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ORIGINAL ARTICLE

Assessment of Degree of Association Between Markers of Oxidative Stress in Lead Poisoned Mice

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ABSTRACT

Lead (Pb) is an abundant and one of the most lethal metals found in the earth's crust. Its use by humans dates back to thousands of year. Even the low doses of lead are responsible for the production of reactive oxygen species which leads to oxidative load. This oxidative stress mitigates production of malondialdehyde (MDA) and down regulates antioxidant enzyme superoxide dismutase (SOD).

Study Design: Quasi experimental Study.

Place and duration of study: Department of Biochemistry, ANMCH, Islamabad, Pakistan in collaboration with NIH, Islamabad from November, 2018 to April, 2019.

Methodology: A total of 40 BALB/c mice were divided into two groups of 20 mice each. Group I was given normal standard diet. Group II was given lead acetate in drinking water with normal diet without any supplementation. Levels of malondialdehyde were measured by using Thiobarbituric acid reactive substances (TBARS) and Superoxide Dismutase (SOD) was estimated by xanthine oxidase method at the end of study.

Results: The results of our study showed increase in MDA and decrease in SOD in lead treated group when compared with the control group. Pearson correlation was applied to assess the degree of association between two parameters, it showed significant negative correlation with value of r = -0.96 and p-value of 0.001

Conclusion: It was concluded from our study that increase in MDA leads to decrease in SOD indicating strong negative correlation in lead poisoned mice.

Key words: Lead poisoning, Malondialdehyde, Oxidative Stress, Superoxide Dismutase

INTRODUCTION

Lead (Pb) is an abundant and one of the most lethal metals found in the earth's crust heavy metal. As a well-known neurotoxicant it is having detrimental effects on the brain by producing increased quantity of reactive oxygen species (ROS) which leads to oxidative stress.[1] Lead use by human dates back to thousands of year. Intoxication of lead produces reactive oxygen species and entails to inactivation of the antioxidant defense mechanism by down regulating the action of antioxidant enzymes like superoxide dismutase (SOD).[2]

Lead as a precarious metal is harmful to both humans and animals. The extensive use of lead in industries and agriculture like batteries and pigments has powerful impact on environment. The crucial aspect of pathogenesis behind lead toxicity is the production of reactive oxygen species and oxidative stress. Immoderation in use of lead consequences in raised malondialdehyde (MDA) and reduced total antioxidant capacity, superoxide dismutase (SOD), and catalase (CAT) activities.[3] Malondialdehyde (MDA), is the end product of auto-oxidation of lipid membranes, and is a good marker of free radical mediated damage and oxidative stress. It has been reported that MDA content increases with increasing heavy metal concentration.[4] Antioxidants wheedle on free radicals and due to this scavenging property they halt the process of oxidative stress and avert the commencement of various diseases. Plants are the major source of exogenous antioxidants such as phytochemicals. Some antioxidants are present endogenously in body and are sufficient enough to scavenge free radicals which are produced during normal physiological processes with a normal turnover. The endogenous antioxidants comprise of antioxidant enzymes which are catalase, superoxide dismutase, glutathione peroxidase and reduced glutathione.[5]

These antioxidant enzymes play elemental and imperative role in scavenging the free radicals in biological system. Among these enzymes SOD is contemplated as the first line of defense against the oxidative load.6 During the normal body metabolism and reactions occurring in cells , the production of super oxide radical, one of the reactive oxygen species is provoked.[6,7]This superoxide radical is catalytically converted to hydrogen peroxide and molecular oxygen by the antioxidant action of superoxide dismutase. Therefore SOD is considered as the significant and indispensable in the entire antioxidant defense system especially in relation to constant yielding of superoxide anion radical through several biochemical pathways.[7]

The rationale of this study was to assess the degree of association of oxidative stress parameters after lead poisoning. The objective of the present study was to observe the correlation of MDA and SOD in lead poisoning

MATERIAL AND METHODS

The BLAB/C mice were procured from animal house of National Institute of Health (NIH) Islamabad. These animals were bred at the NIH and were used in the experiment.

They were adult mice 40 days old weighing 50gms±20gms of either gender. Lead acetate was dissolved in drinking water and given by gauge as described in the grouping section.

