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Elevated Blood Lead level, Iron Deficiency/Anemia and Child Psychomotor Development in Benin, Sub-Saharan Africa

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List of acronyms

ADHD	Attention deficit hyperactivity disorder
BLL	Blood lead level
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CRP	C-reactive protein
DALYs	Disability-Adjusted Life Year
ELC	Early learning composite
HOME	Home observation for the measurement of the environment
ID	Iron deficiency
IQ	Intelligence quotient
MSEL	Mullen Scale of Early Learning
SD	Standard deviation
WHO	World Health Organization

Abstract

BACKGROUND: Lead in childhood is well known to be associated with poorer neurodevelopment. However, the number of studies in children below two years is limited, especially in Sub-Saharan Africa. To date, few authors have specifically tried to answer the question of whether infants with iron deficiency/anemia are more susceptible to the neurotoxic effects of lead.

OBJECTIVES: Investigating the association between post-natal blood lead level and psychomotor functions in Beninese infants. Studying the interaction between blood lead level, iron deficiency/anemia at birth and at one year of life, and psychomotor functions.

METHODS: A cross-sectional study was performed in three health centers located in the district of Allada, South Benin. Psychomotor functions were assessed by Mullen Scale of Early Learning in infants aged 12 months between May 2011 and May 2013. Blood draw was performed to assess BLL, serum ferritin and hemoglobin. Information on socio-economic status and home environment were further gathered during a home visit. Multiple linear regressions were performed to assess the association between BLL and psychomotor functions. Stratified analyses on iron deficiency and anemia status were done to assess the interaction with BLL.

RESULTS: 747 infants were assessed for psychomotor functions at one year. The mean score was 98.6 points (SD± 13.6) for the early learning composite score and 51.2 points (SD±14.3) for the gross motor score. The mean difference in gross motor scores between the highest and lowest quartile of BLL increased by 6.69 points($p < 0.0001$). Among infants with ID at one year, a decrement by -5.18 points ($p = 0.05$) was observed between early learning composite score and BLL.

CONCLUSION: There was an association between gross motor scores and BLL. The effect of BLL on cognitive function was not clear in infants at one year. An interaction was observed on the gross motor scores between BLL and anemia at one year. Reassessment of children at older age should be considered to further investigate potential associations.

Keywords: Neurodevelopment, lead, iron deficiency, anemia, childhood, interaction, Sub-Saharan Africa.

Résumé

Contexte: Le saturnisme dans l'enfance est associé à un retard de développement neurologique. Néanmoins, les études chez les enfants de moins de deux sont limitées, notamment en Afrique subsaharienne. Peu d'auteurs se sont intéressés à savoir si les enfants présentant une carence martiale/ anémie sont plus exposés aux effets neuro-toxiques du plomb.

Objectifs: Explorer le lien entre la plombémie post-natale et les fonctions psychomotrices des enfants béninois, puis entre la plombémie, le déficit en fer/anémie, à la naissance et à un an de vie, et les fonctions psychomotrices.

Méthode: Une étude transversale a été réalisée dans trois centres de santé en Allada dans le Sud du Bénin. Les fonctions psychomotrices ont été évaluées via l'échelle d'apprentissage précoce de Mullen chez des enfants de 12 mois, entre mai 2011 et mai 2013. La plombémie, la ferritine sérique et l'hémoglobinémie ont été mesurées. Des informations sur le statut socio-économique et environnemental ont été collectées sur visite des foyers. La relation entre la plombémie et les fonctions psychomotrices a été rapportée sur des courbes de régressions linéaires. Des analyses stratifiées sur la carence martiale et l'anémie en lien avec la plombémie ont été réalisées.

Résultats: 747 enfants ont été évalués durant une année. La moyenne était de 98,6 points (écart-type de +/- 13,6) pour le score composite de l'apprentissage précoce et 51,2 points (écart-type de +/- 14,3) pour la motricité globale. La différence moyenne sur les scores de la motricité globale entre le quartile le plus élevé et le plus faible de la plombémie augmentait de 6,69 points ($p < 0,0001$). Chez les enfants présentant une carence martiale à un an, une régression de -5,18 points (= 0,05) a été observée entre le score composite de l'apprentissage précoce et la plombémie.

Conclusion: Il existe un lien entre les scores de motricité et la plombémie. Son effet sur les fonctions cognitives n'était pas clair chez les enfants d'un an. Une interaction a été observée sur les scores de la motricité globale entre plombémie et anémie à un an. Une ré-évaluation des enfants à un âge plus avancé devrait être envisagée pour préciser les liens éventuels.

