Vol. 11(7), pp. 84-89, July-September 2019
DOI: 10.5897/JTEHS2019.0442
Article Number: 549260D62016
ISSN: 2006-9820

http://www.academicjournals.org/JTEHS

ACADEMIC JOURNALS
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Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Effect of lead on the activity of antioxidant enzymes and male reproductive hormones

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Received 15 April, 2019; Accepted 14 May, 2019

Environmental exposure to heavy metals such as lead is detrimental to male reproductive system. Lead induced oxidative stress is believed to contribute immensely to male infertility. The study was designed to investigate the influence of environmental lead exposure on the activities of antioxidant enzymes and male reproductive hormones levels in male individuals of Bagega and Kawaye villages of Anka Local Government Area of Zamfara State Nigeria. Sixty male individuals (40 lead exposed and 20 controls) were recruited. Activities of antioxidant enzymes (serum superoxide dismutase [SOD], catalase [CAT] and glutathione peroxidase [GPx]) and malondialdehyde [MDA] levels were determined using standard methods. Blood lead levels and reproductive hormones levels were measured with atomic absorption spectrophotometer and ELISA method, respectively. The lead exposed subjects had mean blood lead levels (BLLs) 208.72 ± 19.89 µg/dl and were within the reproductive age group (15-45 years). The activities of all the antioxidant enzymes were significantly (P<0.05) decreased in lead exposed subjects compared to controls while MDA levels were significantly (P<0.05) increased. Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were significantly (P<0.05) increased in lead exposed subjects compared to controls but testosterone levels remained the same in both lead exposed and control subjects. The study also revealed negative correlation between blood lead levels and reproductive hormones. In conclusion, environmental exposure to lead distorts antioxidant enzymes activity and male reproductive hormones levels perhaps via lead-induced oxidative stress.

Key words: Lead exposure, oxidative stress, reproductive hormones.

INTRODUCTION

Environmental pollution had continued to generate a lot of public health effect in developed, developing and underdeveloped countries (Mohammed et al., 2016). Toxic substances released from industries, heavy automobiles exhaust and municipal discharge sludge had contributed immensely to our environmental degradation. Recently, there is growing concern about the causal relationship between exposure to these toxic

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substances (especially heavy metals) and reproductive health (Sadeghniat et al., 2013).

Occupational and environmental exposures to toxic metals such as lead (Pb) have been reported to induce certain alterations in various sperm parameters and endocrine profile (Sallmen, 2001; Vigeh et al., 2011).

Significant number of workers are exposed to lead in many industries through motor vehicle assembly, panel beating and painting, battery manufacturing, printing, smelting and mining (Hanan et al., 2016). The route of exposure include inhalation, ingestion and dermal. The absorbed lead is mainly stored in bones, soft tissues and to lesser extent in the erythrocytes; once in the system, it exert many health consequences in all of the body organs (Mohammed et al., 2016). These include neurotoxicity (Mason et al., 2014; Chakrabarty et al., 2014), hepatotoxicity (Bharali, 2013), hematotoxicity (Ercal et al., 2001; Sharma et al., 2011) and cardiotoxicity (Sadeghniat et al., 2013).

Lead has generally been implicated on fecundity in both male and female reproductive systems. The toxic effect of lead on male reproductive system has generated more interest since researchers speculate the involvement of exposure to toxic substance such as lead in idiopathic infertility (Hanan et al., 2016). The effect of lead on male reproductive system is believed to involve sperm quality (sperm count, motility and morphology) and reproductive hormones disruption which play a significant role in the regulation of spermatogenesis and sperm development (Sallmen, 2001). Epidemiological and animal studies have indicated that blood lead levels greater than 40µg/dl are associated with decreased sperm count (Bonde et al., 2002), motility and viability (Al-Juboori et al., 2013; Hanan et al., 2016), and greatly affected the hypothalamic-pituitary-testosterone (HPT) axis (Vigeh et al., 2011) which might cause some imbalances in male reproductive hormones (Sallmen, 2001).

In addition, studies had implicated lead in generation of reactive oxygen species (ROS) that cause oxidative stress which may lead to several degenerative diseases (Ercal et al., 2001). Elevated level of lipid peroxide was reported in reproductive organs of rats chronically exposed to lead (Marchlewicz et al., 2007). Consequently, lead-induced oxidative stress is also considered as important mechanism that affects both male reproductive hormones and spermatogenesis.

METHODOLOGY

The study was conducted in environmental lead exposed areas of Bagega and Kawaye located at the coordinates (005° 39.749' E, 11° 51. 858' N and 006° 01. 754' E, 11° 48. 719 N) of Anka local Government Area of Zamfara State, Northwestern, Nigeria. The exposure is connected to the prevalent and persistent illegal gold mining in the area which is confirmed to contain high percentage of lead as impurity (Lar et al., 2014). Forty male individuals with high blood lead levels (>76 µg/dl) and with no history of infertility or had any hormonal therapy were recruited in the study. The study enrolled another twenty male individuals (matched by age with the study subjects), from Kadauri village of Maru local Government Area of the same State who were apparently healthy, with undetected blood lead level or not exposed to any substance known to influence any variable of the study. A consent form was administered, filled and signed after which a questionnaire was also administered to obtain demographic data. After obtaining their consent, a ten milliliters (10 ml) of a venous blood sample was collected from each participant (test and control groups) under aseptic condition into a plain test tubes for lead determination, antioxidant enzymes assay (serum superoxide dismutase [SOD], CAT and GPx), lipid peroxide level (MDA) and hormonal assay (LH, FSH and Testosterone) after centrifuging, serum was collected and stored in freezer (-20°C) until the time for analyses. The study was granted approval by the Zamfara State Ministry of Health Committee on Human Research.

