Prolonged blood coagulation time among occupationally exposed automobile technicians in Abeokuta, Nigeria

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Abstract
Automobile technicians in resource-poor settings often work in poor environments and are exposed to chemicals that put them at risk of ill health and disease. These chemical exposures could affect blood coagulation, leading to bleeding disorders or thrombosis. The present study is aimed at assessing prothrombin test (PT) and activated partial thromboplastin test (aPTT) values, serum zinc (Zn) and copper (Cu) concentrations, and blood lead level (BLL) among occupationally exposed automobile technicians compared to unexposed controls. A total of 140 consenting par- ticipants comprising 70 automobile technicians and 70 unexposed controls were recruited for this case–control study. A 6-mL blood sample was drawn from each participant for estimation of BLL, serum Zn and Cu concentrations, and PT and aPTT values. Blood lead level, and serum Zn and Cu concentrations were determined using atomic absorption spectrophotometry, while PT and aPTT values were determined using Innovin PT and Actin FS Activated PTT reagents on the Sysmex CA-101 coagulation analyser. Data were analysed using t-tests, chi-square tests, and logistic and multiple linear regression analyses with statistical significance set at $p < 0.05$. The mean BLL, serum Zn concentration, and PT and aPTT values were significantly higher in automobile technicians compared to controls. Binary logistic regression showed that automobile technicians had higher odds of elevated PT value (OR = 21.769; p = 0.000), aPTT value (OR = 1.348; p = 0.018), BLL (OR = 1.261; p = 0.000) and serum Zn concentration (OR = 1.063; p = 0.005) than unexposed controls. Linear regression showed significant positive association of PT value with BLL and with serum Zn con- centration. Higher PT and aPTT values reflect prolonged blood coagulation time among automobile technicians, which indicates impairment of extrinsic and intrinsic coagulation pathways associated with work-related exposures.

Introduction
The automotive repair industry comprises automobile mechanics, spray painters, panel beaters, vulcanisers and automobile electricians who form a major part of vocational trade in

Nigeria (Anyadike et al., 2012). These automobile technicians work in poor environments and are exposed to toxic chemicals that increase their risk of health disorders and disease (Adewoyin et al., 2013). Gasoline, diesel, brake fluids, degreasers, lubricants, metal cleaners, benzene, organic solvents, and welding and car exhaust fumes form major sources of chemical toxicants to which automobile technicians may be exposed within their work spaces on a daily basis (Adewoyin et al., 2013; Dongre et al., 2010; Jo and Song, 2001). Occupational chemical exposures have been linked to conditions such as gout, renal failure, encephalopathy and hepatotoxicity (Aylward et al., 2012; Crinnion, 2010). However, there is paucity of information on the effect of these chemical exposures on the blood coagulation status of automobile technicians in Nigeria.

Blood coagulation is an important part of haemostasis necessary to control blood loss due to damaged blood vessels. It involves a cascade that comprises an intrinsic system measured by activated partial thromboplastin time (aPTT), an extrinsic system measured by prothrombin time (PT), and a common pathway. Disorders of coagulation due to defects in any of these pathways can in- crease risk of haemorrhage or thrombosis (Alesci et al., 2008). The work of automobile technicians requires the use of devices and equipment that make them prone to sustaining physical injuries that could involve blood vessel damage. Furthermore, in resource-poor settings where automobile technicians might not use safety gear while working, the chances of such injuries occurring tend to be greater. Hence, blood coagulation disorders among automobile technicians represent a critical health hazard.

The trace metals zinc and copper are involved in many biological functions including homoeostasis (Navarro and Schneuwly, 2017), whereas lead exposure and accumulation alters several biological functions and disrupts homoeostasis (Tuakuila et al., 2013). Hyperzincemia increases coagulability, while hypozincemia is associated with poor platelet aggregation and increased bleeding time (Tubek et al., 2008). Increased levels of lead, zinc and copper have been reported in automobile mechanics compared with individuals involved in desk-bound occupations (Ishola et al., 2017; Jo and Song, 2001). However, it is not clear whether changes in the levels of lead, zinc and copper are associated with blood-clotting time as assessed using PTand aPTT among occupationally exposed automobile technicians.

This study is aimed at assessing PT and aPTT values and blood levels of zinc, copper and lead among occupationally exposed automobile technicians compared to unexposed individuals.

