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Full Length Research Paper

Estimation of lead in urine of school children in south western Nigeria and effect of ascorbic intervention

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This study was carried out to investigate the level of lead in the air and urine of pupils in selected schools in south western Nigeria and ascertain the effect of ascorbic acid, with a view to providing baseline data on lead levels in children. A total of 197 pupils in schools in urban and rural communities of Ife Central Local Government, Osun State in the south west of Nigeria (that is Sabo, Iremo and Ajebamidele (n = 119) and Kajola and Abagboro (n = 78), respectively), The pupils in both communities were randomised into experimental and control group, the experimental group were placed on 500 mg of ascorbic acid daily for five days. Urine samples were collected on the first, third and fifth day of the study from the pupils in experimental and control groups and analysed for lead using Atomic Absorption Spectrophotometer. Air samples were collected using the LaMotte air sampling pump and analysed for lead using the same method. The cross sectional survey indicated that the mean lead levels in the air in the school environment in the urban communities on the first, third and fifth day of the study were (8.69 ± 0.070 µg/m³, 9.27 ± 0.09 µg/m³, 9.27 ± 0.09 µg/m³) respectively and significantly higher than lead levels in rural communities (3.73 ± 0.030 µg/m³). The lead concentration in the urine of the pupils in experimental group in urban (5.51 ± 1.07 µg/m³) and rural communities (5.27 ± 0.98 µg/m³) were similar on the first day of the study. The ingestion of ascorbic acid increased excretion of lead in the urine of pupils significantly (11.22 ± 1.48 µg/m³) on the third day and decreased marginally on the fifth day (9.87 ± 1.20 µg/m³) in the urban communities. Similarly in the rural communities there was a significant increase (11.52 ± 1.41 µg/m³) in the excretion of lead in the urine of pupils on the third day but a marginal increase on the fifth day (12.88 ± 2.27 µg/m³) of the ascorbic acid ingestion. The lead level in the air on the first day of the study had no linear relationship with urinary lead level in the urban and rural communities whilst on the third and fifth day of the study, a linear relationship was observed between lead levels in the air and urinary lead levels of the pupils in the urban and rural communities.

Key words: Urinary lead levels, air lead levels, school children, ascorbic acid intervention

INTRODUCTION

Although lead (Pb) in the atmosphere comes from various sources, leaded-gasoline is probably the primary source of Pb exposure in large cities in most developing countries (Massadeh and Snook, 2002; Odukoya et al., 2000; Von Schirnding and Fuggle, 1996).

Lead poisoning as a health issue has, therefore, been described as a silent epidemic and children have been considered as a special risk group for Pb exposure; they absorb Pb more readily than adults (Centers for Disease Control and Prevention, 1991; Fels et al., 1998).

Childhood Pb poisoning is also an important, preventable environmental disease, affecting millions of children around the world.(Heinze et al., 1998; Schutz et al., 1997; Zejda et al., 1997).

The other sources and pathway of exposure to lead apart from automobile emissions especially for children are ingestion of chips from lead –painted surface and food in lead soldered containers. The risk of exposure

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varies greatly depending on where one lives (McMicheal, 1993). Lead exposure in young children is cumulative and its range of negative health effects appear to be irreversible, the most severe being damage to the central nervous system and death (Bellinger and Dietrich, 1994; Malcoe et al., 2002).

First, it is now well known that chronic exposure to Pb during development is associated with a variety of cognitive and neurobehavioral dysfunctions in children.

Numerous studies have shown that children with only moderately increased blood lead (B-Pb) levels (that is 10 - 15 mg/dL) suffer disproportionately from cognitive and neurobehavioral deficits, including lower intelligence quotient scores and diminished attention span, bilateral co-ordination, visual-motor control, upper-limb speed, dexterity, and fine motor skills (Bellinger and Dietrich, 1999a b; Dietrich et al., 1994; Malcoe et al., 2002, Needleman et al., 1990; Wasserman et al., 1997).

Second, there is mounting evidence of deleterious effects associated with B-Pb even below 5 mg/dL; these include hearing loss, adverse hematological effects, dental caries, and diminished cognitive and academic skills (Goyer 1991; Lanphear et al., 2000; Moss et al., 1999; Schwartz, 1994; Schwartz and Otto, 1991).

