Original Article

DOI: 10.7860/JCDR/2022/53738.16789

Blood Lead Levels in Children Living near Lead Smelting Zone: A Pilot Field Study

Paediatrics Section

KAKALI ROY[1], SURUPA BASU[2], NABENDU MURMU[3], JYOTIRMOY ADHIKARI[4], SUMANTRA ADHIKARI[5], RITABRATA KUNDU[6], APURBA GHOSH[7]

ABSTRACT

Introduction: Children are most susceptible to Lead (Pb) toxicity. Exposure to lead in the environment still exists in various pockets of urban cities due to continued practices of using lead in jewellery making, paints, battery smelting and in cosmetics.

Aim: To evaluate Blood Lead Level (BLL) and its association with haemoglobin, Red Blood Corpuscle (RBC) indices and bone parameters (vitamin D, parathyroid hormone, calcium, phosphorus, and Alkaline Phosphatase (ALP) in children residing near lead battery smelting units of Kolkata.

Materials and Methods: This was a cross-sectional field-based pilot study carried out by Institute of Child Health, Kolkata, West Bengal, India. A camp was organised in the month of August 2015, at a known major cluster of secondary lead smelting area ward no. 66 in the Kolkata metropolitan district. A total of 45 camp attending children were enrolled. BLL was measured using graphite furnace atomic absorption spectrometry, and association with haematological and bone parameters were evaluated.

Results: Mean age of the participants was 5.6±3.3 years, and mean BLL was 3.7±1.9 µg/dL (range:1.3-8.2). About 35 children were found to have low BLL <5 µg/dL (LBLL, 2.88±1.08) while 10 had elevated BLL ≥5 µg/dL (EBLL, 6.59±0.95) (p<0.0001). Red Cell Distribution Width (RDW) was high (p=0.03) and Mean Corpuscular Volume (MCV) was low (p=0.05) in EBLL group; but there was no significant difference in haemoglobin level, compared to LBLL group. The mean vitamin D level was 15.2±8.7 ng/mL, while 23 (51%) were severely deficient without concomitant rise in parathyroid hormone (mean, 37.9±0.7 pg/mL). Calcium, phosphorous and ALP were within normal reference range. None of the bone parameters showed any correlation with BLL.

Conclusion: Overall, 22% children of the cohort had elevated BLL, beyond the permissible safety limit of 5 µg/dL but within 10 µg/dL. Mildly elevated BLL relate to iron deficient haematopoiesis (increased RDW and low MCV) without any apparent affection of bone metabolism.

Keywords: Anaemia, Calcium, Children, Kolkata, Lead toxicity, Vitamin D

INTRODUCTION

With the global phasing out of leaded petrol, there has been steady decline in the BLL, however, man is still exposed to the toxic effects of lead (Plumbum, Pb) mainly due to occupational exposure such as car repair, battery recycling units, smelting, jewellery making, etc. As the air, water and soil around these industries get contaminated with lead, hazards of lead poisoning increase [1,2].

Children are particularly susceptible due to increased risk of exposure from crawling, hand-to-mouth behaviours, pica, and higher respiratory rate. Concomitant iron deficiency anaemia in children increases lead absorption; an immature and developing blood brain barrier leads increased entry of lead into central nervous system resulting greater neurotoxicity such as reduced cognition at low blood levels to death at toxic levels [3]. Environmental and occupational exposure, both constitute major modes of Pb toxicity caused mainly through dermal contact, ingestion and inhalation. Once in the bloodstream, Pb is primarily distributed among three compartments; blood, mineralising tissue like bone and teeth, and soft tissues such as brain, kidney [3,4]. Among the many other effects of lead, metabolism of calcium is affected leading to lower serum calcium levels and concomitant increase in parathyroid hormone and increased synthesis of 1,25-Dihydroxy vitamin D in kidney [5]. There are considerable evidences that BLL affects bone metabolism [6]. Lead causes anaemia by possible impairment of haemsynthesis and an increased rate of red blood cell destruction [7]. Children in Delhi with lead levels ≥10 µg/dL were found to be 1.3 times as likely to have moderate anaemia as children with lead levels <10 µg/dL [8].

