Positive and inverse correlation of blood lead level with erythrocyte acetylcholinesterase and intelligence quotient in children: implications for neurotoxicity

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ABSTRACT

Blood lead level (BLL) is insufficiently sensitive for early detection of Lead-induced neurotoxicity (LIN). This study determined the possible role of the combination of BLL, intelligent quotient (IQ) and erythrocyte acetylcholinesterase (AChE) activity in the early detection of LIN in Children. Apparently healthy children (n=309) from eight public primary schools in Ibadan, Nigeria were recruited and classified into: children with Elevated BLL (EBLL) and children with Acceptable BLL (control) based on CDC cut-off for childhood lead exposure. Neurological indices (speech, memory, cranial nerves and cerebellar functions), IQ, BLL and erythrocyte AChE activity were assessed using standard methods, Standard Progressive Matrices, AAS and HPLC respectively. Statistical analysis involved Student’s t-test, Pearson’s correlation and multivariate regression. p<0.05 was considered significant. There were 169 (54.7%) children with EBLL while there were 140 (45.3%) control children. Both groups exhibited normal speech, memory, cranial nerves and cerebellar functions. However, IQ was lower in EBLL children (85.9±11.6) compared with control (91.5±14.0) while BLL and AChE activity were higher in EBLL children (0.4±0.1 μmol/l; 117.5±25.5 μkat/l) compared with control (0.2±0.0 μmol/l; 59.4±10.2 μkat/l). BLL showed inverse correlation with IQ (r=–0.134, p≤0.001) but positive correlation with AChE (r=0.978, p≤0.001). 16.2% of the observed variation in BLL could be accounted for by AChE using the equation; [BLL=–0.007+0.003 AChE] p<0.05. Elevated blood lead level is prevalent among the school children and appears to have adverse effect on their IQ. Erythrocyte AChE could be a promising marker for early recognition of significant environmental lead exposure and lead-induced neurotoxicity in children.

KEY WORDS: erythrocyte acetylcholinesterase; intelligence quotient; lead-induced neurotoxicity

ABBREVIATIONS:
ACh: Acetylcholine; AChE: Acetylcholinesterase; AAS: Atomic absorption spectroscopy; BLL: Blood lead level; CDC: Centers for Disease Control and Prevention; CNS: Central nervous system; EBLL: Elevated BLL; HPLC: High-Pressure Liquid Chromatography; Ha2O2: Hydrogen peroxide; IQ: Intelligent quotient; Pb: Lead; LIN: Lead-induced neurotoxicity; UI/UCH: University of Ibadan/University College Hospital

INTRODUCTION

Lead (Pb) is a naturally occurring ubiquitous toxic metal whose uncontrolled use in multiple industrial, domestic, agricultural, medical and technological applications has brought about its wide environmental distribution, widespread human exposure and subsequent serious health and intellectual concerns particularly in children in developing countries and Nigeria is not an exception (Ogunseitan & Smith 2007; WHO, 2013; Anetor et al., 2016; Nwobi et al., 2019).

Children are exposed to Pb through contaminated food, water, air and dust which, after being absorbed into the blood, is distributed to virtually every organ and system where it causes broad range of toxicity (WHO,
One of the systems of importance and greatest susceptibility is the central nervous system (CNS) where it has the tendency to cause neurotoxicity particularly in the brain of children because of the high absorption rate of Pb coupled with increased penetration of Pb through the blood–brain barrier that is the most sensitive to damage (Bellinger, 2008; Mason et al., 2014).

One of the mechanisms of lead-induced neurotoxicity (LIN) has been reported to be interference in the neurotransmission function of the CNS and of particular interest is its interference with the cholinergic system which may result in impairments in CNS function including neurocognition (Lidsky & Schneider, 2003; Flora et al., 2012; Sharma et al., 2014). Acetylcholinesterase (AChE) is a key enzyme involved in cholinergic neurotransmission which hydrolyses the neurotransmitter acetylcholine (ACh) in order to terminate normal synaptic transmission of impulse and avoid unnecessary stimulation of the nervous system (Saldanha, 2017). This enzyme is a type-B carboxylesterase found mainly in the neuromuscular junction and cholinergic brain synapses but also in human erythrocytes (Hajjawi 2012; Gupta et al., 2015). Several reports have shown that the activity of erythrocyte AChE does not only correlate positively with the activity of the brain AChE but also reflects neurochemical targets in brain (Ademuyiwa et al., 2007; Lionetto et al., 2013; Gupta et al., 2015).