1. Introduction

1.1. Neurocognitive development

Over 200 million children less than five years old who are living in low and middle income countries are not achieving their developmental potential [1]. Consequently it could be responsible for severe outcomes as they negatively affect the quality of life, diminish scholar and academic achievements and disturb behavior. The consequences could be permanent and have delayed effects later in life. The developing human brain is one of the organs which is very susceptible to the toxic environmental hazards and there are two main periods when the human brain is uniquely vulnerable to these insults: in utero during the developmental stage of the fetus and during the early years of life from one to five years of age [2].

1.2. Risk factors for poor child development

Child development is the ordered emergence of interdependent skills of sensori-motor, cognitive-language, and social-emotional skills. Poverty and socio-cultural factors (Figure 1) augment the exposure of those children to numerous risk factors that affect the development of the children [3].

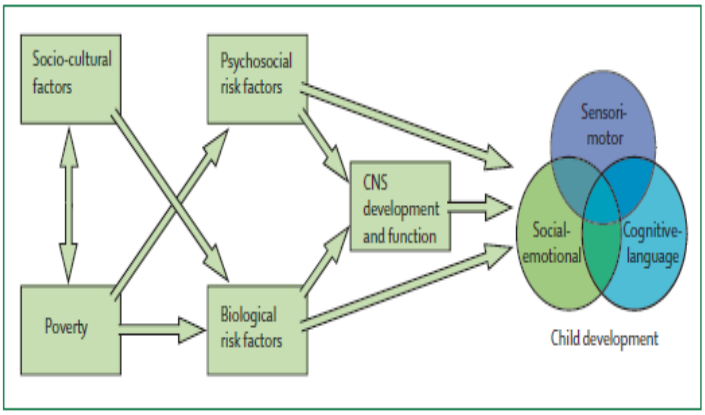

Figure 1: Pathways from poverty to poor child development [3]

These risk factors, which we could refer to as biological, environmental exposures, and psychosocial risks include low birthweight (<2500 g; ≥37 weeks' gestation) which affects 11% of all births in developing countries [3]. Nutrient deficiencies include iodine deficiency, which can cause irreversible mental retardation, iron deficiency, and other micronutrient deficiencies such as zinc, vitamins A and B12 [1,3]. One third of the world's population is estimated to be zinc deficient. Infectious diseases like malaria, intestinal helminthes, HIV/AIDS, and diarrhoeal diseases, affect development through direct and indirect pathways, [2,3]. Annually, there are 300 to 600 million clinical episodes of malaria, with huge burden affectingchildren under five years in Sub-Saharan Africa [3]. HIV/AIDS infection in infancy can cause severe encephalopathy. Many environmental toxins like lead, arsenic, methylmercury, manganese, and fluoride have been proven to be developmental neurotoxins [1-3]. Socio-cultural risk factors include gender inequity, socioeconomic class, low maternal education, and reduced access to services [3]. Psychological risk factors include cognitive stimulation and caregiver-child interaction, which promote age-appropriate language and problem solving skills. Contextual risk factors like

maternal depression with 3% to 60% prevalence rates across developed and developing countries, and exposure to violence specially in developing countries where large number of children are exposed to community and political sectarian violence or to war [3].

1.3. Lead

Lead or Plumbum (Pb) in Latin is a heavy metal from the carbon group [16], with a blood half-life time of nearly 30 days [4]. Lead has no biological role in the human body [7] and it is excreted in urine and bile at a clearance rate of 1-3 mL/min. The half-life time of lead in the bone ranges from 20 to 30 years. Pregnancy, menopause, or lactation are physiological processes during which there is an increased rate of turnover of lead from bone into bloodstream [4]. Measurement of the lead level in body could be assessed in blood in which blood lead level BLL reflects acute exposure, and in bone lead level which better reflects overtime cumulative exposure [4,5].

1.3.1. Acceptable lead levels

The first description of lead poisoning in young children came from Australia over 100 years ago [8,16]. Since then, particularly in Europe and North America, the development of research in this area has been expanded. According to the World Health Organization (WHO), about 800 000 children were affected by exposure to lead each year, and it is the sixth most important contributor to the global burden of diseases measured in disability adjusted life years (DALYs). Sub-Saharan African countries are for the most part responsible for the global DALYs [32,33]. By measuring blood lead level (BLL) in venous blood as an index, the maximum accepted level, set by the American Centers for Disease Control (CDC), for children during the 1960s was 60µg/dl and in the following decades, the level used to define elevated blood lead level by CDC was revised downward several times (Figure 2)[9,10].These changes were based on accumulative evidence from different epidemiological studies showing the adverse effects of lead on children's neurodevelopment. But epidemiological studies have not succeeded to find degree of evidence of a threshold for neurological effects [4]. Economically speaking, US $ 50 billion is the estimated annual cost of childhood lead poisoning in the USA. For every US $1 spent to decrease lead hazards it produces a benefit of US $ 17 to 220, which represents a cost benefit ratio that is even better than that for vaccines [2].

