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Research Article

Chronic Heavy Metal Exposure Causes Alterations in Hemopoises, Hematological Indices and Liver Biomarkers among Artisans and Petrol attendants in Jos, Nigeria

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Abstract

Background: Artisans such as Welders, Battery repairers, Carpenters, Tin miners and Petrol station Attendants generate oxide dusts and fumes of Cadmium (Cd), Lead (Pb) and Chromium (Cr) in the cause of their unregulated activities; and are exposed to these fumes either through inhalation, skin contact or ingestion. This study aims to determine the blood Cd, Pb and Cr levels and their effects on some hematological parameters and liver Biomarkers among Artisans in Jos.

Methods: Blood samples were collected from workers who were all males (n = 400), drawn from Welders, Battery repairers, Carpenters, and Petrol station Attendants with age ranging from 15-60 years, with control group (n = 200) with age range of 28-49 years. The duration of exposure was limited to between 1-8 years only. Fifteen milliliters (15ml) of blood samples were collected into 3.2% Tri sodium citrate tubes and its plasma used for Prothrombin time test and Partial Thromboplastin time with Kaolin; and into Potassium Ethylene diamine tetra acetic acid tubes for Hemoglobin, Erythrocyte Sedimentation Rate, Platelets, and White Blood Cells and Differential counts; and also 10ml of blood into plain tubes for Biochemical markers assay respectively. Serum for Biochemical assays was stored frozen at -20^{o}C pending analysis. Analysis was performed using standard methods. Data was analyzed using statistical package for social sciences version 23 and student t-test to compare means between control and study groups.

Results: Statistically high levels of hematological parameters were seen among exposed participants when compared to the non-exposed (control group);and a concomitant rise in values of serum Biochemical Biomarkers among exposed individuals. All of these findings correspond to significant increase in blood Cd ($p < 0.05$, 1.81 ± 0.008) and $p < 0.05$, 0.34 ± 0.00); Pb ($p < 0.050$, 0.08 ± 0.01, 0.16 ± 0.03 and 0.80 ± 0.04) and Cr ($p < 0.05$, 0.11 ± 0.02) among welders, car painters, battery repairs and petrol attendants respectively.

Conclusion: We therefore concluded that blood Cd, Pb and Cr levels indicate higher absorption of these metals among welders, car painters, battery repairers as well as petrol attendants in Jos,which directly impaired liver function, causing disruption of red cells production and disintegration. Regular monitoring of these heavy metals' levels, liver function tests and routine hematological tests among occupationally exposed individuals is strongly advocated.

Keywords: Heavy Metals; Biomarkers; Artisans; Hematological; Hemopoiesis; Red blood cells.

Abbreviations

Cd: Cadmium; Pb: Lead; Cr: Chromium; PT: Prothrombin Time; PTTK: Partial Thromboplastin time with Kaolin; K_2 EDTA: Potassium Ethylenediaminetetraacetic acid; Hb: Hemoglobin; ESR: Erythrocyte Sedimentation Rate; PLT: Platelets; WBH: White Blood Cells; HCT: Hematocrit MCV: Mean Cell Volume; MCHC: Mean Cell Hemoglobin Concentration; MCH: Mean Cell Hemoglobin; RDW: Red Cells Distribution Width; Neu: Neutrophils; Lym: Lymphocytes; Eos: Eosinophils; Bas: Basophils; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransaminase; ALP: Alkaline Phosphatase; SPSS: Statistical Package for Social Sciences