Participants and method

Participants

This case–control study was reviewed and approved by the University of Ibadan/University College Hospital Research Ethics Committee. The study was carried out between April 2013 and November 2013. A total of 140 consenting participants were recruited. Study participants comprised 70 automobile technicians recruited from five different automobile repair workshops and 70 age-matched controls that were not frequently exposed to automobile workshop environments in Abeokuta, Ogun State, Nigeria. Participants with family history or previous diagnosis of bleeding disorders or liver or renal disease were excluded from this study.

Sample collection

A 6-mL blood sample was aseptically drawn from each participant, with 2 mL dispensed into a plain (non-anticoagulated) bottle, 2 mL into a sodium citrate bottle and 2 mL into a heparinised bottle. The heparinised samples were stored at -20°C until analysis. The citrated samples were centrifuged at 385 × g for 15 min, and plasma was obtained for PTs and aPTTs, which were carried out immediately after the samples were received at the laboratory (within 1 h after sample collection). Samples in plain bottles were allowed to clot and then centrifuged at 246 × g for 10 min. Serum was obtained and stored at -20°C until analysis.

Laboratory analysis

Coagulation parameters

Coagulation parameters (PT and aPTT values) were estimated using the Sysmex CA-101 semi-automated coagulation analyser (Sysmex Corporation, Hyogo, Japan), which is based on the principle of turbo-densitometry. Appropriate volumes of sample and assay reagent (Innovin PT and FS activated PTT reagents for PT and aPTT, respectively) were pipetted into the semi-automated analysers, triggering mixing and clot detection. The mixture was continuously monitored, and the time of clot formation was automatically recorded. Each sample was measured twice and the mean was recorded.

Reference intervals

PT: 10.7–15.3 s
aPTT: 26.8–41.2 s
(Ahmed et al., 2019)

Metals

Serum concentrations of the metals zinc (Zn) and copper (Cu) as well as blood lead level (BLL) were determined using atomic absorption spectrophotometry (PerkinElmer 400 atomic absorption spectrophotometer, Waltham, MA, USA). Samples were diluted 1:10 with 6% (v/v) n-butanol in 0.25 N HCl. Standards of various concentrations and diluted samples were aspirated into the flame, and absorbance was obtained. Absorbance of samples and standards were measured at wave- lengths of 213.9 nm, 324.7 nm and 283.3 nm for Zn, Cu and lead (Pb), respectively. The standard curve obtained for each metal was used to determine serum concentrations of the respective metals in the samples.

Reference intervals

Zn: 80–120 μg/dl
Cu: 63.7–140.12 μg/dl
Acceptable limit for BLL: < 25 μg/dl

Statistics

Data were analysed using statistical package for social sciences (SPSS) version 20.0. The independent student t-test was used to compare the mean values between automobile technicians and unexposed controls. Chi-square test and binary logistic regression analysis were used to test association of occupational exposure with coagulation parameters (PT and aPTT values) and with metal levels. Linear regression analysis was used to test the association between coagulation parameters and metal levels. Statistical significance was set at $p < 0.05$.

Result

There was no significant difference in the mean ages of automobile technicians and controls (Table 1). The mean PT and aPTT values, serum Zn concentration and BLL were significantly higher in automobile technicians compared to controls (Table 1). Prothrombin time and aPTT values and serum Zn concentration were elevated in a significantly higher proportion of automobile technicians (21.4%, 18.6% and 38.6%, respectively) compared with controls (Table 2). Nine (12.9%) automobile technicians had BLL above the acceptable limit (≥ 25 μg/dL), whereas none of the controls had BLL above this limit (Table 2).

Binary logistic regression showed that automobile technicians had higher odds of elevated PT (OR = 21.769; p = 0.000) and aPTT (OR = 1.348; p = 0.018) values, BLL (OR = 1.261; p = 0.000) and serum Zn concentration (OR = 1.063; p = 0.005) than unexposed controls (Table 3). There were significant positive linear relationships between PT value and BLL as well as serum Zn concentration (Table 4).

Table 1. Comparison of mean age, metal concentrations and coagulation characteristics in automobile technicians and unexposed controls.

Variable	Automobile technicians	Controls	t	*p*
Age (years)	33.71 ± 6.07	33.04 ± 5.26	0.691	0.490
BLL (μg/dl)	18.65 ± 5.34	12.13 ± 4.34	7.930	0.000*
Zn (μg/dl)	120.58 ± 26.82	102.53 ± 12.12	5.132	0.000*
Cu (μg/dl)	92.16 ± 22.55	89.04 ± 22.17	0.824	0.411
PT (s)	14.85 ± 0.65	13.90 ± 0.39	10.521	0.000*
aPTT (s)	37.51 ± 3.84	35.84 ± 1.81	3.278	0.001*

BLL: blood lead level; PT: prothrombin time; aPTT: activated partial thromboplastin time. Mean ± Standard deviation.
*Significant at p < 0.05.

