ORIGINAL ARTICLE

Serum Total Antioxidant Status and Oxidative Stress Marker Levels of Subjects in a Lead-Zinc Mining Community of Ebonyi State, Nigeria: Evidence of Lead Intoxication

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Abstract

Background: This study intended to investigate blood lead and serum total antioxidant capacity and oxidative stress marker levels of subjects in a lead-zinc mining community of Ebonyi State, Nigeria.
Methods: Sixty-eight (68) occupationally-exposed (OE) and 62 environmentally-exposed (EE) to lead in Enyigba community and 70 non-inhabitants (controls), were recruited for this study. Blood samples were collected from them for blood lead (BL), malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), assays using standard methods. Data were analyzed with the IBM SPSS software and means were compared using One Way ANOVA and t-test. Statistical differences was considered significant at $p<0.05$.
Result: Mean BL significantly rose in the OE and EE groups ($p<0.0001$ respectively) than the controls. TAC decreased significantly in the OE ($p<0.01$), but increased in EE ($p<0.05$) compared with the control groups, while SOD, CAT, and MDA decreased significantly in the OE ($p<0.01$ respectively) and EE ($p<0.01$ each) versus control group. Significant higher TAC and lower CAT were recorded in the EE compared with the OE ($p<0.05$) subjects. GPx significantly increased ($p<0.05$) in the OE compared with the control group. Only CAT correlated significantly with lead in the EE subjects. BL, MDA, and CAT differed significantly (<0.0001 respectively), and SOD (<0.05) varied with age. Children <10 years recorded the highest BL concentration (34.77±12.46) b young adults aged 21-30 the lowest, which was significantly lower than in the other age categories.
Conclusion: The elevated MDA concentration and reduced antioxidant activities indicated some oxidative stress damage resulting from lead toxicity.

Keywords: Blood Lead, antioxidants, oxidative stress, occupationally-exposed, environmentally-exposed, intoxication

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INTRODUCTION

Alterations in the antioxidant status and induction of oxidative stress have long been associated with lead exposure and toxicity [1, 2] and are important mechanisms underlying lead toxicity [3]. Human exposure to lead may alter the enzyme antioxidants level, and the interaction between prooxidants and blood lead eventually causes oxidative stress [4]. Superoxide dismutase (SOD) catalyzes the dismutation of the highly reactive superoxide anion to O_2 and subsequently to the less reactive species hydrogen peroxide (H_2O_2), which can be broken down to water (H_2O) by catalase (CAT) and glutathione peroxidase (GPX) reactions. The replacement of zinc (a cofactor for many enzymatic reactions) by Pb results in the inactivation of such enzymes. Lead exposure may cause inhibition of glutathione by affecting tissue thiols. Decrease in glutathione and protein-bound sulfhydryl groups and the changes in the activity of various antioxidant enzymes involved in lipid peroxidation have been implicated in lead-induced oxidative tissue damage.

Afaf [5] demonstrated that reduced glutathione (GSH) concentration and SOD activity were significantly decreased in the liver of lead-treated rats, while malonaldehyde (MDA) increased significantly. Some human studies demonstrated a disrupted prooxidant/antioxidant system in lead-exposed workers [6, 7]. As a case in point, Sugawara *et al.*, [6] reported

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decreased activity of SOD, CAT, and GPx in erythrocytes from workers exposed to lead. Chiba [7] reported that the indices of lead exposure in blood and urine of lead-exposed workers correlated significantly with SOD, GSH, and CAT activities. Depressed levels of glutathione reductase, glutathione peroxidase, and glutathione-S-transferase have been shown to correlate with blood Pb concentration in occupationally-exposed lead workers [8].

