Research Article

Occupational Exposure to Toxic Metals Induced Oxidative Stress in Automechanics in Ibadan, Nigeria - Risk of Developing Chronic Kidney Disease

*Akinosun O.M1, Popoola R.A and Bolajoko E.B
Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Received: December, 2016; Accepted: February, 2017

Abstract
Toxic metal induced oxidative stress has been shown to play prominent role in aetiology of chronic kidney disease (CKD) which continues to be a major public health concern. This study investigated levels of these metals, renal function and oxidative stress index (OSI) in automobile mechanics (AM). The study comprised thirty-five AM and thirty-five apparently-healthy participants between 20 and 60 years. Blood (10 ml) and spot urine (5 ml) samples were collected. Plasma creatinine (PCR); urinary albumin, creatinine; total antioxidant capacity (TAC) and total plasma peroxide (TPP) were measured spectrophotometrically. Estimated glomerular filtration (eGFR), OSI and albumin:creatinine ratio (ACR) were calculated. Blood lead (Pb) and mercury (Hg) levels; urinary Pb, Hg and cadmium (Cd) were measured using atomic absorption spectrophotometer. Data were analyzed statistically using Independent T-test and Pearson’s correlation at p < 0.05. The urinary Pb (0.5 ± 0.2 vs 0.7 ± 0.2ug/dl), Cd (0.3 ± 0.1 vs 0.4 ± 0.1ug/dl), Hg (0.05 ± 0.02ug/dl vs 0.07 ± 0.02)and plasma TAC (27353.1 ± 10733.2 vs 32814.3 ± 11328.6) were significantly lower while blood Hg (0.3 ± 0.2 vs 0.2 ± 0.03ug/dl), TPP (174.1 ± 49.6 vs 113.1 ± 56.2) and OSI (0.7 ± 0.4 vs 0.4 ± 0.2)increased significantly in AM than controls. No significant differences in PCR (75.3 ± 8.9 vs 78.3 ± 9.6), ACR (10.7 ± 13.9 vs 12.0 ± 8.4), eGFR (108.2 ± 25.7 vs 118.4 ± 26.3) and blood Pb (9.3 ± 4.8 vs 8.4 ± 1.1ug/dl). Urinary Pb, Hg and Cd correlated significantly with ACR (p<0.01). The urinary levels of Pb, Cd and Hg (p < 0.001); plasma levels of TAC (p = 0.042) were significantly lower while the mean levels of blood Hg, plasma TPP and OSI (p < 0.001) were significantly increased in AM compared with control. However, there were no significant difference in levels of eGFR, ACR and blood Pb between the two groups. Urinary levels of Pb, Hg and Cd correlates significantly with ACR, while there was a negative correlation with blood Hg levels in AM compared with control. In this study, automobile mechanics were found to be at increased risk of developing chronic kidney disease due to their constant occupational exposure to toxic metals.

Key words: Chronic kidney disease, oxidative stress index, toxic metals.

INTRODUCTION

The kidneys are vital to health, thus progressive loss of its functions have serious implications (Afolabi et al., 2009). A 50% to 60% reduction in its functions may occur before the onset of any significant signs and symptoms of kidney failure. This is due to the large functional reserve of the kidneys, which has considerable ability to increase its functional capacity in response to injury (Burtis and Bruns, 2015).Chronic kidney disease (CKD) is a progressive condition which ultimately leads to irreversible kidney damage over time. It is defined as abnormalities of kidney structure or function that is present for more than three months (Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group (2012). It can also be defined as glomerular filtration rate(GFR) of less than 60ml/min/1.73m2 for three months or more (Levey et al., 2005). A reduced GFR, increased urinary albumin excretion or both is definitive of CKD. The major outcomes of CKD, regardless of cause, include progressive loss of kidney function and complications of decreased kidney function (Levey et al., 2005).

The aetiology of CKD are diverse, however, the most common causes accounting for about 70% globally are diabetes mellitus and hypertension (van Blijderveen et al., 2014). Other causes implicated in the aetiology of CKD includes cigarette smoking, ingestion of herbal concoction, abuse of analgesic, HIV infection, sickle cell disease, oxidative stress, toxicants and CKD of unknown origin (Gobe and Crane,2010; Osafo et al., 2015). Heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg) have been implicated as causative agents in kidney damage (Jha et al., 2013). This, it does through the generation of reactive oxygen species (ROS)which can overwhelm the cells’ intrinsic antioxidant defences and activates cell death pathways(Ercal et al., 2001; Kim et al., 2015),thus, producing oxidative stress in the renal proximal tubular cells resulting in CKD (Gobe and Crane, 2010; Reyes et al., 2013).

Chronic exposure to hazardous substances (toxicants) in occupations using solvent based materials is a public health risk of major concern (Ajani et al., 2011; Kamal et al., 2011). An important vulnerable group is automobile mechanics who are frequently exposed to toxicants in the course of repair and maintenance of automobiles which is carried out without the
use of proper protective devices (Wilson et al., 2007). These toxic substances include heavy metals such as those contained in brake fluids, detergents, lubricants, degreasers, paint removers, metal cleaners and antiknock agents (Anetor et al., 2009). Exposure to hazardous substances in automobile workshops occurs through dermal contact, inhalation and accidental ingestion (Prüss-Ustün et al., 2011).

