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INTER RELATIONSHIP BETWEEN BLOOD LEAD CONCENTRATION AND OXIDATIVE CARDIAC RISK PARAMETERS IN ADULTS: EFFECT OF ASCORBIC ACID

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ABSTRACT
It is well known that chronic lead exposure leads to various health hazards. Along with lead, it has also been seen that homocysteine, pyridoxine, cobalamine and folate are linked to cardiovascular complications, oxidative stress and a lot of other lifestyle disorders. In the present study, the relationship between these various parameters and chronic lead exposure was evaluated in lead smelting industry and jewelry industry workers, with an more than 10 years of working experience. Ascorbic acid, a known antioxidant, was administered in controlled doses over a specific period of time to study its effects on the said parameters. **Method:** Samples were analysed for homocysteine using Fluoroscence Polarisation Immunoassay, Vitamin B12 and Folate using ECLIA method, and malondialdehyde and RBC Superoxide dismutase were assayed with kits suppled by Randox Lanoratories. Blood Lead levels were measured by atomic absorption spectrophotometry. **Results:** Results showed a significant decrease in all parameters except malondialdehyde in chronic lead exposed workers. On administration of a controlled dose of ascorbic acid, over a specified period of time, most parameters were seen to be statistically significantly altered towards a healthier side. **Conclusion:** A significant positive association was detected between lead exposure and oxidative stress with cardiovascular effects, which was seen to be improving upon administration of Ascorbic acid over a specific period.

KEYWORDS: Lead, Homocysteine, SOD, Vitamin B12, Ascorbic Acid.

INTRODUCTION
Exposure of lead (Pb) is linked to numerous diseases and negative health outcomes throughout the lifespan. In late life, lead exposure, even at low levels experienced in the community, is related to Cardiovascular Disease (CVD), as measured through hypertension[1], heart rate variability[2], and clinical CVD outcomes.[3]

Studies have also indicated cardiovascular mortality due to Pb.[4] Since Homocysteine (Hcy) is also found to be associated with the risk of CVD, an association between Pb and Hcy in the development of CVD has also been considered a possibility.[4]

Homocysteine (Hcy) is a thiol-containing amino acid that is highly reactive and thus short lived in the body.[5] Though physiologically normal cellular processes produce and require Hcy at low levels, elevated Hcy is associated with toxicity. Accessible cysteinyl residues in cellular proteins can react with free Hcy, forming Hcy-protein thiol-thiol interactions, altering native protein conformation and function. In addition, free Hcy can cleave accessible disulfide bridges, damaging native protein confirmations.[6] Biochemical damage is based on the duration and concentration of exposure to Hcy. Long-lived proteins can accumulate irreversible Hcy-related damages, making these mechanisms especially relevant to the chronic diseases and morbidity of aging.

Elevated Hcy is a risk factor for both CVD and neurodegeneration. In epidemiologic studies, moderately elevated Hcy is associated with CVD. In a meta-analysis of 30 retrospective or prospective studies, reduced plasma Hcy was protective against ischemic heart disease (IHD) (OR=0.89 for 25% lower Hcy), and stroke (OR=0.81).[7] Similarly, a 5-µmol/L increase in Hcy corresponded to elevated odds of IHD (OR=1.23) and stroke (OR=1.42) in a meta-analysis of 20 prospective studies.[8] The suggested mechanisms linking Hcy and cardiovascular outcomes include impaired endothelial elasticity and the production of reactive oxygen species.[9]

The association reported between Pb and Hcy is plausible and supported by compelling data, but it requires further epidemiologic investigation in an additional population. Thus far, only a couple of studies have tested the Pb-Hcy relationship in a community-exposed population of older adults.[10,11] There are currently no studies evaluating the relevant window of Pb exposure's effect on Hcy. Studies of cumulative Pb exposure and Hcy are needed to fill this gap.

Together with Hcy, lipid peroxidation product, namely, Malondialdehyde (MDA), is also an indicator of the oxidative insult in Pb exposed workers. Pb intoxication is often accompanied with severe oxidative damage as reflected in the RBC antioxidant levels, namely Superoxide dismutase, and canbe manipulated by prescribing antioxidant vitamins, like Ascorbic Acid.[12]

In addition, there are no data indicating whether the association of Pb exposure with Hcy can be mitigated (or worsened) with intake of antioxidant vitamins.