From the time solid mineral deposits were discovered in the Abakaliki division of the old Eastern Nigeria, in the forties to the present, Enyigba's environment has reportedly witnessed continuous input of heavy metal pollutants [9, 10]. The indigenes of Enyigba mining community have taken to mining, as a key player in economic empowerment. Thus, solid mineral mining is next to agricultural sector in the economies of many families and is one of the major sources of internally generated revenue to the government [11]. In the Enyigba mining community, there is hardly any household without a heap of lead-zinc metals, resulting in large-scale environmental degradation, unsafe mining conditions, severe environmental pollution, and a cocktail of human health problems [12]. This development has both ecological and human health risks, as recent studies have associated lead-zinc mining activities with high blood lead levels, deranged renal, and hematological indices [10, 13] among inhabitants of Enyigba mining community.

Lead pathways into the body may be through oral, inhalation, or dermal route, particularly when miners and other regulars in the sites do not put on the expected protective wear or adhere to proper preventive and precautionary measures to avoid poisoning. Besides, considering the weather conditions (tropical) in Ebonyi State, Pb could quickly enter the water bodies and the food chain. This could cause water and food pollution, thereby leading to Pb poisoning, as is the case in many mining communities in Nigeria. Lead intoxication is an insidious risk, capable of triggering irreversible health consequences. It interferes with physiological processes and alters systemic and other metabolic functions [14]. Thus, the presence of Pb in environmental matrices, particularly soil, food chain, and water bodies' represents significant health risks to children, adults and the ecosystem.

Studies, so far, on the impacts of lead-zinc mining in Abakaliki lead-zinc mining area have focused mainly on water, soil, and plants metal loads [15, 16] and their speculative health and environmental impacts [9, 11]. The related literature also highlights the impacts of Abakaliki lead-zinc mining on plant physiology, anatomy, and nutrient compositions [12, 17]. However, there is scarcity of research on the impacts of the lead-zinc mining activities on the health status of inhabitants of the Enyigba mining environment, except for few like Tilako *et al* [13] and Shu *et al* [11], which reported the relationship of lead exposure to the renal status and anemia risks, respectively, of the inhabitants of Enyigba lead-zinc mining community. Thus, investigating the serum total antioxidant status and oxidative stress marker levels of subjects in a lead-zinc mining community of Ebonyi State, Nigeria will shed more light on the public health challenges confronting the community.

METHODS

Research Context:

The Enyigba community is the largest and most active mining site in Abakaliki area. It is 14km Southeast of Abakaliki in Southeast Nigeria with latitudes 6º07N and 6º12N and longitudes 8º05'E and 8º10'E. Ezzamgbo, 25km from the study area, with no history of mining activities, was used as a control community.

Participants

One hundred and thirty (130) inhabitants of the lead-zinc mining community were recruited for the study. Among the 130 inhabitants, sixty-two (62) were non-miners, and sixty-eight (68) were artisanal miners, while Seventy (70) volunteers from Ezzamgbo (the control site) with no mining activity history were recruited as the control.

Sample Size:

The sample size was calculated from the study area's population based on the last population census, using Taro Yamane's formula as described by Singh and Masuku [18].

$$n = \frac{N}{1 + N(e)^2}$$

Where n= sample size, N= population size 1= constant

e= sampling error (usually 0.10, 0.05 and 0.01).

Considering a population size of 9000 with a sampling error of 0.10, the minimum sample size for the study was 99 subjects with an attrition rate of 20%, and the minimum sample size was calculated to be 119.

Inclusion and Exclusion Criteria: The participants of this study were consenting, apparently healthy male and female individuals of all ages. It is crucial to mention that pregnant women, individuals with any chronic or debilitating conditions and non-consenting individuals were excluded from the study.

Blood Sample Collection and Preparation for Analysis

Blood (5ml) was collected from each subject through venipuncture.1ml was dispensed into EDTA container (for blood Lead assay) and the rest into a plain tube for antioxidants assay. The blood in the plain tubes were allowed to clot and centrifuged using a tabletop centrifuge (RM-12 Microcentrifuge, REMI, England) at 2000 RPM for 20 minutes. The serum was separated from the spunned blood, dispensed into tubes, and stored in the freezer at -20ºC until assayed for total antioxidant capacity, malondialdehyde (MDA) concentrations, and the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT).