Table 2. Comparison of proportions of automobile technicians and unexposed controls with metal concentrations and coagulation characteristics within respective reference intervals.

Variable	Criterion	Automobile technicians	Controls	$\chi 2$	*p*
BLL	< 25 μg/dl	61 (87.1)	70 (100.0)	9.618	0.002*
	≥ 25 μg/dl	9 (12.9)	0 (0.0)		
Zn	Below RI	0 (0.0)	0 (0.0)	33.451	0.000*
	Within RI	43 (61.4)	70 (100.0)		
	Above RI	27 (38.6)	0 (0.0)		
Cu	Below RI	4 (5.7)	6 (8.6)	1.067	0.587
	Within RI	62 (88.6)	62 (88.6)		
	Above RI	4 (5.7)	2 (2.9)		
PT	Below RI	0 (0.0)	0 (0.0)	16.800	0.000*
	Within RI	55 (78.6)	70 (100.0)		
	Above RI	15 (21.4)	0 (0.0)		
aPTT	Below RI	0 (0.0)	0 (0.0)	14.331	0.000*
	Within RI	57 (81.4)	70 (100.0)		
	Above RI	13 (18.6)	0 (0.0)		

BLL: blood lead level; PT: prothrombin time; aPTT: activated partial thromboplastin time. RI: reference interval. Frequency (Percentage).
*Significant at p < 0.05

Table 3. Binary logistic regression analysis of association between coagulation characteristics, metal levels and occupational exposure.

Variable	β	*p*	Expβ	95% CI
PT	3.080	0.000*	21.769	5.549–85.403
aPTT	0.298	0.018*	1.348	1.052–1.727
BLL	0.232	0.000*	1.261	1.109–1.434
Zn	0.061	0.005*	1.063	1.019–1.110
Cu	0.011	0.415	1.011	0.985–1.037

BLL: blood lead level; PT: prothrombin time; aPTT: activated partial thromboplastin time.
CI: confidence interval
*Significant at $p < 0.05$.

Table 4. Multiple linear regression analysis of relationship between coagulation characteristics and metal levels.

		PT			aPTT	
Variable	β	95% CI	*p*	β	95% CI	*p*
BLL	0.041	0.021–0.061	0.000*	0.065	-0.030–0.160	0.181
Zn	0.006	0.001–0.011	0.022*	-0.003	-0.027–0.022	0.825
Cu	0.001	0.001–0.005	0.831	-0.005	-0.028–0.019	0.686

BLL: blood lead level; PT: prothrombin time; aPTT: activated partial thromboplastin time.
CI: confidence interval
*Significant at $p < 0.05$.

Discussion

Automobile technicians in Nigeria work in polluted environments (Adebayo et al., 2017; Pam et al., 2013) and engage in several hazardous practices that increase their chances of inhalation of, ingestion of and dermal exposure to toxic chemicals (Anetor et al., 2002; Oluwagbemi, 2007; Udonwa et al., 2009). Frequent exposure to these chemicals even at low concentrations favours bioaccumulation and increases their levels in biological fluids. This study found significantly higher mean BLLs in automobile technicians compared to unexposed controls. Automobile technicians also had higher odds of elevated BLL than controls. This finding could account for workplace-related toxic metal exposure among automobile technicians and supports previous reports of elevated blood Pb in automobile technicians compared to controls (Abdulsalam et al., 2015; Omotosho, 2019).

Several repair activities carried out by automobile technicians involve Pb and its products, with the possibility of continued sale of leaded petrol and petroleum products in resource-poor settings including Nigeria (Abdulsalam et al., 2015; Osibanjo and Ajayi, 1989). Given the associated detrimental health effects of Pb exposure and poisoning, workplace-related Pb exposure continues to be a significant public health challenge, particularly for automobile technicians. This is supported by our finding of BLL above the acceptable limit of 25 µg/dL in 12.9% of the automobile technicians recruited for this study. This finding indicates the need for periodic assessment of BLL of automobile technicians and increased enforcement of health and safety policies to control exposure to toxic chemicals within the automobile repair workshops in Nigeria.