Vitamin B12, or Cobalamine, is a cofactor in Methylmalonyl CoA mutase enzyme in the mitochondria, which conducts oxidative degradation of a number of amino acids.[13] This plays a key role in proper cellular metabolism. Folate is also a water soluble vitamin which helps in conversion of Hcys to Methionine, and any impairment in folate metabolism might affect nucleotide polymorphism and cardiovascular diseases.[14]

Studies have shown that both Folate and Vitamin B12 levels are significantly altered in chronic Pb poisoning.[15]

Keeping in mind the lack of data regarding such intervention, the present study has endeavoured to reflect on the effect of Vitamin C on the Pb exposed workers with increased CVD risks.

Objectives

1. To study the effect of lead on the metabolism of lipid peroxidation.
2. To examine the effects of dietary supplementation with ascorbic acid on blood lead concentration and associated cardiovascular parameters.

MATERIAL AND METHODS

Selection of subjects were done on lead smelting industry workers and jewellery workers with cardiovascular involvements. Age and socioeconomically matched controls were tested for similar parameters from same geographical areas.

The study population consisted of 85 workers from a lead smelting factory and a jewelry making factory in Kolkata, India, who have over 10 years of experience working in the same industry, and 60 office staff from same factory were recruited as a control group in this study. Signed consent was obtained from each participants before the samples were taken for subsequent analysis.

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the Institutional research committee.

Whole heparinised blood lead level was analysed by atomic absorption spectrophotometer (Perkin-Elmer, 2380) by complexation of the lead with 2% ammonium pyrrolidine dithiocarbamate and extraction into methyl isobutyl ketone. Constituents of the organic phase were measured at a 283.3 nm wavelength with background correction and calibration by standard addition.

Superoxide dismutase (SOD) was measured in whole heparinised blood washed 3 times in 0.9% NaCl solution to obtain haemolysate. Working standards were prepared using the standard supplied with the kit (Randox Laboratores Ltd., UK), following dilution instructions to produce a standard curve. Samples are pipetted into the respective cuvettes and 2 readings are taken at respective intervals. Value is calculated by dividing standard absorbance rate by respective subject's haemoglobin value in gm/dl.

Malondialdehyde (MDA) was assayed as a marker of lipid peroxidation using a colorimetric reaction which uses 1-methyl-2-phenylindole as chromogen, with kit supplied by Randox Laboratories Ltd., U.K., along with appropriate control.

Homocysteine was measured in plasma by fluorescence polarisation immunoassay. Dithiothreitol (DTT) reduces homocysteine bound to albumin and to other small molecules, homocysteine, and mixed disulfides, to free thiol. S-adenosyl-homocysteine (SAH) hydrolase catalyzes conversion of homocysteine to SAH in the presence of added adenosine. The specific monoclonal antibody and the fluoresceinated SAH analogue tracer constitute the FPIA detection system. Plasma total homocysteine concentrations are calculated by using a machine-stored calibration curve.

Vitamin B12 was measured in serum sample, by electrochemilumenescence immunoassay (ECLIA) method, using Cobas B12 assay kit for Roche diagnostics.

Folate was estimated by electrochemilumenescence immunoassay (ECLIA) method, in haemolysate obtained by treating whole blood with anticoagulants and ascorbic acid and incubated to liberate and release intracellular Folate. The assay was performed by Cobas Folate assay kit for Roche diagnostics.

Statistical significance was assessed using student's t-test. Results were deemed statistically significant where

$p < 0.005$. Also, statistical analysis was conducted using SAS software.

RESULT

85 adult male lead smelting industry workers and jewellery industry workers, aged between 25-55 years, from similar socio economic backgrounds, were selected for the study. Of them 52 were selected on the basis of clinical features of CVD, such as elevated blood pressure, arrhythmia, and imaging techniques. 60 age and sex matched controls were similarly chosen from similar socioeconomic background.

The subjects were then given a regular dose of Vitamin C (ascorbic acid) 500 mg twice daily, for 1 month (30 days), and followed up.

The cardiovascular parameters were measured for the normal population (control), the test population before administration of Vitamin C and after a month of continuous follow up with Vit C administration. The results are computed in Table 1.

Table 1: CVD parameters for controls, cases before Vitamin C and after Vitamin C Supplimentation.

Parameters	Controls (n= 60)	Cases Before Vitamin C Supplementation (n= 52)	Cases After Vitamin C Supplementation (n= 52)
Blood Pb (mg/dl)	15± 4	72±12	54±15
MDA (nmol/ml)	1.08±0.2	3.9±0.4	1.8±0.3
RBC SOD (unit/gm Hb)	1088±90	972±138	1005±82
Folate (ng/ml)	9.89 ± 2.9	6.46 ± 1.2	6.91 ± 1.6
Vit B12 (pg/ml)	384± 31	312 ± 26	321 ± 21
Homocysteine (µmol/l)	11.4±1.9	16.3 ± 2.5	12.6 ± 1.9

Values are represented as Mean ± SD

Table 2 shows the statistical significance between the various parameters between pre and post Vit C administration. The studies are done by Student's t test and $p<0.005$ is considered statistically significant. The calculations are done by using SPS software version 10.