Blood Lead Estimation

Blood lead was measured with the FS240AA Agilent atomic absorption spectroscopy at wavelengths 217.3nm, slit 0.2nm using air-acetylene mixture flame by the American Public Health Association, (APHA) method [19]. On aspiration into the flame, digested sample becomes atomized, and the AAS's light beam, which was directed through the flame into the monochromator, and onto the detector, measures the amount of light absorbed by the atomized element in the flame. The amount of energy of the

characteristic wavelength absorbed in the flame was proportional to the concentration of the element in the sample.

Determination of Superoxide Dismutase (SOD) Activity: SOD was assayed by the colourimetric method of Misra and Fredovich [20]. Adrenaline underwent auto-oxidation at pH 10.2 to form adrenochrome with an absorption maximum of 480 nm. The presence of superoxide dismutase in the reaction mixture inhibited the auto-oxidation of adrenaline. The decrease in the formation of this adrenochrome was proportional to the rate of superoxide dismutase activity in the sample.

Estimation of Catalase: The catalase activity was determined by the method described by Hadwan and Abed [21]. Catalase activity was assessed by incubating the enzyme sample in substrate hydrogen peroxide in sodium-potassium phosphate buffer (pH7.4) at 37 °C for three minutes. The reaction was stopped with ammonium molybdate. The absorbance of the yellow complex of molybdate and hydrogen peroxide was measured at 374 nm against the blank.

Determination of Malondialdehyde (MDA) Level

MDA level was determined by the colourimetric method of Gutteridge and Wilkins [22]. MDA is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline conditions, it formed a pink-coloured product with an absorption maximum of 532 nm. The intensity of the colour generated was directly proportional to the concentration of MDA in the sample.

Estimation of Total Antioxidant Capacity

Total antioxidant activity was estimated by the Ferric Reducing Ability of Plasma (FRAP) method by Benzie and Strain, [23]. At low pH, Antioxidant power causes the reduction of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) that can be monitored by measuring the change in absorption at 593nm. FRAP values are obtained by comparing the change in absorbance at 593 nm in the mixture (test) with those containing ferrous ions in known concentrations (Standard).

Data Analysis: The gathered data were imputed and analyzed via the IBM SPSS software and results were represented as Mean±Standard deviation (S.D.). Differences in means were calculated through One Way ANOVA and t-test. Moreover, correlation was carried out using Pearson's correlation. The obtained results were considered statistically significant at $p<0.05$.

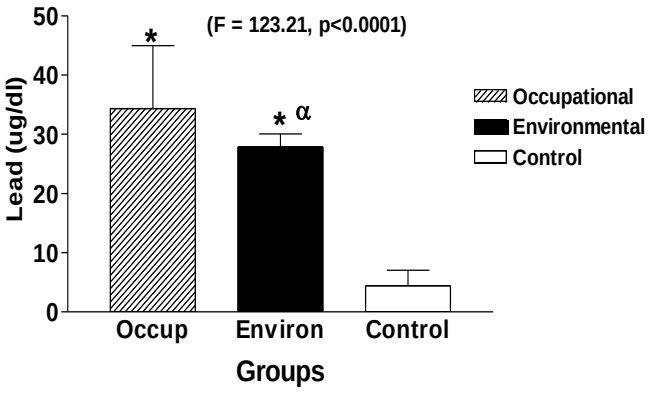
Ethical Considerations:

The study was carried out following the laid down standards of the 1964 Declaration of Helsinki and its later amendments [24]. Ethical clearance for this study was obtained from the Health Research Ethics Committee of the College of Medicine, University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu, Nigeria. Further ethical clearance was obtained from the State Ministry of Health, Abakaliki, Ebonyi State, Nigeria. Informed consent was obtained from each study participant before inclusion in the study.

