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The influence of occupational lead exposure on haematological indices among petrol station attendants and automobile mechanics in Nnewi, South-East Nigeria"

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ABSTRACT

Background: Lead adversely affects a number of organ systems in the body; routine blood count evaluation is an important component of monitoring for organ related toxicity such as leukemia and aplastic anemia. Objective: To evaluate the influence of blood lead levels (BLL) on heamatological parameters among petrol station attendants (PSAs) and auto mobile mechanics (AMs) in Nnewi, South-east Nigeria. Subjects and Methods: One hundred subjects (including 25 PSAs, 25 AMs and 50 normal controls) were prospectively recruited. Five mililiter of blood was collected for full blood count (FBC) and BLL, FBC was done using haematology auto-analyzer (SYSMEX PE 6800), while BLL was determined with atomic absorption spectrophotometer (AAS model: 240FSAA). Results were expressed as means ±SD, while associations between variable were explored using student t-test and analysis of variance. Regression analysis and correlation were used to establish possible link between lead parameters and hematological indices and personal life style habits. Ethical clearance was obtained from our institutional review board and all participants gave informed consent. Results: Blood lead and white blood cell count (WBC) were significantly higher in AMs compared with PSAs and controls (P values < 0.001), while haemoglobin concentration (Hb), haematocrit, mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglobin (MCH) and platelet count were significantly higher in controls, compared to PSAs and AMs (P values <0.001). The MCV and MCH were negatively correlated with BLL in PSAs (P=0.02, respectively) while the Hb, haematocrit, MCV, MCH, and MCHC were negatively correlated with BLL in AMs (P values all <0.05). Conclusion: Lead exposure adversely affects blood count and red cell indices in occupationally exposed groups in Nnewi, South-east Nigeria.

KEY WORDS: Haematocrit; Nnewi; Occupational exposure; Red cell indices; Serum lead levels

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BACKGROUND

Globally, ill health that directly arises as a result of occupational exposure to a number of chemical agents and industrial machines has been recognized by the World Health Organization (WHO) as a significant contributor to mortality and morbidity [1]. The magnitude of this problem was well captured in the WHO report of 2009, in which up to 160 million people globally were said to be affected [1,2]. Interestingly, 0.9% of this global burden is attributable to lead poisoning, out of which between 0.5 and 1.5 million cases have been ascribed to exposure at the work place [3].

Lead (Pb) is a naturally occurring metal found deep within the ground, it can be found in small amounts in ore, along with other elements such as silver, zinc or copper. Levels of lead found in air, food, water, soil and dust vary widely throughout the world and depend upon the degree of industrial development, urbanization and general lifestyle [4]. Occupational exposure to lead has been observed to occur both through inhalation of lead particles in aerosols or by accidental ingestion in the work place [5].

Irrespective of the mode of exposure to lead, systemic effect of intoxication range from acute, life threatening illness, which could result in death, to a progressive damage to a number of organ systems in the body, such

haematopoietic, nervous, cardiovascular, urinary, as and reproductive systems [6]. The central nervous and haematopoietic systems are particularly prone to adverse effects of lead toxicity, as such irreversible learning and behavioural disorders as well as qualitative and quantitative red cell abnormalities have been reported in exposed individuals [7, 8]. Occupational heamatotoxicity arising from exposure to aromatic compounds among AMs and spray painters has been described and includes significant reductions in red blood cell count, haemoglobin concentration and red cell indices. Ajugwo et al showed evidence that PSA and AMs who were exposed for more than two years seemed to show further significant reduction in the above parameters when compared to those exposed for less than two years. They concluded that it appeared that PSA were more at risk compared to auto mechanics and may develop anaemia over time. [9,10] The above effects have been attributed to the cytotoxic effects of aromatic hydrocarbons on haematopoetic precursors in the bone marrow. [11] Long term occupational exposure has been linked with a number of haematological disorders such as leukaemia, myelodysplasia and aplastic anaemia. [12] Screening for these long term complications among occupationally exposed groups could readily be done by doing a full blood count; fortunately nowadays, this test is available even in district laboratories in most countries.

A number of factors have been shown to influence lead toxicity following exposure; these include age, gender, dose and duration of exposure, mode of contact, diet, lifestyle and the individual's pre-morbid health status [7]. In order to further mitigate the adverse effect of occupational lead exposure, current occupational guidelines and regulations in some countries argue for blood lead levels (BLLs) of $40\mu g/$ dL, as the absolute highest which should be permitted [5].