Table 2: Statistical significance between various parameters before and after supplimentation of Vitamin C to lead smelting and jewellery industry workers.

Parameters	Before Vit C	After Vit C	p Value
Blood Pb (mg/dl)	72±12	54±15	<0.0001
MDA (nmol/ml)	3.9±0.4	1.8±0.3	<0.0001
RBC SOD (unit/gm Hb)	972±138	1005±82	<0.0001
Folate (ng/ml)	6.46 ± 1.2	6.91 ± 1.6	0.10
Vit B12 (pg/ml)	312 ± 26	321 ± 21	0.05
Homocysteine (µmol/l)	16.3 ± 2.5	12.6 ± 1.9	<0.0001

Values are represented as Mean ± SD

DISCUSSION

Lipid peroxidation is one of the most important free radical mediated biological processes, and involves both oxygen and carbon cantered free radicals.[2] Blood levels of malondialdehyde, a product of lipid peroxidation (TBARS), were strongly correlated with blood lead concentration in exposed workers with lead concentration higher than 35 µg/dl.[16] In these study the TBARS was increased significantly in comparison to the control group. The data suggest lead may have a role in oxidant/antioxidant system in the body producing lipid peroxidation at the cellular level. Increased TBARS seems to be due to lipid peroxidation of bio membranes. Both the jewelry workers and the lead smelting factory workers were given a test dose of natural antioxidant, 500mg of Vitamin C twice daily for 30 days. There is a growing literature providing evidence that oxidative damage such as lipid peroxidation, protein oxidation and DNA damage play a role in most health problems including cardiovascular disease, cancer and ageing and that antioxidants have critical role in weakness, health maintenance and prevention of degenerative disease.[17,18,19]

The in vitro observation of the protective effects of ascorbic acid against ROS mediated oxidative damage have been substantiated by the in vivo results obtained with guinea pigs The result maybe relevant to human nutrition, because guinea pigs, like humans, are also incapable of synthesizing ascorbic acid. Thus, ascorbic acid is considered to be the most important antioxidant in aqueous phase, such as plasma, cytosol, and other fluids. It efficiently neutralises free radicals like superoxides, hydrogen peroxide, hypochlorites, and hydroxyl and peroxy radicals. The ascorbic acid treated jewellery workers and lead smelting factory workers showed a slight recovery in SOD level probably due to increased

availability of cofactors, such as, copper and zinc, during supplementation.

Lead and homocysteine are considered to have synergistic effect on blood pressure. Mechanisms have been postulated to explain relations between homocysteine and blood pressure which include homocysteine induced arteriolar constriction, renal dysfunction and increased sodium absorption and increased arterial stiffness.[20] Though one cannot conclude whether lead elevates homocysteine or vice versa, it is clear that a relation exists especially for the smelting factory workers. Previous studies have demonstrated a relationship between Pb and Hcy at single time points. In the Baltimore Memory Study, blood Pb and plasma Hcy were significantly correlated with each other (Pearson's unadjusted r=0.27).[8] It was reported that an increase of 1 µg/dL of blood lead level was associated with an increase of 0.35 µmol/L of homocysteine.

There was seen to be significant association between plasma B12 and Folate levels and lead levels in the study population. Mean Hcy concentration was found significantly increased in the exposed workers as compared to the normal population, maybe because Hcy structure is similar to dimercaptosuccinic acid and penicillamine which are known for their ability to chelate lead.

And though Serum Folate deficiency appears to have a major effect on Hcy levels, the values did not change significantly upon Vitamin C supplementation. Further studies are required to establish correlation between the serum B vitamin parameters, Hcys and Vitamin antioxidant supplementation.

CONCLUSION

Considering all aspects, it probably seems that normal defensive mechanism against free radical toxicity was almost fully inhibited by lead toxicity. The observed alterations of the oxidant defence systems suggests that supplementation with antioxidants is a potential therapy in the treatment of jewellery workers and smelting factory workers intoxicated with lead. However, standard dietary recommendation for healthier lifestyle, like consumption of fresh fruits etc, may have the added potential benefits of increased antioxidant intake and helping to protect metabolic functions. In chronic conditions where the plasma lead level remains high for a considerable period of time, a short term antioxidant therapy of antioxidants as used by us probably will not have much beneficial role. A further probe is necessary regarding the role of different combinations of antioxidants and diet therapy on chronically lead exposed individuals.

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Conflict of Interest

The authors declare no conflict of interest in the present study.

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