RESULTS

The results of the study are represented in figure 1 and tables 1-5. Figure 1 shows the mean serum lead levels among the three groups, occupationally exposed, environmentally exposed and non-exposed (control). A statistically significant difference ($p<0.0001$) was observed across the three groups, with the highest mean serum lead concentration observed in the occupationally exposed group (34.67±10.78), followed by the environmentally exposed (27.99±8.51) and the control group (4.41±2.60). Post hoc analysis revealed a significant difference ($p<0.0001$) when the control group was compared with the occupationally and environmentally exposed groups, as well as the occupationally-exposed group when compared to the environmentally-exposed group ($p<0.05$).

The mean serum antioxidant concentration in the occupationally and environmentally exposed and non-exposed (control) groups is represented in table 4.5. There were statistically significant differences in mean TAC ($p<0.0001$), SOD ($p =0.0001$), CAT ($p<0.0001$) and MDA ($p<0.0001$) concentrations across the study groups. Post hoc analysis revealed that TAC was statistically significantly lower in the occupationally-exposed group compared with the control ($p<0.0001$) and environmentally-exposed ($p<0.0001$) group. SOD and CAT activities were significantly lower ($p<0.0001$). At the same time, MDA concentration was significantly higher ($p<0.0001$) in the occupationally exposed (16.63±2.66, 42.55±18.72 and 3.68±0.86) and environmentally exposed (16.49±3.06, 19.88±6.28 and 3.35±0.64) groups when compared with the control group (19.10±2.59, 64.31±13.45 and 2.39±0.42). A statistically significant increase ($p<0.05$) in glutathione peroxidase activity was observed in the occupationally exposed subjects compared to the control individuals. The occupationally-exposed group showed significantly lower TAC ($p<0.0001$) and higher catalase activity ($p<0.0001$) and MDA concentration ($p<0.05$) compared with the environmentally-exposed group ($p<0.05$).



** Compared with the control group p<0.0001, α Compared with occupationally exposed p<0.0001*

Figure 1. mean serum Lead levels amongst occupationally-exposed, environmentally-exposed and the non-exposed (control) group of the study population

Table 1. Mean serum antioxidant and MDA concentrations in the lead-exposed and control subjects.

Parameter	Occupationally exposed (n=68)	Environmentally exposed (n=58)	Control (n=70)
TAC (µmol/ml)	341.70±255.53**	792.33±183.19ᵅᵅ	769.05±136.58
SOD (U/ml)	16.63±2.66**	16.49±3.06**	19.10±2.57
CAT (IU/L)	42.55±18.72**	19.88±6.28** ᵅᵅ	64.31±13.45
GPx (U/ml)	1.02±0.21*	0.99±0.14	0.92±0.24
MDA (nmol/ml)	3.68±0.86**	3.35±0.64** ᵅ	2.39±0.42

*TAC – Total antioxidant capacity, SOD – superoxide dismutase, CAT – catalase, MDA – malondialdehyde, and GPx – glutathione peroxidase. * p<0.05, **p<0.0001 (Compared with the control group). ᵅ p<0.05, ᵅᵅ p<0.001(Compared with occupationally exposed).*

Table 2. Relationship between Lead and antioxidants.

		Occupationally exposed (n=68)	Environmentally exposed (n=58)	Control (n=70)
TAC (µmol/ml)	R	-0.141	-0.274	0.042
	P	>0.05	<0.05	>0.05
SOD (U/ml)	R	0.070	-0.489	0.116
	P	>0.05	<0.0001	>0.05
CAT (IU/L)	R	-0.220	0.659	-0.256
	P	>0.05	<0.0001	<0.05
GPx (U/ml)	R	-0.220	0.104	0.053
	P	>0.05	>0.05	>0.05
MDA (nmol/ml)	R	0.0004	0.330	0.069
	P	>0.05	<0.01	>0.05

Table 3. Age variations in blood lead concentration, MDA levels and antioxidant activities in the exposed group.

