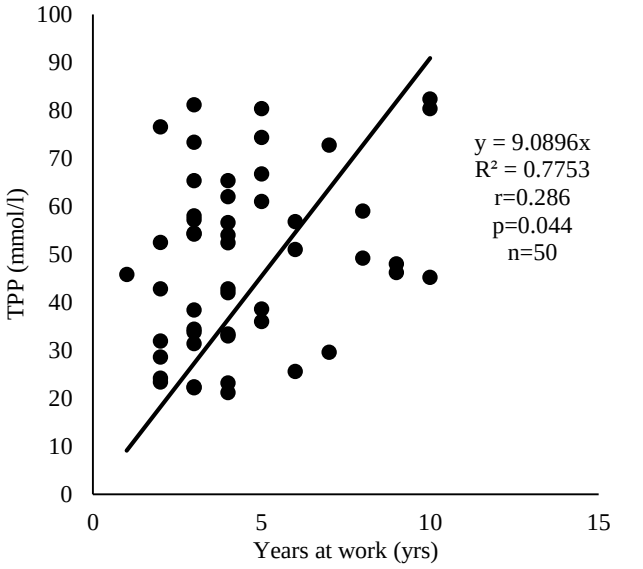

Figure 2. Correlation plot of TPP against numbers of years at work in automobile workers.

indices of OS, inflammation, and oxidative DNA damage in automobile workers and their relative or absolute contributions to the development of chronic health conditions in these workers were assessed in this study.

The present study showed that automobile workers studied had higher urine hippuric acid levels when compared to their control counterparts. Higher uHA in automobile workers may be related to higher level of exposure to toluene which is used as solvent in paints used in automobile workshops. Hippuric acid, an end product of toluene metabolism in the liver is excreted in urine and shows a good correlation with level of exposure to toluene (4). Elevated levels of uHA has been observed after exposure to VOCs at the workplace compared to unexposed state (24). Urine HA levels though higher in automobile workers were below the American Conference of Governmental Industrial Hygiene (ACGIH) recommendations for biological exposure index (1.6g/g creatinine) (25). Urine HA is the most commonly used biological marker for detection and monitoring of toluene exposure levels in occupationally exposed workers and also to assess the conjugatory functions of the liver (26). However, the consumption of food containing benzoate or benzoic acid may significantly contribute to elevated levels of hippuric acid as hippuric acid is also the end product of benzoate metabolism (7). Higher hippuric acid levels in exposed individuals have been associated with acute narcotic and central nervous system effects (26).

Higher Pb levels were recorded in automobile workers compared to controls. Heavy metals as lead, cadmium, and chromium have been described as the major toxic metals in the automobile workshop. Automobile workers are occupationally at increased risk of toxicity from these metals (27, 28). Lead is a constituent of organic solvents as benzene, lubricants, grease, cleaning fluids, and spray paints used in automobile workshop. Therefore, automobile workers have higher level of exposure to lead by virtue of their occupation compared to the general population (29). Poor workplace hygiene such as siphoning of premium motor sprit (PMS) with mouth observed among the automobile workers could also contribute to elevated Pb levels in automobile workers. Other studies have also reported significantly higher levels of Pb in automobile workers when compared to their unexposed counterparts (28, 30). The Pb levels of automobile workers (9.66 versus 4.89 µg/dl) though higher than their control counterparts, are still within the WHO permissible range of 0–10 µg/dl for lead (Pb) (20). Positive correlations were observed between Pb versus TPP, MDA, and OSI only in automobile workers studied. A direct correlation has also been observed between Pb concentration and lipid peroxidation in an animal study (9). Exposure to lead has been associated with increased generation of ROS and consequently increased levels of TPP, MDA and OSI (31). The degree of oxidative stress in blood has been shown to exhibit an upward trend after exposure to Pb (32). Toxic effects of lead on biological systems has been related to lead induced ROS production leading to oxidative stress, oxidation of oxyhaemoglobin to methaemoglobin, and peroxidation of membrane lipids, proteins, and DNA (33).

