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Oxidative Stress Markers in Individuals Environmentally Exposed To Lead

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Abstract: *Environmental exposure to lead is becoming a serious burden afflicting human, animals and environmental quality. Exposure to lead induces oxidative stress and causes deterioration of antioxidant defence system. Interaction between blood lead and endogenous antioxidants eventually cause oxidative stress. Therefore, the present study evaluates the influence of blood lead on antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), antioxidant glutathione molecule (GSH) and end product of lipid peroxidation (MDA) level in individuals environmentally exposed to lead. One hundred individuals from lead polluted villages of Zamfara State, Nigeria, with mean age of 36.17±0.86 years were recruited as study subjects and compared with fifty age-matched from lead free villages as control. The mean blood lead level (BLL) was 312.19 ± 31.14µg/dl with values as high as 1205.2µg/dl. The activities of all the antioxidant enzymes analysed were significantly (P<0.05) decreased in lead exposed subjects compared to controls while MDA concentrations were significantly (P<0.05) increased in individuals exposed to lead. The study observed significant (P<0.05) negative correlation between BLLs and activity of SOD, CAT, GPx and GSH molecule. Therefore, increase BLLs is associated with remarkable decrease activity of antioxidant enzymes, antioxidant molecules and consequent increase of lipid peroxidation. This indicates that high BLLs and decrease activity of antioxidant enzymes, antioxidant molecule and increase lipid peroxidation observed in individuals environmentally exposed to lead in this study might have deteriorated their antioxidant status that eventually caused oxidative stress.*

Keywords: ***Exposure, Blood lead level, Oxidative stress, Antioxidant enzymes***

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I. Introduction

Despite there are existing stringent and legislative regulation on production and use of lead in gasoline, paint and consumable products. Lead continued to be a major environmental and occupational health problem in many developed and developing countries (Jangid *et al*., 2016). In developing countries like Nigeria, anthropogenic activities such as illegal mining, industrial and municipal sludge as well as automobile exhaust continued to aggravate the menace. This could be due to poor implementation of environmental laws and monitoring by appropriate regulatory agencies and use of rudimentary tools, unsafe techniques and unfriendly environmental practices by miners (Lar *et al*., 2014).General population are easily exposed to lead through ingestion of contaminated foods, water, traditional medicinal preparations, some pharmaceutical products and persistent use of cosmetics (Lar *et al*., 2014). Recently, several studies had indicated that environmental and occupational exposure account for high blood lead levels in workers of different industries such as battery manufacturing and recycling, paint, ceramic, mining and smelting, and construction industries (Patil *et al*.,2007; Ghanwat *et al*., 2015).

Illegal mining and metal ore processing in many countries are responsible for some of the largest releases of heavy metals into the environment and are associated with highest environmental exposures and degradation (Ghanwat *et al*., 2015).). Over 300 million tons of lead were estimated to had been released into the environment globally, for just past five millennia due to industrialization and extensive mining activities in developed and developing countries respectively (Singh and Li, 2014) As a consequence, polluting agricultural produce, water bodies, atmospheric air and in turns, causing tenth of thousands premature death and many degenerative disorders (Singh and Li, 2014).Therefore, an overload and gradual accumulation of these toxic metals are highly detrimental to the body and consequently increases the chances of many adverse health issues.

Reports from experimental and epidemiological studies had indicated that exposure to low levels of lead is linked to increased risks of many diseases characterized with reading problem, hearing loss, tooth decay, spontaneous abortions, and cardiovascular disease (Lopes *et al*.,2016; Ma *et al*., 2017).Lead had also been reported to interfere with a number of normal body functions such as the central and peripheral nervous system, haematopoetic, reproductive systems as well as kidneys and liver (Assi *et al.*, 2016; Ma *et al.*, 2017).Under

normal body physiology, reactive oxygen species (ROS) are produced during cell's metabolic processes such as in mitochondrial electron transport chain and reaction of nicotinamide adenine dinucleotide phosphate oxidase nearly in every cell types. The ROS generated are used in regulating cell proliferation and differentiation, and genomic stability (Katsuyama *et al.,* 2012).However, increased ROS production was evidently observed in many experimental and epidemiological studies involving lead toxicity. Lead has been implicated to interfere with pro-oxidant/antioxidant balance which leads to over generation of ROS and reduced cell's antioxidant defense mechanism which eventually leads oxidative stress. This probably, remains the major underline mechanism of lead-induced toxicity (Ghanwat *et al.,* 2015).