Age categories	**Pb**(µg/dl)	**TAC**(µmol/ml)	**MDA**(nmol/ml)	**CAT**(IU/L)	**SOD**(U/ml)	**GPX**(U/ml)
≤10 (n=25)	34.77±12.46***	618.92±259.72	3.59±0.59*	34.34±18.14***	16.18±2.97*	1.02±0.22
11-20 (n=12)	28.43±16.43**	591.83±276.47	3.49±1.10**	40.99±16.71	16.72±1.94	0.95±0.18
21-30 (n=11)	12.25±15.74	732.42±224.68	2.60±0.73	58.25±18.46	18.43±3.09	0.92±0.22
31-40(n=25)	18.56±14.96 ##	713.42±210.22	2.96±0.86	50.66±21.65# β	17.85±2.51	0.98±0.25
41-50 (n=35)	23.97±10.60** #	742.15±229.66	3.36±0.84***	34.81±18.46***	16.72±3.80	1.01±0.15
51-60 (n=12)	30.12±12.12**	618.33±167.86	3.54±0.78***	34.77±26.04**	16.61±1.43	1.00±0.08
>60 (n=6)	22.14±5.44	751.30±137.79	3.00±0.68**	39.36±29.74	17.94±2.87	0.98±0.21
F	8.97	1.93	6.55	8.82	2.77	1.04
P value	<0.0001	>0.05	<0.0001	<0.0001	<0.05	>0.05

<0.05, **<0.01, *<0.0001 In relation to 21-30 age group; #<0.05, ##<0.0001 In relation to <10 age group; β<0.0001 In relation to <41-50 age group*

Table 2 represents the relationship between mean serum antioxidant and lead levels in the study population. TAC and the antioxidants assayed did not correlate significantly with Lead (p>0.05) in the occupationally exposed group. However TAC, MDA, and SOD correlated negatively (r-0.274, p<0.05 ; r = -0.330, p<0.01 and r = -0.49, p<0.0001 respectively) with Pb while CAT had a strong and positive correlation with lead (r = 0.657, p <0.0001) in the environmentally exposed group. Only catalase showed a significant positive correlation with Pb in control exposed group.

The mean±SD serum TAC, MDA, CAT, SOD and GPX concentrations as categorised by age in the exposed group is represented in Table 3. Statistically significant differences were observed in mean blood lead (p<0.0001), MDA (p<0.0001), CAT (p<0.0001), and SOD (p<0.05) concentrations across age categories. Blood lead and MDA

mean concentrations were significantly lowest in the age category 22-30 years (12.25±15.74 and 2.60±0.73) and this was statistically significant compared to age categories ≤10 (34.77±12.46, p<0.0001 and 3.59±0.59, p<0.05), 11- 20 (28.43±16.43, p<0.01 and 3.49±1.10, p<0.01), 41-50 (23.97±10.60, p<0.01 and 3.36±0.84, p<0.001) and 51-60 (30.12±12.12, p<0.01 and 3.54±0.78, p<0.0001). The age category >60 years showed significantly higher mean MDA values than 21-30 years (p<0.01). In contrast, CAT was significantly increased in the 21-30 years age groups compared to the ≤10 (p<0.0001), 41-50 (p<0.0001) and 51-60 (p<0.01) age groups, respectively. In addition, significant differences were observed between the age group ≤10 and 31-40 years in blood Pb (p<0.0001) and CAT (p<0.01) and 41-50 years in blood Pb concentration alone (p<0.05). CAT was significantly lower in subjects within the age range of 41-50 compared to 31-40.

In Table 4, the blood lead concentration in the non-exposed group revealed no significant difference (p>0.05) within the age categories. Likewise, the total antioxidant capacity, MDA, CAT, SOD and GPX did not show any significant variation (p>0.05) in their mean values amongst the age categories.