One of the numerous brain functions include neurocognition (Hasselmo, 2006; Haense et al., 2012). Essential factors in neuro-cognition such as concentration, focus, memory and highly-ordered thought processes have been reported to be facilitated by acetylcholine (Hasselmo, 2006). Several reports have linked Intelligence quotient (IQ), a well recognised and widely used index of neurocognition, to blood lead level (BLL) (Slikker et al., 2000; Haense et al., 2012; Liu et al., 2013).

Blood lead level is the most widely used biomarker of lead exposure and achieving BLL limit of 0 μg/dl in children is rarely feasible (Sommar et al., 2014; WHO, 2015). Consequently, the US Centers for Disease Control and Prevention (CDC) since 2012 has identified BLL ≤5 μg/dl as acceptable and BLL >5 μg/dl as elevated blood lead level (EBLL) that should prompt further medical investigation in children (CDC, 2012). However, the use and interpretation of BLL for the early detection of LIN is not generally accepted owing to its short half-life of about 30 days and its ability to reflect primarily recent exposures without possibility of accurately assessing long term risk which has more insidious impact (Sommar et al., 2014; Nwobi et al., 2019).

Lead-induced neurotoxicity is insidious and has the tendency to go unrecognized especially in the early years (WHO, 2009; WHO, 2013; WHO, 2015). This makes it, and its early detection, an area of exceptional importance and substantial current challenge, and therefore calls for the need for continuous search to identify accessible and simple biomarkers for early identification of LIN in children. However, it is worthy to note that the possibility that Pb-induced perturbation of erythrocyte AChE activity may precede alterations in IQ has been poorly explored in the search of biomarkers for LIN particularly in an environment with high chemical burden such as Nigeria (Anetor et al., 2008; Orisakwe, 2014). This study was therefore designed to determine the possible role of the combination of blood lead level, intelligent quotient and erythrocyte acetylcholinesterase activity in the early detection of lead-induced neurotoxicity in Children.

Materials and methods

Study area, study design and study population

This cross-sectional study involved 309 apparently healthy children (aged 8–10 years) who had been resident in Ibadan, Oyo State, South-West, Nigeria for ≥5 years. The children were selected from 8 public primary schools based on multistage random sampling technique while the recruitment of the children from the schools was based on parental consent, assent and physical presence on the day of sampling.

The participating children were grouped into two; children with elevated blood lead level (EBLL) (n=169; 83 boys and 86 girls) and children with Acceptable Blood Lead Level who served as control (n=140; 71 boys and 69 girls). Elevated blood lead level was defined as BLL >5 μg/dl (>0.2415 μmol/l) while acceptable blood lead level (control) was defined as ≤5 μg/dl (≤0.2415 μmol/l) based on the cutoff for childhood blood lead level recommended by the US Centre for Disease Control and Prevention (CDC, 2012).

Exclusion criteria for the children included history of lead exposure requiring chelation therapy, neurological or neurodevelopmental disorder including mental sub-normalities, such as autism, epilepsy, cerebral palsy, evidence of anaemia, malnutrition, liver dysfunction, renal dysfunction or any obvious pathology, intake of mineral supplements or medications such as anticonvulsants, previous failure or repeat in a class, inapposite age for class and inability to perform the cognitive test for whatever reasons.

This study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Research Ethics Committee, Nigeria with approval number: UI/EC/12/0064 as well as the Ministry of Education, Oyo State, Nigeria. The parents/guardian of all the participating children received oral and written information about the study protocol in both English language and their local dialect and signed written informed consent was obtained from those that agreed that their children should participate in the study. Assent from each participating child was also got.

Assessments and blood sampling

All assessments and blood sampling of the participants were carried out on site during regular school days before the beginning of classes between 8 and 10 a.m.