A recent meta-analysis of BLL in India [9] report that population wide levels still remain elevated in children with a mean of 6.86 µg/dL (95% CI: 4.38-9.35); higher than the Centres for Disease Control and Prevention (CDC, 2012, United States of America) reference value of 5 µg/dL, despite regulatory action to eliminate leaded petrol in 2000 [3,9]. Alarming levels of blood lead with mean BLL of 55.7 µg/dL were reported in children with history of probable exposure, attending the outpatient department of a hospital in Lucknow during 2014-2015, where majority (67.8%) of children did not have any clinical features of lead toxicity [10]. Earlier, Goswami K found children who apply surma (kohl) as a cosmetic had higher BLL compared to a control group [11].

Paediatricians in hospital practice, have encountered sporadic cases of lead poisoning in children living near industries processing lead [12]. A hospital-based study from West Bengal showed higher blood levels in children to be associated with loss of hearing [13]. Significant BLL are found in children residing near informal lead battery manufacturing units near Patna, Bihar [14]. An area in ward no. 66, Picnic Garden of the Kolkata metropolitan district, was known to recycle lead in the several secondary lead smelters [15]. The present study aimed to evaluate children living around these areas for BLL, who were likely to have higher lead concentration in blood. Further effects of elevated BLL on haematological and bone parameters were also evaluated.

MATERIALS AND METHODS

This was a cross-sectional field-based pilot study, carried out by Institute of Child Health, Kolkata, West Bengal, India. The study was approved by Institutional Ethics Committee vide letter number ICH/514/2013. A health check-up camp for children was organised by the research team including senior faculty and paediatricians with the aid of local residents in the month of August 2015 at a

site inundated with battery smelters, situated at Kolkata Municipality ward no. 66, Picnic Garden, close to the hospital [15]. This area is densely populated having around 65,000 inhabitants living mostly in slum-like conditions. This is a low lying area having mixed land use (industrial cum residential). Households in the area use water from public taps supplied by the local municipality [15].

Inclusion criteria: Children of age 1 to 18 years, residing for more than one year in the area around smelters and lead-acid recycling units was included in the study.

Exclusion criteria: Children with known haematological disorder or any chronic systemic illness were excluded from the study.

Among the camp attendees, 45 children who fulfilled inclusion criteria and attendants gave written informed consent to participate in the study. Detailed history and clinical examination including lead toxicity manifestation was documented in a preformed questionnaire (demographic data, source of drinking water, residence period).

Study Procedure

Two milliliters of blood in Ethylenediamine Tetraacetic Acid (EDTA) tubes was collected by venepuncture after thorough cleaning of the site. Samples were transported to the hospital laboratories in ice boxes within 30 minutes of collection. Complete blood count was estimated on cell counter XP Sysmex-100. BLL was determined by the graphite furnace Atomic Absorption Spectrophotometer (AAS) which has high specificity and greatly reduces interference. Vitamin D total, (25-Hydroxy cholecalciferol and ergocalciferol), ng/mL and Parathyroid Hormone intact (iPTH), pg/mL were tested using electrochemiluminiscence. Calcium, mg/dL and phosphorus, mg/dL were tested on roche systems and 5-nitro-5′-methyl-(1,2-bis (o-aminophenoxy) ethan-N,N,N′,N′-tetraacetic acid (NM-BAPTA) and phosphomolybdate complex end point methods, respectively.

Iron Deficiency (ID) was defined on RBC indices of increased Red Cell Distribution Width (RDW) and decreased Mean Corpuscular Volume (MCV) [16]. Anaemia was defined using World Health Organisation (WHO) criteria as Heamoglobin (Hb) <11.0 g/dL for ages 6-59 months, <11.5 g/dL for ages 5-11 years, and <12.0 g/dL for ages 12-14 years and for girls ≥15 years <13.0 g/dL for boys ≥15 years of age [17]. Vitamin D deficiency and insufficiency was defined as serum 25 (OH) D, <20 mg/mL and <30 ng/mL respectively [18]. The more restrictive definition of deficiency as 25 (OH)D, <15 ng/mL used by others was utilised as a severe deficiency. The BLL was defined using the CDC, United States of America (USA) recommended reference value of <5 µg/dL [9,19].

STATISTICAL ANALYSIS

Statistical analysis were done using Graph-Pad Prism version 5.0 software. Data were expressed as mean±standard deviation (SD) or median and Interquartile Range (IQR) as appropriate. Fisher's-Exact test was used for categorical variables. Independent t-test (for parametric data) and Mann-Whitney U test (for non parametric data) were used to compare the available data. Spearman's rank correlation test was used to determine the correlation between BLL and other parameters. The p-value <0.05 was taken to be statistically significant.

