RESEARCH ARTICLE

Evaluation of blood lead levels by atomic absorption spectrometry in school going children of northern areas of Pakistan

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Abstract

Objectives: To evaluate blood lead levels and assess their correlation with academic performance in school children.

Methods: The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January to December, 2017, and comprised school children in Rawalpindi, Pakistan, and Hajira, Azad Jammu and Kashmir. About 5 ml of venous blood was collected in potassium-ethylenediaminetetraacetic acid tubes for lead levels, haemoglobin and plasma ferritin. The preceding three years' academic performance, rated as excellent, good, fair and poor, of the subjects was categorised on a specifically-designed proforma. Data was analysed using SPSS 24.

Results: Of the 400 subjects, 205 (50.25%) were females. The overall mean age was 9.15±2.8 years. Mean level of lead was 4.3±2.6 µg/dL, and difference in values was significant between children of Hajira and Rawalpindi ($p<0.05$). Mean haemoglobin was 12.9±0.919g/dL and mean ferritin was 49.17±16.0ng/ml. Of the total, 85(21.3%) showed excellent school performance, 162(40.5%) good, 113(28.3%) fair and 40(10%) showed poor performance. Significant negative association was found between school performance and mean blood lead level ($p>0.05$). No significant difference was found in terms of gender and age with school performance of both regions.

Conclusion: Majority of school going children in northern Pakistan were found to have lead levels ≤ 5µg/dL which is a level of concern as it is inversely related to academic performance.

Keywords: Lead, Pakistan, Rawalpindi, Academic performance. (JPMA 70: 1714; 2020)

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Introduction

Lead poisoning being among the dominant environmental disease can have life-lasting adverse health effects. Children are more vulnerable to the deleterious effects of this exposure than the adults. However, in countries where leaded gasoline has been banned, there has been a significant decline in average blood lead levels (BLLs). But unsuspecting lead threats, like improper dumping of electronic gadgets and toys defiled with lead, keep emerging. Lead poisoning in kids is a preventable and essential environmental concern and contributes to childhood diseases and maladies.[1] Studies have confirmed biological and neurological impairment associated with behavioural and cognitive damage even at low BLLs.[2,3] In 2012, the threshold was lowered further to 5µg/dL from 10µg/dL by the United States Centre for Disease Conntrol and Prevention (CDC)[4] and Needleman et al. concluded that significant harm happens even at BLLs <5µg/dL.[2] Lead exposure at an early age can induce a wide variety of problems in children. Among the impacts, the most usual and commonly occurring are subclinical impacts on the behavioural complications, cognitive impairment, central nervous system (CNS),[5,6] and lower intelligence quotient (IQ) levels.[3,7] Students with elevated BLLs are more often found to suffer from decreased attention span, lower IQ, absentmindedness and violent behaviour.[3,8-10]

Even low levels of lead in children are connected with conduct and learning deficit.[4,10] While various examinations have featured the impact of early youth lead introduction on intellectual and behavioural deficits, few have associated early childhood lead exposure to school performances universally. One study showed that early youth lead introduction eminently impacted the probability of getting a higher academic degree which is another reason behind achievement and accomplishment gap.[11]

Lead exposure poses a major environmental health threat in Pakistan and various studies have were done in this regard between 1990 and 2010 (Table-1). However, discrete recent studies on a large scale have not yet been performed in recent times. The current study was planned

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to determine BLL of school going children in two cities of Pakistan, and to document the impact of early childhood lead exposure on academic performance.

Subjects and Methods

The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi (RWP), Pakistan, from January to December, 2017, and comprised schoolchildren in Rawalpindi, Pakistan, and Hajira, Azad Jammu and Kashmir (AJK). Subjects of either gender were enrolled using non-probability consecutive sampling technique after approval from the institutional review board and written informed consent. Simple randomisation procedure was employed in which if subjects are assigned to groups A and B purely on a random basis. This procedure is the most basic way, and if the total number of subjects is small, then the groups are are likely to be unequal, and, therefore, this method is recommended when the sample is >100.[12] The sample size was calculated using the equation:[13]

Sample size= $1.96^2x(0.5)x(1-0.5)^{\circ} / 0.05^2$, Sample size $\simeq$ 385

In the equation, z=z value (e.g. 1.96 for 95% confidence level), p = percentage population proportion, expressed as decimal, and c = error (1-x) = 95%, = 0.05 expressed as decimal.

