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Heavy Metal Bioaccumulation, Urinary Microalbumin and Some Haematological Variables among Artisans in Jos Metropolis

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Abstract

Prolonged exposure to some materials used by artisans has been reported to be harmful to health. However, there is a lack of information regarding their associations with functional biomarkers among artisans in Jos. To establish this, we collected urine and blood samples from 400 artisans and 200 age-matched non-artisan control group. Urinary microalbumin, heavy metals and some haematological variables were analysed using standard methods and haematology autoanalyser (MYTHIC 22 CT) respectively. Data were analysed using Statistical Package for Social Sciences (SPSS) version 23 and student t-test was used to compare mean values between the control and study groups. We reported significant differences in average values of MCV, per cent monocytes & neutrophils, total WBC counts, ESR and platelet counts among welders, petrol hawkers, and battery repairers compared to the control group. Significantly higher PTT and PTTK average values were observed among test subjects (24.23±2.45, 42.45±9.79) when compared to the control group (18.97±3.26, 35.05±15.61) respectively (p=0.00). Also, urinary microalbumin levels were significantly higher (P<0.05) among petrol hawkers (16.11±15.63), car painters (13.10±5.00) compared to the control (5.10±4.97). Our study revealed significantly (P<0.05) high levels of WBC, Monocytes, Neutrophils, ESR, Platelets, and Microalbumin among welders, car spray workers, battery repairers and petrol hawkers in Jos when compared to control. Data suggest a high probability of abnormal coagulation and susceptibility to metabolic diseases such as Diabetes mellitus, atherosclerosis among others in the test population.

Keywords: *Artisans, Haematological variables, Urinary Microalbumin, and Heavy Metals*

Introduction

Aside from the compounding issues of emerging and re-emerging infections, itis also known that the battle with the communicable disease, hygiene and other third world challenges has re-emphasised hazards posed by environmental heavy metals. Also, due to poor documentation processes, poisoning cases arising from the effect of such metals are not adequately documented. In Jos Plateau State (Nigeria). There are several petrol Hawkers, motor vehicle mechanic workshops (involved in car spray/painting activities), lead/cadmium battery repair-workshops and a host of other artisans whose activities pose a serious threat to human lives since many of them are located in residential areas. It is due to paucity of published work in Plateau State on occupational heavy metal exposure among-petrol Hawkers, battery repairers, and automobile paint workers that this study seeks to investigate some of the alterations in urinary and Hematological parameters associated with heavy metal exposure among artisans and petrol station Hawkers in Jos metropolis.

Materials And Methods

Study Area

The study was conducted at building materials, Kuru Jenta, Dilimi and Farin Gada Settlement areas of Jos South and North Local Government area of Plateau State, Nigeria.

Jos has a land area of about 26, 899 square Kilometers (Km^2). It is the most densely populated area in Plateau State with about 900,000 inhabitants[1].

It is located between 80^0 24'N and Longitude 80^0 32'and 100^0 38' East. It lies on a Plateau with the altitude that ranges from around 1,200 meters (about 4000 feet) to a peak of 1,829 meters above sea level.