These toxic metals accumulate slowly overtime in different organs including the kidney due to chronic exposure to low levels (Sabath and Robles-Øsorio, 2012; Kim et al., 2015). Screening of “at-risk” people is important because it facilitates early detection, evaluation and prompt treatment to delay the progression of CKD (Plantenga et al., 2010; Ulasi et al., 2011). Early diagnosis of CKD especially in those at risk will help in prompt interventional management and delay its irreversible progression to end stage renal disease (ESRD).

**MATERIAL AND METHODS**

**Participant’s selection:**
A self-administered questionnaire was adopted as a tool for obtaining data from participants who gave consent to participate in this study. Seventy (70) participants aged 20-60 years who were apparently healthy and not on any medications were recruited into this study. Thirty-five (35) were occupationally exposed participants (mainly automobile mechanics) from Oulyole and Ibadan North Local Government Area, Oyo state. The control participants (35) were recruited from amongst the members of staff and students of UCH Ibadan.

**Blood and urine sample collection:**
Ten millilitres of venous blood was collected aseptically from each participant into heparinised bottles. About 5 ml of it was centrifuged at 4000rpm for 10 minutes at room temperature and the supernatant was decanted to generate plasma. Five millilitres of spot urine was also collected into plain universal containers without any preservatives. All samples were stored at -20°C prior to analysis. Total plasma peroxide (TPP), total antioxidant capacity (TAC) and creatinine were measured in heparinised plasma, lead (Pb) and mercury (Hg) were measured in heparinised whole blood while Pb, cadmium (Cd), Hg, creatinine and microalbumin were measured in urine. Estimated glomerular filtration rate (eGFR), albumin:creatinine ratio (ACR) and oxidative stress index (OSI) were calculated using the following formula respectively.

**Assay methodology:**
Plasma levels of TAC were measured using ferric reducing antioxidant power (FRAP) method as described by Benzie and Strain (1999). The method is based on the reduction of 2, 4, 6-tripirydyl-s-triazine (TPTZ)-ferric complex at low pH to ferrous form, to give a colour change that can be monitored by measuring the change in absorbance at 593nm.

Total plasma peroxide concentrations were measured spectrophotometrically at 560nm using FOX-2 assay according to the method described by Miyazawa (1989) with minor modifications (Harma et al., 2005; Adedapo et al., 2014). This method is based on the oxidation of ferrous ion to ferric ion by various types of peroxides contained within the plasma samples to produce a coloured ferric-xylenol orange complex.

Creatinine in plasma and urine was determined by modified Jaffe's method (Junge et al., 2004) using DIALAB reagent kit. In this method, creatinine forms a yellow-orange complex with picric acid in alkaline solution. The intensity of the colour produced is directly proportional to the concentration of creatinine in the samples which was measured spectrophotometrically at 500nm.

Immunoturbidimetric method as described by Molnár and Schaefer (2000) using DIALAB reagent kit was used to determine urinary microalbumin. The method is based on the formation of antigen-antibody insoluble immune complexes which is turbid. The intensity of turbidity produced is inversely proportional to the concentration of microalbumin in urine and it was measured spectrophotometrically at 340nm.

The trace elements were determined using atomic absorption spectrophotometric (AAS) method. In this technique, the atoms of the element aspirated into the AAS vaporize and absorb light of the same wavelength as that emitted by the element when in the excited state. The procedure includes thawing of frozen plasma samples and diluting with 0.1N hydrochloric acid (1:20) to release bound trace metals in order to enhance accurate measurement. The digested samples were aspirated directly into the AAS for analyses. Working standard solutions were prepared in part per million (ppm) and used for the standardization of the corresponding trace elements. Cadmium, and Pb in both whole blood and urine were determined by the technique described by Trzcinka-Ochocka et al. (2014) using 210/211 VGP and 220 GF AAS (Buck Scientific, USA). The cold vapour atomic absorption spectrophotometric (Hg-CVAAS) method using 210/211 VGP and 220 GF AAS (Buck Scientific, USA) was used to measure the Hg concentration by replacing GF with the cold vapour unit. This was after treating the samples with nitric and sulphuric acids followed by further oxidation with potassium permanganate to reduce the loss of Hg through volatilization. The cold vapour for Hg uses the same graphics technique as GF, but a chemical reaction in the reaction vessel causes the peak as opposed to using the furnace or furnace programme in GF at a wavelength of 253.65. (Manufacturers Manual)

**Statistical analysis:**
Data was analysed using statistical package for social science (SPSS) version 22. Data was presented as mean ± SD (standard deviation). The Student independent t-test was used to determine significant differences between the means. Correlation among data was performed using the Pearson’s correlation coefficient test and p < 0.05 was considered to be statistically significant.

**Ethical approval and consent to participate:**
This study was approved by the Joint University of Ibadan and the University College Hospital Institutional Review Committee (UI/UCH IRC) (approval number UI/EC/15/0186).