Assessments of anthropometry and neurological indices

Anthropometric, blood pressure and neurological indices such as speech, memory, cranial nerves and cerebellar functions were assessed by a Paediatric Neurologist using standard procedures before blood sampling.
Assessment of intelligence quotient

First, intelligence capacity was assessed by a Clinical Psychologist (before blood sampling) using Raven’s Standard Progressive matrices (Raven et al., 2000). This test is a widely used non-verbal test of intelligence capacity that relies on non-verbal problems that require abstract reasoning. It involves visuo-spatial reasoning, abstract thinking, deductive reasoning and general intelligence and covers widest range of mental ability of individuals (Raven et al., 2000). The Raven’s Standard Progressive matrices has been reported as one of the best instruments for the assessment of IQ in sub-Saharan Africa and has also been validated for use for Nigerian children (Daramola et al., 2010; Iloh et al., 2017).

Instrument: The Raven’s Standard Progressive Matrices consisted of 60 problems divided into five sets (Set A, B, C, D and E), each made up of 12 problems. Each set consisted of matrices of increasing difficulty. While the earlier series required accuracy of visual discrimination, the later ones involved two-dimensional analogies which demanded permutation, alteration of pattern and perception of other logical relations for successful solution. Intelligence capacity was finally converted to Intelligence quotient (IQ) by a Psychometrist using the validated formula; IQi = (100+Zi) × 15 as described by Wicherts et al. (2010).

Blood sampling

Non-fasting blood samples (5 ml) of the participants were collected by a trained Paediatric Phlebotomist into heparinised tubes between 8 and 10 a.m. on site during regular school days, before the beginning of classes. Percentage hematocrit was determined immediately on site using Hawksley micro-HCT reader using Hawksley micro-HCT reader, Hemocue (Angelholm, Sweden). Aliquots of blood samples (2 ml) were separated for lead analysis and the remaining blood samples (3 ml) were for erythrocyte AChE analysis. The obtained blood samples were stored in cooler boxes and transferred from the point of collection to the laboratory either for immediate analysis and/or storage at −20°C.

Biochemical analyses

Lead

Lead analysis was based on the method of Miller et al., 1987, using a graphite furnace Atomic Absorption Spectrometer Perkin-Elmer AAnalyst 800 with Zeeman-effect background correction (Norwalk, U.S.A). Sample preparation involved simple dilution (1+9) with a matrix modifier which contained 0.5% V/V Triton X-100, 0.2% V/V 16 M nitric acid and 0.2% m/V dibasic ammonium phosphate (Miller et al., 1987).

Erythrocyte Acetylcholinesterase Activity

The blood sample was centrifuged at 2500 RPM for 10 minutes to separate plasma and erythrocyte. Erythrocyte were washed twice with phosphate buffered saline before being used for AChE assay. Erythrocyte AChE activity was determined by the method of Miller & Blank. 1991, using Waters 616/626, High Pressure Liquid Chromatography (HPLC) analyser with electrochemical detector system (Young Lin, Seoul, South Korea). The reaction mixture was prepared by mixing 500 µl of 0.1 M phosphate buffer (pH 7.2), 50 µl of Ethylhomocholine bromide (internal standard), 25 µl of 500 mM acetylcholine and 25 µl of the hemolysate (prepared by diluting the washed cells 1:4 with deionized water). Acetylcholine as a substrate, was acted upon by cholinesterase to produce choline and acetate. The product choline was eluted into a post column reactor containing immobilized choline oxidase, where it was oxidized to produce electrochemically active H2O2. The H2O2 produced was directly proportional to the activity of AChE activity (Miller & Blank, 1991).

All reagents and standards used were of analytical grade. Internal controls, certified reference standards as well as samples spiked with known concentrations of the reference material were included in each batch of 20 samples. Calibration curves were obtained using 6 points with the certified standard. After each analytical run, calibration curves were obtained again in check for linearity and replication. A mean recovery rate of >95% was obtained for each element after two determinations. The samples were all analysed in one day and results were only acceptable when data obtained fell within expected quality control samples (X±2SD).

List of chemicals used

Reagents. Acetylcholine chloride, choline chloride, acetylcholinesterase, choline oxidase, diisopropyl phosphorofluoridate, and sesame oil were all purchased from Sigma Chemical Co., Ltd. (St. Louis, MO); potassium phosphate monobasic was purchased from Fisher Scientific Co. (Fair Lawn, NJ); tetramethylammonium chloride, sodium azide, tris(hydroxymethyl) aminomethane (Tris), and ethylenediaminetetraacetic acid, disodium salt, dihydrate (EDTA) were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI). Bromoethane and 3-dimethylaminol-propanol (Aldrich), were employed to synthesize the internal standard, ethylhomocholine bromide (N,N-di-methyl-N-ethyl-3-amino-1-propanol bromide), Octyl sodium sulphate was purchased from Eastman Kodak Co. (Rochester, NY). All other chemicals used were of analytical grade and of the highest available purity and used without purification.