Mice were randomized into two groups Group I, Group II. Each group contained 20 mice. They were given standard mice chow along with the supplemented diet.

Group I served as a control group and contained 20 mice. Group II was treated with normal mice chow for 8 weeks and was given plain tap water along with 0.5 ml plain water by gauge tube

Group II was treated with normal mice chow and lead acetate 30mg/kg body weight in drinking water for 8 weeks[8]. All the samples were taken at the end of the study by intracardiac puncture.

To check the serum levels of MDA in mice TBARS (Thiobarbituric acid reactive substances) method was used. Thiobarbituric acid (TBA) is used to check the MDA as a product of lipid peroxidation in plasma, serum, urine, tissue homogenates and cell lysates. This method required high temperature ranging between 95-100C and acidic conditions to generate a pink coloured complex. This pink coloured complex was detected colorimetrically at wavelength of 530- 540nm.[9]

The average absorbance of standard and that of each sample was calculated. Absorbance of the standard A was subtracted from all to get value of corrected absorbance. By plotting the corrected absorbance value against MDA concentration standard curve was generated. Results were obtained from the standard curve.

The antioxidant capacity of Super Oxide Dismutase was calculated by Super Oxide Dismutase assay kit purchased from Abcam. This kit utilizes water soluble tetrazolum-1 (WST-1) salt by xanthine oxidase and produces water soluble formazan dye (WST-1 formazan).[10] In this assay superoxide dismutase reduces superoxide anion and has a linear relationship with the activity of xanthine oxidase. The SOD assay measures activity all three types of SOD (Cu/Zn, Mn, and Fe-SOD) and provides a simple fast tool for assaying SOD activity in serum, plasma, erythrocyte lysates, tissues etc. All the solutions were mixed and were incubate at 37□C for 20 minutes. Absorbance was calculated for each well at 450nm. SOD percent inhibition of each sample was measured with the help of formula:

Inhibition rate % = (A blank1 – A blank3) – (A sample – A blank2) X 100
(Ablank1 – Ablank3)

Statistical Analysis: Data obtained from the above procedures were analyzed on SPSS version 21. The arithmetic mean and standard deviation of mean of all samples was calculated. Difference in mean among the control and treated groups was calculated by independent 't test' for two group comparisons. The difference was considered significant if p value was found less than 0.05. To measures the strength and direction of linear relationships between variables, bivariate pearson correlation was applied.

RESULTS

The total number of mice included in the study were 40 (N=40). They were divided into two groups with 20 mice in each group (n=40). Administration of lead in group II significantly increased the lipid peroxidation which was calculated by estimation of serum malondialdehyde levels as shown in Table-I. This table shows that the MDA levels in control group were 1.46±1.21whereas the MDA levels in group II after administration of lead acetate for 8 weeks were significantly raised (p-Value < 0.01) and were 38.06±2.99.

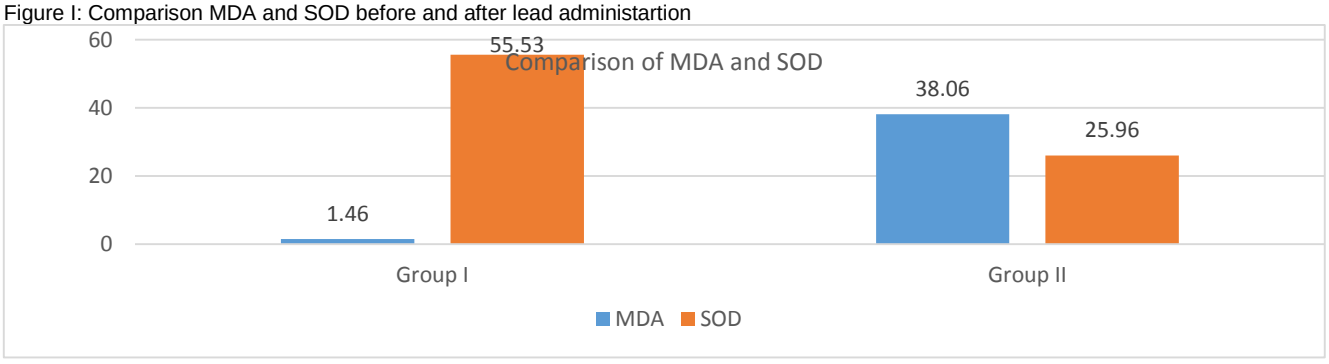
Table – I Effect of lead acetate administartion on Malondialdehyde and serum superoxide dismutase levels in mice.

