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Elevated Blood Lead Results in oxidative stress and Alters the Antioxidants of Silver Jewellery Labors

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ABSTRACT

This study is designed to find out the present status of PbB level and its adverse effect on oxidative stress and antioxidant of Silver Jewellery Workers (SJW). Forty-two SJW of having an age range of 20-45 years were included for this study and compared with 50 healthy male subjects of the same age-matched. All biochemical investigations were determined by using standard methods. In this study, we found significantly increased PbB level (325%), serum lipid peroxide (191%) and significantly decreased antioxidant enzymes like superoxide dismutase (-37.83%), Catalase (-27.64%), Ceruloplasmin (-10.2%), Nitric oxide (-36.54%) in silver jewellery workers as compared to control subjects. PbB level positively correlated with lipid peroxide (0.46) and negatively correlated with superoxide dismutase (-0.31), Catalase (-0.30), Ceruloplasmin (-0.20) and Nitric oxide (-0.28) were observed in study subjects. Therefore we have planned this study to know the oxidative stress and antioxidant status of silver jewellery workers and we found that blood lead level results in oxidative stress and alters antioxidant enzymes of silver jewellery workers and to reduce this oxidative stress it is indispensable to decrease PbB level of silver jewellery workers by taking vitamins, minerals and using activated carbon fabric mask which can prevent the health hazards of lead.

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INTRODUCTION

Lead is a heavy metal, soft, very malleable, ductile, pliable, dense, easily recycled, easy workability, excellent antifriction and inexpensive metal. Because of this, it is used in colour pigments, acid battery manufacturing, making silver jewellery ornaments, petrol additives, soldering water distribution pipes, and traditional practices such as folk remedies (ATSDR, 2005). During smelting, mining, processing, recycling it enter into the environment.

Lead is absorbed by the GIT. Increased blood lead level causes adverse effects on organ systems, including the cardiovascular, hematopoietic, reproductive, renal, immune and nervous system. Lead is mutagenic in mice have been reported in the literature (Waalkes *et al.*, 1995). The effects of Pb depend on the duration of exposure and its level.

Indian silver jewellery manufacturers have been observed to have high PbB levels. They were engaged in silver purifying by smelting and mining lead.

Lead results in the oxidative stress and changes the concentration of antioxidant enzymes. For this reason, we have designed this study to see the PbB of silver jewellery workers and its effects on their health.

MATERIALS AND METHODS

For this project, forty-two male silver jewellery workers from Hupri, District Kolhapur located in Western Maharashtra and fifty healthy male subjects who were not - occupationally exposed to lead were taken.

Silver jewellery workers and control subjects were included having age between 20-45 years; Subjects taking medicine for any major disorders were excluded. Socio-economic status of both groups was same. The occupational, demographic and clinical data were included by asking questions. All the subjects were informed about the health hazards of lead exposure before taking informed consent. Institutional ethical approval was taken from the institutional ethical committee.

7 ml blood was collected in EDTA and heparin tube for analysing biochemical investigations. By using standard methods all the biochemical investigations were estimated.

Blood lead levels were quantified by using blood lead analyzer (lead care II) and is based upon an Anodic Stripping Voltammetry (ASV) to quantify the lead levels from the blood. Lead care treatment reagent was used to lyses the red blood cells and to release the lead from RBC cell wall. Lead particles accumulate on the test electrode to which a negative potential was applied. The potential was quickly reversed which releases the lead particles. The current formed was directly proportional to the quantity of lead particles in the blood (Ghanwat *et al.*, 2016).

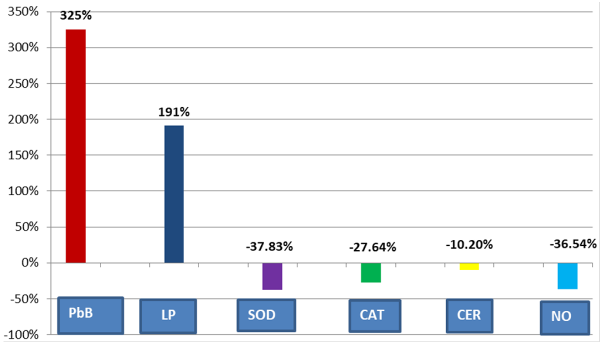
Kei Satoh method was used to measure serum lipid peroxide (Kei, 1978). Superoxide Dismutase (SOD) activity was measured by Marklund and Marklund (1988) method.

Catalase was determined by the method of Aebi 1983 (Aebi, 1974). Plasma ceruloplasmin was determined by the method of Herbert (Ravin, 1961). Nitric oxide was estimated by the cadmium-reduction method (Cortas and Wakid, 1990).

Student's t-test was used for statistical analysis using Instat GraphPad software.

