

Impact of Chronic Lead and Cadmium Exposure on Redox Status in Industrial Workers: Evidence from a Case-Control Study

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Abstract

Background: Chronic occupational exposure to heavy metals — particularly lead and cadmium — is a recognised public health hazard in industrialising countries such as India. These metals generate reactive oxygen species (ROS) by Fenton-type catalysis, displacement of essential metal cofactors, and depletion of thiol-based antioxidants, thereby producing systemic oxidative stress and progressive depletion of the endogenous antioxidant defence network. **Aim:** The present case-control study aimed to evaluate the activity of erythrocyte antioxidant enzymes and the levels of selected oxidative damage markers in workers chronically exposed to lead and cadmium in a battery and lead-acid recycling industry, and to compare these with non-exposed controls drawn from the same socio-demographic milieu. **Materials and Methods:** A hospital-based case-control study was conducted in the Department of Biochemistry, South India, between July 2016 and March 2017. Seventy (n=70) male battery industry workers chronically exposed to lead and cadmium and 70 age-matched non-exposed male office workers were recruited. Blood lead, blood cadmium, urinary delta-aminolevulinic acid (δ -ALA), erythrocyte SOD, catalase, glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH), total antioxidant capacity (TAC), serum MDA, protein carbonyls, and 8-OHdG were measured using validated atomic absorption spectrophotometric, kinetic spectrophotometric, and ELISA methods. Independent samples t-test, Pearson correlation, and multivariate regression were used. **Results:** Exposed workers showed significantly elevated blood lead (28.4 vs 4.6 μ g/dL), cadmium (3.84 vs 0.84 μ g/L), and oxidative damage markers (all $p < 0.001$), accompanied by significantly reduced antioxidant enzyme activities and TAC (all $p < 0.001$). Blood lead correlated negatively with SOD ($r = -0.612$, $p < 0.001$) and positively with MDA ($r = 0.586$, $p < 0.001$). **Conclusion:** Chronic occupational lead and cadmium exposure produces a profound depletion of erythrocyte antioxidant

enzymes coupled with extensive lipid, protein, and DNA oxidative damage. Routine biological monitoring of redox indices is recommended for early detection and risk mitigation in exposed Indian workers.

Keywords

Environmental toxin; Lead exposure; Cadmium; Antioxidant enzymes; Oxidative stress; Occupational health; Glutathione.

1. Introduction

Heavy metal pollution has emerged as one of the most pressing environmental and occupational health challenges of the twenty-first century. Among the heavy metals, lead and cadmium are particularly relevant in low- and middle-income countries owing to extensive use in battery manufacture, automotive recycling, paint, electroplating, electronic waste, and informal smelting industries [1,2]. The unregulated nature of much of this work in India produces significant occupational and environmental exposure, with measurable elevations of biomarkers in workers, family contacts, and surrounding communities [3,4].

Lead exerts its toxicity primarily through three mechanisms: (i) inhibition of enzymes containing sulphhydryl (–SH) groups, including delta-aminolevulinic acid dehydratase (δ -ALAD) of the haem biosynthetic pathway; (ii) displacement of essential divalent cations such as calcium, iron, and zinc from metalloenzymes; and (iii) Fenton-type generation of reactive oxygen species [5,6]. Cadmium, although not directly redox-active, depletes glutathione, displaces zinc from metallothionein, and impairs mitochondrial function, leading to indirect oxidative stress [7,8].

The cumulative consequence of chronic exposure to these metals is a sustained increase in ROS production coupled with progressive depletion of enzymatic and non-enzymatic antioxidants. The principal enzymatic antioxidants — superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase (GR) — collectively neutralise superoxide, hydrogen peroxide, and lipid peroxides, while reduced glutathione (GSH) serves both as a thiol scavenger and as a cofactor for GPx [9,10]. Sustained oxidative load damages cellular lipids, proteins, and DNA, manifesting as elevated malondialdehyde (MDA), protein carbonyls, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) [11].

Although several international studies have characterised oxidative stress in lead- and cadmium-exposed workers, integrated biochemical data from the Indian informal industrial sector remain limited. Workers in unorganised battery, smelting, and recycling units are often outside the purview of formal occupational health monitoring and represent a vulnerable population. The present study was undertaken to characterise the antioxidant enzyme response and oxidative damage profile in such workers from a South Indian industrial belt.