RESULTS

The demographic details, clinical features, and laboratory data of the 45 children was described in [Table/Fig-1]. The mean age of the population was 8.1 years (range 2-19 years), with a higher proportion of males 27 (60%). Medical examination of all children did not show any florid signs of lead toxicity but 3 (6%) of the study population had pica, complaints of pain abdomen, while 2 (4%) had complaints of nausea/vomiting and irritability. Maximum BLL found in the cohort was 8.2 µg/dL. At these mildly elevated BLLs, florid clinical signs of lead toxicity are not present, although milder symptoms are reported but these were not investigated systematically as the focus was to study the association with haematological and bone parameters.

Demographic features	Values
Male	27 (60%)
Female	18 (40%)
Age {years, mean±SD (range)}	8.1±4.1 (2-19)
Height {cm, mean±SD (range)}	117.6±20.65 (63-58)
Weight {kg, mean±SD (range)}	22.9± 1.6 (8-50)
BMI {kg/m^2, mean±SD (range)}	22.9±10.5 (8-50)
Median years of stay near the factory (IQR)	5.8 (2-7.5)
Clinical features	
Constipation	1 (2%)
Nausea/Vomiting	2 (4%)
Pain abdomen	3 (6%)
Pica	3 (6%)
Irritability	2 (4%)
Delayed neurodevelopmental milestones	0
Pallor	0
Laboratory features	
Haemoglobin {g/dL, mean±SD (range)}	12.3±1.1 (9.7-15.2)
Haematocrit {%, mean±SD (range)}	36.4±2.5 (30.8-40.9)
MCV {fL, mean±SD (range)}	78.9±7.6 (60.3-91.1)
MCH {pg, mean±SD (range)}	26.6±3.4 (18.0-31.5)
MCHC {gm/dL, mean±SD (range)}	33.7±1.5 (29.9-37.2)
RDW {%, mean±SD (range)}	14.0±1.4 (11.7-17.2)
RBC count x10^6 {mean±SD (range)}	4.7±0.6 (3.8-6.5)
Calcium {mg/dL, mean±SD (range)}	9.9±0.5 (8.5-10.9)
Phosphorous {mg/dL, mean±SD (range)}	5.0±0.7 (3.3-6.2)
ALP {U/L, mean±SD (range)}	264±81.7 (69-577)
Vitamin D {ng/mL, mean±SD (range)}	15.2±8.7 (4.7-48.3)
PTH {pg/mL, mean±SD (range)}	37.9±20.7 (12.6-106.7)
BLL {µg/dL, mean±SD (range)}	3.7±1.9 (1.3-8.2)

[Table/Fig-1]: Demographic, clinical and laboratory data.
BMI: Body mass index; MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; RBC: Red blood corpuscle; ALP: Alkaline phosphatase; iPTH: Intact parathyroid hormone; BLL: Blood lead level; SD: Standard deviation; IQR: Inter quartile range

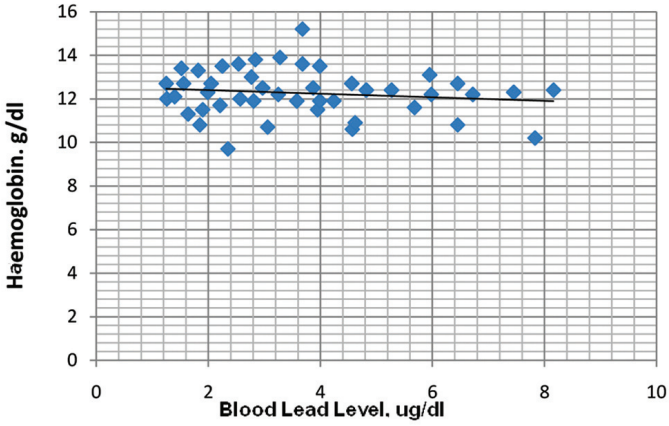
The BLL had a range of 1.3-8.2 µg/dL with mean BLL value of 3.7±1.9 µg/dL. According to BLL, study population was divided into two groups: Elevated BLL of ≥5 µg/dL (EBLL, n=10, mean±SD, 6.59±0.95) and low BLL of <5 µg/dL (LBLL, n=35, mean±SD, 2.88±1.08). The difference of BLL between the two groups was significant (p<0.0001). Difference of variables in these two groups is showed in [Table/Fig-2].