RESULTS AND DISCUSSION

Blood lead level and lipid peroxide significantly increased while SOD, catalase, ceruloplasmin and nitric oxide significantly decreased in SJW as compared to controls.



Blood lead (Pb), Lipid peroxide (LP), Superoxide Dismutase (SOD), Catalase (CAT), Ceruloplasmin (CERU), Nitric oxide (NO)

Figure 1: Percentage Change of biochemical Parameters of Silver Jewellery Workers with Respect to Control

The correlation coefficient (r) was calculated to see the correlation of blood lead with biochemical investigations of silver jewellery workers.

Blood lead levels of silver jewellery workers were significantly increased (325 %, $p<0.001$,) when compared with controls (Table 1, Figure 1), which indicate the lead absorption was more in silver jewellery workers. Such increased levels have also reported in other studies (Patil *et al.*, 2006b).

In the silver jewellery industry, lead is mainly used for making silver rings, an ornament usually worn around ankles/toes. Lead melts at a high temperature which is then poured in 3 feet mould thereby making lead wires. These wires are then stretched to 15 feet by machine and coated with silver plating. When re-heated, lead melts leaving behind silver wires which are made into rings. The molten lead is then reused. The lead smelting process releases fumes/vapours which are inhaled by workers and their family members. Most of this work are done in their household without adequate protective gears.

The silver jewellery manufacturing is mainly carried out at Hupari, Western Maharashtra (Dist. Kolhapur, India) and silver jewellery workers are smelting old silver ornaments and manufacturing the new silver jewellery at high temperatures in congested place without an adequate exhaust system. The workers involved in the production barely use any proper protective gears, such as a mask or special aprons. In addition, such works are conducted in their home, so the family members are also exposed to lead. Silver jewellery workers are directly exposed to lead oxide dust and lead fumes, which results in increased lead absorption by ingestion, inhalation and direct skin contact and affects all the organs and systems. The clinical symptoms like reduced appetite, irregular abdominal pain, muscle pain, diarrhea, nausea and constipation were

Table 1: Biochemical investigations of Silver Jewellery Workers and Control Group

Sr. No.	Biochemical Investigation	Controls (n =50)	Silver Jewellery Workers (n =42)
A	Blood Lead(μg/dl)	5.46 $\pm$ 2.58 (3.3 – 11.9)	23.23 $\pm$5.91*** (11.9 - 37)
B. **Oxidative Stress**			
1.	Serum Lipid peroxide (nmol/ml)	0.89 $\pm$0.79 (0.5 - 1.7)	2.59 $\pm$0.62*** (1.5 - 4.1)
C. **Antioxidants Status**			
1.	Superoxide Dismutase (units/ml of hemolysate)	12.9 $\pm$3.3 (6.9 – 20.8)	8.02 $\pm$3.01*** (3.6-14.5)
2.	Catalase (mM H2O2 decom/mg Hb/min)	23.3 $\pm$ 5.22 (12.6-29.8)	16.86$\pm$8.96*** (4.2-33.8)
3.	Ceruloplasmin (mg/dl)	32.25 $\pm$ 6.53 (18.5-43)	28.96 $\pm$ 5.89* (14.1 - 43)
4.	Nitric oxide (μmol/lit)	37.03 $\pm$10.18 (17-64.6)	23.5 $\pm$7.01*** (13 - 38)

Figures mention Mean $\pm$ SD values and the range of values shown in parenthesis.
Significancelevels - *** P< 0.001, ** P< 0.01, * P< 0.05.

Table 2: Correlation coefficient (r) value between blood lead and biochemical parameters of silver jewellery workers

Sr. No.	Biochemical Parameters	Correlation Coefficient (r)	P-Value
A. **Oxidant**			
1.	LP (nmol/ml)	0.46	P< 0.001
B. **Antioxidants**			
2.	Superoxide Dismutase (units/ml of hemolysate)	-0.31	P< 0.001
3.	Catalase (mM H2O2 decom/mg Hb/min)	-0.30	P< 0.001
4.	Ceruloplasmin (mg/dl)	-0.20	P< 0.05
5.	Nitric oxide (μmol/lit)	-0.28	P< 0.01

reported by the majority of silver jewellery workers because of elevated blood lead level. The poor hygiene, insufficient ventilation and lack of suitable protection lead to increased blood lead level in silver jewellery workers.

We observed significantly elevated serum lipid peroxide (P<0.001, 191%) in silver jewellery labours when compared with controls, which shows more generation of free radicals (Patil *et al.*, 2006a). Lead increases the generation of reactive oxygen species by the following reasons.

