SUMMARY
Lead pollution is a global concern because of its ubiquity in the environment and more so in developing countries where few environmental regulations are adopted. Children are more vulnerable to lead exposure than adults because of their hand to mouth activity, increased respiratory rates and gastrointestinal absorption per unit body weight, and increased susceptibility of the developing brain to the neurotoxic effects of lead. After measures to control lead pollution were implemented in the U.S. beginning in 1970, blood lead levels (BLLs) in children have declined by >80%. Conversely, lead pollution remains a public health concern in developing countries such as India. To reduce environmental lead burden, government of India had introduced unleaded petrol with coverage of the entire country in year 2000. Yet, the extent and real magnitude of problem associated with environmental exposure to lead to in and around Lucknow, the capital of most populous state, Uttar Pradesh in India have neither been duly emphasized nor systematically studied to generate a base-line data. In Lucknow, leaded petrol (0.56 g/L) was used as vehicular fuel in 1994. There was a drastic reduction in mean lead concentration in the air from 1.6 µg/m³ in 1994 to 0.2 µg/m³ in 2002, reflecting the impact of unleaded petrol in reducing the atmospheric lead concentrations of Lucknow. Although motor vehicle emissions from the consumption of leaded petrol has been the chief source of wide spread environmental lead pollution, other sources such as leaded pipes for water supply, lead based paints, use of leaded ceramics & canned food, and lead in cosmetics & folk remedies are still good sources of lead exposure, in addition
to lead smelters and industrial processes following the phasing out of leaded petrol.

Experimental and human data indicate that there are persistent and deleterious effects of low level lead exposure on brain function, lowered intelligence, behavioral problem and diminished school performance. In 1991 the U.S. Centre for Disease Control and Prevention (CDC) established 10 μg/dL blood lead set as the intervention level for children. A growing body of evidence, however, suggests that a still lower intervention level (BLL 2 μg/dL) is required. This issue is complicated by the fact that there is no demonstrated biological function of lead in human. In a limited number of studies, researchers have investigated the possible adverse effects of moderate to high level lead exposure on growth of children. However, evidence that low level lead exposure impairs growth of children remains ambiguous.

Oxidative stress appears to be a possible mode of the biochemical mechanism of lead toxicity. The depletion of glutathione and protein bound sulfhydryl groups and changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in lead-induced oxidative tissue damage. Several oxidant/antioxidant molecules such as malondialdehyde (MDA), glutathione (GSH) and glutathione disulfide (GSSG) levels, and antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities are the most commonly used parameters to evaluate lead-induced oxidative stress. The delta-aminolevulinic
Acid dehydratase (δ-ALAD) is the second enzyme in heme biosynthetic pathway and catalyzes the condensation of two molecules of delta-aminolevulinic acid (δ-ALA) to a porphobilinogen (PBG), with the thiol (SH) group essential for its activity. Due to its affinity for SH group, lead is known to inhibit δ-ALAD activity, resulting in the accumulation of δ-ALA. The latter has been shown to undergo metal catalyzed auto-oxidation and give rise to the formation of free radicals as superoxide ion (O$_2^-$), hydroxyl radical (HO$^*$) and hydrogen peroxide (H$_2$O$_2$). This possibility implies that δ-ALAD inhibition, in addition of being a biochemical indicator of lead toxicity, might also be a promising indicator of lead-induced oxidative stress.

Until now, evidence on lead-induced oxidative stress has been based mostly on in vitro experiments or animal studies. Several studies among workers with high occupational exposure to lead have reported associations between lead exposure and oxidative stress parameters. Recent epidemiological studies have reported that environmental lead exposure has a graded association with several disease outcomes such as hypertension, peripheral artery diseases, kidney diseases, neurodegenerative diseases and cognitive impairments. Although all these diseases include components of oxidative stress, the relevance of oxidative stress to lead-related diseases in general population with low exposure has been criticized because most of the mechanistic studies have been conducted at moderate to higher doses than the concentrations observed in general population.
This is first comprehensive study among children from India describing environmental exposure to lead and its association with sociodemographic characteristics, growth, neurobehavioral development, relevant biochemical indices and hematologic & neurologic disorders. In collaboration with Department of Pediatrics, King George's Medical University (KGMU) and Analytical Toxicology Division, Industrial Toxicology Research Centre (ITRC), Lucknow, the present study was designed:

- To determine blood lead levels among children.
- To evaluate the association between exposure to lead and children's health with regard to physical growth and neurobehavioral development.
- To examine the impact of blood lead on relevant biochemical parameters as a mechanism of lead toxicity.
- To examine the role of lead-induced oxidative stress in the pathophysiology of neurologic disorders among children.
- To assess the environmental lead exposure as a risk for childhood aplastic anemia.