Variables	LBLL (<5 ug/dL) (n=35)	EBLL (≥5 ug/dL) (n=10)	p-value
Age (years, mean±SD)	8.30±4.18	6.71±3.93	0.51
Sex (M:F)	21:14	6:4	1.0
Hb (g/dL, mean±SD)	12.34±1.12	11.99±0.88	0.5
PCV (%, mean±SD)	36.46±2.63	36.19±2.18	0.7
MCV (fL, mean±SD)	80.13±9.06	74.66±6.79	0.05
MCH (pg, mean±SD)	26.39±5.18	24.87±4.18	0.21
RBC count x10^6 (mean±SD)	4.59±0.56	4.92±0.79	0.25
RDW (%, mean±SD)	13.69±1.24	14.94±1.61	0.03
Weight (kg, mean±SD)	27.26 ±14.38	25.22±16.76	0.64
Calcium (mg/dL, mean±SD)	9.97±0.43	9.63±0.59	0.13
Phosphorous (mg/dL, mean±SD)	5.00±0.67	5.11±0.73	0.66

ALP (U/L, mean±SD)	256.5±59.17	265.7±86.8	0.96
Vitamin D (ng/mL, mean±SD)	15.24±7.52	15.17±9.04	0.76
PTH (pg/mL, mean±SD)	33.09±12.65	39.13±22.33	0.82
BLL (µg/dL, mean±SD)	2.88±1.08	6.59±0.95	<0.0001

[Table/Fig-2]: Comparison of haematological indices and bone parameters in the LBLL and EBLL groups.
PCV: Packed cell volume (Haematocrit); MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width, RBC: Red blood corpuscle; ALP: Alkaline phosphatase; iPTH: Intact parathyroid hormone; BLL: Blood lead level; SD: Standard deviation
Mann-Whitney U test used for non parametric data, p<0.05 considered significant

Mean Hb% (g/dL) in EBLL group was 11.99±0.88 in comparison to 12.34±1.12 in LBLL group (p=0.5) but the difference was not statistically significant. Mean RDW (%) and MCV (fL) in EBLL group was 14.94±1.61 and 74.66±6.79 respectively whereas in LBLL group was 13.69±1.24 and 80.13±9.06, respectively. RDW (p=0.03) was increased and MCV (p=0.05) was decreased significantly in EBLL group compared to LBLL group, suggesting iron deficient erythropoiesis in children with elevated BLL. However, haemoglobin level did not reveal any linear dose-response relation with lead level using scatter plot (r=-0.1146, p=0.45) as shown in [Table/Fig-3].The study found a relationship between mildly elevated BLL (between 5-10 µg/dL) with iron deficient erythropoiesis, but yet not anaemia.



[Table/Fig-3]: Scatter plot of correlation between BLL (µg/dL) and Haemoglobin (g/dL). r=-0.1146, p=0.45

The mean vitamin D of the cohort was 15.2 ng/mL (SD-8.7), which is lower than the standard cut-off for deficiency (20 ng/mL). A total of 9 (20%) and 32 (71%) children were vitamin D insufficient (20-30 ng/mL) and deficient (<20 mg/mL) respectively. There were 23 (51%) children with severe vitamin deficiency (<15 ng/mL). The iPTH remained in the normal range with mean of 37.9 pg/mL (SD-20.7); high values >80 pg/mL were observed in only three children who had levels of vitamin D <10 ng/mL. Calcium, phosphorous and ALP were normal for almost all children; reflecting on normal bone metabolism in the cohort as given in [Table/Fig-1]. BLL was not correlated with vitamin D (r=0.0.008; p=0.95), calcium (r=0.05, p=0.72), phosphorous (r=-0.17, p=0.91) or ALP (r=-0.05, p=0.75).

DISCUSSION

The present study demonstrated that the mean BLL of children residing inward no. 66, Picnic Garden, Kolkata Metropolitan area close to lead smelting units was 3.7 µg/dL (range: 1.3-8.2). The mean BLL found in our study agrees with the mean BLL of 4.9 µg/dL found in healthy children (control group) residing in Kolkata and lower than the national average of 6.86 µg/dL [9,12]. The most significant source of leaded petrol was eliminated in 2000. This was followed with significant decrease in mean BLL from urban areas [20]. There seems to be no data reported from Kolkata before the phasing out of leaded petrol.