Introduction

As human population increases, so also urbanization, industrialization, mining operations, increased vehicular traffic and agricultural activities ranging from use of fertilizers and pesticides which ultimately cause environmental pollution affecting the air, soil and water. These bear negative consequences on the health of the people engaged in them. Although such activities notwithstanding, have contributed positively to economic development of nations, thus enhancing the quality of life of people [1]; workers face many problems in such work environments especially amongst industries engaging in heavy metals production (e.g Ferrous and non-ferrous foundries, welding or cutting of old painted metal battery manufacturing/repairs, recycling, scrap-metal handling and automobile repair shops). As such, workers in those industries/factories are often exposed (by other means) to metal contamination due to work processes, unhygienic personal behaviours. Additionally, accumulated work place dust or inhalation of metal fumes and their health can be affected by metal contamination in several ways leading to ailments such as headache, chest pain, respiratory trouble, high blood pressure. Also, heavy metal poisoning has been established to induce oxidative stress among Artisans exposed to it [2-4]. Such heavy metal induced oxidative stress can result to liver injury following the release of nitric oxide and reactive oxygen species like super oxide all of which can induced hematoxicity, hepatoxicity alongside lipid peroxidation and carcinogenesis [5]. The potential health hazards associated with exposure to these ubiquitous pollutants in the environment has attracted the attention of the general public and the scientific community; more so that occupational exposure to heavy metals particularly lead still occurs in many countries of the world, especially in many developing countries like Nigeria where occupational heavy metals exploration and miningis entirely unregulated and no monitoring of exposure exist [6].

Materials and Methods

Study area

The study was conducted among Artisans doing business at building materials market Jos and Kuru Jenta in Jos South Local Government Area of Plateau State, Nigeria; and in Dilimi and Farin Gada areas of Jos North Local Government area of Plateau State, Nigeria. Jos North has a land area of about 26, 899 square Kilometers (Km^2). It is the most densely populated Local Government area in Plateau State with about 900,000 inhabitants. It is located between Latitude 80° 24'North and Longitude 80°32'and 100°38' East. It lies on a Plateau with altitude that ranges from around 1,200 meters (about 4,000 feet) to a peak of 1,829 meters above sea level.

Study population

The study population was drawn from the study areas. All participants were Artisans involved in welding, battery repairs, selling of petrol, Tin mining and car painting. 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 were recruited. The study population which was stratified into welding, petrol station attendants, car painters and Tin miners had eighty (80) each, and the control served for all the groups of artisans.

Inclusion and exclusion criteria

Apparently healthy participants, between the ages of 18 and 60 years that consented to take part in the study, and had been on the job for a minimum of six (6) months, were included in the study. On the other hand, smokers, persons with signs of one ailment or the other and those who refused consent, were excluded from the study.

Ethical consideration

The Plateau State Ministry of Health granted the ethical clearance for this work. The leaders of the various occupations were met and their co-operation and support solicited for in mobilizing their members. Participants' informed consent was obtained.

Sample Collection

Fifteen (15) and 10 ml of blood samples were collected into 3.2% trisodium citrate tubes for Prothrombin Time (PT) and Partial Thromboplastin time with Kaolin (PTTK) tests; K_2EDTA tubes for Hemoglobin (Hb), Erythrocytes Sedimentation Rate (ESR), Platelets count, Hemoglobin concentration (Hb), White Blood Cells count (WBC) and Differential count); and into plain tubes for Bio-

chemical assay respectively. Plasma was used for the PT and PTTK tests. The EDTA samples were mixed properly using a blood mixer and analyzed immediately. Blood for Biochemical markers and heavy metals was allowed to stand for 15 minutes, thereafter; centrifuged at 3,000 rpm for 20 minutes and the sera separated using a Pasteur pipettes into clean, dry cryovials. Sera samples were stored frozen at -20°C pending analysis.

Laboratory procedures

All reagents used were commercially purchased and the manufacturers' SOPs/instructions were followed strictly.

Determination of hematological parameters

The hematological parameters WBC, RBC, HCT, Hb and Platelets were analyzed using the MYTHIC 22 CT hematology auto analyzer (5-part differential auto analyzer). PT and PTTK were measured using Quick one stage analysis method [7].

Determination of liver biomarkers (AST, ALT and ALP)

This was done using COBAS CIII Chemistry auto analyzer.

Statistical analysis

Data generated from the questionnaire through interviews and Laboratory analyses of blood samples for heavy metals, hematological indices and biochemical parameters (liver indices), were analyzed using statistical package for social science (SPSS) version 23. The comparison between control and study group was analyzed student t-test. Pearson's correlation was used to evaluate correlation between various parameters in control and study group for the hematological indices. The values were expressed as mean standard deviation (± SD). Results are presented in tables and figure below.