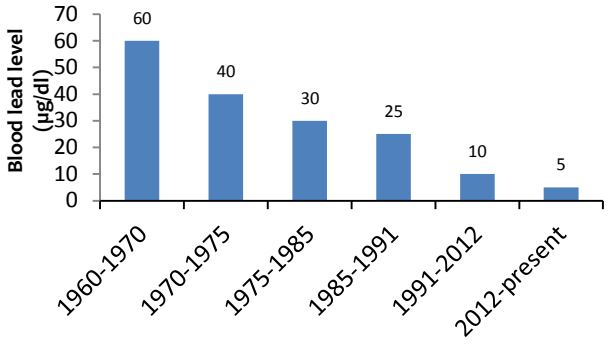

Figure 2: Lowering of CDC definition of elevated BLL (µg/dl) over time. Graph based on data reported in references 10 & 14

1.3.2 Hazards of lead in children

Pediatric exposure has been shown to cause far more severe outcomes than adult exposure and could cause long term consequences especially in learning and overall intelligence [2]. In low and middle income countries, old painted walls, mining, smelting, battery recycling, piped water and electronic waste are the principal sources of lead [7]. The elimination of lead from the brain is very slow because of its long half-life time (two years) [13]. Moreover, once in the brain, it cannot be removed by chemical chelating agents (Rogan et al., 2001) [13]. Accordingly, even though the BLL is reduced to seemingly low concentrations, the deposited lead in the brain keeps to cause its neurotoxic effects. Consequently, once a high BLL is detected, it is too late to stop or prohibit the harmful effects of lead on the growing brain. Thus, the only way to prevent the harmful effects of lead on brain is by preventing it from getting into the infant's blood especially during the critical stages of brain development [13].

High BLL is associated with decreased IQ level, decreased hearing, impaired peripheral nerve function and decreased growth [10]. It is also associated with different forms of behavioral changes from hyperactivity, attention deficit hyperactivity disorders (ADHD), to aggression, delinquency in schoolchildren, and higher rate of arrest in adolescents and young adults. There is also increasing evidence of early lead exposure connecting to a higher rate of antisocial behavior including violence [7].

However, the number of studies in very young children (below 2 years of age) is limited, especially in sub-Saharan Africa.

1.3.3 Mechanisms of lead toxicity

There are many mechanisms involved in lead toxicity which could be summarized as competition with and substitution for calcium (Ca^{2+}). This mechanism is an important factor responsible for neurotoxicity as lead's ability to pass through the blood brain barrier is due to its ability to substitute for calcium ions [4,13]. Other mechanisms are incriminated in lead toxicity like stimulation of calcium release from mitochondria, inhibition of anti-oxidative enzymes (e.g. superoxide dismutase), alteration of lipid metabolism, substitution for zinc, accumulation in brain by astrocytes, sequestration and mobilization of lead from bone stores, long half-life in brain (two years) and slow release from sites of accumulation [13].

Lead can manifest its neurotoxic characteristics through direct and indirect effects : the direct effects through neuronal death by apoptosis (programmed cell death) and mitochondrial damage, effects on intraneuronal regulatory mechanisms, effects on neurotransmission by alteration of neurotransmitter release and changes in neurotransmitter receptor density. The most affected neurotransmitters are noradrenaline, dopamine, acetylcholine, and dopamine, which have an important role in the regulation of our emotional, cognitive, locomotive responses [18]. In addition to, lead e affects the glia impairing the development of the

oligodendrocytes, disrupting myelin morphology which leads to irregular and loose sheaths and membrane fluidity [4,13]. The indirect neurotoxic effects through causing anemia at a BLL of 40µg/dl [10,15] by both disrupting with haem biosynthesis in the bone marrow, reducing erythropoietin (EPO) levels in adults and children, decreasing the life span of the red blood cells and by lowering iron absorption in the duodenum. Other indirect effect is through disruption of thyroid hormone transport into the brain [4,13,19].