**RESULTS**
Significant decrease in the plasma levels of TAC, urinary levels of Pb, Cd and Hg were found in the automobile mechanics (AM) compared with the control participants. However, OSI, blood Hg and TPP were significantly increased in the AM compared with the control participants.
There were no significant differences between blood Pb levels, estimated glomerular filtration rate and albumin:creatinine ratio in both groups. Urinary levels of Pb, Hg and Cd correlates significantly with ACR, while there was a negative correlation with blood Hg levels in AM compared with control at p < 0.05.

**DISCUSSION**

The increasing incidence and prevalence of CKD is of public health concern worldwide. Due to its silent and asymptomatic nature in the early stages, its diagnosis is commonly made at the advanced stage (Arogundade and Barsoum, 2008; Vassalotti et al., 2010). The asymptomatic nature of CKD underlies the need for routine screening of the general population especially in those at high risk.

In this study, biomarkers of renal functions (estimated glomerular filtration rate (eGFR), albumin: creatinine ratio (ACR) and plasma creatinine) were found to decrease in automobile mechanics (AM) compared with the controls but the decrease was not statistically significant. This observation might indicate that there is a silent deterioration of the renal function in AM. Chronic low exposure to toxic metals has been associated with a decline in GFR that can cause renal damage (Thijssen et al., 2007; Li et al., 2010).

There was no significant difference in blood Pb levels between AM and control participants in this study. This observed finding agrees with the study of Imah-Harry and Olorunsogo (2014) who reported that there was no significant difference between the blood Pb levels in AM and control. Although, these levels were higher than 5µg/dl in both groups but below 25 µg/dl, which is the preferred target levels for exposed adults as reported by occupational guidelines and regulations worldwide for blood Pb levels (Saliu et al., 2015). In Nigeria, electricity supply is not steady leading to a dependence on generators; which uses gasoline for constant power supply. Likewise, traffic congestion is on the rise due to increase in number of personal automobiles coupled with bad roads. Exhaust fumes from these generators and automobiles releases Pb into the environment leading to its inhalation. This could probably explain why both the control participants (unexposed) and AM (exposed) have similar blood Pb levels in this study.

Similarly, in this study, the blood Hg levels were observed to increase significantly in AM compared with the control. The observed increase in the levels of Hg may be due to constant and recent exposure of the AM to various sources of Hg such as brake fluids, detergents, lubricants, degreasers, paint removers, metal cleaners and antiknock agents (Anetor et al., 2009) compared with the controls because most forms of Hg in the blood decrease by one-half every three days if source of exposure is removed (Nuttall, 2004). This author also reported that Hg levels in the blood provide more useful information of recent exposure.

The kidney is the main organ involved in the excretion of these toxic metals. Urinary levels of Pb, Cd and Hg were found to be significantly decreased in the AM compared with the controls. This observed reduction could indicate that there is impaired excretion of these metals in AM. This is further supported by the earlier observed elevated blood levels of Pb and Hg in AM. Also, the observed decrease in eGFR in AM compared to controls further buttresses this finding, although the decrease was not significant.

Lead, Cd and Hg induce oxidative stress by increasing free radical generation and depleting antioxidant levels. The observed significant increase in the levels of total plasma peroxide (TPP) and oxidative stress index (OSI) in automobile mechanics (AM) compared with the control agrees with the report of Imah-Harry and Olorunsogo (2014). This observation suggests that AM are exposed to chemicals that contain toxic substances including heavy metals, that can increase the generation of free radicals and oxidative stress.

Total antioxidant capacity (TAC) is a dynamic equilibrium influenced by the synergy in the activities of all plasma antioxidants (Al-Fartosy et al., 2014), thus, protection against attacks by free radicals is thought to be more effective with the cooperation of total antioxidants in human plasma than only one antioxidant (Koracevic et al., 2001). In this study, there was a significant decrease observed in TAC of AM compared
to the control. This depletion in TAC is a reflection of the increase in circulating TPP which may be aggravated by the chronic exposure to metal toxicants from the routinely used chemicals in the AM workshops. This observation is in agreement with the study of Ferrari (2012) that reported an association between chronic exposure to heavy metals and a decrease in TAC.

This study also reveals significant inverse association between blood Hg and urinary levels of Pb and Cd in AM compared with controls. This may be as a result of their accumulation in the blood due to reduced excretion in the urine. The earlier observed reduced urinary excretion of these metals and increased blood levels of Pb and Hg as well as OSI further buttress this observation.

In conclusion, this study showed that automobile mechanics have increased risk of developing chronic kidney disease resulting from heavy metal induced oxidative stress. This may be attributed to the constant exposure to these toxic metals arising from the nature of their job. Therefore, automobile mechanics should be educated and encouraged on the use of protective gadgets during the course of carrying out their automobile repair works.

Authors Contribution
This work was carried out in collaboration between all authors. Author OMA designed the study, wrote the protocol and interpreted the data. Author RAP anchored the field study, gathered the initial data and performed preliminary data analysis. Authors RAP and EBB managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

REFERENCES
Oxidative stress in automechanics