Lead is known to generate ROS via inhibition of δ-aminolevulinic acid dehydratase(ALAD), the second enzyme in the heme biosynthesis pathway which catalyses the condensation of aminolevulinic acid (ALA) to a porphobilinogen (Hassan *et al*., 2014) the accumulated δ-ALA is known to generate ROS through metal-induced auto oxidation (Ercal *et al*., 2001).Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and reduced glutathione that are known to participate actively in scavenging free radicals generated in lead-induced oxidative stress. However, the activities of these enzymes are compromised due to lead's strong affinity for sulfhydryl group (-SH)and inhibitory characteristic to important trace metals, particularly zinc, copper, and selenium which serve as cofactors for these antioxidant enzymes (Kasperckzyk *et al*., 2012; Ghanwat *et al*., 2015; Assi *et al*., 2016). Consequently, this results into deterioration of cellular redox balance and the antioxidant defense system of the cell became overwhelmed (Rahman, 2007).

Several studies had shown that lead induces oxidative stress through depletion of glutathione (Sadhana *et al*., 2011; Muhammad *et al*., 2008), interfering with essential metals and vitamins (C and E) (Mehta and Flora,2001;Herman *et al.,* 2003; Khodamoradi *et al*., 2015), inhibiting sulfhydryl dependent enzymes (Kasperczyk *et al*., 2004) or antioxidant enzymes activity or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acids composition (Sharma *et al.,* 2011). Increased MDA was observed in many animals and epidemiological studies, as a marker of lipid peroxidation, concentration of MDA increase as a result of the interaction between ROS and cell membrane (Klaunig *et al*., 2011). More importantly, it is one of the sensitive and reliable oxidative markers used in determining the degree of damage to cell membranes in the human body (Guichardant *et al*., 2004). Correlation between occupational exposure to lead and increased MDA levels were observed in many studies (S´ciskalska *et al*., 2014).The aim of this study was to evaluate the effect of environmental exposure to lead on the activity of antioxidant enzymes, minerals and vitamins and extent of LPO as well as potential correlation between these parameters and blood lead levels.

II. Materials and Methods

Study Design

The study was conducted in Bagega , Kawaye and Dareta (005^0 39.749′ E, 11^0 51. 858′ N and 006^0 01. 754′ E, 11^0 48. 719 N and $005^0$57.398′E, $12^0$02.330′N respectively) towns of Anka Local Government Area and Yargalma ($005^0$30.897′E, $11^0$58.108′N) town of Bukkuyum Local Government Area of Zamfara State in North-western Nigeria that have been linked to lead poisoning due to artisanal mining of gold-rich ore (Dooyema *et al*., 2012).The places were chosen because studies have indicated that the environment is highly polluted due to high rate of mining activities and the lead outbreak continues to linger in the area.

Ethical Clearance and Informed Consent

The study proposal was granted approval by Zamfara State Committee on Human Research (ZSCHR). Informed consent was obtained from the Emir, Village heads, civil authority council Chairmen and respective participants to conduct the research. Advocacy visits were conducted in the area to inform them about the study objectives, potential benefits and risk if any for their involvement in the study.

Questionnaire for Lead Exposure and Risk Awareness

Questionnaire was administered and filled in by the participants with the help of the research group in some cases. It contained data about demographics, work history, occupation, workplace, duration of exposure; risk information, intervention, if any and blood lead status. The questionnaire was carried out in the villages of Dareta, Bagega and Kawaye of Anka and Yargalma of Bukkuyum and Kadauri of Maru Local Government Areas of Zamfara State Nigeria.