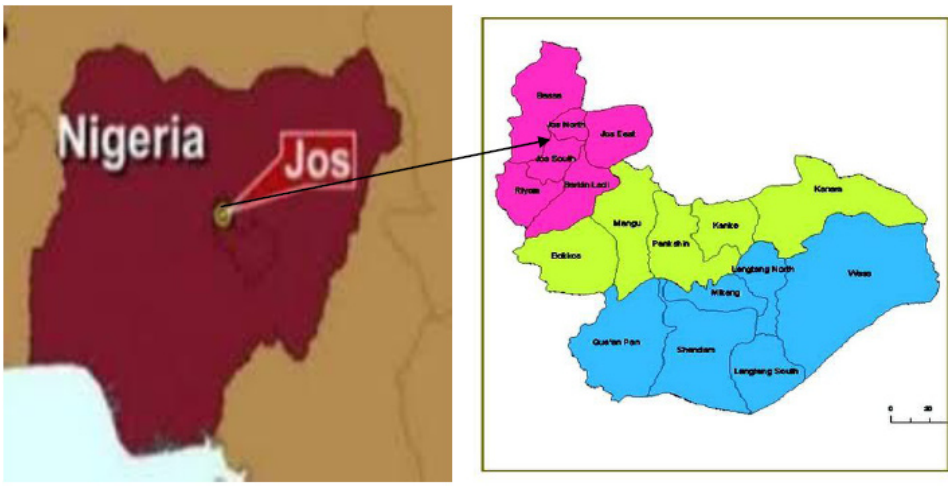

Fig 1. *Map of Plateau State showing the Study area. Source: JMDB, 2009*

Study Sample Size

Stratified random sampling technique was adopted, and a minimum of four hundred (400) individuals were recruited. This was obtained using Atchley's formula[2].

$$n=\frac{z^2pq}{d^2}$$

where n= the desired sample size (target population)

z=Standard normal deviate at the required confidence level

p= proportion in the target population estimated to have the measured character.

Q=1-p

D=the level of statistical significance set.

In this study, z=statistics was 1.96, and the desired accuracy was at 0.05 level.

Hence, $n=\frac{(1.96)^2 \times (0.5) \times (0.5)}{(0.05)} = 384$

Study Population

The study population was drawn from Building Materials, Kuru-Jenta, Dilimi and Farin-Gada settlements of Jos North and Jos South Local Government Areas of Plateau State-Nigeria. All participants were artisans involved in welding, battery repairs, selling of petrol, Tin mining and car painters. Two hundred (200) control subjects were students from the University of Jos. All subjects recruited into the study were between the ages of 18 and 60 years. Hence, a total of 600 subjects comprising of welding workers, petrol hawkers, car painters, and tin miners had eighty (80) each was recruited.

Advocacy

The Plateau State Ministry of Health granted the ethical clearance for this work. The members and leaders of the various occupations were met and informed about the study that was to be carried out in their workplaces.Their co-operation and support were solicited in mobilizing their subjects, and informed consent was obtained from the subjects recruited into the study.

Inclusion and Exclusion Criteria

Subjects who were between the ages of 18-60 that consented to the study and have spent a minimum of six (6) months on the job with no prior history and treatment for hypertension and diabetes mellitus or renal disease were included.

Those subjects who had a prior history of tobacco smoking and treatment for hypertension and diabetes mellitus and a previous history of renal disease, as well as those who refused consent, were excluded from the study.

Sample Collection

Blood samples were collected by venous puncture usingpyrogen-free sterile disposal syringes in the presence of a trained Phlebotomist according to the procedure described by [3]and analysed for heavy metals, microalbumin and haematological indices

Samples for evaluation of Heavy metals were collected into plain tubes. They were allowed to stand for 15 minutes after which they were centrifuged at 3000rpm for 20 minutes and the serum separated with the help of a sterile Pasteur's Pipette into cryovial containers. Samples were later stored frozen at -20^{0}C pending analysis.

Random Urine samples (5mls) was also taken. Samples for measurement of haematological parameters (HCT, ESR, Platelets, Hb, prothrombin time test (PTT), partial thromboplastin time test with kaolin (PTTK), WBC and Differential Counts) were collected into 3.2% trisodium citrate at a ratio of 4 parts of blood to 1 part of 3.2% trisodium citrate for (PTT and PTTK) and into K_3EDTA for (HCT, ESR, Platelets, Hb, WBC and Differential Counts) anticoagulated plastic bottles. This was mixed thoroughly by gentle repeated turning using a blood mixer. Plasma was used for the PTT and PTTK testing.

Laboratory Procedures

All reagents used were commercially purchased,andthe manufacturer's SOP's were followed strictly.

Measurements of Cd, Sn, Zn, Hg, As, Pb and Cr

AAS-HITACHI 180-80 Polarised-zee man model Atomic Absorption Spectrophotometer was used for the measurements of these metals.

Determination of Hematological Parameters

WBC, RBC, HCT, Hb & PLT Was carried out using the MYTHIC 22 CT Haematology auto analyser (5- part differential autoanalyser). Whereas clotting time was measured using the PTTK and PTT method of Quick one-stage analysis by Laffan. M. and Manning.