In Nigeria it was estimated that 15 - 30% of the children in urban areas have blood lead levels higher than 25 µg/dl (Nriagu, 1996) which is above 10 µg/dl that is considered by the United State of America CDC as an action level. This level is associated with about two and a half decrease in intelligent quotient (I.Q).

The Advisory Committee on Childhood Lead Poisoning Prevention reviewed the evidence for dietary intervention in lead-exposed children (Centres for Disease Control and Prevention, 2002). They concluded that there is no trial data supporting dietary interventions aimed specifically at preventing lead absorption or modulating the effects of lead. However, there are laboratory and clinical data suggesting that adequate intake of iron, calcium, and vitamin C are especially important for these children. Adequate iron and calcium stores may decrease lead absorption, and vitamin C may increase renal excretion. Recent children studies in urban population in Karachi indicate that treatment with vitamin C (500 mg)causes increased urinary excretion of lead in the first few days of treatment and brings the urinary lead concentration to normal levels within two weeks (Gilani et al., 2005).

The above concerns apply to children in every society and there has been an increasing recognition of the importance of focusing on children everywhere by World Health Organisation (WHO, 2002) and many national and international organisations (Suk et al., 2003). In Nigeria, one of the most rapidly industrialising countries in Africa is currently facing a challenge of a clean environment. Protecting the health of the children from both physical and psychological harm and toxic exposures may ensure a healthy population in the next generation. In addition, the curative potential of Vitamin C in lead detoxification is yet to be ascertained in this part of the world despite the fact, fact, lead levels are unusually high particularly in urban areas. This study was therefore carried out to estimate the air and urinary level of lead in children in urban and rural communities in Osun State, in the southwest Nigeria and the effect of vitamin C supplementation.

METHODOLOGY

The study was a cross sectional survey conducted in two communities of Ife Central local Government Area. Aba Gbooro and Kajola (that is rural site located about 5 and 1 km away from national road) and Sabo, Iremo and Ajebamidele Ile-Ife (that is urban site characterised by heavy motor vehicle traffic). The five schools (three urban and two rural) were selected from the communities by random sampling technique. Two hundred pupils in primary 3 and 4 were recruited from the selected schools using probability by propionate sampling technique that is the pupils were recruited from the urban and rural schools in proportion to their estimated population. One hundred and twenty pupils in urban schools and eighty pupils in rural schools. In order to be included in the final analysis, the children had to fulfil the following criteria: aged 6 - 12 years and present no obvious pathology. The present epidemiological study was conducted in accordance with national and/or institutional guidelines for the protection of human subjects. The purpose and risks of the study protocol were explained to parents, and their consent was obtained prior to sample collection.

Air samples were collected in the school environments using the air sampler LaMotte Air Sampling pump (Model BD) on first, third and fifth day of the study in the mornings. The sampler was placed outside the classrooms and allowed to run for 2 h as indicated in the manual. Lead absorbing solution is placed in impinge tube and a flexible tube connects the impinge to the air sampling pump which is operated with batteries. The lead absorbing solution after each days ' visit to the schools was poured into a plastic tube and 10 mls of 1% concentrated nitric acid was added and the labelled sample is sent to the central laboratory for lead analysis using the Atomic Spectrophotometer.

Spot urine samples of children recruited were collected directly into 100 ml disposable bottles on first day of visit in the morning. The pupils were randomised into experimental and control group in the urban and rural schools by dividing pupils into half. One half which are the experimental group were given 500 mg of ascorbic daily, the drugs were taken by the pupils in the morning under the supervision of the investigators and the teachers. Spot urine samples were collected at close of school on days, 3 and 5 of the study. The samples were diluted with 10 mls of one percent nitric acid and sent for further analysis using the Atomic Absorption Spectrophotometer. Blood studies could not be carried out for lack of cooperation due to cultural belief of the people within the environment.

After dilution of the urine samples, the samples are labelled and taken to central laboratory for analysis. A blank reagent was prepared with 2.00 mls of distilled water and a calibration curve was prepared using standard solution and 10 and 5 ppm level of concentration (µg/100 ml). The absorbance of the working standard corrected for the blank reagent was plotted. The most sensitive absorption line was listed first followed by measurement of the less sensitive line. For the measurements, two determinations were made from each sample and the mean lead rate obtained.