The present study found that almost 1/4th (22%) children of the cohort having elevated BLL and these children also had longer periods of stay near smelters. Though majority (78%) of this study population had BLL below CDC cut-off value of 5 µg/dL [3]. There is no safe level of lead in blood. Any detectable amount of lead in children can cause neurocognitive deficit affecting Intelligence Quotient (IQ), academic performance, ability for attention and no lower limit of BLL for this yet established [21]. While EBLL has definite risk of toxicity but LBLL even present a major concern.

A retrospective data study in Mumbai and Delhi during 1998-1999 had shown BLL between 5-20 µg/dL in majority of children; increasing age being the strongest correlate; probably due to the use of leaded petrol [22]. In 2013, another study by Kalra V et al., in Delhi school children revealed high BLL, while they were between 4.23-9.86 µg/dL in children from Lucknow [23,24]. Present study from Kolkata finds BLL between 1.3-8.2 µg/dL with one quarter children having higher than the current international action levels.

Sporadic incidents of toxic levels have been reported previously by this study centre and others in infants and new born babies [25], and in plastic industry workers of Kolkata [13,14,26]. This indicates the presence of high lead levels in the community amongst those who are exposed to heavy metal, while the general population may not be at high risk. West Bengal has not reported high BLL in a 2015 pan India study [27]. Population based studies need to confirm the above findings.

Lead and iron compete at the common receptor of the intestine for absorption. So, ID enhances absorption of lead, thus, predisposing to lead poisoning and vice versa that lead poisoning also predisposes to ID. Early ID causes iron deficient erythropoiesis denoted by high RDW (anisocytosis) followed by low MCV (microcytosis) but haemoglobin level remains yet in the normal range and subsequently, land up in frank anaemia, if, ID progresses further. ID without anaemia can also have a detrimental effect on the neurodevelopment of children [16]. On the other hand, lead interferes with heme adsorption and can cause red blood cell destruction, thus anaemia. Children with elevated BLL did have statistically significant higher RDW and lower MCV values denoting iron deficient erythropoiesis but, did not have lower haemoglobin concentration.

Enzymes of heme biosynthesis get affected at lower BLL and thus, haemoglobin synthesis, while anaemia is seen in very high BLL [8,28,29]. In the study by Jain NB et al., lead levels ≥10 µg/dL, including values ≥10-19.9 µg/dL, were significantly associated with moderate and severe anaemia [8]. Froom P et al., found that haemoglobin levels did not correlate well with BLL and further suggested that anaemia is not related to lead at low BLL [28]. Although elevated blood Pb can suppress Hb production and is thus an important risk factor for anaemia, this possible interaction was not tested in our sample since, according to CDC, blood Pb does not begin to suppress Hb, until it reaches 25-40 µg/dL [4], which is far higher than even the highest BLL as seen in the index sample. The present study's finding of anisocytosis and microcytosis in children with BLL between 5-10 µg/dL indicates that mildly elevated BLL may cause iron deficiency anaemia by its influence on iron metabolism and/or haembiosynthesis; which may later manifest as frank anaemia. The present findings are similar to those by Wright RO et al., who showed iron deficiency is significantly associated with low-level lead poisoning in children [29].

Being biochemically similar deficiency of iron, calcium and zinc increases lead absorption [3]. Lead also interferes with calcium and phosphorous metabolism through its inhibitory effects on 1-α-hydroxylase that affects synthesis of the active form of vitamin D (calcitriol) and thus, affects the growth and mineralisation of bone, teeth [5]. It was observed that bone parameters; calcium, phosphorous, and ALP levels were within reference limits. The mean vitamin D levels indicate generalised deficiency of vitamin D in the cohort, which is not much different than regional estimates in eastern India such as those found by the present group [30]. In comparison, there was no difference in the vitamin D levels of the EBLL group from the LBLL group. Overall, the vitamin D level is in an insufficient-deficient range, warranting emphasis on vitamin D and calcium supplementation in the population. BLL did not correlate with vitamin D or with any other bone parameter. The findings corroborate with the observations of

Kersey M et al., who reported that while vitamin D deficiency was far more common in children, Hb and Pb were not predictors of vitamin D status [31]. Himani et al., found that in adults (including lead-exposed battery workers), BLL was weakly negatively correlated with vitamin D and calcium levels but not with phosphorous [6]. This association may be attributed to the higher number of adults having BLLs in the toxic range (mean BLL was 39.5±31.5 µg/dL), compared to children in the present study having a mean BLL well below the toxicity mark.