Determination of Microalbumin in Urine

Thiswas done using Latex particles coated with specific antibodies anti-human albumin.

Statistical Analysis

Data collected from the interview and the laboratory results of blood levels of heavy metals, blood count, and urinary microalbumin were analysed using Statistical Package for Social Sciences (SPSS) version 23. Both the mean and standard deviations were computed. The comparison between the control and study group wereanalysed using Student t-test. The values were expressed as mean and standard deviation (±SD). Results were presented in tables, graphs and charts.

Results

The comparison of means and standard deviation of heavy metals in the study population and control are presented in Table 1. Welders had the highest level of Cd (1.81±0.08) and Cr (0.11±0.02) at $p<0.05$; the highest level of Hg was seen among Battery Repairers (0.36±0.00) at $p<0.05$. However, Pb was found among all categories of artisans at statistically significant levels ($p<0.05$), except Tin Miners and Welders. Zn was also detected among all artisans except among Tin Miners. However, As was not detected in any of the groups. Changes in the mean and standard deviation values of Arterial blood pressure and weights of the study population according to the duration of exposure (career) in years are presented in Table 2. The highest level of Arterial Blood Pressure among was reported among Welders (Systolic, 156 ± 6 and Diastolic 95 ± 5) while the least was seen among Tin Miners (Systolic 125 ± 4 and Diastolic 84 ± 3). The control group had Systolic 120 ± 4 and Diastolic 80 ± 2. Data showed significantly higher arterial blood pressure among Welders ($p=0.012$) and Car Painters ($p=0.026$) compared to the control group. Car painters had the highest weight (88 ± 4 while Tin Miners had the least (76±4). A significant difference was only observed among Car painters when compared to the control ($p=0.044$).

Table 1. *Mean and standard deviation of Heavy Metals in Artisans and Petrol Hawkers*

Heavy Elements	Welders Mean±SEM	Battery Repairer Mean±SEM	Petrol Hawkers Mean±SEM	Car Painters Mean±SEM	Tin miners Mean±SEM	Control Meant±SEM
Cd(ppm)	1.81±0.08 p = 0.000*	0.24±0.03 p = 0.389	0.23±0.03 p = 0.519	0.04±0.00 p = 0.000*	0.20±0.02 p = 0.575	0.21±0.02
Sn(ppm)	0.03±0.00 p = 0.151	0.03±0.00 p=0.827	0.02±0.00 p=0.336	0.03±0.00 p=0.763	0.02±0.00 p = 0.503	0.03±0.00
Zn(PPnl)	11.25±0.13 p= 0 288	11.23±0.14 p = 0.203	10.94±10.12 p = 0.001*	10.69±0.12 p = 0.000*	9.93±0.07 p = 0.000*	11.41±0.08
Hg(ppm)	0.08±0.00 p=0.186	0.06±0.00 p = 0.005*	0.07±0.00 p = 0.060	0.07±0.00 p = 0.059	0.08±0.00 p = 0.172	0.09±0.01
As(PPN)	0.02±0.00 p=0.053	0.02±0.00 p=0.066	0.02±0.00 p = 0.074	0.02±0.00 p = 0.082	0.02±0.00 p = 0.068	0.03±0.00
Pb(ppiu)	0.06±0.00 p = 0.06S	0.08±0.01 p = 0.036*	0.16±0.03 p = 0.000*	0.80±0.04 p = 0.000*	0.06±0.00 p = 0.081	0.07±0.00
Cr(ppm)	0.11±0.02 p = 0.000*	0.06±0.00 p = 0.059	0.06±0.00 p 4.074	0.06±0.00 p = 0.068	0.06±0.00 p = 0.055	0.07±0.00

p< 0.05 is significant, * implies significant

Table 2. *Mean and standard deviation of Arterial Blood pressure (mmHg) and weights of the study population according to the duration of exposure*