Children suffering from anaemia because of iron deficiency, and those who had zinc supplementation in the preceding six months and those with known instances of thallasaemia characteristics were excluded. Zinc supplementation is considered to lessen lead toxicity.[14]

Detailed clinical history, including demographic data, sources of lead, symptoms and signs of lead poisoning, and preceding 3 years' academic performance, was recorded on a specially-designed proforma. About 5ml venous blood sample was collected under aseptic measures in potassium-ethylenediaminetetraacetic acid (EDTA) tube for lead level, plasma ferritin and haemoglobin (Hb). Whole blood lead analysis was performed using atomic absorption spectrometery (Agilent Technologies, Malaysia). Lead levels were measured in batches of 50 samples interspersed with calibration checks using ClinChek Controls provided by the manufacturer. Hb analysis was done on Sysmax Haematology analyser (Kobe, Japan). Ferritin was checked using Immulite 2000 (Siemen incorporation, United Kingdom).

Data was analysed using SPSS 24. Data was expressed frequencies and percentages as well as mean ± standard deviation (SD) for qualitative and quantitative variables respectively. Test of associations, like Chi-square, and their significance at $p<0.05$ were used to compare BLL school performance, age group, gender and regional distribution. Analysis of variance (ANOVA) tests were used to assess statistical significance of difference of mean BLL for two or more than two groups, like age, region and school performance. It was followed up with Least Significant Difference (LSD) post-hoc test.

Results

Of the 400 subjects, 205(51.25%) were females, and 240(60%) were from RWP (Table-2). The overall mean age was 9.15±2.8 years. Mean BLL was 4.3±2.6 µg/dL. Mean haemoglobin was 12.9±0.919g/dL and mean ferritin was 49.17±16.0ng/ml. Of the total, 85(21.3%) showed excellent school performance with mean BLL 3.5±1.7µg/dL, 162(40.5%) good with mean BLL 3.6±2.2µg/dL, 113(28.3%) fair with mean BLL 5.50±3.4µg/dL, and 40(10%) showed poor performance with mean BLL 5.20±2.6µg/dL (Table-3).

Significant negative association was found between school performance and rising mean BLL ($p<0.05$). When further analysed, four of the six comparisons were statistically significant ($p<0.05$) but no major difference was observed in academic performance (Table-4).

There were 324(81%) children with BLL ≤5µg/dl, and, among them, mean BLL was the greatest in children with poor school performance 4.18±0.84µg/dl, while the lowest in those with good school performance 2.9±1.3µg/dl. Among the 76(19%) children with BLL >5µg/dl, the mean BLL was greatest in children with poor school performance 10.0±2.82µg/dL, while the lowest was in those with excellent school performance 6.7±2.3µg/dL. Mean BLL in boys was 4.32±2.9µg/dL compared to 4.30±2.4µg/dL for girls ($p>0.05$). Mean BLL in 221(55.25%) pre-pubert group was 4.18±2.6 µg/dL compared to 4.47±2.8 µg/dL in 179(44.75%) subjects in the peri-puberty groups ($p>0.05$). Mean BLL values were significantly different between children of Hajira 3.5±1.2µg/dL and RWP 4.8±3.2µg/dL ($p<0.05$). However, the academic performance of the two region based groups was not significantly different ($p>0.05$). In AJK, 68(42.5%) children showed good school performance with lowest mean BLL of 3.2±1.18µg/dL, and 19(11.87%) showed poor school performance with greatest mean BLL of 4.3±0.74µg/dL. In RWP, 46(19.16%) children showed excellent performance with lowest mean BLL of 3.5±2.1µg/dL and 79(32.91%) showed fair school performance with greatest mean

Table-1: Results of studies regarding blood lead levels (BLLs) in Pakistan.