An assessment of BLL among automotive garage workers in the town of Jimma, south west Ethiopia revealed high blood levels (range of $11.7 - 36.52 \mu g/dl$), with a significant proportion of the workers at risk of lead related organ toxicity.[13] We had earlier reported that blood count and red cell indices were significantly different in automobile technicians in Nnewi metropolis, compared with normal controls; this study was however limited by the fact that serum lead levels of subjects were not evaluated [8]. Even though leaded gasoline has been phased out in a number of countries, an earlier study reported the average lead content of gasoline in Nigeria to be 0.66 g/L. [14] This is therefore a potential source of lead toxicity to occupationally exposed groups.

The objective of the present study was therefore to evaluate the effects of lead exposure on hematological parameters in two related groups (automobile mechanics (AMs) and petrol station attendants (PSAs)) who were exposed to lead by virtue of their work and to explore the influence of personal lifestyle habits on the risk of exposure.

SUBJECTS AND METHODS:

Research design:

This was a prospective study which involved a total of 100 participants (aged between 18 and 60 years), 50 of these were apparently normal, occupationally unexposed subjects (control group) recruited from amongst students while 50 were occupationally exposed subjects (test group, consisting of 25 PSAs (20 males and 5 females) and 25 AMs (all males)). The PSAs become occupationally exposed while dispensing petrol into automobiles while the AMs suck petrol from vehicle fuel tanks and similarly wash car components such as engine parts with same during the course of their work which lasted between 8 and 12 hours on each work day. Both categories of study subjects also come in contact with exhaust fumes from automobiles which is a potential cause of inorganic lead poisoning. To qualify for inclusion into the study, each test subject would have spent a minimum of one year on his/her respective job; there was no history of previous or current use of personal protective equipments (PPEs) in any of the participants.

The presence/ absence of signs and symptoms of ill health, including that which could suggest lead toxicity were obtained by using a short structured questionnaire, relevant social history such as alcohol consumption and/or tobacco smoking was similarly collected. We stratified subjects into those that consumed alcohol or tobacco products and those who did not with a view to understanding the influence(s) of social habits on occupational exposure to lead. Significant alcohol consumption was taken as up to 280g/week (for men) and 168g/week (for women). [15] Exclusion criteria included the presence of any sign/symptom of lead toxicity and any form of ill health, particularly fever or any other feeling of unwell.

Study Area

The study town of Nnewi is cosmopolitan, (comprising of 2 local government areas; Nnewi north and south) and located in Anambra State, South-east Nigeria. It is well known for its high industrial nature and expertise in imports, sales and distribution of automobiles and spare parts and has been documented to have a significant amount of soil and dust particle lead contamination. [15]

Sample Collection/ Analyses

Each participant who satisfied the inclusion criteria had 5ml of whole blood collected in the morning hours following standard protocols for venesection and dispensed into Ethylene Diamine Tetracetic Acid (EDTA) specimen container for full blood count (FBC) estimation and whole blood lead level. FBC was done within 6 hours of collection using automated cell counter (SYSMEX PE 6800). The remaining was stored at -20°C until blood lead level (BLL) testing, which was carried out using atomic absorption spectrophotometer (AAS model: 240FSAA). The manufacturer's instructions were strictly adhered to while carrying out each test. All blood samples for BLL were determined under fume hood to avoid unwanted contamination with any external source of lead from the environment or dust.

Statistical analysis

The Statistical package for Social Sciences, version 16.0 (SPSS Inc., Chicago IL, USA) was used for all data analyses. Results were expressed as means \pm SD while comparisons were made between variables by using the student's t-test and Analysis of Variance (ANOVA). Regression analysis and correlation were used to establish possible link between lead parameters and hematological indices and personal life style habits and the level of statistical difference was set at P < 0.05, at 95% confidence interval.

Ethical approval

Ethical clearance for this research was sought and obtained from our institutional review board and all participants gave informed consent.

RESULTS

Blood lead levels (BLL) and white blood cell count (WBC) were significantly higher in auto mechanics (AMs) compared with petrol station attendants (PSAs) and controls (P values < 0.001, Table 1). Correspondingly, the

haemoglobin concentration (Hb), haematocrit, mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglobin (MCH) and platelet count were significantly higher in controls, compared to PSAs and AMs (P values <0.001, Table 1). The means of Hb, haematocrit and MCH were significantly higher in PSAs compared with AMs, while the BLL was significantly higher in AMs compared with PSAs (P values of, 0.001, 0.001 and 0.002 respectively).