Study group	Arterial BP Systolic	Diastolic	Weight
Welders (n=80)	156 ± 6 P=0.012*	95 ± 5 P=0.028*	86±4 P=0.239
Battery repairers (n=80)	132 ± 4 P=0.117	85 ± 2 P=0.313	82 ± 2 P=0.372
Petrol hawkers (n=80)	144 ± 4 P=0.043*	89 ± 2 P=0.144	84 ± 3 P=0.118
Car painters (n=80)	152 ± 6 0.026*	93 ± 3 P=0.36*	88 ± 4 P=0.044’
Tin Miners (n=80)	125 ± 4 0.374	84±3 P=0.255	76±4 P=0.617
Control (n = 200)	120 ± 4	80 ± 2	78±3

p<0.05 is significant, * implies significant

The urinary microalbumin data is shown in Table 3. Study participants within the age bracket of 36 and 40 had a significantly highest level of mean urinary microalbumin (14.66±9.70) compared to the control group (p=0.000). Conversely, the least values were seen in those between the ages of 26 and 30 (7.81±6.33). Table 4 shows significantly higher mean levels of microalbumin amongst welders (13.01±5.16), battery repairers (12.58±5.24), petrol hawkers (16.11±15.63) and Car painters (13.10±5.00) when compared to the control group (5.10±4.97) (p=0.000).

The data on haematological parameters among test and control populations are depicted in Table 5. We reported significantly elevated values of ESR (27.66±18.52), Lymphocytes (50.79±18.54), Eosinophils (3.51±3.28), MCV (89.23±57.26), PTT (24.23±2.45), PTTK (42.45±9.79) and RDW (14.94±2.05) among artisans compared to the control group. Also, significantly low levels of WBC (5.82±4.01), Monocytes (4.31±3.92), RBC (4.44±0.76), MCHC (30.61±6.67) and Platelets (176.90±73.28) were seen in artisans when compared with the non-artisans.

Table 3. *Mean and standard deviation of Urinary Micro Albumin (mg/L) among studypopulation (exposed) and control group (non exposed)*

Age group	Non exposed (n = 400) Mean ± SD	Exposed (n = 200) Mean ± SD	T	p-value
15-20	-	6.02 ±5.69	-	-
21-25	-	15.42 ±12.30	-	-
26-30	6.46 ±4.50	7.81 ±6.33	-0.865	0.390
31-35	1.03 ±0.88	10.98 ±6.49	-12.411	*0.000
36-40	4.54 ±3.60	14.66 ±9.70	-8.478	*0.000
41-45	11.78 ±1.39	13.38 ±6.44	-1.064	0.293
46-50	12.13 ±3.65	9.42 ±5.14	2.033	*0.048
51-55	-	13.63 ±3.62	-	-
56-60	-	13.66 ±12.08	-	-
Total	5.10 ±4.97	11.99 ±9.01	-10.078	0.000

p<0.05 is significant, * implies significant

Table 4. *Mean and standard deviation of Urinary Micro Albumin (mg/L) among study population by Enterprise*

Enterprise	No of Workers	Micro albumin (mg/L) Meant ± SD	T	p-value
Control	200	5.10±4.97	-	-
Welders	80	13.01±5.16	-11.919	*0.000
Battery Repairers	80	12.58±5.24	-11.207	*0.000
Petrol Hawkers	80	16.11±15.63	-6.183	*0.000
Car Painters	80	13.10±5.00	-12.162	*0.000
Tin Miners	80	5.15±4.45	-0.088	0.933

p<0.05 is significant, * implies significant

Table 5: *Mean and standard deviation of Hematological Parameters among artisans (exposed) and control (non-exposed)*