Authors	Study population	Year published	Sample	City	n	Mean (µg/dL)	SD
Manser[25]	Children	1990	School children	Karachi	-	38.2	-
Sadaruddin[26]	Adolescents 13-19 years	1995	School children	Islamabad	170	2.38	-
Hafeez and Malik[27]	Children <5 years	1996	Population based	Rawalpindi	92	18.8	
Malik and Hafeez[27]	Children 1-5 years, surma users Pica eating None	1999	OPD patients	Rawalpindi	30 (surma users), 29 (pica eating), 33 (none)	21.2 (surma users), 21.2 (pica eating), 14.55 (none)	-
Rahbar[28]	Children (30-60 months)	2002	Population based	Karachi (saddar with high traffic)	83	16.46	15.72
				Karachi (suburban with low traffic)	52	12	4.50
				Karachi (city with moderate traffic)	107	14.3	5.30
				Karachi (city with moderate traffic)	105	14.9	5.76
Rahman[29]	Children 6-10 years	2002	School children	Karachi	138	16.8	6.29
Zakir[30]	Children (7-14 years)	2002	Automobile workers	Peshawar	60	38.2	-
Rahman and Hakeem[31]	Pregnant women	2003	OPD patients	Karachi	73	9.91	4.44
Khalil[32]	Children 1-6 years	2004	OPD patients	Lahore	50	9.95	5.77
Agha[33]	Adolescents 13-19 years	2005	Population based	Islamabad	88	3.22	0.20
Khan DA[34]	Workers were exposed to lead from 3- 28 years and controls	2009	Lead exposed workers and controls	Wah	87 62	291 108	-
Khan DA[35]	Children 1-6 years	2010	Children of lead-related occupational workers	Wah/ Gujranwala	123	8.1	-
				Controls (30 km away from industrial area)	123	6.7	

Table-2: Region-wise distribution of study population.

Subjects	Gender		Age Category		City
Total (n= 400)	Male (n=195)	Female (n=205)	Pre-puberty (4-9 Years)	Peri-puberty (10-14 Years)	
240 (60 %)	129 (66.1%)	111 (54.1%)	130 (58.5%)	110 (61.4%)	Rawalpindi, Pakistan
160 (40 %)	66 (33.8%)	94 (45.8%)	91 (41.1%)	69 (38.5%)	Hajira, Azad Jammu & Kashmir (AJ&K)

Table-3: Comparison of mean blood lead level (BLL) with school performance.

Summary	Excellent	Good	Fair	Poor
N	85	162	113	40
Means	3.55	3.66	5.50	5.20
Standard Deviation (SD)	1.79	2.22	3.44	2.61
F- value	15.35			
P-value	0.000*			

*Significant at 1% level of significance (P<0.05).

Table-4: Results associated with Least Significant Difference Post-Hoc multiple comparisons.

School Performances		p-values
Excellent	Good	.99
Excellent	Fair	.000*
Excellent	Poor	.006*
Good	Fair	.000*
Good	Poor	.005*
sFair	Poor	.920

*Significant at 1% level of significance (P<0.05).

BLL 6.3±3.6µg/dL.

Discussion

The current study found a negative impact of high BLLs on academic performance of children in two cities of Pakistan. The relationship between BLLs and school performance suggests that the higher a child's BLL, the more likely is the chance of poor academic performance. In 2013, a study among elementary and junior high school children in Detroit, Michigan, evaluated the longstanding impact of lead exposure on the performance of children academically in subjects like mathematics, science and reading skills.[15] The current study, along with previous studies,[2,15-18] emphasises the importance of further lowering the BLLs in order to protect our students from adverse health effects. A review article in 2008, focussing on previous studies conducted in Pakistan, suggested that lead levels in Pakistani children were beyond the acceptable limits, probably interfering with the school performance of the coming generation.[19] Due to limited information on the status of lead exposure and its effects on children, it is difficult to formulate a complete picture of the extent of this issue in Pakistan and in other developing countries. Studies conducted in Pakistan between 1989 and 2010 had a small sample size due to possibility of selection bias and imprecision in results as concisely mentioned in the review article.[19]