Limitation(s)

Sample was likely too small in size. However, there are very few studies reported from eastern India in children and the present research was motivated by sporadic cases of lead poisoning reported in children at the study hospital and reports of nearby pockets of lead smelting areas, which could have exposed children to high environmental lead. The study did not assay markers like iron profile, ferritin for defining iron deficiency, bone X-ray, bone mineral density as a part of bone parameters, and future studies with these markers are needed to verify the present study findings.

CONCLUSION(S)

In conclusion, 22% children of the cohort living near the lead smelting zone in Kolkata had elevated BLL. Iron deficient erythropoiesis was significant in the elevated BLL group, but there was no correlation among anaemia, vitamin D, and lead status in the cohort. The cohort had widespread vitamin D deficiency but iPTH and other bone parameters were within normal limits. The present study reports a pocket of high risk area for lead toxicity in children, and suggests exploration of other zones of higher lead exposure in future studies to understand the prevalence of lead toxicity in children along with mass screening and awareness programs; as even lower BLL can have subtle, but irreversible toxic effects among children.

Acknowledgement

Institute of Child Health, Kolkata for granting fund. Chittaranjan National Cancer Institute (CNCI), Kolkata for providing infrastructure and support to perform lead estimation.

REFERENCES

[1] Singh AK, Singh M. Lead decline in the Indian environment resulting from the petrol-lead phase-out programme. Sci. Total Environ. 2006;368:686-94.
[2] Ghose MK, Paul R, Banerjee RK. Assessment of the status of urban air pollution and its impact on human health in the city of Kolkata. Environ Monit Assess. 2005;108:151-67.
[3] Hauptman M, Bruccoleri R, Woolf AD. An update on childhood lead poisoning. Clin Pediatr Emerg Med. 2017;18(3):181-92.
[4] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Lead, US Department of health and human services. Atlanta, Georgia, USA: US Government Printing. August 2020; pp. 1-583. https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf accessed 09.12.2021.
[5] Kristal-Boneh E, Froom P, Yerushalmi N, Harar G, Ribak J. Calcitropic hormones and occupational lead exposure. Am J Epidemio. 1998;147(5):458-63.
[6] Himani, Kumar R, Ansari JA, Mahdi AA, Sharma D, Karunanand B, et al. Blood lead levels in occupationally exposed workers involved in battery factories of Delhi-NCR region: Effect on vitamin-D and calcium metabolism. IJCB. 2020;35(1):80-87.
[7] Waldron HA. The anaemia of lead poisoning: A review. Occupational and Environmental Medicine. 1966;23:83-100.
[8] Jain NB, Laden F, Guller U, Shankar A, Kazani S, Garshick E, et al. Relation between blood lead levels and childhood anemia in India. Am J Epidemiol. 2005;161(10):968-73.
[9] Ericson B, Dowling R, Dey S, Caravanos J, Mishra N, Fisher S, et al. A meta-analysis of blood lead levels in India and the attributable burden of disease. Environ Int. 2018;121(1):461-70.
[10] Chaudhary S, Firdaus U, Ali SM. Factors associated with elevated blood lead levels in children. Indian Pediatr. 2018;55:38-40.
[11] Goswami K. Eye cosmetic 'surma': Hidden threats of lead poisoning. Indian J Clin Biochem. 2013;28(1):71-73.
[12] Maji B, Ganguly N. Lead poisoning in an infant. Indian Pediatr. 2014;51(4):319-20.
[13] Santra B, Raychowdhury R, Roychoudhury A, De M. Heavy metal blood levels and hearing loss in children of West Bengal, India. Noise and Health. 2019;21(102):189-93.
[14] Ansari JA, Mahdi AA, Malik PS, Jafar T. Blood lead levels in children living near an informal lead battery recycling workshop in Patna, Bihar. J Health Pollut. 2020;10(25):200308.
[15] Site Assessment of Lead Pollution at Picnic Garden Kolkata, India. https://www.coursehero.com/file/p1uvq9o/Excessive-exposure-also-leads-to-death-It-has-been-foundthat-lead-has/ accessed 8.07.20221.