Parameter	Group I Control (n=20) Mean±SD	Group II Lead acetate (n=20) Mean±SD	p value*
Malondialdehyde	1.46±1.21	38.06±2.99.	< 0.001
Serum Superoxide dismutase % inhibition	55.53±3.84	25.96±3.56	< 0.001

Similarly administration of lead in group II significantly increased the lipid peroxidation by decreasing natural antioxidant levels, which in this case was calculated by estimation of superoxide dismutase activity as shown in Table-I. This table shows that the Serum Superoxide dismutase activity in control group was 55.53±3.84 whereas in group II after administration of lead acetate for 8 weeks it was decreased significantly (p-Value < 0.01) and was 25.96±3.56.

The MDA levels of group II were significantly increased (p-Value< 0.01) when compared to the SOD levels of group II which were significantly decreased (p-Value< 0.01) as shown in Fig: I. This bar graph shows the comparison of MDA levels with the activity SOD before and after administration of lead for 8 weeks. The significant rise in MDA levels in group II resulted into significantly decreased activity of SOD due to lead poisoning.

Figure I: Comparison MDA and SOD before and after lead administartion



Pearson correlation was applied to obtain degree of association between our study parameters. It showed highly significant p value of< 0.01 and represented strong negative correlation with value of r -0.96, between MDA and SOD.

DISCUSSION

The present study was designed to assess the degree of association between MDA and SOD in lead poisoned mice. MDA is the product of lipid peroxidation owing to the oxidative stress which in case of our study was produced by lead poisoning. The MDA levels of our control group were similar to the study conducted on hypertensive subjects in human population. In the same study SOD was also measured in normotensive patients and its activity was same as the activity of SOD in control group of our study.[11]

Lead use has been banned in gasoline but still it is used in many industries. A study conducted on Wistar rats in 2018 observed the nephrotoxic effects of lead. This study reported that on exposure to lead, kidneys showed increase in MDA and decrease in SOD when compared with the control group similar to the conclusion made by our study.[12]

Mercury is a heavy metal comparable to lead and produces oxidative stress when ingested. A study was conducted on mice by Zhao Y et al, where mice were exposed to toxic doses of mercury to produce oxidative stress. When measured, the MDA concentration was significantly higher and the activity of SOD was significantly lower showing negative correlation as in our group of lead poisoned mice.[13] Another study conducted on Murrah buffaloes of heavy metals exposed areas showed results in accordance with our study. The MDA was significantly raised whereas the SOD activity was significantly declined indicating strongly negative correlation.[14]

One of the experimental study done on plants in green house at the Sichuan agricultural university, in which the oxidative stress was introduced by the high concentration of salt in the soil. Due to high salinity plants showed increase in MDA and decrease in SOD. Which showed significant results. Similar conclusion was withdrawn from our study with strong negative correlation between the two parameters.[15]

Aging is a multifactorial process associated with physiological decline. The process of aging results in the progressive decline in antioxidant function combined with increased mitochondrial ROS (reactive oxygen species) generation and increased accumulation of oxidant products leading to oxidative stress. An experimental study was conducted on mice model in China. In this study aging was introduced by exposure to d- galactose for 28 days. This exposure resulted in oxidative stress and caused significant increase in MDA and decrease in SOD similar to our study groups.16

Increasing concentration of heavy metals in aquatic system is of great concern. Lead is one of the toxic heavy metals polluting marine waters. An experimental study conducted on fish revealed that on exposure to lead for 21 days resulted in increased antioxidant enzyme activity.[17] These results were against the results withdrawn from our study possibly due to less exposure time which in case of our study was 8 weeks.

CONCLUSION

It was concluded from our study that increase in MDA leads to decrease in SOD indicating strong negative correlation in lead poisoned mice.

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