In this study, it was also found that automobile workers had higher Cd levels compared to controls. Cadmium is a common component of toxic chemicals (welding fumes and car exhausts) present in the automobile workshop. Occupational exposure to these chemicals in the automobile workshop may be responsible for higher Cd levels observed in automobile workers compared to unexposed controls. Our observation is in agreement with the findings of a previous study that reported elevated levels of Pb, Cd, Cr, Zn and Cu in mechanics compared to unexposed controls (34). Higher levels of cadmium, lead chromium, and zinc in auto mechanics, spray painters, and battery recyclers compared to unexposed controls have also been reported by a previous study (27). However, in contrast to our finding, comparable blood levels of Cd have been demonstrated in auto repair workers and their control counterparts (28). The Cd levels of automobile workers (1.76 µg/l) studied were higher than the WHO reference level of 0.03–0.12 µg/dl for cadmium (Cd) (20). Positive correlations were observed between Cd and TPP, MDA, and OSI, respectively only in automobile workers. Exposure to cadmium has been shown to increases lipid peroxidation in mammalian systems. Though the exact mechanism underlying this association is not known, the induction of free radicals and reactive oxygen species production and depletion of intracellular GSH content leading to oxidative stress has been implicated (35, 36). Exposure to cadmium have been associated with genotoxicity, mutagenicity, and carcinogenesis accruing from cadmium induced OS, inhibition of DNA repair, and suppression of apoptosis even at very low concentrations (29, 37).

Automobile workers had elevated TPP, OSI, and MDA and lower TAC and GSH compared to controls studied. The mixed chemical components of exhaust gas, paints, heavy metals, and other volatile organic compounds (VOC) present in automobile workshop have been shown to modulate

antioxidant defense system and cause oxidative damage in exposed population by the generation of ROS (6). Oxidative metabolism of toluene produces cytosolic NADH. The oxidation of NADH by mitochondria electron transport chain results in the generation of ROS that are capable of inducing multiple organ toxicities. These ROS including superoxide, hydrogen peroxide (H_20_2), hydroxyl (OH-) reacts with proteins and membrane lipids leading to the peroxidation of bio-molecules and structural abnormalities (3). Thus, higher TPP and OSI observed in automobile workers is a consequence of increased ROS generation and oxidative stress associated with exposure to toluene, HM, exhaust gas, and other VOCs in these workers. Elevated levels of MDA, OSI, and H_2O_2 have also been reported in automobile spray painters compared to non-painters (38). Increased ROS generation associated with toluene biotransformation and consequent membrane peroxidation has been linked to elevated MDA observed in automobile workers (7). Moreover, a positive correlation was observed between TPP and years at occupation in automobile workers studied. A similar observation has also been made between TPP and years at occupation in auto mechanics (38). Toluene and its metabolites are lipophilic in nature and can, therefore, accumulate in cellular membranes and fatty tissues depending on duration of exposure (38). In a similar manner, heavy metals have been indicated to bio-accumulate in specific tissues and organs, with level of accumulation being dependent on duration and degree of exposure (28). Bio-accumulation of toluene and HM in tissues and organs and associated peroxidation of lipid and protein membranes may be responsible for the positive association observed between total plasma peroxidation products and years at occupation in automobile workers. Lower TAC and GSH were also observed in this group which may be the result of their depletion in the neutralization of excess ROS to avert oxidative stress. Significant decreases have also been observed in reduced and oxidized glutathione levels in automobile painters compared to controls. Alterations in GSH levels have been observed in Cd toxicity (9).

Additionally, in this study, the NO levels of automobile workers were significantly higher than those of controls. Higher NO in automobile workers could be attributed to high concentration of nitrogen oxides (NO_x) (often referred as nitrogen oxide (NO) and nitrogen dioxide (NO_2)) in automobile exhaust gas (39). Moreover, exposure to toluene has been shown to induce inflammatory response leading to the generation of ROS and reactive nitrogen species. Up regulation in the activities of inducible NO synthase (iNOS) by inflammatory mediators results in increased production of NO (40). Increased production of NO has been observed with exposure to toluene diisocyanate in rat bronchoalveolar lavage cells (41). NO which constitutes 85–95 % of NO_x can directly or indirectly lead to the formation of free radicals and oxidative stress (39). At physiological concentration, NO mediates many beneficial biological functions including blood pressure regulation, vasodilatation, inflammation, non-specific immunity, and neurotransmission while higher concentrations of NO may promote lipid peroxidation and DNA damage (42). The ambivalent role of NO in the regulation of cellular functions has been described to be a function of not only intracellular NO concentration but also on the chemical redox environment and duration of exposure to NO (43).