Statistical analysis

The SPSS statistical software program version 21.0 (IBM Inc, Chicago, IL) was used for the statistical analysis. Values were assessed for normality by checking for skewness. Results were expressed as mean ± SD. Independent sample t-test was used to determine differences between children with EBLL and control. Pearson’s product moment correlation analysis was used to evaluate the relationship among BLL, erythrocyte AChE activity and IQ. Step-wise Multiple Regression Analysis was used to model cause-effect relationship between BLL (independent variable) and the erythrocyte AChE activity and IQ (dependent variables). All tests were 2-tailed and p-value <0.05 was considered as statistically significant.
Results

The study population was stable with no attrition and all participants had normal neurological indices such as speech, memory, cerebellar and cranial nerves functions. Children with elevated blood lead level (EBLL) and control were properly matched for gender, age, haematocrit level, anthropometric and blood pressure indices that could be confounders. Thus, suggesting that both groups were comparable because they had relative homogeneity (p>0.05) (Table 1). Out of the 309 children that participated in this study, 169 (54.7%) exhibited elevated blood lead level, while 140 (45.3%) exhibited acceptable blood lead level which also served as the control (Table 1).

Blood lead level (BLL) and erythrocyte AChE activity were significantly increased in children with EBLL compared with control (p<0.05) (Table 2, Figure 1, Figure 2). Remarkably, IQ was significantly decreased in children with EBLL compared with the control (p<0.05) (Table 2 and Figure 2). Blood lead level showed strong significant positive correlation with AChE activity (p<0.05) but significant negative correlation with IQ score (p<0.05) (Table 3).

Multiple regression analysis between BLL (dependent variable) and erythrocyte AChE activity and IQ

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### Table 1. Age, haematocrit levels, anthropometric and blood pressure indices and in children with elevated blood lead level and control.

| Indices               | Participants with EBLL (n=169) | Control (n=140) | t-value | p-value |
|-----------------------|--------------------------------|-----------------|---------|---------|
| Age (years)           | 8.6±1.6                        | 8.9±1.5         | -1.711  | 0.088   |
| MUAC (cm)             | 17.2±1.6                       | 17.1±2.6        | 0.376   | 0.707   |
| OFC (cm)              | 51.3±2.4                       | 51.6±1.6        | -0.865  | 0.388   |
| Weight (kg)           | 23.8±4.8                       | 24.5±4.8        | -1.015  | 0.311   |
| Height (cm)           | 125.0±9.2                      | 126.7±10.1      | -1.285  | 0.200   |
| BMI (kg/m²)           | 15.1±1.5                       | 15.3±1.8        | -0.539  | 0.590   |
| Heart rate/minute     | 92.2±13.2                      | 91.9±10.8       | 0.134   | 0.894   |
| SBP (mm/Hg)           | 96.5±13.4                      | 96.8±11.1       | -0.159  | 0.874   |
| DBP (mm/Hg)           | 55.9±9.0                       | 54.2±7.3        | 1.381   | 0.168   |
| Haematocrit (%)       | 36.6±3.3                       | 36.7±2.5        | 0.867   | 0.387   |

Results are presented as mean ± standard deviation, EBLL = elevated blood lead level, MUAC = mid upper arm circumference, OFC = occipital frontal circumference, BMI = body mass index, SBP = systolic blood pressure, DBP = Diastolic blood pressure.

### Table 2. Blood lead level, erythrocyte acetylcholinesterase activity and intelligence quotient in children with elevated blood lead level and control.

| Indices | Participants with EBLL (n=169) | Control (n=140) | t-value | p-value |
|---------|--------------------------------|-----------------|---------|---------|
| BLL (μmol/l) | 0.4±0.1 | 0.2±0.0 | 17.133 | <0.001* |
| AChE (μkat/l) | 117.5±25.5 | 59.4±10.2 | 17.545 | <0.001* |
| IQ | 85.9±11.6 | 91.5±14.0 | 2.884 | 0.004* |

Results are presented as mean±standard deviation, EBLL = elevated blood lead level, * = Significant at p<0.05 (2-tailed), BLL = blood lead level, AChE = erythrocyte acetylcholinesterase activity, IQ = intelligence quotient.