Study Subjects

One hundred and fifty two (152) subjects were recruited for the study. One hundred (100) subjects had blood lead levels >5µg/dl. They included 94 males and 6 females who were either actively involved in illegal gold mining or resided within the polluted areas. The control subjects consisted of fifty two (52) persons (all males), from Kadauri village of Maru Local Government area that were matched for age, sex and with no history of exposure to lead, to any substance or medication known to influence the variables of the study were

purposively selected for the study. Ten millilitres (10mL) of blood sample were collected by venepuncture under aseptic condition; five millilitres (5mL) were transferred into heparinised tubes from each participant and centrifuged at 5000 rpm for five minutes. The serum was stored at -20^{o}C in Chemical pathology of Usmanu Danfodiyo University Teaching Hospital until analysis.

Chemicals and Reagents

All the glass wares used were made cleaned and metal free under standard protocol. Chemicals and reagents used were of analytical grade. Activity of antioxidant enzymes was determined using commercial kits (Cayman Chemical Company, USA) with the following item numbers: superoxide dismutase (706002), catalase (707002), glutathione peroxidase (703102) and MDA (10009055). Hormonal estimation was carried out using radioimmunoassay (ELISA) method.

Serum lead determination

Serum lead level was determined using Atomic Absorption Spectrophotometer (AAS Perkin Elmer, 6300 model USA). A standard wet digestion procedure was carried out on the serum samples, 1.0 ml of blood was transferred into test tube and 2.0 ml concentrated (HN03) was added slowly and heated at 130°C in fume cup board until yellow fumes disappear. The test tube was allowed to cool for about 10 minutes, made to 5 ml with deionized water and stored until analyses.

Determination of serum antioxidant status

The activity of serum superoxide dismutase (SOD) was measured according to the method of Marklund (1980). The activity of serum glutathione peroxidase (GPx) was measured according to the method of Paglia and Valentine (1967). The activity of serum catalase (CAT) was measured according to the method of Johansson and Borg (1998). Serum malondialdehyde (MDA) levels were measured according to the method of Niehans and Samuel (1968). The estimation of ascorbic acid was done by the method of Natelson (1971). Vitamin E was assayed by the method of Hashim and Schuttringer (1966)

Determination of some minerals (Zn, Cu and Fe)

The concentrations of Zn, Cu and Fe were determined using Atomic Absorption Spectrophotometer (AAS Perkin Elmer, 6300 model USA).The calibration curve was prepared for each element by running different concentration of standard solutions. The machine was set to zero by running reagent blank. Three replicate results were taken for each sample and average values were obtained.

Data analysis

The data generated were expressed as the Mean ± standard error of mean (SEM). Parameters were analysed statistically by one way analysis of variance (ANOVA) using statistical software Instat 3 version (San Diego, USA) and Tukey Kramer multiple comparison test was used to establish the significance of the observed difference among various groups. Differences were considered significant when $P<0.05$. Pearson's correlation was carried out to investigate degree of relationship between blood lead levels and oxidative stress parameters.

III. Results

The demographic characteristic and risk awareness of 100 lead exposed populations is presented in Table 1. The mean age of study population was 36.17±0.86; 56% of the exposed individuals were within the age range of 16 – 30years. Majority (68%) had formal education and 56% were artisanal gold miners. The mean blood lead level (BLL) was 312.19 ± 31.14 with values as high as 1205.2µg/dl.

Table 2 summarizes the number of individuals sampled for each village and Kawaye P.S had the highest participants of the study and their mean blood lead levels (927.4±37.8)were significantly ($p<0.05$) high compared to other villages.

All the 100 lead exposed individuals in the study were divided into two major groups based on:(i) age range and (ii) blood lead concentrations. Based on age range, individuals were divided into three subgroups (a) 0 – 15, (b) 16 – 30 and (c) 31 – 45years as shown in Table 3. The individuals within the age range of 0 -15 (11%) had mean BLLs 115.78±55.67µg/dl and this group had the lowest BLLs, though far greater than CDC and WHO safe limits. The second group (16 – 30, 54%), the mean BLLs were 444.22±55.27µg/dl and the last group 31-45 (35%) was 370.46±56.27µg/dl.