The comparison of the male and female subjects' mean blood Pb concentration and antioxidant status and their corresponding controls is represented in Table 5. Blood lead and MDA concentrations were significantly higher (p<0.0001) in the exposed male and female subjects compared to their corresponding controls. On the other hand, GPx also revealed a higher mean value in the exposed males and females as against the control male and females; however, this was not statistically significant (p>0.05). In contrast, TAC and the antioxidant enzymes CAT and SOD values were significantly decreased in the exposed male (p<0.05, p<0.0001 and p<0.05, respectively) and female (p<0.05, p<0.0001 and p<0.05, respectively) subjects compared to their controls. No significant differences (p>0.05) were observed in blood lead concentration and antioxidant parameters between male and female subjects of the same group.

DISCUSSION

Lead poisoning has been recognized as a significant public health risk causing severe effects, particularly in developing countries. Several cases of lead poisoning have been and are still being reported in Nigeria [25, 26]. In the study area, lead-zinc mining activities have been associated with some diseases of public health interest [10, 13]. Some of these studies recommended that adequate sensitization of the inhabitants on the health implications of unregulated and unprotected mining activities be embarked upon and necessary intervention measures implemented, by appropriate government agencies to forestall the progression of lead-related diseases. However, these interventions will be more effective if the relationships between subjects' blood lead levels, antioxidants status and oxidative stress parameters, are known. This study was designed to provide this information.

Table 4. Mean blood Lead, TAC, MDA, CAT, SOD, and GPX concentration in the Control group as stratified by age.

Parameter	11-20 (n=6)	21-30 (n=30)	31-40 (n=24)	41-50 (n=6)	51-60 (n=2)	>60 (n=2)
Pb (µg/dl)	4.40±0.28	3.42±2.80	4.77±2.44	7.20±1.00	7.20±0.00	4.60±0.00
TAC (µmol/ml)	613.60±11.46	794.69±166.20	737.74±102.63	771.80±48.27	935.10±0.00	871.20±0.00
SOD (U/ml)	2.60±0.57	2.36±0.37	2.33±0.40	2.47±0.23	3.60±0.00	1.90±0.00
CAT (IU/L)	57.50±9.33	65.69±12.36	61.33±14.93	60.63±11.14	89.3±.000	77.60±0.00
GPx (U/ml)	18.25±2.47	19.09±3.31	19.25±2.19	19.33±1.44	19.50±0.00	19.10±0.00
MDA (nmol/ml)	0.93±0.28	0.90±0.22	0.91±0.30	0.88±0.16	0.97±0.00	1.23±0.00

Table 5. Gender differences in Lead, TAC, MDA, CAT, SOD and GPX concentrations amongst the exposed and control groups.

	MALES		FEMALES	
	Exposed n(=57)	Control (n=20)	Exposed (n=73)	Control (n=50)
Pb (µg/dl)	34.03±9.94***	4.87±2.69	31.88±10.88***	3.40±2.26
TAC (µmol/ml)	617.06±280.13*	771.80±110.79	591.55±255.55*	762.19±196.73
SOD (U/ml)	3.56±0.78***	2.42±0.45	3.56±0.93***	2.34±0.38
CAT (IU/L)	37.82±18.66***	65.98±14.17	35.91±19.48***	60.14±10.99
GPx (U/ml)	17.08±2.49*	19.52±2.86	16.10±3.12*	18.05±1.50
MDA (nmol/ml)	1.02±0.20	0.91±0.21	1.02±0.19	0.94±0.32