Results

Table 1 shows Comparison of means and standard deviation of heavy metals in the study population and the control. The mean levels of Cadmium, Lead and Chromium as depicted indicates that welders had the highest level of Cd (1.81 ± 0.08) and Cr (0.11 ± 0.02) $p < 0.05$ level of significance. The highest level of Pb was seen among Car painters (0.16 ± 0.03) $p < 0.05$. Whereas Pb was detected in significantly high levels among battery repairers, petrol station attendants and car painters. Elevated Cd levels were seen among welders and car painters only. Cr was only detected among welders.

Table 2a shows changes in the comparison of the mean and standard deviation values of biochemical parameters (ALT, AST and ALP) of the study population according to the duration of exposure in years**.** There was significant difference in mean values ($p < 0.05$) of AST, ALT and ALP among Welders, Battery repairers, petrol attendants and car painters.

Parameters in study group (n = 400)	Cd		Pb		Cr	
	Control Mean ± SD	Test Mean ± SD	Control Mean ± SD	Test Mean ± SD	Control Mean ± SD	Test Mean ± SD
Welders (n = 80)	0.21 ± 0.02	1.81 ± 0.08 p = 0.000*	0.07 ± 0.00	0.06 ± 0.00 p = 0.068	0.07 ± 1.00	0.11 ± 0.02 p = 0.000*
Battery repairers (n = 80)	0.21 ± 0.02	0.24 ± 0.03 p = 0.389	0.07 ± 0.00	0.08 ± 0.01 p = 0.036*	0.07 ± 1.00	0.06 ± 0.02 p = 0.059
Petrol Attendants (n = 80)	0.21 ± 0.02	0.23 ± 0.03 p = 0.519	0.07 ± 0.02	0.16 ± 0.03 p = 0.000*	0.07 ± 1.00	0.06 ± 0.00 p = 0.074
Car Painters (n = 80)	0.21 ± 0.02	0.04 ± 0.00 p = 0.000*	0.07 ± 0.00	0.80 ± 0.04 p = 0.000*	0.07 ± 1.00	0.06 ± 0.00 p = 0.068
Tin Miners (n = 80	0.21 ± 0.02	0.20 ± 0.02 p = 0.575	0.07 ± 0.00	0.06 ± 0.00 p = 0.081	0.07 ± 1.00	0.06 ± 0.00 p = 0.055

Table 1: Levels of Heavy Metals (ppm) in the Study Population.

Key: SD = Standard Deviation, n = Number of samples, * = Significant ($p < 0.05$).

Parameters in study group (n = 400)	AST		ALT		ALP	
	Test Mean ± SD	Control Mean ± SD	Test Mean ± SD	Control Mean ± SD	Test Mean ± SD	Control Mean ± SD
Welders (n = 80)	161.29 ± 5.81[a]	42.21 ± 6.11	88.54 ± 7.65[a]	41.48 ± 1.97	98.06 ± 7.97[a]	25.04 ± 1.14
Battery repairers (n = 80)	152.44 ± 7.83[a]	42.21 ± 6.11	77.75 ± 1.57[a]	41.48 ± 1.97	105.36 ± 1.61[a]	25.04 ± 1.14
Petrol Attendants (n = 80)	150.66 ± 4.60[a]	42.21 ± 6.11	78.90 ± 1.49[a]	41.48 ± 1.97	99.83 ± 8.49[a]	25.04 ± 1.14
Car Painters (n = 80)	155.74 ± 7.11[a]	42.21 ± 6.11	91.40 ± 1.72[a]	41.48 ± 1.97	115.06 ± 1.88[a]	25.04 ± 1.14
Tin Miners (n = 80	37.71 ± 5.52[b]	42.21 ± 6.11	38.70 ± 1.12[b]	41.48 ± 1.97	32.46 ± 1.83[b]	25.04 ± 1.14

Table 2a: Level of Biochemical Parameters (ppm) in the Study Population.

Key: a = Significant increase ($p < 0.05$) compared to control, b = Increase not statistically significant ($p > 0.05$).

Table 2b shows a comparison between the group mean values of biochemical parameters among non-exposed and exposed groups. The mean values of AST, ALT and ALP were statistically ($p < 0.05$) significant.