Zn is an essential metal that plays an important role in several metabolic pathways (Kambe et al., 2015); however, excessive exposure to Zn also has detrimental health effects (Plum et al., 2010). Zn is a component of many additives and lubricating oils (Abenchi et al., 2010), with substantial amounts released into the environment from combustion of engine oil and vehicle tyres (Matthews-Amune and Kakulu, 2013). Fumes formed during welding of galvanised materials is another major source of Zn within the automobile workshop (Adejumo et al., 2017). These sources lead to increased work-related exposure to Zn among auto- mobile technicians within their workspace.

In this study, a significantly higher mean serum Zn concentration was observed in automobile technicians compared to controls, with 38.6% of the automobile technicians showing serum Zn concentration above the reference interval. The odds of elevated serum Zn concentration were also greater in automobile technicians than in controls. This result corroborates the finding of Adejumo et al. (2017) who reported significantly higher serum Zn concentration in occupationally exposed automotive workers in Benin City, Nigeria. Excessive exposure to Zn resulting in elevated Zn concentrations is associated with lethargy, focal neural deficits, respiratory disorder, metal fume fever, nausea/vomiting, epigastric pain, diarrhoea and elevated risk of prostate cancer (Plum et al., 2010). In a previous study, dizziness was reported by more than half of the automobile technicians enrolled. This may be associated with increased work-related exposure to Zn and Pb, which has proven neurotoxic effects. Further studies assessing the association between health problems commonly encountered by automobile technicians with their blood Zn and Pb concentrations could help elucidate the link between workplace Zn and Pb exposure and detrimental health outcomes among automobile technicians.

Toxic metals affect the haematopoietic system in diverse ways and through different mechanisms that tend to alter several homoeostatic processes including coagulation (Aleemuddin et al., 2015; Barman et al., 2014; Handin et al., 2003). An imbalance in the coagulation system can lead to bleeding disorders or thrombosis, which could represent a disruption in the extrinsic or intrinsic coagulation pathways. Prothrombin time is an assay that screens for defects in fibrinogen; prothrombin and factors V, VII, and X and thus measures activities of the extrinsic pathway of coagulation, while aPTT measures the action of fibrinogen; prothrombin and factors V, VIII, IX, X, XI, and XII, which make up the intrinsic coagulation pathway. Functional or structural defects of any of these factors could lead to prolonged PT or aPTT (King, 2011).

In this study, mean PT and aPTT values were significantly higher in automobile technicians compared to controls, and automobile technicians showed greater odds of elevated PT and aPTT values than did controls. Likewise, prolonged PT was observed in 15 (21.4%) automobile technicians, while 13 (18.6%) automobile technicians had prolonged aPTT. These prolonged times may indicate that automobile technicians are more likely to develop coagulation abnormalities than unexposed controls, which may be linked with chemical exposures within their workspaces. This result supports the finding of (Adu et al., 2018) who reported significantly reduced haematopoietic output with significantly lower mean platelet count in automobile mechanics and sprayers compared to controls. A previous study of workers involved in a lead acid battery manufacturing plant also reported significantly decreased platelet count, plateletcrit and mean platelet mass and increased BLL in the workers (Barman et al., 2014). Those authors suggested that Pb exposure might impair coagulation function through endothelial tissue injury and reduction of nitric oxide (Barman et al., 2014). The present study also found a positive association of BLL and of serum Zn concentration with PT value, which could also be suggestive of exposure-related changes in coagulation characteristics.

Prolonged PT and aPTT values, in isolation or combination, suggest deficiencies or inhibition of coagulation factors or liver disease (Loizou et al., 2018). Odongo et al. (2019) suggested that automobile repair artisans in Nakuru town, Kenya, were occupationally exposed to Pb and risked chronic pathological effects on liver function. Significantly higher PT and aPTT values observed in this present study could also be an indication of liver injury due to chronic workplace exposure to metals. The liver plays a pivotal role in haemostasis through the synthesis of clotting factors. Hence, impaired hepatic synthetic ability results in clotting factor deficiency, which could in turn lead to prolonged PT and APTT (Kujovich, 2005). Further studies are required to assess the state of the liver of automobile technicians in relation to coagulation characteristics as well as to investigate specific coagulation factors to establish the possible cause of prolonged PT and aPTT observed in this group.

In conclusion, the results of this study indicate that work-related toxic metal exposure leading to increased circulating levels of Pb and Zn among automobile technicians may impair the extrinsic and intrinsic coagulation pathways causing prolonged PT and aPTT.

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