In Pakistan, lead poisoning still remains a problem[5-7] as depicted in a recent study conducted in Greece[20] where a large proportion of children with BLLs $\geq$5 µg/dL were of Pakistani descent (17.8%), which was much more than anticipated. Another study conducted in New York City showed that 12% of children aged <18 years were of Pakistani descent with BLLs $\geq$45µg/dL.[21] The review article[19] mentioned that in Peshawar, children aged 7-14 years working at automobile workshops as auto mechanics or helpers had mean BLL of 38.2µg/dL, the mean levels for Islamabad were 22.8 µg/dL, whereas in Karachi, much higher lead levels were recorded ranging between 21.3 and 52.2µg/dL with a mean of 38.2µg/dL. A school in Karachi's posh locality reported none of the children having lead levels <20µg/dL. Mean BLLs found in the current study is low (4.2µg/dL) compared to the aforementioned data, but even low BLLs (<5µg/dL) severely affect academic performance.[17]

Studies in the recent past showed higher BLLs in boys compared to girls, but our results showed no significant difference between genders. The difference can possibly be due to the role of sex hormones etc.[22] Evaluation of how different sources of lead contribute and the exposure influences academic performance is complex, which is likely to differ between areas and population groups. Studies recommend that for every 10µg/dL of blood lead, IQ is decreased in any event by 1-3 points.[3,7] At the individual level, this drop may appear to be little and unimportant, but at the population level, little consequences for some people might be a noteworthy burden on society, with decrements in general intellectual performance and economical misfortunes and losses.

A study conducted in 1995 showed a decline in BLL concentrations conducted in schoolchildren of Chak Shehzad, Islamabad. The results reflected very little or no risk to the health of children, indicating that the area is relatively free from lead pollution.[19] A population-based study conducted in Islamabad in 2005 in adolescents aged 13-19 years[19] showed similar results with mean BLL of 3.22µg/dL.

Our findings are parallel with this descending pattern in BLL, but it ought to be noticed that the Ecuadorian newborn children and youthful kids in 2003-2013 still showed elevated BLLs, with a geometric mean of 15.9µg/dL, which is as much as three times higher than the present CDC reference estimation of 5µg/dL.[23] Information gathered in the current study showed that newborn children and youthful kids, who are more prone to clinical and neuro-developmental impacts of lead poisoning, ought to be observed intently by health experts for lead exposure. Values in the study were practically identical to the amount of absorptions found in Karachi (22µg/dL).[24] The current study showed no significant difference between school performances of the two regions. And there was no difference between mean BLL in pre-puberty (4.18 µg/dL) and peri-puberty categories of children (4.47 µg/dL). In our study, 19% had BLLs >5µg/dL, indicating a correlation between the BLLs in children and environmental exposure to lead. We hypothesised that the hoisted BLLs in kids residing in Rawalpindi might be simply caused by the exposure of lead. Fresh studies ought to be done to decide the connection between BLLs in children and the genuine lead tainting in the surroundings.

Being first of its kind, the current study consists of findings from RWP, an urban area, and Hajira, a rural area of Pakistan. The results from the rural area can be used as a benchmark for future studies. Indications from rural area results could be from a direct result of improvement on economic background, education level, infrastructure, especially roads, and water supply.

Finally, the results depict the inverse relationship between school performance and BLLs. Children with poor performance had higher BLLs. This relationship additionally proposes that the higher the student's BLL was, the more probable it was that the student would perform poorly. On the basis of the findings, we screened out group of children who showed poor performance which will then be followed up using this criterion in a future study that is being planned.

It must be noted that no studies were found on the association of BLL with school performance in children in Pakistan. Also internationally, majority of studies were related to determination of BLL in children, and a few with blood lead health effects in children.[5,6,8,10] Very few studies with blood lead affecting the IQ level of children were found.[7,9,16] Also, no specific questionnaire was found assessing a child's association of BLL with school performance.