Based on blood lead concentrations, individuals were subdivided into three groups as low exposed individuals (LEI), medium exposed individuals (MEI) and high exposed individuals (HEI) as presented in Table 4. The LEI group (32%) had BLLs 51.19 ± 4.56 µg/dl, the mean BLLs of second group MEI (24%) was 136.62 ± 6.07 µg/dl and that of HEI (44%) 579.62±41.24µg/dl.

Table 5 summarizes mean values of antioxidants enzymes, GSH and MDA levels in lead exposed individuals and control. The activity of serum SOD, CAT and GPx in lead exposed group decrease significantly ($P<0.05$) compared to control subjects. A statistically notable decrease in serum GSH concentration was demonstrated ($P< 0.05$). However, significant ($P<0.05$) increase MDA level (+ 70.04%) among lead exposed subjects was noticed compared to control.

Mean values of Antioxidants Enzymes activity (SOD, CAT and GPx), GSH and MDA Levels in different age groups and control are shown in Table 6. In all the three age ranged groups, the lead exposed individuals had significantly ($P<0.05$) low activity of SOD, CAT, GPx and GSH level compared to control. The lowest activity of these enzymes was recorded in 16 – 30 age group and CAT activity was significantly ($P<0.05$) decrease among the age groups. Significant ($P<0.05$) increase of MDA level was demonstrated in all the age groups compared to control. In fact, high lipid peroxidation was observed in 16 – 30 age ranged group.

Table 7 shows mean values of Antioxidants Enzymes activity, GSH and MDA Levels in different lead concentrations and control. SOD activity was significantly ($P<0.05$) decrease in all the groups compared to control. The decrease activity was pronounced in HEI group. Similarly, significant ($P<0.05$) decrease activity of CAT and GPx were observed in all groups. The study recorded statistically notable ($P<0.05$) decrease GSH levels when compared to control. Lipid peroxidation (MDA) increase significantly ($P<0.05$) in all groups compared to control.

The relationship between BLLs and oxidative stress markers has been presented in Table 8. Negative correlation between BLL and activities of CAT ($r = - 0.1924$) and GPx ($r = - 0.0764$) were recorded in the present study. A significant ($P<0.05$) negative correlation between BLL and activity of SOD ($r = - 0.2579$) was observed. However, a significant ($P<0.05$) positive correlation between BLL and MDA ($r = 0.4352$) was observed.

Table 9 summarizes correlation between BLL and oxidative stress markers in various age groups. In (0 – 15yrs) age group, non-significant ($P>0.05$) positive correlation was observed between BLL and activity of CAT ($r = 0.4016$), GSH level ($r = 0.3952$) and negative correlation with MDA ($r = - 0.0704$). Significantly ($P<0.05$) negative correlation between BLL and SOD ($r = - 0.3558$ in 16- 30yrs and $r = - 0.5041$ in 31- 45yrs) were observed. Positive correlation was recorded between BLL and MDA level was observed in the study.

In addition, relationship between BLL in different blood lead concentrations and oxidative stress markers are depicted in Table 10. In LEI group, significant positive correlation between BLL and activities of CAT ($r = 0.4135$) and SOD ($r = 0.4253$) were recorded while in HEI group negative correlation between BLL and activities of CAT ($r = - 0.0377$), SOD ($r = - 0.1136$) and GPx ($r = - 0.3745$) and significantly ($P<0.05$) positive correlation between BLL and MDA level was recorded.

Table 1: Demographics and Risk Awareness among Lead Exposed Individuals

Variables		Percentage distribution (%)
Sex	Males	94
	Female	6
Age	0-15 years	11
	16-30 years	54
	31-45 years	35
Level of Education	Primary	68
	Secondary	23
	Tertiary	2
	None	7
Occupation	Civil Servant	10
Business		12
Artisanal Mining		56
	Farming	22
Duration of Exposure	≤ 5 years	25
≤ 10 years		48
>10 years		27
Risk Awareness	Yes	52
	No	48