** p<0.05, **p<0.01 and ***p<0.0001 (Compared with the corresponding control group).*

In this study, the observed significant ($p < 0.05$) higher values of blood lead concentrations in the occupationally and environmentally-exposed subjects in comparison with the control has similarly been reported by Tilako et al, [13] and Shu *et al* [10], implicating lead-zinc mining activities in blood Pb contamination. Giving the recommended permissible limit (<10ug/dL for adults) of the United States (U.S.) Centers for Disease Control [27], the blood lead concentrations of the subjects in the mining community were above the allowable limits for elevated blood lead levels, while those of the control subjects were below the permissible limit. This raises the suspicion that the subjects in the mining community are at the risk of lead intoxication. The mean serum lead concentration differed significantly ($p<0.05$) between the occupationally and environmentally exposed groups and between the exposed and the control groups. This finding agrees with several other studies [28, 29, 30, 31], which reported that occupational exposure was the major source of adult and children blood-lead contamination.

Experimental and epidemiological studies have indicated that antioxidant/oxidant balance can be disrupted through induction and generation of reactive oxygen species due to high lead levels [32]. Based on their response to the general free radical invasion, the first-line defense antioxidants, which include SOD, CAT, and GPX, play an effective and indispensable role in the entire defense strategy of antioxidants [33]. Superoxide radical is formed during various chemical reactions by adding an extra electron to the oxygen molecule and making it highly reactive. It reacts with multiple molecules and results in either direct damage or the generation of potentially harmful products like H_2O_2. The antioxidant enzyme SOD is responsible for scavenging superoxide free radical (O_2-), catalyzing a dismutation reaction, and converting it into H_2O_2. The load of H_2O_2 synthesized by SOD activity is removed constantly and efficiently by another enzymatic antioxidant glutathione peroxidase, present in the same sub-cellular compartment as SOD [5]. CAT prevents the conversion of H_2O_2, through the Fenton reaction, to the deleterious hydroxyl radical (*O.H.) by breaking down the H_2O_2 into water and molecular oxygen [33].

Our study revealed a significant reduction in the antioxidant activities of SOD and CAT in the Pb-exposed groups compared to the control. TAC decreased significantly while glutathione peroxidase rose significantly in the occupationally exposed group compared with the control group, indicating possibilities of lead intoxication. Reduction in the antioxidant activities of SOD CAT and TAC was associated with lead intoxication [2, 3].

In addition, the mean serum concentration of MDA increased significantly in the two lead exposed groups compared to the control group. Eshginia and Marjani [34] showed a decrease in antioxidant enzyme activities of SOD, GPX, and glutathione reductase (GR_X) in blood and Moreira *et al*. [35] reduced SOD activity in the hypothalamus of rats following exposure to lead. Some studies have shown that blood Pb concentration increased and decreased the erythrocyte antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase [6, 7]. Evidence shows that such changes in the activities of these antioxidant enzymes depend on the lead concentrations [34].

Workers with high (>40 μg/dL) and low blood lead levels (25-40 μg/dL) were observed to have significantly decreased and increased blood glutathione peroxidase levels, respectively, in a study by Kasperczyk *et al*. [36]. Though our work showed a higher GPX concentration in the exposed groups compared to the control group, the mean blood lead concentration recorded in this study was within the range of low lead concentration in the work by Kasperczyk et al. [36]. The result of this study is consistent with that of Rabiu *et al*. [32], which was conducted on lead environmentally-exposed subjects in Northern Nigeria as well as Jangid *et al*. [4], that was run in an apparently healthy urban population set with an increase in MDA levels and decrease in SOD, GPX and CAT concentrations. Enzymatic antioxidants like SOD, CAT, and GPx are produced in the cells. Reports indicate that decreased activities of these antioxidants in erythrocytes from lead-exposed workers may play a part in the increased membrane lipid peroxidation, as observed by an increase in oxidative parameters such as MDA in lead-exposed workers [4, 37]. Santhosh and Asha [38] reported that lead accumulation in the cells severely affects the mitochondria, altering its normal function by inducing oxidative stress resulting in the formation of reactive oxygen species (ROS) like superoxide and hydroxyl radicals. The presence of the ROS results in damage to corpuscular cell materials, modification of cell genetics and oxidative cell damage [38]. The higher concentration of MDA in the lead–exposed groups in the present study indicated that there could be some level of oxidative damage, which was corroborated by decreased antioxidant enzyme activities.