This study is first of its kind from India that can be of wider environmental and societal importance. Our data may help regulatory agencies adopt measures resulting in reduction of exposure and thereby adverse health impact among children.

A cross-sectional study was conducted to determine BLLs and its association with sociodemographic characteristics, growth, intelligent quotient
(IQ) and relevant biochemical indices in children from general population. In order to be included in the final data analysis, children had to fulfill the following criteria (i) children of age group \( \leq 12 \) years, (ii) residing in and around Lucknow since birth and (iii) present no obvious pathology unrelated to lead.

Children suffering from mental retardation, chronic disease(s) or under any medication were excluded from the study. For final analysis 200 children (3-12 years, 147 boys and 53 girls) were included in the study. Information such as sociodemographic characteristics, growth parameters (head circumference, height, weight & BMI) and IQ score of children were recorded. Their BLLs and relevant biochemical indices including δ-ALAD, MDA, GSH, SOD, CAT and GPx were determined. Mean (SD) BLL of the present study was 9.3 μg/dL (6.3 μg/dL). This is comparable to BLLs of children from other cities of the country such as Varanasi (12.0 μg/dL), Hyderabad (13.3 μg/dL), Mumbai (8.0 μg/dL) and Amritsar (7.3 μg/dL). BLL of the present study was higher as compared to children from certain countries including Sweden (2.1 μg/dL), U.K. (3.4 μg/dL), U.S.A. (3.6 μg/dL), Taiwan (5.5 μg/dL), Poland (6.3 μg/dL) & South Africa (6.4 μg/dL), and lower than those reported from Pakistan (15.6 μg/dL), Bangladesh (15.0 μg/dL) & Saudi Arabia (10.4 μg/dL). In the present study, 36% of children crossed the CDC intervention level of 10 μg/dL blood lead. This data is comparable to BLLs of newborns and children when leaded petrol was in practice in the country. Fifty four percent newborns from Lucknow and >50% children from other metropolitan cities of India then exceeded the CDC intervention level. The lowest percentage of children with BLLs ≥10 μg/dL was
reported in U.S. (4.4%) while highest reported in Bangladesh (90%) and Pakistan (81%). Palestine, Israel, Indonesia, South Africa, Poland and China have 5.2% to 34% of children with BLLs ≥10 μg/dL.

In multivariate regression analysis low socioeconomic status (p = 0.066), distance of their residence from high traffic density (p = 0.066) and mother’s illiteracy (p = 0.085) were found to be inversely correlated with BLLs (log transformed). We found significant negative monotonic relationship between BLLs and IQ score of children after accounting for known confounders (adjusted β = -0.993, SE = 0.398, p = 0.014). However, in final regression model growth parameters (head circumference, height, weight & BMI) were not significantly associated with BLLs. Although we could not explore the possible biologic mechanism that could explain this finding, the ability of lead to substitute for calcium is a factor common to many of its toxic actions including influences on neurotransmitter storage and release, second messengers, cerebrovascular endothelial cells and glial cells. For example, lead’s ability to pass through the blood–brain barrier is due in large part to its ability to substitute for calcium ions (Ca$^{2+}$). Lead also suppresses activity-associated Ca$^{2+}$-dependent release of acetylcholine and dopamine neurotransmitters.

To examine the effects of BLLs on biochemical indices, children were categorized into five groups; GI had BLLs <5 μg/dL (2.94±1.45), GII 5-10 μg/dL (7.65±1.39), GIII >10-15 μg/dL (11.95±1.67), GIV >15-20 μg/dL (17.50±1.54) and GV >20 μg/dL (22.61±2.17). Blood δ-ALAD activity was
significantly lower in groups of children with higher BLLs in comparison with groups of children with lower BLLs (p<0.05/0.01/0.001). Blood MDA level was significantly higher while blood GSH level was significantly lower in groups of children with higher BLLs as compared to children of lower BLLs (p=0.05/0.01/0.001). We proposed that lead could enhance membrane lipid peroxidation of erythrocytes through the promotion of membrane physical changes, which would favor the propagation of oxidative stress. By increasing lipid peroxidation rates, lead could affect membrane-related processes such as the activity of membrane enzymes, endo- and exocytosis, the transport of solutes across the bilayer, and signal transduction processes. GSH can act as a non-enzymatic antioxidant by direct interaction of the SH group with free radicals, or it can be involved in the enzymatic detoxification reactions for free radicals, as a cofactor or coenzyme.