2. Materials and Methods

2.1 Study Setting

This case-control study was conducted in the Department of Biochemistry, South India, between July 2016 and March 2017.

2.2 Participants

Seventy (n=70) male workers aged 25–55 years employed in lead-acid battery manufacturing and recycling units in the Varanasi industrial belt for at least three years were recruited as cases (the 'exposed' group). Seventy male office workers (clerks, accountants, administrative staff) of comparable age, BMI, and socioeconomic status, with no history of occupational exposure to heavy metals, were recruited as controls. Inclusion criteria comprised male sex, age 25–55 years, and willingness to provide informed consent. Exclusion criteria comprised current smoking, alcohol use, diagnosed chronic disease (diabetes, hypertension, cardiovascular, hepatic, renal, or pulmonary disease), regular medication use including antioxidant supplements, occupational radiation exposure, and family history of haemoglobinopathy.

Only male participants were enrolled to control for sex-related differences in haematological and antioxidant parameters and because the exposed cohort in the catchment industrial units was almost exclusively male. Demographic and lifestyle characteristics are summarised in Table 1.

Table 1. Demographic and Lifestyle Characteristics of Participants

Variable	Exposed (n=70)	Controls (n=70)	p-value
Age (years)	38.6 ± 8.2	37.9 ± 7.8	0.612
Male, n (%)	70 (100.0)	70 (100.0)	—
Duration of exposure (years)	8.2 ± 4.1	—	—
Daily working hours	8.6 ± 1.2	—	—
BMI (kg/m ²)	23.4 ± 2.6	23.1 ± 2.5	0.498
Smoking, n (%)	0 (0.0)	0 (0.0)	—

Alcohol use, n (%)	0 (0.0)	0 (0.0)	—
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2.3 Sample Collection

After overnight fasting, 8 mL of venous blood was collected aseptically into trace-metal-free EDTA tubes for blood lead, cadmium, and erythrocyte enzyme analyses, plain red-cap tubes for serum biochemistry and oxidative stress markers, and lithium heparin tubes for plasma glutathione. A 10 mL random morning urine sample was collected in trace-metal-free containers for delta-aminolevulinic acid (δ -ALA) estimation. Samples were centrifuged at 3000 rpm for 10 minutes; aliquots were stored at -80°C until analysis. Erythrocytes for SOD, catalase, GPx, and GR were washed three times in cold normal saline before lysis.

2.4 Biochemical Estimations

Blood lead and cadmium were quantified using a graphite furnace atomic absorption spectrophotometer (Analytik Jena ContrAA 800, Germany) following acid digestion and matrix modification, with internal quality control using SeronormTrace Elements Whole Blood standards (Sero AS, Norway). Urinary δ -ALA was estimated colorimetrically using the modified Mauzerall and Granick method [12]. Erythrocyte SOD activity was measured by the pyrogallol autoxidation method of Marklund and Marklund [13]; catalase activity by the method of Aebi [14]; GPx by the method of Rotruck et al. [15] using glutathione and t-butyl hydroperoxide; and glutathione reductase by the method of Carlberg and Mannervik [16].

Reduced glutathione was estimated using DTNB (Ellman's reagent) [17]. Total antioxidant capacity was estimated using the FRAP assay [18]. Serum MDA was estimated by the thiobarbituric acid method [19]; protein carbonyls were estimated by the DNPH method [20]; and 8-OHdG was estimated by competitive ELISA (Cell Biolabs, USA). Haemoglobin was measured on a Sysmex XN-1000 automated haematology analyser. All biochemical measurements were performed in duplicate by a single trained analyst, blinded to participant group assignment.

2.5 Statistical Analysis

Data were analysed using IBM SPSS Statistics v26.0. Continuous variables were summarised as mean $\pm$ SD. Differences between exposed and control groups were assessed using independent samples t-test. Pearson correlation coefficient was used to evaluate associations between exposure indices and antioxidant/damage markers. Multivariate linear regression with stepwise variable

selection was performed with serum MDA as the dependent variable and blood lead, blood cadmium, age, and BMI as predictors. A two-tailed p-value of less than 0.05 was considered statistically significant.

3. Results

Demographic characteristics were comparable between exposed and control groups (Table 1). Both groups were exclusively male and matched for age, BMI, and absence of confounding lifestyle factors. The mean duration of occupational exposure was 8.2 ± 4.1 years, with a mean working day of 8.6 hours.