1.3.4 Moderating factors

A general principle of toxicology is that numerous elements either increase or decrease the vulnerability to a toxin [13]. Even at low levels of lead exposure, many variables including time of exposure, dose, and individual susceptibility and other moderating variables interact in a complex way [18]. The vulnerability of the brain to lead's toxicity is influenced by such diverse factors as genes and socioeconomic status. At least there are three known genes which could augment the accumulation and toxicokinetics of lead in the human body; δ-amionlevulinic acid (ALAD) gene, Vitamin D receptor (VDP) gene and haemochromatosis gene [17]. The socioeconomic status is one of the factors which has a high influence on the vulnerability of the body to lead toxicity. Considering socioeconomic status simply as a confounder might underestimate its influence. Rutter, (1983) hypothesized that children with low socioeconomic class, as a neuropsychological status rendered fragile by environmental influences, might be highly vulnerable to the neurotoxic consequences of lead. In addition to, the presence of several concomitants of poverty that increase the chances of the poor children to be exposed to lead, and once exposed, the absorption of lead will be more [13]. Bellinger (2000) said that socioeconomic status plays a modulating effect, at 24 months children from low socioeconomic class did more poorly on cognitive tasks than children with equal high lead in cord blood but from high socioeconomic class. This effect was not apparent in younger children [13].

1.4. Iron deficiency/Anemia

Iron or ferrum (Fe) in latin is an abundant element on the earth and it is an essential biological component of every living organism. For a very long time, iron has been recognized to have a role in health and disease. It was used as a medicine by the Egyptians, Hindus, Greeks and Romans [19].

1.4.1. Iron forms and absorption

The body needs iron for the formation of hemoglobin and myoglobin (oxygen transport proteins), heme enzymes synthesis and other iron containing enzymes needed for electron transfer and oxidation reductions [19]. Two thirds of the iron in the body is found in hemoglobin

which is present in the red blood cells. 25% is found in a readily mobile iron stores in form of insoluble ferritin (one ferritin molecule contains 1000 or more iron atoms) [20] present primarily in the liver, spleen, and bone marrow, and the rest is bound to myoglobin and in different forms of enzymes [19,22]. There are many ways to assess the amount of iron stores in the body, serum ferritin being the most convenient way to measure it [19,21,22].

When it comes to iron absorption there are several factors that could influence it, some factors inhibit iron absorption like calcium, phytates and some proteins, other factors promote iron absorption like ascorbic acid, citrate, meat and fish. There are also some competitors with iron at the level of the absorption site at the duodenum as heavy metals like lead, zinc, manganese and cobalt [19].

1.4.2. Iron body needs

The body iron needs are markedly increased after 4 to 6 months of age [23]. The daily requirement for iron is about 0.7-0.9 mg/day during the remaining part of the first year of life [19]. Iron deficiency is a state in which there is no mobilization of the iron stores and the signs of a compromised iron supply to different body tissues are noted. The primary causes of iron deficiency include non-sufficient iron intake, high iron requirements as during early childhood due to rapid growth, and during pregnancy, some pathological causes due to infections, as hookworm and whipworm which cause gastrointestinal blood loss [19,22]. WHO estimates that two billion individuals have anemia worldwide and 50% of all anemia could be attributed to iron deficiency which is considered the most common micronutrient deficiency [19,23]. The highest prevalence of anemia is during infancy and early childhood. Global prevalence of anemia (Hemoglobin < 110 g/L) in young children is 41.8% [23]. In developing countries ID is nearly 2.5 times more prevalent than anemia [7,23].

1.4.3. iron deficiency and psychomotor development

The impact of ID on children Psychomotor development has been reviewed extensively. The researchers have found that ID with anemia in infants affects mental, motor and language development [7]. One of the mechanisms that explain these effects is that IDA is associated with slower neural processing. Capillary endothelial cells in the brain take iron through transferring receptors (TfRs). Iron is transported to astrocytes and neurons via different mechanisms, including divalent metal transport 1 (DMT1), and to oligodendrocytes through ferritin and transferrin [7,19]. ID is associated with a reduction in myelin components including proteins, lipids, and cholesterol, and also associated with altered nerve conduction and disruption of neurotransmission [7,24].

1.5. Lead and iron deficiency/anemia interaction

In a real world scenario humans are exposed to multiple hazards and environmental factors. Usually people with low socioeconomic status are a disadvantaged population and have higher risk of exposure to a mixture of these hazards [25]. Simultaneous exposure to multiple chemicals may have more synergistic effects on the developing brain and on cognition than exposure to each chemical alone (Wright et al. 2006) [26]. The hazards of lead are augmented by diverse dietary states (i.e. iron, calcium, zinc or protein deficiencies) which are more common in economically disadvantaged infants. (Chisolm, 1996 ; Cheng et al., 1998) [26].