Evidence for lead-induced oxidative stress in the present study also arises from the significant increase in the erythrocyte SOD, CAT and GPx activities in groups of children with higher BLLs than those of children with lower BLLs (p<0.05/0.01/0.001). Increased SOD, CAT and GPx activities can be explained as a defense mechanism of erythrocytes against increased fluxes of free radicals during lead-induced oxidative stress. SOD dismutates $O_2^+$ into $H_2O_2$. CAT has been suggested to provide an important pathway for $H_2O_2$ decomposition at higher steady state of $H_2O_2$ concentrations, while GPx is believed to play a more important role in $H_2O_2$ decomposition under lower steady state levels of $H_2O_2$. Enhanced lipid peroxidation (increase in
MDA) and increased activity of antioxidant enzymes (SOD, CAT & GPx) together with depletion of GSH as observed in the present study suggest the possible contribution of lead-induced oxidative stress in children through the direct participation of lead in membrane lipid peroxidation and/or inhibition of δ-ALAD, accumulation of δ-ALA that triggers the process of oxidative stress.

BLLs had significant negative correlations with δ-ALAD ($r = -0.338$, $p<0.001$) & GSH ($r = -0.316$, $p<0.001$), and positive correlations with MDA ($r = 0.311$, $p<0.001$), SOD ($r = 0.305$, $p<0.001$), CAT ($r = 0.318$, $p<0.01$) & GPx ($r = 0.308$, $p<0.001$). Interestingly, δ-ALAD had significant negative correlations with MDA ($r = -0.176$, $p<0.05$), SOD ($r = -0.180$, $p<0.05$), CAT ($r = -0.239$, $p<0.01$) & GPx activity ($r = -0.189$, $p<0.05$), and positive correlation with GSH ($r = 0.314$, $p<0.001$). These may suggest the possibility of the potential use of these oxidative stress parameters as biomarkers of lead intoxication and/or inhibition of δ-ALAD, as an additional indicator of lead-induced oxidative stress. Alteration in these parameters as a function of blood lead might elucidate some of the mechanisms involved in the lead-induced oxidative stress among children, wherein many diseases are thought to be associated with free radicals generation.

Central nervous system is at risk to suffer oxidative damage because of high oxygen consumption, enriched in more easily peroxidizable fatty acids and relatively low levels of protective antioxidants. Many lines of evidence suggest that free radicals generation play a pivotal role in the neurologic disorders. We examined the effects of BLLs on oxidative stress markers in
children suffering from neurologic disorders. Thirty children (3-12 years) with neurologic disorders; cerebral palsy (n=12), seizures (n=11) and encephalopathy (n=7) belonged to in and around Lucknow, were recruited in the study group. We wanted more numbers but could not do so because of practical difficulty in getting such cases. Sixty healthy children (3-12 years) from similar socio-economic environment and not suffering from any chronic disease(s) were taken as the control group. Their BLLs and oxidative stress markers were determined. Age, sex, body size (height, weight & BMI), area of residence and socioeconomic status between the study and control groups were not different statistically. Mean BLL was significantly higher in children with the seizures (15.5 μg/dL), cerebral palsy (17.9 μg/dL) and encephalopathy (24.5 μg/dL) when compared with children of the control group (10.4 μg/dL) (p<0.05 each). Delta-ALAD activity was significantly lower in the study group as compared to the control group (p<0.05) leads to accumulation of δ-ALA that also has a potential of neurotoxicity; interferes with gamma-aminobutyric acid neurotransmission, a process that might be implicated in neurologic disorders. MDA level was significantly higher while GSH level was significantly lower in the study group as compared to the control group (p<0.05 each). Activity of antioxidant enzymes; SOD and CAT were significantly higher in the study group than those of the control group (p<0.05 each). There were significant negative correlations of BLLs with δ-ALAD (r = -0.352, p<0.05) & GSH (r = -0.321, p<0.05), and positive correlations with MDA (r = 0.370, p<0.05), SOD (r = 0.528, p<0.05) & CAT (r = 0.528, p<0.05)
0.314, p<0.05). In turn, δ-ALAD had significant negative correlations with MDA (r = -0.292, p<0.05), SOD (r = -0.344, p<0.05) & CAT (r = -0.279, p<0.05), and positive correlation with GSH (r = -0.324, p<0.05). Our results revealed that environmental lead exposure was associated with oxidant/antioxidant imbalance in the blood of children with neurologic disorders.