Haematological Parameter	Exposed (n = 400) Mean ± SD	Non-exposed (n = 200) Mean ± SD	T	P-value
ESR (mm/br)	27.66±18.52	14.48±4.64	-13.412	*0.000
WBC ($x10^3/\mu L$)	5.82±4.01	6.65±1.72	3.537	*0.000
LYM (%)	50.79±18.54	29.92±9.00	-18.560	*0.000
MONO (%)	4.31=3.92	5.154±2.50	3.742	*0.000
NEUT (%)	38.14±17.78	59.22±7.44	20.412	*0.000
EOS (%)	3.51±3.28	2.42±2.06	-4.968	*0.000
BAS (%)	1.52±1.23	1.18±0.58	-0.558	0.577
RBC ($10^{12}/L$)	4.44±0.76	4.67±0.47	4.470	*0.000
HGB (g/dL)	12.40±2.23	12.20±0.96	-1.553	0.121
HCT (%)	39.99±8.03	39.13±3.85	-1.770	0.077
MCV (FL)	89.23±57.26	77.50±7.30	-2.885	*0.004
MCH (Pg)	26.29±3.70	26.07±2.15	-0.937	0.349
MCHC (g/dL)	30.61±6.67	31.86±1.12	3.645	*0.000
PLT ($10^3/\mu L$)	176.90±73.281	209.32±23.82	8.039	*0.000
PTT (Sec.)	24.23±2.45	18.97±3.26	22.069	*0.000
PTTK (Sec)	42.45±9.79	35.05±15.61	7.090	*0.000
RDW	14.94±2.05	13.57±0.72	-12.033	*0.000

p<0.05 is significant, * implies significant

Legend

ESR=Erythrocyte Sedimentation Rate, WBC=White Blood Cells, LYM=Lymphocytes, Mono=Monocytes, Neut.=Neutrophils, Eos. =Eosinophils, Bas. =Basophils, RBC. =Red Blood Cells, HGB. =Hemoglobin, HCT. =Hematocrit, MCV=Mean Cell Volume, MCH. =Mean Cell Hemoglobin, MCHC=Mean Cell Hemoglobin Concentration, PLT. =Platelets, PTT. =Prothrombin time test, PTTK=partial thromboplastin time test with kaolin.

Discussions

This study was designed to test the hypothesis that states that artisans are exposed to certain chemicals and substances that are capable of altering some haematological and biochemical parameters in individuals due to sustained exposure, which can lead to poor health. After obtaining ethical clearance and informed consent, we recruited a total of 400 artisans, comprising of 80 subjects each from welders, battery repairers, petrol hawkers, car painters and tin miners to test the hypothesis. Two hundred age-matched non-artisan control individuals were also recruited into the study.

Our data showed significantly higher values of Cd among welders, Pb among Battery repairers, petrol hawkers and car painters, as well as Cr among welders, compared to the control group. On the other hand, significantly lower values of Znwas reported in petrol hawkers, car painters and tin miners, Hg among battery repairers and Cd in car painters in relation to the non-artisan control. In line with our hypothesis, the data revealed that the majority of artisans had altered levels of heavy metals compared to the control. The results are in agreement with the observation made by Johri et al.whichimplicated heavy metals, particularly Cd and Pb as occupational and environmental toxicants[4].The bioaccumulation of Cd as seen in the blood samples of welders and car painters in relation to the control group was far above the recommended reference value of 0.06 ppm [5]. These results indicate that sustained exposure to chemicals and substances in a work-related environment is capable of affecting the health status of practitioners. The increased levels of Cd, Pb and Cr found in the blood samples of welders, and car painters could be attributed to the fact that inhalation of an oxide of these metals might have resulted in increased metal uptake. Such oxides are released in huge quantities into the environment continually through the activities of welding or car painters in the form of nanoparticles which areeventually inhaled as metal dust, paint dust or ingested as a result of poor hygiene [6]. Notably, Pb levels in the petrol hawkers who have practised the petrol selling career for between 3and 8 years (data not shown) were also higher than the permissible level of 0.1 ppm [5]. Also, the high level of Cd as observed among welders and battery repairers is in tandem with the findings of Abdull Wahab who reported that galvanized and ungalvanized iron pipe products used in mechanical industries and vehicle construction industries contains various types of metals like Cr, Pb, Zn, Cu, Mn and Ni [7]. Chromium levels among the five study groups were higher only among welders in limits far beyond the permissible and acceptable level of 0.05 ppm [5]. However, in this study Tin, Arsenic and Mercury were not detected among the study group.