Ruff & Bijur, (1989) proposed a model showing how nutrition deficiency and lead exposure may interact to produce behavioral deficits [7]. Iron and lead in particular could be studied together because both are divalent metals, both are absorbed through the same intestinal mechanism, both exposure to lead and ID occur disproportionately in disadvantaged populations. They often occur simultaneously in infants during the window period for brain development, and both could cause potentially irreversible cognitive insults in children [7].

As iron deficiency often coexists with elevated BLLs, this interaction can lead to serious medical complications especially in children [19]. From the available evidence it is difficult to conclude that ID raises the susceptibility of children to the neurotoxic effects of lead due to the shortage of studies that addressed this question. Our research will try to answer this question.

1.6. Hypothesis

Children with ID or anemia may be more susceptible to the neurotoxic effects of lead.

1.7. Objectives

Our study aims:

a. To study the association between post-natal blood lead level (BLL) and psychomotor function in Beninese infants aged 12 months.
b. To study the association between anemia and iron deficiency (ID) at birth and at one year of age and psychomotor function.
c. To study the interaction between anemia and ID at birth and at one year of age, BLL and psychomotor function.

The results are expected to add understanding on the potential role of ID with lead on cognitive and motor outcomes in childhood.

2. Methods

2.1. Study design, site and population

TOVI which means “child of the country” in the Fon local language, is a cross sectional study, conducted between May 2011 and May 2013. The study population was composed of the inclusion of 747 singleton infants to women who were enrolled in the MiPPAD trial in Benin. MiPPAD “Malaria in Pregnancy Preventive Alternative Drugs” [27] is a multi-center trial (Benin, Gabon, Mozambique and Tanzania) comparing sulfadoxine-pyrimethamine and mefloquine, given for intermittent preventive treatment in pregnancy (IPTp) to protect the women from malaria between 2009 and 2013.

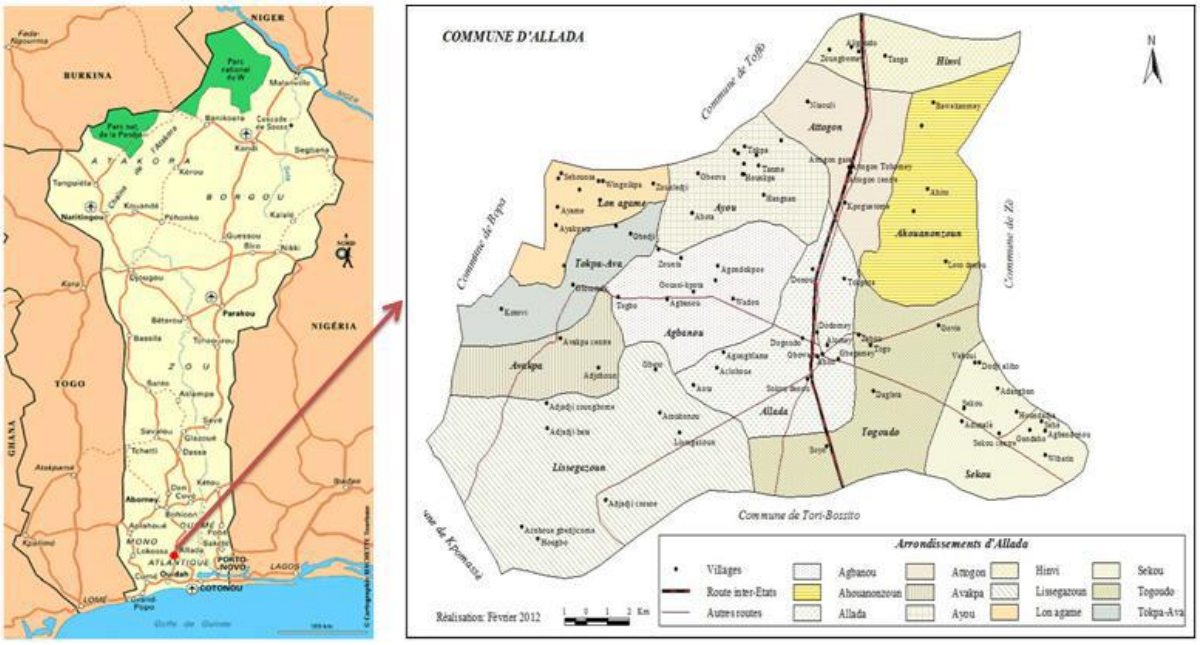

Figure 3: Map of Benin[1] and district of Allada[2]