Aplastic anemia is a rare hematologic disorder in which all cellular components of bone marrow origin are deficient. Relatively little is known about its possible etiologies and risk factors. Lead interferes with heme biosynthesis, and affects the formation and function of erythrocytes. The oxidation phenomena and/or the formation of free radicals have been suggested to be causally related to various hematologic disorders including aplastic anemia. If lead were indeed toxic to hematologic system, one would expect the risk of aplastic anemia might be associated with the lead exposure. A hospital based case-control study was conducted to determine the incidence of childhood aplastic anemia in and around Lucknow and to elucidate the possible lead-induced free radical mediated mechanisms for the disease of unknown etiology. A total of 25 cases of childhood aplastic anemia (3-12 years) were identified as per established criteria and enrolled at the Department of Paediatrics, KGMU, Lucknow. Eight cases out of 25 were excluded from the study because they declined to participate and therefore, were not interviewed. Seventeen healthy children (4-12 years) not suffering from any chronic disease(s) were taken as the control group. Age, sex, body size (height, weight & BMI), area of residence and socioeconomic status in two
groups of subjects were almost similar. We could not match two cases by sex
and one case by age due to practical problems in the collection of blood in the
control group. Their BLLs and oxidant/antioxidant status were determined. The
annual incidence of childhood aplastic anemia in and around Lucknow was
found to be 6.8 cases per million which is higher than many countries [e.g.
France (1.5 cases/million), Brazil (2.4 cases/million), UK (2.3 cases/million),
Thailand (3.7 cases/million), US (5.0 cases/million)] but lower than those
reported in Sweden (24.6 cases/million), China (20.0 cases/million) and
European-Israeli collaborative study (20.5 cases/million). Mean BLL was
significantly higher (9.9 μg/dL vs. 6.0 μg/dL, p<0.05) while δ-ALAD was
significantly lower (p<0.01) in aplastic anemia cases when compared with the
control group. Blood MDA level was significantly higher while blood GSH level
was significantly lower in aplastic anemia cases as compared to the control
group (p<0.05 each). Lead-induced erythrocyte membrane lipid peroxidation
as observed in the present study leads to alterations in the biological
properties of the membrane, such as the degree of fluidity, and can lead to
inactivation of membrane-bound receptors or enzymes, which in turn may
impair normal cellular function, increase tissue permeability, and shortening
the life span of blood cells. Furthermore, significantly higher CAT activity in
aplastic anemia cases than those of the control group (p<0.05) suggests the
defense mechanism of blood cells against the increased fluxes of H₂O₂. There
were significant negative correlations of BLLs with δ-ALAD (r = -0.406, p<0.05)
& GSH (r = -0.380, p<0.05), and positive correlations with MDA (r = 0.352,
p<0.05) & CAT (r = 0.391, p<0.05). Results of the present report suggest that lead-induced oxidative stress may be one of the possible underlying mechanisms for childhood aplastic anemia.

In conclusion, our data indicate a declining trend of BLLs among children after unleaded petrol came into being in the region, however, that level of blood lead seems to be enough for adverse health consequences of children. Low socioeconomic status, distance of residence from high traffic density and mother illiteracy were the risk factors influencing the BLLs of children. We found negative monotonic relationship between BLLs and IQ score after accounting for known confounders and suggest that no threshold exists for intellectual impairment of children. Results of the present study suggest that there may be two independent sources of lead-induced oxidative stress; the first is the prooxidative effect of δ-ALA, and the second is connected with the direct effect of lead on membrane lipids. In the present study, higher production of free radicals may lead to increased lipid peroxidation with concomitant decrease in antioxidant molecules and activity of antioxidant enzymes also increases to scavenge these free radicals. This appears to be the possible underlying mechanism of lead-induced oxidative stress. Impaired oxidant antioxidant balance as observed in blood children suffering from aplastic anemia disease and neurologic disorders suggest that lead-induced free radicals generation may be one of the underlying mechanisms for these diseases. BLLs were also significantly correlated with parameters of oxidative stress that have the potential to be used as
biomarkers of lead intoxication. This is a first comprehensive study from a particular region of the vast country and is a pointer to the perils of environmental exposure to lead among children and may address the attention of regulatory agencies to curtail the input of lead from sources other than petrol as well. A national programme involving different regions of the country to monitor BLLs and associated health risks including lead-related diseases among children is warranted to generate a base-line data that might aid planners, regulators and public at large, because there is no common approach for the treatment of environmental lead toxicity.