The liver enzyme activities ALP, GGT, and ALT in automobile workers were higher than those of the controls. Exposure to organic solvents has been associated with changes in the oxidative metabolism of the liver and hepatotoxicity because of its role in the metabolism of xenobiotics (6). Hepatocellular damage results in increase in the activities of alanine and aspartate aminotransferase enzymes, while cholestatic damage is associated with increase in alkaline phosphatase and gamma glutamyl transferase activities. These enzymes can leak into the circulation in response to hepatocellular injury caused by reactive metabolism in the liver leading to increase in the observed activities of these enzymes. Hence, serum activity of these enzymes is a reflection of the physiological state of the liver and their activity in the blood depends on the severity of the cellular damage (44). Increased liver enzyme activities as a result of exposure to mixed organic solvents and HM have also been reported in automobile workers. Elevated activities of GGT and aminotransferases indicating cholestasis and hepatic necrosis has been demonstrated in workers exposed to organic solvents containing toluene (45, 46). However, the findings of this study did not show any indication of hepatotoxicity in the automobile workers studied as the activities of these enzymes in exposed workers, though higher than those of unexposed controls were within the normal reference range. Since these workers are constantly exposed to organic solvents from paints and exhaust gas and are vulnerable to associated adverse effects, routine medical check-up may be important to avert multiple organ toxicities in these occupationally exposed individuals. The duration of working hours, also, correlated positively with ALP activity in automobile workers. An earlier study had also reported a significant increase in the activities of serum aspartate and alanine aminotransferases and alkaline phosphatase enzymes in petrol station attendants with long-term exposure (6-10 years) to petrol vapors containing toluene in comparison to the control group (47).

The TNF-α levels of automobile workers though higher than that of the controls, were not statistically significant. A previous study also observed no significant differences in blood TNF- α levels in auto mechanics exposed to low levels of particulate matter (PM) (25 ìg/ml of PM2.5) compared to unexposed controls. However, higher mean levels of TNF-α was demonstrated in the mechanics group exposed to higher levels of PM (250 ìg/ml of PM2.5) when compared to the unexposed control group (48). Exposure to toluene in gasoline was associated with elevated levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) relative to the nonexposed group. Increased ROS generation associated with biotransformation of toluene has been shown to contribute to elevated IL-1β, IL-6, TNF-α, and IFN-γ in occupationally exposed individuals (8).

Automobile workers had significantly higher urine levels of 8-OHdG compared to controls. Higher 8-OHdG levels seen in automobile workers may be related to increased

generation of ROS associated with exposure to HM and toluene. Increased ROS accruing from the biotransformation of toluene to toluene epoxide results in OS and consequently oxidative DNA damage and increased levels of 8-OHdG, which is a widely used biomarker of oxidative DNA damage. In vitro studies have revealed that transition metals induce the formation of 8-OHdG, while in vivo studies have demonstrated an association between concentrations of certain transition metals and urinary 8-OHdG concentrations (49). Persistent exposure to relatively low levels of HM has been linked to higher oxidative DNA damage and levels of 8-OHdG (50). Strong associations between 8-OHdG levels and exposure to benzene and toluene have been demonstrated in the residents of industrial areas polluted with benzene and toluene (45). In addition to its use as a biomarker of mutagenesis and carcinogenesis, 8-OHdG may also be a useful marker of environmental health risk associated with exposure to environmental pollutants (51). Oxidation of the DNA has potential genotoxic and mutagenic consequences and has been implicated in the ethio-pathogenesis of chronic illness including neurodegenerative disorders and cancer (7).

CONCLUSION

This study has shown that chronic exposure to heavy metals and toluene present in automobile workshop is associated with increased excretion of hippuric acid, increased heavy metals level, liver enzyme activities, lipid peroxidation, oxidative DNA damage, and the depletion of antioxidants which may predispose individuals to oxidative stress and hepatotoxicity. Higher levels of Cd and Pb in automobile workers signify the need for the implementation of requisite safety and hygienic measures by automobile workers to protect themselves against adverse health effects of the automobile workshop environment. Mandatory use of personal protective devices is also necessary to avert heavy metal and toluene induced organ and systemic toxicity among automobile workers. Furthermore, a regular check-up is recommended for automobile workers.

LIMITATIONS

The study is limited by small sample size

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Author's contribution: All authors contributed to the conception and design of the research, data analysis and approval of final manuscript.

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