The MCHC was significantly lower in PSAs that gave a history of alcohol consumption compared to those that did not $(297.50 \pm 15.66 \text{ g/L vs.} 311.64 \pm 9.19 \text{ g/L}$, respectively, P=0.01, Table 2). Similarly, the WBC was significantly higher in AMs that gave a history of alcohol use, compared to those that did not $(5.00 \pm 1.38 \text{ x}10^{9}/\text{L vs}. 6.48 \pm 1.50$ x10⁹/L, respectively, P=0.03, Table 3). There were no significant differences in other haematological parameters compared between AMs and PSA that gave a history of alcohol use and those who did not (P values all >0.05, Tables 2 and 3). Haematological parameters were also not significantly different among AMs and PSAs who had a history of smoking and those who did not.

The MCV and MCH were negatively correlated with BLL in PSAs (P=0.02, respectively, Table 4) while the haemoglobin, haematocrit, MCV, MCH, and MCHC were negatively correlated with BLL in AMs (P values all < 0.05, Table 5).

Following logistic regression, alcohol consumption significantly affected WBC count of both the PSAs and AMs (odds ratio=2.39, 95% confidence interval (CI) = 1.05-5.42, P=0.04), while smoking did not show any significant influence on measured parameters (BLL and haematological indices).

Parameters		Test	Control	E volue	Duralua			
	PSA	Auto mechanics	Control	F- value	P value			
BLL (µg/dl)	15.11 ± 6.50	36.11 ± 14.52	13.61 ± 3.48	66.29	*<0.001			
RBC (x 10 ¹² /L)	5.60 ± 0.72	5.93 ± 0.88	5.93 ± 1.44	0.75	0.48			
HGB (g/L)	128.09 ± 21.61	99.56 ± 33.03	142.96 ± 36.79	14.68	*<0.001			
HCT (L/L)	42.30 ± 6.31	33.12 ± 11.12	51.82 ± 12.36	25.67	*<0.001			
MCV (fL)	75.50 ± 7.53	58.38 ± 24.03	87.37 ± 7.95	37.32	*<0.001			
MCH (pg)	22.94 ± 2.71	17.64 ± 7.27	25.11 ± 9.16	8.12	*<0.001			
MCHC (g/L)	277.72 ± 14.81	270.40 ± 16.86	301.4 ± 14.06	32.19	*<0.001			
WBC (x10 ⁹ /L)	5.88 ± 1.41	6.06 ± 1.59	4.92 ± 1.20	7.71	*<0.001			
Platelet Count (x10 ⁹ /L)	220.48 ± 85.49	210.52 ± 75.41	236.17 ± 79.92	1.07	0.35			
*significant P values. Abbreviations:	HCT - Haematocrit MCV – Mean Cell Volume							

PSA- Petrol Station Attendants.

RBC - Red Cell Count

HGB - Haemoglobin

MCV – Mean Cell Volume

MCH - Mean Cell Haemoglobin

MCHC - Mean Cell Haemoglobin Concentration. WBC - White Cell Count. BLL - Blood Lead Level

Table 2. Comparison of means of haematological indices and blood lead levels in Petrol Station Attendants that consumed Alcohol.

Parameters	Alcohol Consumption Non-Alcohol consumers (n=11)	Alcohol consumers (n=14)	P - value	t - value		
BLL (µg/dl)	14.33 ± 7.12	15.73 ± 6.10	0.60	-0.53		
RBC (x 10 ¹² /L)	5.92 ± 0.59	5.35 ± 0.74	0.05	2.11		
HGB (g/L)	137.15 ± 13.73	120.97 ± 24.46	0.06	1.96		
HCT (L/L)	44.60 ± 4.26	40.49 ± 7.19	0.12	1.68		
MCV (fL)	75.33 ± 4.90	75.64 ± 9.28	0.92	-0.10		
MCH (pg)	23.43 ± 1.38	22.56 ± 3.42	0.44	0.78		
MCHC (g/L)	311.64 ± 9.19	297.50 ± 15.66	*0.01	2.65		
WBC (x10 ⁹ /L)	5.72 ± 1.05	6.01 ± 1.67	0.61	-0.51		
Platelet Count (x10 ⁹ /L)	199.00 ± 55.89	237.36 ± 101.95	0.28	0.28		
Abbreviations: RBC – Red Cell Count HGB – Haemoglobin HCT - Haematocrit MCV – Mean Cell Volume	Sell Count MCHC – Mean Cell Haemoglobin Concentration. loglobin WBC – White Cell Count. atocrit BLL – Blood Lead Level					