Chemical and reagents

All plastic wares including test tubes, semen collection containers and pipettes were cleaned and made metal free, following a standard protocol. Chemicals and reagents used were of analytical grade. Antioxidant enzymes were assayed 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 determination was carried out using Atomic Absorption Spectrophotometer (AAS Perkin Elmer, 6300 model USA). Wet digestion was carried out on the serum samples, 1.0 ml of blood was transferred into test tube and 2.0 ml concentrated ($HN0_3$) was added slowly and heated at 130°C until yellow fumes disappear. The test tube was allowed to cool, 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).

Determination of hormones

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone concentrations were determined for each participant (test and control), using accubind ELISA microwells kits.

Table 1. Demographic for lead exposed and control subjects.

Parameter	Lead exposed	Control
Age		
15-25	12	5
26-35	27	9
36-45	21	6
Education		
Primary	37	11
Secondary	8	6
Tertiary	2	1
None	13	2
Occupation		
Civil servant	4	-
Artisanal mining	38	-
Farming	18	14
Business	10	6
Duration of exposure		
≤ 10 years	48	-
> 10 years	12	-

Table 2. Antioxidant enzyme activities and MDA levels in lead exposed and control subjects.

Parameter	Lead exposed	Control	P-value
SOD (U/mL)	2.81±0.11*****	5.85±0.21	0.0001
GPx (nmol/ml/min)	11.05±0.18*****	21.02±0.51	0.0001
CAT (nmol/ml/min)	45.90±1.31*****	86.01±1.73	0.0001
MDA (µM)	5.43±0.91*****	1.23±0.87	0.0001

Values are expressed as mean ± SEM, *values differ from the control subjects significantly at p< 0.05. SOD: Superoxide dismutase, GPX: glutathione peroxidase, CAT: catalase and MDA: malondialdehyde.

Data analysis

Results were expressed as the Mean ± standard error of mean (SEM). Differences were considered significant when P<0.05. Parameters were analyzed statistically by unpaired t-test, using statistical software Instat 3 version (San Diego, USA). Pearson's correlation was carried out to investigate degree of relation between blood lead levels of exposed subjects and oxidative parameters/hormones.

RESULTS

The demographic data of the study subjects is shown in Table 1. All the subjects analyzed were within the reproductive age group (15-45 years). The lead exposed subjects had mean blood lead level (BLLs) of 208.72 ± 19.89 µg/dl. The mean ages of lead exposed and control subjects were 36.17 ± 0.86 and 35.13 ± 0.79, respectively. Investigation of their educational status indicated that 78% of the lead exposed subjects had formal education and 90% for control subjects. Majority (80%) of the subjects were exposed to lead through artisanal mining as their major source of livelihood.

Antioxidant enzymes activities and the degree of lipid peroxide formation (MDA levels) in lead exposed and control subjects are shown in Table 2. The activities of all the antioxidant enzymes analyzed were significantly (P< 0.05) decreased in lead exposed subjects compared to the control subjects. Serum concentrations of lipid peroxide (MDA) in lead exposed subjects were significantly (P< 0.05) increased compared to the control subjects.

Table 3. Hormonal levels of lead exposed and control subjects.

Parameter	Lead exposed	Control	P-value
LH (mIU/ml)	2.91±0.37*	1.05±0.21	0.0001
FSH (mIU/ml)	6.45±0.36*	5.65±0.67	0.0001
Testosterone(mIU/ml)	5.02±1.74	5.25±2.29	0.6385

Values are expressed as mean ± SEM, *values differ from the control subjects significantly at p< 0.05. LH: Luteinizing hormone, FSH: follicle stimulating hormone.

Table 4. Spearman correlation coefficient between blood lead levels and oxidative markers.

Parameter	r- value	P-value
SOD (U/mL)	0.0592	0.6531
GPx (nmol/mL/min)	0.1139	0.5716
CAT (nmol/mL/min)	0.2695	0.1926
MDA (µM)	0.4279*****	0.0001

n = 60, r: Correlation coefficient, *significant at p < 0.05. SOD: Superoxide dismutase, GPX: Glutathione peroxidase, CAT: Catalase, MDA: Malondialdehyde.

Table 5. Spearman correlation coefficient between blood lead levels and hormones.

Parameter	r- value	P-value
LH (mIU/ml)	-0.3570	0.0798
FSH (mIU/ml)	-0.3419	0.0944
Testosterone (mIU/ml)	-0.2751	0.1833

n = 60. r: Correlation coefficient, LH: Luteinizing hormone, FSH: Follicle stimulating hormone.