Table 2: Number of Exposed Individuals Sampled in Different Villages

Status	Bagega	Dareta	Kawaye	Kawaye P.S	Yargalma
No. Sampled	42	11	17	67	37
Exposed	30 (71.43%)	9 (81.82%)	11 (64.70%)	35 (52.24%)	15 (40.54%)
Unexposed	12 (28.57%)	2 (18.18%)	6 (35.30%)	32 (47.76%)	22 (59.46%)
Mean BLLs (µg/dl)	594.8±62.2[a]	108.5±20.5[b]	95.5±7.2[b]	927.4±37.8	50.7±14.6

Exposed = Blood Lead Levels Above 10µg/**Unexposed** = Below Detection Limit (1.0 µg/dl)

Table 3: Blood Lead Levels of Exposed Individuals in Various Age Groups

Age range (years)	Range (µg/dl)	Mean BLLs (µg/dl)	Portion of the total (%)
0-1	515.9 – 613.7	115.78±55.67	11
16-30	40.2 – 1205.2	444.22±55.27	54
31-45	27.4 – 1046.4	370.46±56.42	35
TOTAL -	-	-	100

Table 4: Mean Blood Lead Levels Distribution of the Exposed Individuals

Group	Range (µg/dl)	Mean BLLs (µg/dl)	Portion of the total (%)
LEI	15.9 – 99.1	51.19 ± 4.56	32
MEI	101.6 – 194.4	136.62±6.07	24
HEI	205.2 – 1205.2	579.65±41.24	44
TOTAL	-	-	100

BLLs: Blood Lead Levels

Table 5: Mean values of Antioxidants Enzymes, GSH and MDA Levels in Lead Exposed Individuals and Control

GROUP	GSH(µg/dl)	GPx(nmol/min/ml)	SOD (U/ml)	CAT(nmol/min/ml)	MDA(µM)
Lead Exposed Individuals	13.51±0.21	346.47±24.31	2.81±0.11	27.59±1.67	4.84±0.25
Control	87.24±3.10[a]	2026.67±46.18[a]	5.85±0.21[a]	85.62±2.20[a]	1.45±0.09[a]
% Diff	(- 84.51%)	(- 82.90%)	(- 51.97%)	(- 67.78%)	(+ 70.04%)

Values are expressed as mean ± SEM. Values bearing superscripts on the same column differ significantly (P<0.05) when compared by unpaired test using Instat 3 software (San Diego, USA).
GSH: glutathione, **GPx**: glutathione peroxidase, **SOD**: superoxide dismutase, **CAT**: catalase, **MDA**: malondialdehyde

Table 6: Mean values of Antioxidants Enzymes, GSH and MDA Levels in Different Age groups and Control

AGE	GSH(µg/dl)	GPx(nmol/min/ml)	SOD(U/ml)	CAT(nmol/min/ml)	MDA(µM)
0-15	13.71 ± 0.88	235.17 ± 76.36	2.60 ± 0.38	24.74 ± 2.16[a]	4.70 ± 0.45a
16-30	12.33 ± 0.19	394.08 ± 33.70	2.56 ± 0.13	23.81 ± 1.77[ab]	5.36 ± 0.41[ab]
31-45	14.31 ± 0.37	305.14 ± 36.89	3.18 ± 0.19	34.81 ± 3.56[ac]	4.19 ± 0.28[ac]
Control	87.24 ± 3.09[a]	2026.67 ± 46.18[a]	5.85 ± 0.21[a]	85.62 ± 2.20[d]	1.45 ± 0.09[d]

Values are mean ± SEM. Values bearing different superscripts on the same column differ significantly (P<0.05) when compared by Tukey-Kramer multiple comparison test using Instat 3 software San Diego, USA).
GSH: glutathione, **GPx**: glutathione peroxidase, **SOD**: superoxide dismutase, **CAT**: catalase, **MDA**: malondialdehyde.