A copy of the proposal was sent to the Commissioner for Education in the state who sent it to the appropriate unit for thorough scrutiny and the accompany application for permission to carry out the project was endorsed for approval by the commissioner and liaison education officer in the local government. The purpose of the study was explained to the school managements who were use of represented by the head teachers and pupils were given informed consent form home to be presented to the parents and parents to

Table 1. Demographic characteristics of the pupils.

Characteristics	Urban n = 119		Rural n = 78	
	Experimental group	Control group	Experimental group	Control group
Age				
6 - 9 yrs	23	37	25	15
10 - 12yrs	35	24	13	25
Sex				
Female	21	16	11	13
Male	37	45	27	27

Mean age of experimental group 9.13 ± 1.82, Mean age of control group 9.40 ± 1.89.

Table 2. Concentration of lead (µg/m³) in air in the school environments.

Location	Lead concentration (Day 0)	Day 3	Day5
Urban	8.69 ± 0.070	9.27 ± 0.09	9.27 ± 0.09
Rural	3.73 ± 0.030	3.73 ± 0.03	3.73 ± 0.03
T test	21.9	48.9	48.9
P value	0.001 (sig)	0.001(sig)	0.001 (sig)

Mean ± S.E.M

Table 3. Concentration of lead (µg/dl) in urine before ascorbic acid administration.

Treatment	Urban	Rural	t test	p value
Experimental group	5.51 ± 1.07	5.27 ± 0.98	0.16	0.87
Control group	6.11 ± 0.99	8.01 ± 1.51	1.09	0.28

Values are in mean ± S.E.M.

sign. The consent form was written in English and the local language.

The statistical analysis of the data collected employed the STATA 8 software. The mean concentration of lead in the air environment and urinary lead levels of pupils in the urban and rural schools were compared using the student t test .The concentration of lead in the air environment were also correlated with urinary lead levels of the pupils. The level of significance was determined at p less than 0.05.

RESULTS

In the final analysis, 119 pupils (58 in the treatment group and 61 in the control group) were included in the cross sectional study as one of the pupils in the treatment opted out of the study and another pupil crossed from the treatment group to the control group. In the rural schools, 80 pupils (38 in treatment group and 40 in the control group) were included in the cross sectional study as two pupils in the treatment group were lost to follow up, didn't show up in school the last day of the study.

Table 1 summarises the group demographic characteristics. Most of the pupils that took part in the study were males and were equally distributed in the age groups 6 - 9 years and 10 - 12 years.

Table 2 depicts the concentration of lead in the air of urban and rural schools. Lead concentration in the air at urban schools was more than 130% higher than that of the rural schools ($p < 0.05$).

The children in rural schools excreted similar concentrations of lead in the urine when compared to children in the urban schools (Table 3).The ascorbic acid increased urinary excretion of lead significantly in the urban and rural schools on the third day of the study in the experimental group ($p < 0.05$). There was no significant difference in the urinary lead levels in both schools ($p > 0.05$). Urinary lead levels were higher in the experimental groups in both schools (Table 4).The concentration of lead in the urine of pupils in urban schools dropped marginally by 12% on the fifth day while there was an increase of 12% in the urinary lead concentration in the rural schools. There was no significant difference in the lead levels in the urine of pupils in the experimental and control groups however the level is still higher in the experimental group (Table 5). The lead level in the air in the school environment showed a non linear relationship with the urinary lead levels in both schools on day 0 whilst it demonstrated a linear relationship on 3 and 5 days (Table 6).

Table 4. Concentration of lead (µg/dl) in urine on the third day after ascorbic acid ingestion

Treatment	Lead concentration			
	Urban	Rural	t-test	p value
Experimental group	11.22 ± 1.48	11.52 ± 1.41	0.14	0.89
Control group	8.51 ± 1.29	7.99 ± 1.51	0.26	0.80

Values are in mean ± S.E.M.

Table 5. Concentration of lead (µg/dl) in urine on the fifth day of ascorbic acid ingestion.

Treatment	Lead concentration			
	Urban	Rural	t-test	p value
Experimental group	9.87 ± 1.20	12.88 ± 2.27	1.27	0.21
Control group	9.29 ± 1.30	9.83 ± 1.92	0.24	0.81

Table 6. Result of analysis of relationship between mean concentration of lead in the air and urine of pupils in the schools.