Hormonal estimation in both lead exposed and control subjects is shown in Table 3. Lead exposed subjects were found to have significantly (P< 0.05) high levels of LH and FSH compared to the control subjects. However, there was no significant (P> 0.05) difference in testosterone levels between lead exposed and control subjects.

Table 4 shows the correlation between antioxidant enzymes activities including lipid peroxide levels and blood lead levels of the exposed subjects. The relationship between blood lead levels and the antioxidant enzymes (SOD, GPx and CAT) is not quite significant. MDA showed a significant (P< 0.05) correlation to blood lead levels.

The correlation between hormones (LH, FSH and Testosterone) and blood lead levels of lead exposed individuals is shown in Table 5. All the hormones analyzed demonstrated negative relationship to blood lead levels, though, not quite significant.

DISCUSSION

Occupational and environmental exposures to toxic metal lead have been implicated to reduce sperm functional indices and male reproductive hormone. Reports from both experimental and epidemiological studies (Sallmen, 2001; Al-Juboori et al., 2013) have indicated that high levels of lead (>40 µg/dl) induces generation of reactive oxygen species (ROS) that invariable disrupt antioxidant/oxidant balance. Moreover, progressive generation of these free radicals can equally affect both spermatogenesis and reproductive hormones (Benoff et al., 2000; Darbandi et al., 2018). In the present study, effect of environmental exposure to lead on antioxidant enzymes activities, lipid peroxide levels and reproductive hormones concentration was assessed in both lead-exposed and control subjects. The study recruited eighty individuals made up of forty lead-exposed and twenty 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 lead-exposed subjects had high mean blood levels of 208.72 ± 19.89 µg/dl which is far above US center for disease control and prevention (CDC) limit of 10 µg/dl. This shows that the subjects under study have been exposed to lead through gold mining activities over time.

The activities of antioxidant enzymes decreased significantly in lead-exposed subjects compared to the control subjects while the mean serum concentration of MDA increased significantly in lead-exposed subjects compared to the control subjects (Table 2). Superoxide dismutase (SOD) and catalase are enzymatic antioxidants which scavenge the superoxide anion (O_2^{-2}) and peroxide (H_2O_2) radicals by converting them into water and oxygen. Both SOD and CAT are present within both sperm and seminal plasma (Zini et al., 2000). The high concentration of MDA in lead-exposed subjects indicated that there is element of oxidative damage which supported by decreased antioxidants enzymes activities. In fact, this can expose the sperm cell to oxidative onslaught which might have detrimental effect on sperm quality (Ghosh et al., 2002). The findings of this study are similar to that of Alkan et al. (1997), Sanocka et al. (2003) and Ghosh et al. (2002) conducted in infertile men. Similarly, some studies conducted in infertile women show similar reduction of antioxidant enzymes activities (Veena et al., 2008; Majid et al., 2013; Rajeshway et al., 2016; Panti et al., 2018).

Human and animal studies have indicated the effect of exposure to lead on reproductive hormones. The results of this study revealed significant increase in LH and FSH in lead-exposed subjects compared to the control subjects (Table 3) and are consistent with the results reported by Ng et al. (1991), Ronis et al. (1996) and Grattan et al. (1996). There was no significant difference in testosterone concentrations in both lead-exposed and control subjects. Some studies reported increased serum concentration of testosterone in men exposed to lead (Gustafson et al., 1989; Telisman et al., 2007). High serum concentration of LH and FSH are usually associated with normal testosterone concentrations in acute lead-exposed subjects and literature has indicated that systemic hormones such as LH, FSH and testosterone may play an antioxidant role to safeguard sperm and other testicular cells from oxidative damage induced by toxic metal lead (Chainy et al., 1997; Meucci et al., 2003; Shang et al., 2004; Mancini et al., 2008).

Correlation between antioxidant parameters and blood lead levels is shown in Table 4 and there was no establish significant relationship between the blood lead levels and antioxidant enzymes. Though, there was slight increase activity of those enzymes which could be due to an adaptive mechanism of the antioxidant defense system in response to lead-induced oxidative stress. Significant positive relationship was observed between blood lead levels and MDA. This clearly indicates to some extent, the degree of lipid peroxidation.

The means of sex hormones levels were correlated with blood lead levels of exposed subjects (Table 5). The circulating sex hormones were inversely associated with blood lead levels though not quite significant. The findings of this study are contrary to those reported by Meeker et al. (2010) and Chen et al. (2016) whom reported none and positive correlation, respectively. Thus could be due to high blood lead level and longer duration of exposure observed in this study.

Conclusion

The study showed that environmental exposure to lead could induce generation of reactive oxygen species which eventually decreased the activity of antioxidant enzymes known to protect the integrity of cells (including sperm cells) from oxidative onslaught. Lead-induced oxidative stress could as well affect the hypothalamic-pituitary-testosterone axis. Antioxidant enzymes, sex hormones and lipid peroxidation are affected as blood lead levels raise. Therefore, environmental exposure to lead may result to unexplained male infertility.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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