The current study has its limitations as it only included a few schoolchildren in two selected areas of the country. BLL of children in other areas may differ. The study did not measure lead in potential exposure sources, such as soil, dust and air. Lead concentrations were measured only once, rather than serial monitoring throughout the entire time of childhood period. Only subjective evaluation of the children's academic performance by the teachers was used. While the teachers were not aware of the children's BLL, an assessment, such as an IQ test, would have been a better measure of the effects of lead on the children's intellectual capacity, but a validated IQ test was not available in Pakistan during the study period. No physical parameters were measured, while a more thorough medical examination could have revealed other areas of health affected by lead exposure. Since very elaborative and time-consuming scales were used in previous studies for the evaluation of school performance with blood lead in children, such scales could not be used in the current study due to time constrains.

In the light of the findings, we recommend adopting lead administrative techniques to lessen lead outflows from various sources. Usage of institutionalised lead screening programme for kids, particularly those living in lead-defiled zones, ought to be established. Also, lead education targeting public health authorities, paediatricians as well as parents need to be initiated to increase awareness about the problem.

Conclusion

Among the subjects, 81% had BLL ≤5µg/dL which is comparable or equivalent to the CDC level of concern. The BLL was inversely related to academic performance. Every single developing country should take all the possible measures to control or dispose of all the possible lead hazards in surroundings of the children before they are exposed to it.

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References

1. Raymond J, Brown MJ. Childhood blood lead levels-United States, 2007-2012. Morb Mortal Wkly Rep. 2015; 62:76-80.
2. Needleman HL, Landrigan PJ. What level of lead in blood is toxic for a child? Am J Public Health. 2004; 94:23-43.
3. Canfield RL, Henderson Jr CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. New Eng J Med. 2003; 348:1517-26.
4. Control CfD Prevention. Response to advisory committee on childhood lead poisoning prevention recommendations in low level lead exposure harms children: A renewed call for primary prevention. US Department of Health and Human Services: Atlanta, GA, USA. Morb Mortal Wkly Rep. 2012; 61:1-383.
5. Surkan PJ, Zhang A, Trachtenberg F, Daniel DB, McKinlay S, Bellinger DC. Neuropsychological function in children with blood lead levels< 10 µg/dL. Neuro Toxicol. 2007; 28:1170-7.
6. Bellinger DC. Very low lead exposures and children's neurodevelopment. Curr Opin Pediatr. 2008; 20:172-7.
7. Chen A, Cai B, Dietrich KN, Radcliffe J, Rogan WJ. Lead exposure, IQ, and behavior in urban 5-to 7-year-olds: does lead affect behavior only by lowering IQ? Pediatrics. 2007; 119:e650-e8.
8. Kaiser MY, Kearney G, Scott KG, DuClos C, Kurlfink J. Tracking childhood exposure to lead and developmental disabilities: examining the relationship in a population-based sample. J Public Health Manag Pract.2008; 14:577-80.
9. Liu J, Li L, Wang Y, Yan C, Liu X. Impact of low blood lead concentrations on IQ and school performance in Chinese children. PLoS One. 2013; 8:e65230.
10. Bellinger DC. Neurological and behavioral consequences of childhood lead exposure. PLoS Med. 2008; 5:e115.
11. Miranda ML, Maxson P, Kim D. Early childhood lead exposure and exceptionality designations for students. Int J Child Health Hum Dev. 2010; 3:77-84.
12. Kim J, Shin W. How to do random allocation (randomization). Clin Orthop Surg. 2014; 6:103-9.
13. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. Gastroenterology and Hepatology from bed to bench. Gastroenterol Hepatol Bed Bench. Winter. 2013; 6:14-7.
14. Prasanthi RP, Reddy GH, Reddy GR. Calcium or zinc supplementation reduces lead toxicity: assessment of behavioral dysfunction in young and adult mice. Nutr Res. 2006; 26:537-45.
15. Zhang N, Baker HW, Tufts M, Raymond RE, Salihu H, Elliott MR. Early childhood lead exposure and academic achievement: evidence from Detroit public schools, 2008–2010. Am Econ J Appl Econ. 2013; 103:e72-e7.
16. Aizer A, Currie J, Simon P, Vivier P. Do low levels of blood lead reduce children's future test scores? Am Econ J Appl Econ. 2018; 10:307-41.
17. Blackowicz M, Hryhorczuk D, Rankin K, Lewis D, Haider D, Lanphear B, et al. The impact of low-level lead toxicity on school performance among Hispanic subgroups in the Chicago public schools. Inter J Environ Res Public Health. 2016; 13:774.
18. Evens A, Hryhorczuk D, Lanphear BP, Rankin KM, Lewis DA, Forst L, et al. The impact of low-level lead toxicity on school performance among children in the Chicago Public Schools: a population-based retrospective cohort study. Environ Health. 2015; 14:21.
19. Kadir MM, Janjua NZ, Kristensen S, Fatmi Z, Sathiakumar N. Status of children's blood lead levels in Pakistan: implications for research and policy. Public health. 2008; 122:708-15.
20. Tanaka M, Petsios K, Dikalioti S, Poulopoulou S, Matziou V, Theocharis S, et al. Lead Exposure and Associated Risk Factors among New Migrant Children Arriving in Greece. Int J Environ Res Public Health. 2018; 15:1057.
21. Keller B, Faciano A, Tsega A, Ehrlich J. Epidemiologic characteristics of children with blood lead levels≥ 45 µg/dL.J Pediatr. 2017; 180:229-34.
22. Shamsudin SB, Majid AA. Association of blood lead levels and working memory ability of primary school children surrounding ex-copper mining area in Ranau, Sabah (Malaysia). Acta Sci Malaysia. 2017; 1:01-3.