1. Official site of Beninese government. Administrative map. http://www.gouv.bj/tout-sur-le-benin/histoire
2. Allada district map. National institute of statistics and economical analyses of Benin.2012

Infants were assessed for psychomotor development at the age of 12 months. The study took place in the district of Allada, which is located in southern Benin, a semirural area, 50 km north of Cotonou (Figure: 2), the economic capital of Benin. The entire district consists of 12 sub-districts, which in turn consist of 84 villages, and a total of 91,778 inhabitants who represent different ethnic groups. The most common groups are Aïzo and Fon, and each ethnic group has its own language with slight differences. The study was done at three health centers: Allada, Attogon, and Sékou.

2.2. Data collection

2.2.1. At birth

The clinical and laboratory information of the mothers and infants were collected within the MiPPAD trial: maternal weight, and maternal height at inclusion in the trial, infants birth-

weight in grams by weekly-calibrated scales, Ballard score at birth and fundal height at delivery for estimating the gestational age. Weights were measured to the nearest 0.1 kg by using an electronic scale (to ± 100 grams; Seca Corp., Hanover, MD) and heights to the nearest 0.1 cm by using a bodymeter device (Seca 206 Bodymeter; Seca Corp.). Weights and heights were measured by two nurses, and the mean of the two measurements was calculated for each participant. At delivery, women's peripheral blood and cord blood were collected for hematological and parasitological evaluation. Anemia and ferritin levels in cord blood were measured, and placental malaria status was checked on stained placental smears.

2.2.2. At 12 months of age

Infant psychomotor development was assessed by research nurses trained specifically in the use of the Mullen Scales of Early Learning. Blood draw was performed at the end of the MSEL assessment. Eight milliliters of venous blood was obtained from each participant, of which 4 mL was collected into a tube containing dipotassium EDTA. Further 4 mL were collected into an iron-free dry tube. Three days later, a home visit was made by another nurse or a psychologist. During this visit, a face-to-face questionnaire to the infant's mother/caregiver was administrated. The questions were designed to explore the family's socio-demographic status, disease symptoms, and potential sources of lead exposure in the home environment. The collected data included maternal age, language, ethnic group, marital status, number of siblings, number of persons living in the same household, type of housing, maternal education, maternal IQ, post-partum depression, HOME score, maternal and parental working status, and the possible sources of lead (painted walls, animals killed by bullets and piped water).Blood draw was performed to assess blood lead level, hemoglobin level, serum ferritin level, serum C-reactive protein (CRP) level, and malaria status.

2.3. Laboratory methods

Most analyses were performed in the field or in Cotonou. The hemoglobin level was measured with a HemoControl® photometer (EKF Diagnostics, Germany). Daily calibration of the Hemo-Control device was performed by laboratory technicians. In addition, an external quality control was made by sending one of 10 consecutive samples to the Allada Central Hospital laboratory, where dosages were determined by using a hematology analyzer (Erma Laboratory, Tokyo, Japan). Serum ferritin concentrations were measured by using an AxSym Immuno-Assay Analyzer (Abbott Laboratories, Abbott Park, IL) with 500 mL of serum. C-reactive protein (CRP) concentrations were determined by using a rapid slide test (CRP Latex; Cypress Diagnostics Inc., Campbellville, Ontario, Canada) to correct for increased ferritin levels associated with inflammatory conditions. The Lambaréné method was used to

assess malarial infection. Ten microliters of blood was spread on a rectangular area of 1.8 cm2 (1.8 cm + 1 cm) of a slide. The slide was stained with Giemsa and read at a magnification of 1,000 + with an oil immersion lens. A multiplication factor was applied to the average parasitemia/field to determine the number of parasites/microliter, The Lambaréné method detection threshold has been estimated to be 5 parasites/mL. Whole blood samples were assessed at the University of Laval in Canada for lead level. Blood Lead Level (BLL) were measured by analyzing the blood samples using inductively coupled plasma mass spectrometry which is capable of detecting metals at a very low concentrations (one part per trillion) through ionizing the sample with inductively coupled plasma and then a mass spectrometry to separate and quantify those ions.

2.4. Definitions

Elevated lead level (ELL): defined as blood lead level higher than 5µg/dl, as recommended by the CDC [14].

Anemia at birth: defined as hemoglobin level in cord blood below 140 g/L according to the WHO guidelines [28].