Exposure to environmental toxins was confirmed objectively. Blood lead in exposed workers (28.4 ± 8.6 µg/dL) was almost six times higher than in controls (4.6 ± 1.8 µg/dL, $p < 0.001$) and exceeded the OSHA action threshold of 25 µg/dL in a substantial proportion. Blood cadmium was elevated more than fourfold (3.84 vs 0.84 µg/L). Urinary δ-ALA, a biological indicator of haem biosynthesis disruption by lead, was elevated more than fivefold (16.4 vs 3.2 mg/g creatinine; $p < 0.001$).

Antioxidant enzyme activities were significantly depleted in the exposed group (Table 2). Erythrocyte SOD activity declined by 36% (3.12 vs 4.86 U/mg Hb), catalase by 35%, GPx by 33%, and glutathione reductase by 32%. Reduced glutathione fell by 43%, and total antioxidant capacity by 42%, indicating a substantial cumulative deficit in both enzymatic and non-enzymatic arms of the antioxidant defence.

Table 2. Antioxidant Enzyme Activities and Total Antioxidant Capacity

Antioxidant Enzyme / Marker	Exposed (Mean ± SD)	Controls (Mean ± SD)	p-value
Erythrocyte SOD (U/mg Hb)	3.12 ± 0.68	4.86 ± 0.82	<0.001
Erythrocyte Catalase (U/mg Hb)	54.6 ± 12.4	84.2 ± 14.8	<0.001
GPx (U/g Hb)	32.4 ± 7.6	48.6 ± 9.2	<0.001
Glutathione Reductase (U/g Hb)	4.62 ± 1.18	6.84 ± 1.42	<0.001
Reduced Glutathione (mg/dL)	16.2 ± 4.4	28.6 ± 5.8	<0.001
Total Antioxidant Capacity (mmol/L)	0.94 ± 0.21	1.62 ± 0.28	<0.001

Oxidative damage markers were correspondingly elevated (Table 3). Serum MDA increased more than twofold (4.62 vs 2.18 nmol/mL), protein carbonyls more than twofold (3.18 vs 1.46 nmol/mg), and 8-OHdG more than twofold (19.4 vs 8.2 ng/mL), confirming widespread lipid, protein, and DNA oxidative injury. Haemoglobin was significantly lower in the exposed group (12.4 vs 14.2 g/dL), suggesting biologically meaningful haematotoxicity from chronic lead exposure.

Table 3. Toxin Exposure Indices, Haematological, and Oxidative Damage Markers

Toxin / Damage Marker	Exposed (Mean $\pm$ SD)	Controls (Mean $\pm$ SD)	p-value
Blood Lead ($\mu\text{g/dL}$)	28.4 $\pm$ 8.6	4.6 $\pm$ 1.8	<0.001
Blood Cadmium ($\mu\text{g/L}$)	3.84 $\pm$ 1.42	0.84 $\pm$ 0.32	<0.001
Urinary δ -ALA (mg/g creatinine)	16.4 $\pm$ 4.6	3.2 $\pm$ 1.1	<0.001
Serum Malondialdehyde (nmol/mL)	4.62 $\pm$ 0.96	2.18 $\pm$ 0.54	<0.001
Protein Carbonyls (nmol/mg)	3.18 $\pm$ 0.84	1.46 $\pm$ 0.42	<0.001
8-OHdG (ng/mL)	19.4 $\pm$ 4.8	8.2 $\pm$ 2.1	<0.001
Hb (g/dL)	12.4 $\pm$ 1.4	14.2 $\pm$ 1.2	<0.001

Pearson correlation revealed strong negative associations of blood lead with SOD ($r=-0.612$, $p<0.001$), catalase ($r=-0.564$, $p<0.001$), GSH ($r=-0.498$, $p<0.001$), and TAC ($r=-0.534$, $p<0.001$), and strong positive associations with MDA ($r=0.586$, $p<0.001$), 8-OHdG ($r=0.512$, $p<0.001$), and protein carbonyls ($r=0.498$, $p<0.001$). Blood cadmium showed similar but weaker correlations. Multivariate regression confirmed that blood lead was an independent predictor of MDA ($\beta=0.482$, $p<0.001$) after adjusting for age, BMI, and cadmium.