Biochemical Parameters	non-exposed (n = 400) Mean ± SD	exposed (n = 400) Mean ± SD
AST	42.21 ± 6.11	50.37 ± 11.94[a]
ALP	26.62 ± 0.1	109.26 ± 0.9[a]
ALT	38.40 ± 0.2	82.10[a]

Table 2b: Comparison of Mean and Standard Deviation of Biochemical Parameters in the Exposed and Non-exposed.

Key: a = Significant increase ($p < 0.05$) compared to control.

Table 3 shows the data on hematological parameters among test and control populations. There were 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. There was a significant difference between exposed and non-exposed group by using independent t-test ($p < 0.05$).

Figure 1 indicates Neutrophils: r = -0.171, p = 0.00; Eosinophils: r = 0.275, p = 0.000; Basophils: r = 0.017, p = 0.736; Monocytes: r = -0.182, p = 0.000; Lymphocytes: r = 0.142, p = 0.005.

Discussion

There was bioaccumulation of Cd, Pb and Cr in the blood samples of Welders, Battery repairers, Car painters and Petrol station Attendants in significant levels ($p < 0.05$) as shown in table 1 which also appeared to be far above recommended standard reference ranges: Cd (0.06), Pb (0.1), Cr (0.05) mg/l [8]. This is in line with similar observations of [9]. In their study, they pointed out that heavy metals particularly Cd and Pb were implicated as occupational and environmental toxicants and their major route of entry into the system was either by inhalation, ingestion or both.

We observed high rate of exposure in the work places, which is often due to high concentration of chemicals in the environment, although the duration of exposure is also limited to the time at

Hematological Parameters	Exposed (n = 400) Mean ± SD	Non-exposed (n = 200) Mean ± SD	t	p-value
ESR (mm/hr)	27.66 ± 18.52	14.48 ± 4.64	-13.412	*0.000
WBC (x10^3/µL)	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
Hb (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/ µL)	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

Table 3: Mean and Standard Deviation of Hematological Parameters in Exposed and Non-Exposed Groups.

Key: ESR = Erythrocyte Sedimentation Rate, WBC = White Blood Cells, LYM = Lymphocytes, Mono = Monocytes, Neut = Neutrophils, Eos = Eosinophils, Bas = Basophils, RBC = Red Blood Cells, Hb = Hemoglobin, HCT = Hematocrit, MCV = Mean Cell Volume, MCH = Mean Cell Hemoglobin, MCHC = Mean Cell Hemoglobin Concentration, PLT = Platelets, PTTK = Prothromboplastin time test with kaolin. * = Significant ($p < 0.05$).

work. The high levels of Cd, Pb and Cr found in this study could be attributed to the fact that inhalation of oxides of these metals might have resulted in increased metal uptake. As the oxides of these metals are released in very large quantities into the environment continually through the activities of welding or car painters in form of Nano particles, they are later inhaled as metal dust, paint dust or ingested due to poor hygiene habit by such workers who often engage in eating without washing their hands [10].

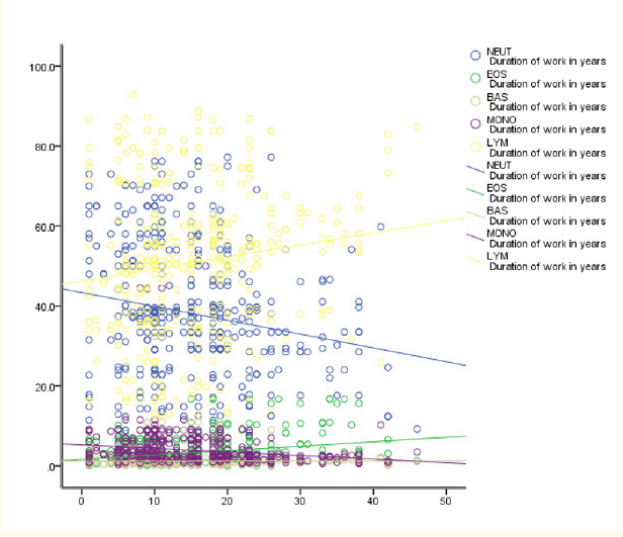

Figure 1: Correlation Between Differential WBC Count Ratios (%) And Duration of Exposure.