23. Counter SA, Buchanan LH, Ortega F. Blood lead levels in Andean infants and young children in Ecuador: an international comparison. Journal of Toxicology and Environmental Health Part A. 2015;78:778-87.
24. Rahman MA, Rahman B, Ahmad MS, Blann A, Ahmed N. Blood and hair lead in children with different extents of iron deficiency in Karachi. Environ Res. 2012; 118:94-100.
25. Manser W, Lalani R, Haider S, Khan MA. Trace element studies on Karachi populations. Part V: Blood lead levels in normal healthy adults and grammar school children. J Pak Med Assoc. 1990; 40:150-4.
26. Sadaruddin A, Agha F, Khatoon N, Sultana K. Blood lead levels in young children in Chakshahzad, Islamabad. J Pak Med Assoc. 1995; 45:215-7.
27. Hafeez A, Malik QU. Blood lead levels in preschool children in Rawalpindi. J Pak Med Assoc. 1996; 46:272-3.
28. Rahbar MH, White F, Agboatwalla M, Hozhabri S, Luby S. Factors associated with elevated blood lead concentrations in children in Karachi, Pakistan. Bull World Health Organ. 2002; 80:769-75.
29. Rahman A, Maqbool E, Zuberi HS. Lead-associated deficits in stature, mental ability and behaviour in children in Karachi. Ann Trop Paediatr. 2002; 22:301-11.
30. Zakir S, Ahmad B, Hussain T, Bashir S, Ismail M, Badshah A. Lead poisoning in children working at automobile workshops in Peshawar (Pakistan). OnLine J Biol Sci. 2002; 2:390-91.
31. Rahman A, Hakeem A. Blood lead levels during pregnancy and pregnancy outcome in Karachi women. J Pak Med Assoc. 2003; 53:529-33.
32. Khalil M, Rashid J, Khan M, Iqbal M. Determination of serum lead levels in children aged 1–6 years with or without history of pica. Pakistan Ped J. 2004;28:26-30.
33. Agha F, Sadaruddin A, Khatoon N. Effect of environmental lead pollution on blood lead levels in traffic police constables in Islamabad, Pakistan. J Pak Med Assoc. 2005; 55:410.
34. Khan DA, Qayyum S, Saleem S, Khan FA. Evaluation of lead body burden in occupational workers by lead mobilization test. J Pak Med Assoc. 2009; 59:350-4.
35. Khan DA, Qayyum S, Saleem S, Ansari WM, Khan FA. Lead exposure and its adverse health effects among occupational worker's children. Toxicol Ind Health. 2010; 26:497-504