4. Discussion

The principal observation of this study is that workers chronically exposed to lead and cadmium in unorganised North Indian battery manufacturing and recycling units exhibit a profound and biologically coherent oxidative stress profile, characterised by depletion of antioxidant enzymes and accumulation of lipid, protein, and DNA damage markers. The strong correlations between exposure indices and oxidative biomarkers reinforce a dose-response relationship and lend mechanistic plausibility to the observed associations.

Mean blood lead in our exposed cohort (28.4 µg/dL) substantially exceeded the Centers for Disease Control reference value of 5 µg/dL [21] and the Occupational Safety and Health Administration action level of 25 µg/dL [22]. Such elevations are consistent with previous Indian biomonitoring studies of battery and smelter workers reported by Patil et al. [23], Ahamed et al. [24], and Mishra et al. [25], all of whom documented mean blood lead between 25 and 40 µg/dL in similar workplaces. The persistence of these elevated levels nearly two decades after the introduction of unleaded petrol underscores the public health failure of unregulated occupational exposure.

The 35–45% reduction in erythrocyte antioxidant enzyme activities observed in the present series mirrors the findings of Hsu and Guo [9], Kasperczyk et al. [26], and Patil et al. [23]. Several mechanisms underlie the depletion. Lead binds to sulphhydryl groups in catalase and GPx, causing direct conformational inactivation. Glutathione reductase, dependent on functional NADPH and –SH integrity, is similarly inhibited. The fall in reduced glutathione reflects both consumption during ROS scavenging and impaired regeneration secondary to GR inhibition. SOD, although not a thiol-dependent enzyme, may decline through indirect mechanisms including impaired transcription, reduced copper/zinc availability, and substrate-induced inactivation under sustained superoxide load [27].

Cadmium, although less abundant in our cohort than lead, exerts complementary oxidative toxicity. By displacing zinc from metallothionein and competing with calcium and iron at metalloenzyme sites, cadmium depletes thiol antioxidants and disrupts mitochondrial membrane potential, generating additional ROS [7]. The combined exposure to both metals therefore produces a synergistic oxidative challenge greater than either metal alone, as documented in animal models by Wang et al. [28].

The accumulation of MDA, protein carbonyls, and 8-OHdG demonstrates that the depletion of antioxidant defences is not merely biochemical but translates into measurable cellular damage. Elevated 8-OHdG, in particular, indicates oxidative attack on guanine residues of DNA, which has been linked to the increased incidence of cancer and accelerated cellular ageing in long-term exposed workers [29]. The accompanying mild anaemia observed in our exposed cohort is attributable to lead-induced inhibition of δ -ALAD and ferrochelatase, two essential enzymes of haem biosynthesis, and to oxidative damage of the erythrocyte membrane and intracellular redox milieu [30].

These findings carry urgent implications for occupational health policy in India. The substantial blood lead burden documented here, despite years of formal regulation, suggests that compliance and surveillance in informal industrial sectors remain inadequate. Periodic biological monitoring should incorporate not only blood lead but also at least one antioxidant enzyme (preferably SOD or GSH) and one oxidative damage marker (preferably MDA or 8-OHdG), as these provide a more sensitive integrative readout of internal oxidative load. Workplace controls — engineering ventilation, personal protective equipment, dietary supplementation with vitamin C, vitamin E, and zinc, and access to chelation therapy when clinically indicated — should be reinforced [31].

The strengths of the present study include its case-control design, sex-matched cohort, comprehensive biomarker panel covering exposure, antioxidant defence, and damage indicators, and the use of NABL-accredited methodology. Limitations include the cross-sectional design that precludes causal inference, the focus on male workers only (necessary to control for hormonal influences but limiting generalisability), and the absence of clinical outcome data. Future longitudinal studies should integrate clinical, neurobehavioural, and renal end-points and should evaluate the effect of antioxidant supplementation on biomarker trajectories.

5. Conclusion

Chronic occupational exposure to lead and cadmium in North Indian battery manufacturing workers produces marked depletion of erythrocyte antioxidant enzymes — SOD, catalase, GPx, and glutathione reductase — together with significant accumulation of lipid, protein, and DNA oxidative damage markers. The strong correlations of antioxidant decline and damage accumulation with internal exposure indices support a causal mechanistic link. Routine inclusion of antioxidant enzyme assessment in the biological monitoring of exposed Indian workers is recommended to enable early identification of toxicity and to inform protective workplace interventions.

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