### Table 3. Correlation of lead with erythrocyte acetylcholinesterase activity and intelligence quotient in the study participants.

| Variables | Correlating pair BLL (μmol/l) | r-value | p-value |
|-----------|--------------------------------|---------|---------|
| IQ score  | -0.134                         | 0.019*  |         |
| AChE (μkat/l) | 0.978           |         | <0.001* |

* = Significant at p<0.05, BLL= blood lead level, AChE= erythrocyte acetylcholinesterase activity, IQ= intelligence quotient.

### Table 4. Relationship between blood lead, cholinesterase activity and intelligence quotient in the study participant.

| β         | t-value | p-value |
|-----------|---------|---------|
| Constant | -0.007  | -1.519  | 0.130   |
| AChE (μkat/l) | 0.003       | 82.888  | <0.001* |

Excluded variable

| IQ         | -0.002  | -0.133  | 0.894   |

Where, BLL= independent variable, AChE and IQ= dependent variables. R²= coefficient of determination, β0= intercept, β=slope of each variable. BLL=-0.007±(0.003) AChE; BLL in μmol/l, erythrocyte AChE in μkat/l; R²=0.162, p=0.005. *= Significant at p<0.05, BLL= Blood lead level, AChE= erythrocyte acetylcholinesterase activity, IQ= intelligence quotient.
(independent variables) is shown ($R=0.162$, $R^2=0.026$, $p=0.005$) (Table 4). Blood lead level showed significant positive relationship with AChE (Model Coefficient: $B=-0.007; \beta=-0.003; p<0.001$) (Table 4) which implies that 16.2% of the observed variation in BLL could be accounted for by AChE activity using the linear equation between BLL and AChE; BLL ($\mu$mol/l) = $-0.007+0.003$ AChE ($\mu$kat/l) (Table 4 and Figure 3). However, the model did not show significant relationship with IQ (Model Coefficient $B=-0.007; \beta=-0.002; p>0.05$) (Table 4).

Discussion

Lead-induced neurotoxicity (LIN), a condition whose insidious effect could be unrecognised particularly in the early years, remains a topic of substantial concern and interest particularly in developing countries where about 99% of 600,000 new cases of children with intellectual disabilities as a result of lead exposure reside (WHO, 2013; Sharma et al., 2015). Thus, this study attempted to determine the possible role of the combination of blood lead level, intelligent quotient and erythrocyte acetylcholinesterase activity in the early detection of lead-induced neurotoxicity in Children.

The study showed that 54.7% of the total participants had blood lead levels greater than the current CDC cut-off for acceptable limit (5 μg/dl) (0.2415 μmol/l) (CDC, 2012), which implies a high prevalence of increased lead exposure in the children. The prevalence of childhood lead exposure in Nigeria was reported as 25% in 2008 (Nriagu et al., 2008). However, this prevalence was determined when BLL of 10 μg/dl (0.4830 μmol/l) was the accepted as the lowest level of medical concern for children (CDC, 991). The difference between observed prevalence in this study and the documented prevalence could at least in part, be explained by the recent reduction in the cut-off value for elevated blood lead level from 10 μg/dl to 5 μg/ dl (CDC, 2012). Consequently, applying this recent CDC recommended cut-off in a chemical laden environment could make the burden of lead toxicity much greater especially given the fact that the already estimated health and educational cost of every 1 μg increase in blood lead level in Nigerian children is as high as US $0.38–$1.15 billion (Ogunseitan & Smith, 2008).

Lead toxicity may be explained by its interference with the activity of the most enzymes. Lead could bind to the thiol-groups of these proteins or displace some essential metal ions that are necessary for their normal functioning (Sharma et al., 2015). However, the mechanism by which lead alters AChE activity is still incompletely understood as this enzyme does not contain free thiol groups in its structure to which Pb could bind (Rosenberry & Soggin, 1984; Ademuyiwa et al., 2007). However, the mechanism may be dependent on calcium, a nutritionally essential element involved in the regulation of many neurological processes, with which Pb competes at protein binding sites (Florea et al., 2013; Brini et al., 2014; Nwobi et al., 2019).