Table 3. The Means of haematological indices and blood lead levels in Auto mechanics that consumed alcohol.
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Parameters	Non- Alcohol consumers (n=6)	Alcohol consumers (n=19)	P- values	t- values			
BLL (µg/dl)	38.87 ± 15.51	35.04 ± 14.43	0.57	0.58			
RBC (x 10 ¹² /L)	5.92 ± 1.04	5.93 ± 0.85	0.97	-0.04			
HGB (g/L)	95.71 ± 33.45	101.10 ± 33.71	0.73	-0.36			
HCT (L/L)	(L/L) 32.06 ± 10.98		0.77	-0.29			
MCV (fL)	56.69 ± 24.66	59.04 ± 24.47	0.83	-0.22			
MCH (pg)	17.31 ± 7.89	17.77 ± 7.26	0.89	-0.14			
MCHC (g/L)	297.71 ± 6.90	302.83 ± 15.95	0.43	-0.81			
WBC (x10 ⁹ /L)	5.00 ± 1.38	6.48 ± 1.50	*0.03	-2.26			
Platelet Count (x10 ⁹ /L)	207.43 ± 92.93	211.72 ± 70.55	0.90	-0.13			
Abbreviations: RBC – Red Cell Count HGB – Haemoglobin HCT - Haematocrit MCV – Mean Cell Volume	ed Cell Count MCHC – Mean Cell Haemoglobin Concentration. aemoglobin WBC – White Cell Count. aematocrit BLL – Blood Lead Level						

MCV – Mean Cell Volume

Table 4. Correlation of blood lead level with haematological indices in Petrol Station Attendants.

Parameters	Correlation Coefficient	RBC	HGB	нст	MCV	МСН	МСНС	WBC	Platelet Count
	R value	0.03	-0.26	-0.28	-0.47	-0.46	-0.27	-0.01	0.06
BLL (µg/dl)	P value	0.87	0.18	0.18	*0.02	*0.02	0.19	0.96	0.78
	Ν	25	25	25	25	25	25	25	25
*significant P values.	MCV – Mean Cell Volume MCH – Mean Cell Haemoolobin								

Abbreviations:

RBC - Red Cell Count

HGB – Haemoglobin

HCT - Haematocrit

MCH – Mean Cell Haemoglobin MCHC - Mean Cell Haemoglobin Concentration. WBC - White Cell Count. BLL - Blood Lead Level

Table 5. Correlation of blood lead level and haematological indices in Auto mechanics.

Parameters	Correlation Coefficient	RBC	HGB	нст	MCV	МСН	мснс	WBC	Platelet Count
BLL (µg/dl)	R value	0.31	-0.53	-0.45	-0.47	-0.50	-0.45	0.03	0.08
	P value	0.14	0.01*	0.02*	0.02*	0.01*	0.02*	0.88	0.72
	Ν	25	25	25	25	25	25	25	25

*significant P values. Abbreviations: RBC - Red Cell Count

HGB – Haemoglobin

HCT - Haematocrit

MCV - Mean Cell Volume

MCH – Mean Cell Haemoglobin

MCHC - Mean Cell Haemoglobin Concentration.

WBC - White Cell Count.

BLL - Blood Lead Level

DISCUSSION

This study observed that BLL was significantly higher in AMs compared with PSAs and normal controls (Table 1). The BLL remained significantly higher when levels were compared in AMs PSAs alone $(36.11 \pm 14.52 \mu g/dl \text{ vs.})$ $15.11 \pm 6.50 \mu g/dl$, respectively, P=<0.001). This is an important observation and may reflect the fact that AMs do have other potential sources of contact with lead during the course of their duty (such as from car batteries, paints, radiators and probably have closer contact with car exhaust fumes) compared with PSAs. There is also evidence that auto technicians in Nigeria aspirate petrol in their mouths and even use same to wash off remnants of grease and engine oils from their hands [17,18]. These behaviours could significantly increase the chances of contact with inorganic lead (from exhaust fumes) as well as increase the risk of accidental ingestion and mucosal contamination. In addition, most mechanic workshops in Nigeria are located in enclaves, known as mechanic villages/garages, where car welding and soldering, painting, radiator repairs and other activities that potentially cause lead contamination occur simultaneously [19]. The above factors probably combine to increase both the duration and magnitude of exposure of AMs to lead and may therefore explain the higher blood levels observed in them in this study.