Table 7: Mean values of Antioxidants Enzymes, GSH and MDA Levels in Different Lead Concentrations and Control

GROUP	GSH(µg/dl)	GPx(nmol/min/ml)	SOD(U/ml)	CAT(nmol/min/ml)	MDA(µM)
LEI	14.17±0.64	323.06±46.28	3.05±0.26	35.52±3.90 ^{b}	3.32±0.25^{a}
MEI	13.59±0.35	400.99±54.37	3.32±0.17	24.59±2.99	4.24±0.39^{ab}
HEI	13.17±0.24	334.32±34.07	2.29±0.09	23.36±1.46	5.81±0.35^{c}
Control	87.24±3.09^{a}	2026.67±46.18^{a}	5.85±0.21^{a}	85.62±2.20^{a}	1.45±0.09^{d}

Values are mean ± SEM. Values bearing different superscripts on the same column differ significantly (P<0.05) when compared by Tukey-Kramer multiple comparison test using Instat 3 software (San Diego, USA).
GSH: glutathione, **GPx**: glutathione peroxidase, **SOD**: superoxide dismutase, **CAT**: catalase, **MDA**: malondialdehyde.

Table 8: Spearman correlation coefficient between blood lead levels and oxidative stress markers

Parameter	r- value	p – Value
GSH	- 0.1857	0.0643
SOD	- 0.2579*	0.0096
CAT	- 0.1924	0.0552
GPx	- 0.0764	0.4500
MDA	0.4352**	0.0001

n = 100, r: Correlation coefficient, *significant at p < 0.05 and ** very significant at < 0.001. GSH: glutathione SOD: Superoxide dismutase, GPX: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde.

Table 9: Correlation between Oxidative Stress Markers and BLLs in Different Age groups of Exposed Individuals

Age group	GSH	SOD	CAT	GPx	MDA
0 – 15	0.3952	0.6725	0.4016	- 0.1739	- 0.0704
	P(0.2290)	P(0.0234)	p(0.2208)	p(0.6091)	p(0.8371)
16 - 30	- 0.1569	- 0.3558	- 0.0166	- 0.1634	0.2013
	P(0.3537)	p(0.0307)*	p(0.9323)	p(0.3334)	p(0.2323)
31 – 45	- 0.1260	- 0.5041	- 0.2916	0.0346	0.2533
	P(0.4994)	p(0.0038)***	p(0.1115)	p(0.8533)	p(0.1692)

r: Correlation coefficient, significant level * P<0.05 and ** very significant at < 0.001
GSH: glutathione, **GPx**: glutathione peroxidase, **SOD**: superoxide dismutase, **CAT**: catalase, **MDA**: malondialdehyde

Table 10: Correlation between Oxidative Stress Markers and BLLs in Different Lead Concentrations of Exposed Individuals

Group	GSH	SOD	CAT	GPx	MDA
LEI	0.3065	0.4253	0.4135	- 0.2992	- 0.3216
	P(0.0935)	p(0.0171)*	p(0.0207)*	p(0.0617)	p(0.0777)
MEI	0.1580	0.0019	0.1080	- 0.3003	0.2601
	P(0.4714)	p(0.9928)	p(0.6238)	p(0.1639)	p(0.2306)
HEI	- 0.0377	- 0.1136	- 0.1682	- 0.2750	0.3745
	P(0.8123)	p(0.4629)	p(0.2752)	p(0.0708)	p(0.0123)*

Significant level * P<0.05
GSH: glutathione, **GPx**: glutathione peroxidase, **SOD**: superoxide dismutase, **CAT**: catalase, **MDA**: malondialdehyde

Discussion

Occupational and environmental exposure to lead is common in underdeveloped and developing countries (Saeed *et al*., 2017). This could be due to use of rudimentary tools, improper/ non-use of personal protective equipment (protective clothes, masks, and goggles)and lack of stringent environmental regulations (Dobrakowski *et al*., 2017) The main source of lead in the affected environment is the gold ore mining activities of gold ore which releases dust containing lead to the atmosphere. Lead has been for long implicated to induce oxidative stress through increase generation of reactive oxygen species (ROS) and decrease activity of the antioxidant enzymes and depletion of antioxidant molecules. These antioxidant enzymes and molecules have been evaluated in both clinical and experimental studies to analyse lead-induced OS (Wang *et al*., 2007).OS

befalls when the free radicals generated exceeds the cell's antioxidant system. Studies have shown that lead affects antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)and other components of the antioxidant system like reduced glutathione (GSH) (Malekirad *et al.,* 2010). Thus, the lead's effect depends largely on concentration and duration of exposure (Jangid *et al.,* 2016). The present study investigates the influence of environmental exposure to lead on markers of oxidative stress.