1. In red blood cell lead interact with oxyhemoglobin and generate O2- formation.

2. An elevated blood lead level inhibits the heme biosynthesis and increases the δ-ALA, which may undergo enolization and autoxidation at pH 7.0–8.0, results in more generation of superoxide anions (Al-Ubaidy *et al.*, 2006).

3. δ -ALA/oxy-haemoglobin-coupled oxidation results in the generation of ROS (Monteiro *et al.*, 1989).

4. Lead get accumulated in RBC's (since it having high affinity) and changes the structure and functions of RBC membrane, enzyme activity and proteins composition, which increases the osmotic and mechanical susceptibility of RBCs, finally leads to more oxidative damage (Waldron, 1966).

Therefore, increased blood lead levels may induce the oxidative stress in silver jewellery workers and such findings are also reported in the earlier study (Patil *et al.*, 2006b). However, we are unable to clarify the exact reason for the increased oxidative stress in this study. It might be due to more accumulation of lead and ALA in RBC. Also in an earlier study the interaction of heavy metals with oxyhemoglobin considered as an important source of superoxide formation in RBCs (Carrell *et al.*, 1975).

The antioxidant parameters like erythrocytes-super oxide dismutase (P< 0.001, -37.83%), erythrocytes - catalase (P< 0.001, -27.64%), plasma ceruloplasmin (P< 0.05, -10.2%), serum nitric oxide (P< 0.001, -36.54%) were reduced in silver jewellery workers as when compared with controls, which shows the generation of ROS.

Erythrocyte- SOD activity was significantly reduced in silver jewellery workers when compared with control, which is because of more superoxide radical generation (ATSDR, 2005) or decreased serum copper level. In earlier studies, it was reported that lead reduces the absorption of copper across the gastrointestinal tract since lead is divalent and due to copper deficiency, the RBC-SOD activity decreases because this enzyme is a Zn-Cu-containing enzyme. Therefore the exact reason for the decrease in the RBC- SOD remains unexplained; it may be due to increased superoxide formation or decreased serum copper level. However, the decreased erythrocyte-SOD may be due to scavenging superoxide radicals and these results are consistent with the earlier study (Kshirsagar *et al.*, 2020).

The decreased RBC- catalase activity may be due to more generation of H_2O_2 free radicals or decreased heme level, resulting in reduced heme synthesis and heme pool. It indicates that lead is responsible for the reduction in erythrocytes-catalase activity since it is a heme containing enzyme. Decrease in superoxide dismutase, catalase and ceruloplasmin also shown by other study (Kshirsagar *et al.*, 2020).

A serum nitric oxide was significantly reduced in silver jewellery workers as compared to control, because of more production of H_2O_2 and O_2^-. Nitric oxide protects against cell death caused by H_2O_2, alkyl hydroperoxides and xanthine oxidase since it acts as an antioxidants. Nitric oxide interacts with thiol groups also it decomposes the OH- free radicals.

Therefore, reduced serum nitric oxide levels in the study group, might be due to more generation of hydrogen peroxide, O2- and OH- free radicals. However, the decreased serum nitric oxide levels may be due to scavenging the H_2O_2, O_2- and OH- free radicals and our finding are supported by an earlier study (Vaziri and Sica, 2004).

Ceruloplasmin is an important extracellular antioxidant. During the conversion of Fe++ to Fe+++ simultaneous production of H_2O from H_2O_2 take place We observed decrease in ceruloplasmin levels and this may be because of replacement of iron by lead and it also scavenges superoxide anion radical (• O_2−).

We found that blood lead was positively correlated with serum lipid peroxide and negatively correlated with other antioxidants (Table 2). These results clearly indicate that elevated PbB creates the oxidative stress in almost 46% of lead-exposed silver jewellery workers and which may deplete 20% to 31% antioxidant status. In silver jewellery workers, more free radicals were generated due to long duration of lead exposure and to scavenge these free radicals antioxidants are used. An earlier study also reported that a high duration of lead exposure increases lipid peroxide (Sanders *et al.*, 2009).

From the past reports and present results, it is tempting to speculate that the increased blood lead levels in silver jewellery workers, may interrupt the balance between oxidative stress and antioxidants. Increased PbB induces the oxidative stress and decreases antioxidants enzymes or alters the components of antioxidants enzymes, which may induce oxidative stress. Therefore, the adverse effects of lead can be prohibited by the supplementation of natural antioxidants like vitamin E, C, multivitamins, and multi-minerals. Taking precautionary measures like use of activated carbon fabric (ACF)mask to decrease the lead exposure, which can prevent the health hazards of lead.

CONCLUSION

Increased blood lead level results in oxidative stress and alters antioxidant enzymes of silver jewellery workers and to reduce the oxidative stress it is indispensable to decrease PbB level of silver jewellery workers by taking vitamins, minerals and using activated carbon fabric mask which can prevent the health hazards of lead.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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