While the mean BLL observed in AMs in this study (36.11 \pm 14.52 µg/dl) was lower than the 40µg/dl accepted as the critical cut-off allowable for adults in some countries, it is however higher than the $25\mu g/dl$ which has been adopted as the preferred target level for any occupationally exposed group [5]. Similarly, the BLL in this study was higher than previously reported among company workers in Enugu, South-east Nigeria ($36.11 \pm 14.52 \ \mu g/dl$ vs. $7.00 \pm 0.07 \ \mu g/dl$) [20]. The lower BLL reported in the Enugu study may be due to the fact that it involved company workers whose extent of exposure to lead may not be as much as either AMs or PSAs. It is never the less important that efforts be intensified towards promoting the routine use of personal protective equipments (PPEs) as well as encouraging the establishment of other safe work place habits among AMs in Nnewi, with a view to obviating the potential adverse effects of lead toxicity.

A number of studies had previously enunciated the deleterious effects of lead exposure and other aromatic compounds in both occupational and non occupational settings [8, 20, 21]. Ibeh et al had reported a significant decrease in red cell count, haematocrit, MCV and MCH in auto motive technicians compared to normal controls and equally observed that red cell count and MCV significantly decreased with duration of exposure [8]. Similarly, Ukaejiofor et al evaluated BLL in 81 male subjects in 3 manufacturing companies in Enugu metropolis and reported a significant reduction in haematocrit, haemoglobin concentration and reticulocyte count in the occupational group, compared with controls [20]. The present study appears to emphasize the above observations as haemoglobin concentration, haematocrit and red cell indices were significantly higher in controls, compared with study subjects. More so, among PSAs, MCV, and MCH were negatively correlated with serum lead levels (Table 4), while haemoglobin concentration, haematocrit, MCV, MCH and MCHC were negatively correlated with serum lead levels in AMs (Table 5). This observation therefore further establishes the adverse effects of lead on red blood cell lines and AMs appeared to be more severely affected than PSAs. A number of mechanisms have been proposed to explain the deleterious effect of lead on cells of the haematopoietic system, particularly red cells. These include lead induced oxidative stress, inhibition of red blood cell adenyl and mono oxygenase enzyme systems and interference with enzymes of the haem biosynthetic pathway. [22,23,24] Alcohol appeared to worsen the adverse haematological effect of lead in PSAs, as the MCHC was significantly lower in those with a history of alcohol consumption (297.50 \pm 15.66 g/L vs. 311.64 ± 9.19 g/L, P=0.01, Table 2). Udonwa et al studied the effect of exposure of PSAs and AMs to Premium Motor Sprit Fumes in Calabar, South-south Nigeria and reported higher levels of met-haemoglobin and lower haematocrit in test subjects compared with normal controls [25]. This effect was related to the toxic effect of components of gasoline (mainly benzene) on cellular elements of blood, the authors proposed that assessing for methaemoglobin and haematocrit could be a useful biomarker in determining the level of exposure to benzene in petrol vapour.

The WBC was significantly higher in test subjects (highest in auto mechanics) compared with controls (Table 1). This is in agreement with the earlier reports in Nigeria and has been attributed to occupational inhalation of lead fumes (inorganic lead) from the exhaust of automobiles by test subjects with resultant reactive leukocytosis [21,26]. This phenomenon was even more marked among AMs who gave a history of alcohol consumption in this study (Table 3), on further multiple regression analysis, alcohol consumption was significantly related with WBC in all participants (odds ratio=2.39, 95% confidence interval (CI) = 1.05-5.42, P=0.04). The reason for this observation could be because alcohol impairs judgment and the ability to observe simple precautionary measures against potential lead poisoning, such as avoiding excessive inhalation of automobile exhaust fumes and application of adequate hygienic measures, thereby potentially increasing exposure to lead. Even though efforts were made at recruitment of study subjects to ensure that all was free from any symptom of ill health (particularly fever) it is however not impossible that subclinical inflammation may be present in some of them, which may account for the high WBC.

CONCLUSION

Occupational lead exposure has adverse effects on the haematopoietic system, particularly Hb, haematocrit, MCV, MCH and MCHC (in AMs) and MCV and MCH (in PSAs), this in keeping with earlier reports. Auto mechanics, probably because of their higher levels of exposure and involvement in other jobs which could potentially increase lead contamination, appear to be more at risk of toxicity, compared to PSAs. Alcohol consumption appears to exacerbate the haematotoxicity of lead exposure.

RECOMMENDATION

We recommend regular blood lead analysis for all occupationally exposed groups in Nnewi, particularly AMs, as well as the provision and encouragement of the use of PPEs to further protect those at risk.

LIMITATION OF THE STUDY

This study is limited by the lack of markers for acute phase reaction and inflammation, such as C-reactive protein, which would have helped to objectively rule out subjects with subclinical inflammation.

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