The present study recruited 150 individuals made up of 100 lead-exposed and 50 control subjects with mean age of 36.17 ± 0.86 and 35.13 ± 0.79, respectively (Table 1). Indeed, both the test and control subjects are within the reproductive age group thus formed a good base for comparison. The study further demonstrates occurrence of oxidative stress in lead exposed individuals with 312.19 ± 31.14µg/dl mean blood lead concentration. The observed high BLLs in the exposed individuals may be attributed to lead pollution caused by intensive gold mining activities in the area under study. In all the individuals analysed, those from Kawaye P.S had significantly ($p<0.05$,Table 2) high mean BLLs compared to other villages. The elevated lead concentration in blood in individuals (16 – 30 years, Table 3) can be related to increased exposure to lead due to their active involvement in all the gold ore mining processes. About 44% of the exposed individuals had blood lead concentrations within the range of 205.2 – 1205.2µg/dl (Table 4).

The antioxidant enzymes analysed were significantly ($p<0.05$, Table 5) decreased in lead-exposed individuals compared to control. The tripeptide glutathione molecule that acts both as a direct scavenger of reactive oxygen species and as a cofactor in metabolic detoxification was significantly ($p<0.05$, Table 5) reduced in lead-exposed individuals compared to control. The MDA concentration which is an indicator of lipid peroxidation was significantly ($p<0.05$, Table 5) increased in lead-exposed individuals compared to control. These are strong clinical indices of lead toxicity suggesting a possible contribution of lead-induced oxidative damage.

Superoxide dismutase is considered most crucial enzyme involved in the antioxidant defense system of the cell and primarily responsible for immediate scavenging of superoxide free radicals (Sharma *et al*., 2011; Jangid *et al*., 2016). This enzyme discharges its protective role against the detrimental effects of superoxide free radical via dismutation reaction there by generating another toxic substance hydrogen peroxide (H_2O_2).

The present study indicates significant ($p<0.04$, Table 6 and 7) decrement in activity of SOD in all aged group of exposed individuals compared to control and was proportional to increment in blood lead concentration of the exposed subject. The lowest activity of the SOD observed was found in groups (16 – 30years, Table 6 and HEI Table 7) compared to control. A significant ($p<0.05$) negative correlations were observed between SOD activity and BLLs (Table 8, 9 and 10). The finding of this study is corroborated by the reports of Patil *et al*. (2007) in Battery manufacturing workers (BMW) of Western Maharashtra India, Jangid *et al*. (2016) in lead exposed subjects of Jaipur, India and Conterato *et al*. (2013) in painters and battery workers. However, the findings is contrary to that of Ahmad *et al*.(2006) who showed increased activity of SOD among urban adolescents and Shraideh *et al*. (2018)in Jordanian automobile workers. Lead has negative influence on the activity of SOD. Perhaps, by either continuous generation of radicals that overwhelmed the enzyme antioxidant activity, or through competitive displacement of its cofactors (Cu and Zn) which lower or inhibits its catalytic functionality.

The H_2O_2 generated by dismutation reaction of SOD is readily converted to water and oxygen molecule by another important enzyme catalase: a heme protein that equally protects tissues from oxidative damage (Chelikani *et al*., 2004;Ercal *et al*., 2001). Significant ($p<0.05$, Table5, 6 and 7) reduction in CAT activity was observed with corresponding in increased blood lead concentration in all the groups of exposed individuals compared to control. The study noted alteration of CAT activity within the age groups (Table 6).Inconsistent results regarding CAT activity in lead exposed individuals had been documented in literature (Nehru and Kanwar, 2004; Farmand *et al*., 2005; Ahmad *et al.,* 2008; Ahmad *et al*., 2011). The reduction in activity of CAT observed in the lead exposed individuals may be due to lead's interference with iron (serving as cofactor for catalase enzyme) absorption and haem biosynthetic pathway (serving as prosthetic group of the enzyme)(Patil *et al*., 2006).Negative correlation was observed between blood lead level and CAT activity in the exposed subjects (Table 8,9 and 10). This suggest that CAT activity decrease with increase blood lead concentration.