Anemia at one year: defined as hemoglobin level below 110 g/L. Mild, moderate and severe anemia was defined as hemoglobin level between 109-100 g/L, between 99-80 g/L and less than 80 g/L, respectively. according to the WHO guidelines [29].

Iron deficiency (ID) at birth: In the absence of international definition of iron deficiency at birth[30], we divided our sample into quartiles and considered the lowest quartile as ID at birth.

ID at one year: Iron deficiency was defined as a serum ferritin concentration less than 12 mg/L or as serum ferritin concentration of 12–70 µg/L in the context of inflammatory syndrome [21].

Inflammation: defined as positive CRP result, (i.e. CRP concentration > 5 mg/L) [21].

Post-partum depression: defined by a score of 10 or more on Edinburgh Postnatal Depression Scale. The maximum score is 30 [31].

Body Mass Index (BMI) at inclusion: It is defined as the weight in kilograms divided by the square of the height in metres (kg/m²).

2.5. Measurement of outcome

The Mullen Scales of Early Learning (MSEL) is a well-validated (in English), individually administered, comprehensive measure of cognitive functioning for infants and preschool children, from birth through 68 months [34]. It has good correspondence validity to the Bayley Scales of Infant Development and has been widely used in high-income Anglophone countries [10-13]. For the purpose of the study, the MSEL was translated and adapted in 4 steps: linguistic translation, training, pilot testing and review [34]. The MSEL covers five

domains to assess the psychomotor development of the children. The five Mullen scales are fine motor, visual reception, receptive language, expressive language, and gross motor. For each scale a raw score is computed, and then these raw scores are converted into a normative score called the T score based on age at assessment. The T scores for the fine Motor, visual reception, receptive language, and expressive language scales are transformed into the early learning composite score. The outcomes of interest in our study were the early learning composite score and the gross motor score.

2.6. Measurement of exposures

2.6.1. Main exposure

The level of lead in blood in one year old infants was the main exposure of interest in our study. For the purpose of data analysis, the blood lead level was treated as both continuous (µg/dl) and categorical (lowest to highest in quartiles).

2.6.2. Other exposures

Anemia and iron status at birth were the other exposures of interests. Iron status at birth was assessed by measuring the ferritin concentration in cord blood. Ferritin at birth was coded as a categorical variable (lowest to highest in quartiles). ID at one year was coded as a dichotomous variable (present/absent). Anemia at birth was coded as a dichotomous variable (present/absent), and anemia at one year treated as continuous (g/L), dichotomous (present/absent), and categorical (no anemia, mild, moderate, and severe anemia).

2.7. Other variables

Other variables considered as potential confounding factors included maternal non verbal Intellectual Quotient (IQ) which was assessed by the Raven Progressive Matrices (RPM). Maternal IQ was treated as a continuous variable. The Home Observation for the Measurement of the Environment (HOME) Inventory was used to assess the offered stimulation and learning opportunities by the home environment and parent-child interaction. HOME score was treated as a continuous variable. The Edinburgh Postnatal Depression Scale (EPDS) was used to assess the maternal depressive symptoms. Post-partum depression was treated as a dichotomous variable (present/absent). Socio-economic status was assessed by a score of family possessions and by maternal education. The score of family possession was treated as a continuous variable, and maternal education was treated as a dichotomous variable (none/ primary or more). Other maternal variables include maternal age, marital status, ethnicity, parity and occupation. Maternal age was coded as a dichotomous variable (≥21 years: yes/no), marital status coded as a categorical variable (monogamous, polygamous), ethnicity coded as a categorical variable (Fon, Aïzo, or other),

parity coded as a dichotomous variable (primigravidae/multigravidae), and occupation treated as a dichotomous variable (employed: yes/no). BMI (Kg/m²) at inclusion was treated as a categorical variable (<18.5 Kg/m², 18.5-24.9 Kg/m², and ≥25 Kg/m²).

Birth weight was treated as a dichotomous variable (low birth weight: yes/no). Gestational age estimated by Ballard score at birth was treated as a dichotomous variable (preterm: yes/no). Language spoken at home was treated as a categorical variable (For, Aïzo, or other). As five different nurses assessed the infants using the MSEL, the examiner was treated as a categorical variable.