The observations in this study is in tandem with the findings of [11] and [12] who reported the presence of Cr, Pb, Zn, Cu, Mn, and Ni in mechanical, industries and vehicle construction industries,as well as the presence of nephropathy (kidney damage), gastro intestinal disturbances, anemia and neurological effect characterized by weakness, fatigue, irritability, high blood pressure, mental deficiencies or even associated infertility in both sexes as well as fetal damages respectively.

The result of our study showed that activities of serum (ALT, AST, and ALP) in both groups except among Tin Miners, increased when compared to control group. ALT, AST and ALP increase in enzyme levels confirmed hepatocytes cell membrane damage.

The liver is a target site of many toxic chemicals and high level of AST, ALT and ALP is associated with liver damage. ALT is a liver enzyme which is released into blood when liver cells have been damaged [13]. All of these correlated with blood pollutant (heavy metals) concentrations by standard deviation analysis. This might suggest that the liver may have been damaged by the heavy metals' presence. The bioaccumulation of Pb is particularly worrisome because its accumulation rate was greatest among the other metals tested and it can reduce reproductive function [14].

This study also showed a decrease in the mean values of WBC (5.82 ± 4.01), Monocyte (4.30 ± 3.92), RBC (4.44 ± 0.76), MCHC

(30.61 ± 6.67) and Platelets (176.90 ± 73.28). However, as reported earlier on by Bot et al., 2019 [15]. Values of ESR, lymphocytes eosinophils, basophils, MCV, PT and PTTK and RDW appears raised among Artisans compared to control while other parameters fall within normal reference values.

The spikes in RDW seen among Artisans could suggest a predisposition to macrocytic anemia due to vitamin B_{12} and or folate deficiency as suggested by a corresponding high MCV. While the raised ESR values reported here among study subjects could be an indication of possible inflammatory reaction as the system responded to the effect of exposure generated sequel to heavy metal entrance into the system. Increase in white cells types (Lymphocytes, eosinophils and basophils) as reported in this study may not suggest any complications since total WBC count is within normal limits. However, the prolonged PTT (24.23 ± 2.45) and PTTK (42.45 ± 9.79) reported among the exposed population as compared to non-exposed group, may be an indication of the effects of heavy metals on the liver. This could be suggestive of the likelihood of metal induced oxidative stress causing impairment of the liver cells leading to the prolongation of PTT and PTTK. The Liver is the sole organ for the synthesis of all clotting factors (vitamin K, fibrinogen, factor V), except factor VIII which is also synthesized in the endothelial cells. As the clotting factor with the shortest half life is factor VII, this factor becomes deficient at an early stage and the PT is prolonged first. Later, the other clotting factors fall and the PTTK or TT also gets prolonged. The liver also clears the activated clotting factors, fibrin and Fibrin degraded products (FDPs) as well as plasminogen activator. Thus in liver derangement (advanced liver disease) the FDPs may be raised, platelets may be low (due to hypersplenism or marrow dysfunction) and the picture is similar to Disseminated Intravascular Coagulation (DIC).

Furthermore, the decrease in RBC counts among Artisans as against the control may likely be as a result of Hb-auto oxidation sequel to inducement of oxidative stress which must have led to peroxidative hemolysis in RBC membrane [15]. This might also be a contributory factor to the rising concentrations of AST levels reported in this study.

Conclusion

This study suggests that chronic and prolonged exposure to heavy metal particularly Cadmium, Lead and Chromium (Cd, Pb, and Cr) is capable of altering both hematological and biochemical parameters negatively among Artisans who are exposed to them. Thus, this calls for regular monitoring of these heavy metals, liver function tests and routine hematological tests among occupationally exposed individuals which will help prevent severe health hazards due to their effects.

Acknowledgement

The authors acknowledge Plateau State Ministry of Health for granting Ethical clearance for the work. We also acknowledge leaders of the various occupations for their co-operation and support in mobilizing their members, and the willingness of the artisans to take part in the research work.

Conflict of Interest

The authors declare that there is no conflict of interest in this work.

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