GPx is a selenium-containing enzyme that catalyzes the reduction of H_2O_2 and lipoperoxides generated in the tissues to water and molecular oxygen using GSH as a reducing agent (Matés *et al.* 1999; Gurer and Ercal. 2000). Significant decreased GPx activity was established in individuals exposed to lead ($P<0.05$, Table 6 and 7) compared to control. The decreased activity of this enzyme is probably due to the high affinity of lead for –SH group containing enzymes and formation of an insoluble lead-selenium-complex and reduced selenium absorption (Matés *et al*., 1999). Several studies have shown that the enzyme is considered most sensitive to lead impact among antioxidant enzymes when exposed to different concentration of lead (Monteiro *et al*., 1985; Sugawara *et al*. 1991; Solliway *et al*., 1996). However, epidemiological studies from different population indicate non alteration of GPx activity with respective to blood lead concentration (Jin *et al*., 2006; Ahamed *et al*., 2008).

Glutathione is a tripeptide cysteine-based molecule containing reactive thiol group (-SH) that plays a major role in protecting cell against oxidative stress (Nemsadze *et al*., 2009). Its functional group –SH plays an important role in heavy metal binding, detoxification and excretion (Ding *et al.,* 2000; Sharma *et al*., 2011). Findings from our study demonstrate that red GSH concentration decreased significantly ($P<0.05$, Table 6 and 7) in lead exposed individuals compared to control. A significant (Table 8, 9 and 10) negative correlation was observed between GSH concentration and blood lead levels. Similarly, Mohammad *et al*. (2008) reported decrease GSH concentration in lead-exposed subjects, Martinez-Haro (2011) reported decline GSH concentration in experimental animals. However, Conterato *et al*. (2013) reported elevated GSH levels in lead-exposed workers with low lead concentrations. Therefore, decrease concentration of this important endogenous antioxidant molecule may invariably disrupt the antioxidant defence capacity of the cell. Studies have shown that GSH synthesis tend to increase immediately when oxidative stress occurs but as time goes on, the GSH level declined with increased oxidative stress.

Lead toxicity has been known to inflict damage to cell membranes, by promoting LPO through overproduction of free radicals, depletion of antioxidant molecules), thus leading to an increase in peroxides as MDA (Lopes *et al* 2016). Increase MDA concentration has been reported in many epidemiological and animal studies involving low and high exposure to lead (Gurer and Ercal 2000; Bokara *et al*., 2008; Jia *et al*., 2012; Sirivarasai *et al.,* 2015).The present study indicates a significant increase in MDA concentration was observed in lead exposed individuals compared to control ($P<0.05$, Table 6 and 7) and positive correlation was observed between MDA levels and blood lead levels (Table 8, 9 and 10). A number of researches has shown increased concentration of MDA in both experimental animals and human exposed to lead (Patil *et al*., 2006; Adegbesan and Adenuga, 2007; Bokara *et al*., 2008; Sirivarasai *et al*., 2015). Therefore, an elevated concentration of MDA observed in this study may be a reflection of increased membrane lipid peroxidation associated with decreased activity of antioxidant enzymes induced due exposure to lead.

Conclusion

The study recorded high blood lead concentration among individuals environmentally exposed to lead and was linked to intensive mining activities observed in the affected area under study. This has invariably cause significant decrease activity of antioxidant enzymes and GSH concentration in the lead exposed individuals. The study also observed significant increase MDA concentration in the lead exposed subjects. This is a strong indication of lipid peroxidation due to generation of ROS and depletion of antioxidant defence mechanisms. In nutshell, the study concludes that environmental exposure to lead induced oxidative stress. The data generated from this study provide reference values for public health authorities to identify and mark affected communities and individuals for necessary medical intervention.

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