The correlation analysis in the present study showed no correlation between Pb and TAC, SOD, CAT, and GPx in the occupationally exposed and the control groups. However, in the environmentally-exposed group, blood lead correlated negatively with TAC, SOD and CAT and positively with MDA suggesting that increased blood Pb levels were directly related to enhanced lipid peroxidation and decreased antioxidant enzyme activities. The decreased antioxidant enzyme activities could be due to Pb's displacement of essential metals such as copper, zinc (cofactors for SOD) and iron (necessary for heam formation, a prosthetic group in CAT enzyme). The result is similar to the study of Jangid *et al*. [4], who recorded a negative and significant correlation between blood lead levels and the antioxidant enzymes SOD, GPx, and catalase.

In the present study, as stratified by age, all the age groups had mean blood lead concentrations above the recommended tolerable limits of 10ug/dl. Some prospective epidemiological studies in children and adults have shown that low levels of lead exposure (5-25ug/dl) in blood resulted in intellectual impairment. The CDC [27] in the United States has reduced the tolerable amount of lead in children's blood from 25 to 10ug/dl and recommended universal lead screening for all children. The cutoff value of <10ug/dl, defined by the Center for Disease Control and Prevention as a limit for elevated blood lead levels, is primarily based on neurological toxicity [40]. The lowest blood lead concentration was observed in the 21-30 age groups, significantly lower than in the other age categories. This could be explained in that the age bracket of 21-30 years represents

the most active period of human life, often characterized by hyperactivity. Activeness has been associated with improved metabolic rate and positive health outcomes, including detoxification of xenobiotic [41]. At the same time, children <10 years recorded the highest blood lead concentration. Studies show that children are at a higher risk of lead toxicity, especially at sites where Pb-related occupations are nearby [30]. The high levels in the children is likely because young children absorb a higher percentage of ingested Pb, around 40-50%, compared to 10% in adults [26] and are more vulnerable to organ damage. The control group observed no significant differences in blood lead concentration, total antioxidant capacity, antioxidant enzymes, and MDA concentration amongst the age categories.

In the exposed population, males had higher lead, TAC, CAT, and SOD concentrations than the females, but this was statistically not significant. Compared to their corresponding controls, the exposed male and female subjects observed a substantial rise in blood lead concentration and greater evidence of lipid peroxidation, as demonstrated by the higher MDA value. While TAC and the first line defense antioxidant enzymes, CAT and SOD, levels declined significantly, indicating increased oxidative stress and hence, lead toxicity. Though reports [10, 29] pointed out female dominated mining activities in Enyigba mining community, the higher blood Pb levels observed in the male subjects could be attributed to the fact the males are relatively more exposed to lead contamination through various lifestyle characteristics such as tobacco smoking and snuffing. Besides, males are more involved in excavation of shafts and adits to reach buried ore bodies, so they become exposed to lead contamination more than women do.

CONCLUSION

It is widely accepted that Lead is a principal lethal metal toxicant. Its toxicity through both occupational and environmental exposure has caused significant public health problems. Lead, has been shown to disrupt antioxidant/oxidant balance through the induction and generation of reactive oxygen species at high blood concentration**.** In this research study, the blood lead concentration and antioxidant status of inhabitants of a mining community in Ebonyi State, Nigeria was investigated. Based on the results, significantly high blood lead levels with concomitant reduced antioxidant activities and increased evidence of lipid peroxidation in the inhabitants suggesting Pb toxicity and the occurrence of some oxidative damage. This calls for a comprehensive health check of the test community's inhabitants and public enlightenment on the potential risk of lead and possible measures of prevention and mitigation of its toxicity. Further studies are therefore recommended to investigate other probable health effect as well as concomitant presence of other heavy metal toxicity.

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