Welders, petrol hawkers and car painters were equally seen to have significantly higher levels of Arterial blood pressure (table 2) compared to control. This is in line with the report of Proctor which showed that toxic metal exposure could result in nephropathy (kidney damage), gastrointestinal disturbances, anaemia and neurological effect and such effects may be felt as weakness, fatigue, irritability, high blood pressure, mental deficiencies or even associated infertility in both sexes as well as fetal damages[8]. It has also been reported that lead may elevate blood pressure in susceptible adults population at blood lead levels as low as 14 µg/dl [9]. Bener and colleagues also confirmed this statement by reporting a positive correlation between lead exposure and high blood pressure among lead-exposed workers in the United Arab Emirates[10]. Similarly,a high level of Cd as observed in this study has been reported by Vallee *et al.* as a cause of emphysema and proteinuria in occupationally exposed individuals[11].

Looking at the artisans collectively as exposed (to work-related chemicals and substances) against the non-artisan control group as presented in table 3, the average levels of urinary microalbumin were seen to be increased significantly among the age groups 31 - 40 years and 46 – 50 years and thus suggesting the possibility of individuals within these age groups to be prone to kidney related problems. Also, the data on

the study population by enterprise in table 4 showed significantly high levels of urinary microalbumin among welders, battery repairers, petrol hawkers and car painters compared to the control.

Furthermore, we have assessed and reported on some haematological parameters of the exposed and non-exposed groups as depicted in table 5. Data showed increased mean values of ESR (27.66±18.52), Lymphocytes (50.79±18.54), Eosinophils (3.51±3.28), MCV (89.23±57.26), PTT (24.23±2.45), PTTK (42.45±9.79) and RDW (14.94±2.05) among the study population compared to the non-artisan control. Contrarily, we observed decrease in the mean values of WBC (5.82±4.01), Monocytes (4.31±3.92), RBC (4.44±0.76), MCHC (30.61±6.67) and platelets (176.90±73.28) among the test group when compared with the non-exposed (control group). Although we have reported a significant increase in the values of ESR, lymphocytes, Eosinophils, basophils, MCV, PTT, PTTK and RDW among artisans compared to control, most values fall within the normal reference ranges. However, the high RDW seen in artisans could suggest a predisposition to macrocytic anaemia due to Vitamin $B_{12}$and/or folate deficiency as suggested by high MCV. Also, the high mean ESR value reported among the study subjects could be indicative of possible inflammatory reactions as the system tries to respond to the effect of exposure to chemical substances following the entrance of heavy metals into the system. Since the mean total WBC count falls within the normal limits, the increased white cell types (lymphocytes, eosinophils and basophils) in the study population are relative and may not suggest any complications. The significantly high mean values of PTT (24.23±2.45) and PTTK (42.45±9.79) recorded among the exposed population as compared to the non-exposed group may be an indication of the effects of heavy metals on the liver. It is most likely that the metal-induced oxidative stress had impaired the functions of the liver cells which include the production of the prolongation of PTT and PTTK.

Furthermore, the decreased in RBC count among artisans as against the control is in tandem with the findings of Ribarov and Bochev who demonstrated that Pb exposure could lead to Hb – autoxidation due to inducement of oxidative stress by interacting with Oxyhemoglobin, leading to peroxidative hemolysis in RBC membrane [12]. In our study, WBC, and other FBC parameters (monocytes, neutrophils, basophils and eosinophils) including MCHC were found to be statistically lower in artisans ($P<0.05$). Similarly, while some workers reported a reduction in WBC [13]others reported an increase [14, 15]. The bioaccumulation of heavy metals in these artisans could be responsible for the variations, which could have caused suppressed immunity as well as coagulation disorders as evident by the significantly lower levels of WBC, platelets and longer Prothrombin time s seen in the exposed group. Therefore, this study reveals inadequate production of RBCs and other formed elements such as WBC, monocytes, neutrophils, basophils including some red cell indices (MCHC) among artisans. This suggests that the presence of heavy metals, especially Pb in the system of car painters and petrol hawkers maybe acting not only as poison but also as a suppressant to the bone marrow capacity.

Conclusion

This study suggests that prolonged exposure to heavy metals may portend a negative impact on haematological and urinary microalbumin of artisans who are exposed to these substances. This study suggests a routine haematological and biochemical screening of artisans to prevent the onset of ailments due to the effects of heavy metals.

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