Calcium normally activates Protein Kinase C (PKC), a phospholipid-dependent enzyme that is found in high concentrations in neuronal tissues where it participates in several signal transduction cascades by regulating neurotransmitter release and neuronal ion channels such as calcium channels involved in cholinergic neurotransmission (Hwang et al., 2002; Brini et al., 2014). However, reports have shown that Pb, even at picomolar concentrations, can substitute calcium in the activation of this enzyme possibly resulting to abnormally increased PKC activity (Long et al., 1994; Hwang et al., 2002; Brini et al., 2014). The observed increased erythrocyte AChE activity in the children with EBLL coupled with its positive relationship with blood Pb may be accounted for, at least in part, by the breakdown in normal homeostatic function of calcium resulting to abnormally sustained lead-induced PKC activation. This possibly impacted the presynaptic calcium ion channel and increased the level of acetylcholine released into the synaptic cleft which subsequently resulted to the concomitant increase in AChE activity observed in this study (Florea et al., 2013; Brini et al., 2014).

Acetylcholine, a key factor in neuro-cognition (Soreq & Seidman, 2001; Hasselmo 2006), is a cholinergic neurotransmitter which binds briefly to the postsynaptic acetylcholine receptors for the chemically-gated ion channels in the postsynaptic membrane to open for impulse transmission. The observed reduced IQ in children with elevated blood lead levels and the inverse relationship between blood lead levels and IQ may, at least in part, be
accounted for by the lead-induced acetylcholine receptor desensitisation resulting to decreased responsiveness of the receptors to acetylcholine, diminished stimulatory effect of acetylcholine at the post-synaptic membrane and reduced efficiency of cholinergic neurotransmission which possibly manifested as the observed reduced IQ. Several reports have shown that lead not only desensitises but also reduces the operations and the aggregation of acetylcholine receptors (Morley et al., 2003; Chen et al., 2005; Badawoud & Hassan, 2013). This inhibits the action of acetylcholine at the postsynaptic membrane and reduce ion flow (Quick & Lester, 2002) which regulates impulse transmission and inadvertently negatively impact intelligent quotient. This observation is in line with other reports that emphasised the existence of an inverse relationship between blood lead level and different measures of neuro-cognition (Canfield et al., 2005; Tellez-Rojo et al., 2006 and Surkan et al., 2007).

The reduced IQ may have some adverse societal consequences such as learning difficulties and increase in the number of children who are school drop-outs because they cannot cope well with intellectual abilities in school. The consequences may be worrisome to the affected families because of extra counselling and financial burden as these children may require several attempts, special education and remedial programs before they can succeed in their study. These children may also not contribute fully to the development of the society when they become adults resulting to societal poor economic development and leadership. Thus, there is need for sensitive, reliable early indicator of lead-induce neurotoxicity in the paediatric population particularly in our environment.

The significant multivariate regression analysis involving blood lead level, erythrocyte acetylcholinesterase and intelligent quotient showed that blood lead level could be predicted by erythrocyte acetylcholinesterase activity based on the equation (BLL (μmol/l) = –0.007 + 0.003 AChE (μkat/l)) (R²=0.026, p=0.005). This relationship appears to suggest that erythrocyte AChE activity could account for BLL and may imply that erythrocyte AChE activity, if measured regularly in children exposed to similar duration and level of lead, might act as a surrogate index for LIN.

Taken together, elevated blood lead level is prevalent among the school children in Ibadan, South-West, Nigeria and appears to have adverse effect on their intelligence quotient. However, the multivariate regression analysis involving blood lead level, erythrocyte acetylcholinesterase and intelligent quotient showed that blood lead level could be predicted by erythrocyte acetylcholinesterase activity based on the equation (BLL (μmol/l) = –0.007 + 0.003 AChE (μkat/l)) (R²=0.026, p=0.005). Thus, the activity of this enzyme appears to be a promising marker for early recognition of significant environmental lead exposure and lead-induced neurotoxicity in children. This study provides a valid scientific basis for the possible role of the combination of blood lead level, erythrocyte acetylcholinesterase activity and intelligence quotient in the early detection of lead-induced neurotoxicity in the paediatric population.

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