[16] Bessman JD, Gilmer PR, Gardner FH. Improved Classification of Anemias by MCV and RDW. Am J Clin Pathol. 1983; 80(3): 322-26.
[17] WHO Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. WHO/NMH/NHD/MNM/11.1, pages 1-6. https://www.who.int/vmnis/indicators/haemoglobin.pdf accessed 09.12.2020.
[18] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Endocrine Society. Evaluation, treatment, and prevention of Vitamin-D deficiency: An endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.
[19] Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and associations of 25-hydroxyVitamin-D deficiency in US children: NHANES 2001-2004. Pediatrics. 2009;124:e1-e9.
[20] Nichani V, Li WI, Smith MA, Noonan G, Kulkarni M, Kodavor M, et al. Blood lead levels in children after phase-out of leaded gasoline in Bombay. India. Sci Total Environ. 2006;363:95-106.
[21] Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. Low-level environmental lead exposure and children's intellectual function: An international pooled analysis. Environ. Health Perspect. 2005;113: 894-99.
[22] Jain NB, Hu H. Childhood correlates of blood lead levels in Mumbai and Delhi. Environ Health Perspect. 2006;114:466-70.
[23] Kalra V, Sahu J, Bedi P, Pandey RM. Blood lead levels among school children after phasing-out of leaded petrol in Delhi, India. Indian J Pediatr. 2013;80:636-40.
[24] Ahamed M, Akhtar MJ, Verma S, Kumar A, Siddiqui MK. Environmental lead exposure as a risk for childhood aplastic anemia. Biosci Trends. 2011;5(1):38-43.
[25] Mazumdar I, Goswami K. Congenital lead poisoning: An unusual presentation. Indian Journal of Clinical Biochemistry. 2014;29(2):257-59.
[26] Mazumdar I, Goswami K. Chronic exposure to lead: A cause of oxidative stress and altered liver function in plastic industry workers in Kolkata, India. Indian J Clin Biochem. 2014;29:89-92.
[27] Iyer S, Sengupta C, Velumani A. Lead toxicity: An overview of prevalence in Indians. Clin Chim Acta. 2015;451:161-64.
[28] Froom P, Kristal-Bonch E, Benbassat J. Lead exposure in battery-factors workers is not associated with anemia. J Occup Environ Med. 1999;41:120-23.
[29] Wright RO, Shannon MW, Wright RJ, Hu H. Association between iron deficiency and low-level lead poisoning in an urban primary care clinic. Am J Public Health.1999;89:1049-53.
[30] Basu S, Gupta R, Mitra M, Ghosh A. Prevalence of Vitamin-D deficiency in a pediatric hospital of Eastern India. Indian J Clin Biochem. 2015;30:167-73.
[31] Kersey M, Chi M, Cutts DB. Anaemia, lead poisoning and Vitamin-D deficiency in low-income children: Do current screening recommendations match the burden of illness? Public Health Nutr. 2011;14(8):1424-28.

PARTICULARS OF CONTRIBUTORS:
1. Clinical Tutor, Department of Paediatrics, Nil Ratan Sagar Medical College and Hospital, Kolkata, West Bengal, India.
2. Associate Professor and Head, Department of Biochemistry, Institute of Child Health, Kolkata, West Bengal, India.
3. Senior Scientific Officer, Gr.-I, Department of Signal Transduction and Biogenic Amines, Chittaranjan National Cancer Institute, Kolkata, West Bengal, India.
4. Junior Scientific Assistant, Department of Signal Transduction and Biogenic Amines, Chittaranjan National Cancer Institute, Kolkata, West Bengal, India.
5. Junior Scientific Assistant, Department of Signal Transduction and Biogenic Amines, Chittaranjan National Cancer Institute, Kolkata, West Bengal, India.
6. Professor, Department of Paediatrics, Institute of Child Health, Kolkata, West Bengal, India.
7. Professor, Department of Paediatrics, Institute of Child Health, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Surupa Basu,
11, Dr. Biresh Guha Street, Kolkata, West Bengal, India.
E-mail: basusurupa@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 04, 2022
- Manual Googling: Jun 09, 2022
- iThenticate Software: Jun 14, 2022 (13%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Feb 23, 2022**
Date of Peer Review: **Apr 08, 2022**
Date of Acceptance: **Jun 10, 2022**
Date of Publishing: **Aug 01, 2022**