Regression analysis	Area					
	Urban			Rural		
	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5
ß coefficient	0.0060	-0.0357	-0.1217	-0.119	0.0178	0.0229
Standard Error	0.1530	0.5022	0.4623	0.3408	0.3525	0.4990
P value	0.94	0.71	0.79	0.30	0.89	0.85

DISCUSSION

Blood lead concentration are the internationally accepted measure of lead exposure, this study had to be carried out on urine samples due to inaccessibility to blood samples. Studies have shown that urine is a potentially useful exposure indicator and can be used to assess total lead excretion as a function of the dose of ascorbic acid given. However, it has received limited attention because of potential contamination during sampling and analysis.

The concentration of lead in the urine of pupils in experimental group in the urban and rural schools before ascorbic acid were below the ATSDR lead levels in urine for children age 6 years and above (0.677µ/L). However the values in the control group in both schools were similar or slightly the ATDSR lead level in urine for the age groups (ATSDR, 2007). There was no significant difference in the lead concentration in the urine of pupils in rural and urban schools before ascorbic acid intake ($p > 0.05$). This finding was not in agreement with the finding of urine lead concentrations being significantly higher in urban school children than in rural school children in the city of Mazini in Swaziland (Okonkwo et al., 2001). The presence of lead in the children in rural schools could be due to contributory factors such as overcrowding, hygienic habits in the household, wood fires used for cooking, presence of local smelters and nutritional status of the children.

Ascorbic acid ingestion increased urinary lead excretion significantly in experimental group on the third day in both urban and rural schools This finding supported the report of an increase in the mean urine lead concentration after treatment with 500 mg vitamin C (Gilani et al., 2005) The increase in the urinary lead levels in the control group in both schools suggests an interplay of multiple factors such as nutritional status of the pupils, regularity of meals and micronutrient deficiencies in addition to exposure to multiple sources of lead. In addition, the fact that there are unrestricted accesses to natural fruits such as oranges, and mango intake rich in vitamin C among rural dwellers suggest that the excretion of Pb may be continuous.

The reduction in the concentration of lead in urine in experimental groups in the urban schools on fifth day supported the report of a previous study that ascorbic treatment causes increase urinary lead concentration the first few days and brings the concentration back to normal levels later (Gilani et al., 2005).

The concentration of lead in the air in the urban and rural schools observed was far above the recommended WHO air quality guideline for lead (0.5 µg/m^3). The lead concentration in the air in the urban schools was far above the lead concentration of 5 µg/m^3 reported in Lagos, an urban city in Nigeria (Nriagu, 1992). The significant difference in the mean lead concentration in air in urban and rural schools supported the report that the concentration of lead in air varies from 2 - 4 µg/m^3 in large cities with dense automobile traffic to less than 0.2 µg/m^3 in most suburban areas and still less in rural areas. The finding in the study was also supported by the report of high lead concentration in air and soil in urban areas due to ever increasing automobiles especially those using leaded gasoline (Nriagu et al., 1996)

The study observed a non linear relationship between the lead levels in air and urine on day 0 before ascorbic acid ingestion in both schools; this might be due to similarity in the lead levels. The estimates of the slope were between 0.006 and -0.119. This showed a lower estimate as compared with estimates of the slope between 0.072 and 0.144 µmol/L / µg/m^3 reported by Chamberlain et al. (1983). A linear relationship was observed on third and fifth day of the study, this might be as a result of increased excretion of lead in the urine and minimal or no increase in the level of lead in the air in urban and rural schools. This report was further corroborated by a previous study that describes a linear relationship between air lead concentrations of 0.1 - 2.0 µg/m^3 and blood lead levels less than 30 µg/dl (Colombo, 1985).

The high value of lead in the air in the school environment and high values of lead in urine of children in urban and rural schools observed in this study points to the public health implication of lead exposure and its effects especially for children and needs attention. The increase in urinary lead concentration after ascorbic acid intake suggested that ascorbic acid ingestion could be a useful preventive action by increasing lead excretion in the urine after exposure. Hence high dietary intake of ascorbic acid could be encouraged.

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