2.8. Strategy of analyses

1. Description of the study population:
   - The descriptive analysis of the general and clinical characteristics of the mothers and infants was done by using χ^2 test for the qualitative variables and analysis of variance (ANOVA) for the quantitative variables. Pearson's correlation was used to assess the correlation between 2 continuous variables.
2. Study of the relation between the outcome variables (early composite score and gross motor score) and the maternal and infants characteristics:
   - Univariate linear regression analyses
3. Study of the relation between the outcome variables (early composite score and gross motor score) and blood lead level at one year, anemia and iron deficiency at birth and at 12 months by:
   - Univariate linear regression analyses.
   - Multivariate linear regression analysis (backward elimination according to 5% level of significance of the variables selected in step 2 (according to 20% level of significance) and relevant significant variables in the literature). Cord blood parameters (hemoglobin and ferritin) in association with gross motor scores are adjusted for maternal BMI at inclusion
4. Assessment of interaction:
   - Stratified analysis on anemia and ferritin level at birth and on anemia and ID at 12 months of age.

The analyses were performed with Stata® version 11 for Windows (Stata Corp, College Station, TX, USA).

2.9. Ethical considerations

Ethical clearance for the TOVI study was obtained from the Ethics Committee of the Faculty des Sciences de la Santé of Cotonou, Benin, New York University, Michigan State University

Institutional Review Boards, and the French Institut de Recherche pour le Développement (IRD) Consultative Ethics Committee. Before each inclusion, full study explanation in local language was provided, and a voluntary consent was obtained from the infant's parent. If the parent was illiterate, a neutral witness was involved in the process.

3. Results

3.1. Baseline characteristics of the study population

747 mother-child pairs were included in the analyses. The psychomotor assessment was performed in three health centers at the age of 12 months post-partum, 462 in Sékou, 217 in Attogon, and 68 in Allada. Table 1 shows the general characteristics of infants and mothers included in the analysis. The study cohort consisted of 51% males and 49% females. The mean birth weight for the study population was 3034 g (standard deviation, ±SD: 422), among which 9.6% were low birth weight (<2500 g). The mean gestational age at birth was 38.4 weeks (±SD: 1.4), and 11.5% of the infants were preterm. The mean age of the mothers during the study was 27 years old (±SD: 5.8). Most mothers (61.5%) were from Fon ethnic group and Approximately 92% of these mothers were working at time of testing. More than half of the mothers (61.2%) completed primary education or higher. There were significant differences in number of educated mothers between the three cities – Attogon (49%), Sékou (30%) and Allada (67%), which were correlated with the early composite scores of the infants (Spearman's *rho* = 0.19; $p< 0.0001$). The majority of the population (84.5%) lives in compounds. There wasn't a correlation between HOME inventory score and score of family possession, and the place of residence, but there was a significant correlation with maternal IQ and place of residency (Spearman's rho = 0.16; $p< 0.0001$) because of the differences in maternal education. These measures indicate that there wasn't a significant difference in socio-economic status between the three cities. And also indicate that the children were mainly from poor families ("Middle Class" would be a misnomer in the Beninese society where people are either rich or very poor and few are in between).

3.2. Prevalence of high BLL, anemia, and ID in the study area (Tables 2 and 3)

The geometric mean blood lead concentration at 12 months of age for the 673 assessed infants was 5.9µg/dl (range, 0.8;63 µg/dl). According to the new CDC guidelines, 58% of the infants had high BLL (≥ 5 µg/dl). 45% of the infants were anemic at birth, compared to 65.9% at 12 months of age, which is higher than the national prevalence of anemia (58%) for the age group 6 to 59 months, according to the latest demographic health survey in Benin. The BLLs was correlated to ID status at one year (Spearman's rho = 0.13; $p< 0.0001$), but ferritin, anemia at birth and anemia at 12 months of age were not correlated.

Table 1. Maternal and infants characteristics at twelve months post-partum

Characteristics	N Total 747	Percentage %
Maternal characteristics		
Health center		
Attogon	217	29.1%
Sékou	462	61.8%
Allada	68	9.1%
Maternal age (years)		
< 21	95	12.7%
≥ 21	652	87.3%
Continuous (mean [SD])	27	[5.8]
Ethnic group		
Fon	390	61.5%
Aïzo	169	26.7%
other	75	11.8%
Language spoken at home		
Fon	383	51.8%
Aïzo	336	45.4%
other	21	2.8%
Marital status		
Monogamous	467	63.5%
Polygamous	269	36.5%
Gravidity		
Primigravidae	117	18.5%
Multigravidae	517	81.5%
Education		
Never schooled	453	61.2%
Primary or more	287	38.3%
Occupation		
Housewives	60	8%
Employed	685	92%
Pre-pregnancy BMI (Kg/m²)		
<18.5	41	6.5%
18.5-24.9	498	78.5%
≥25.0	95	15%
Score of family possession (median [SD])	5	[2.77]
EPDS score (median [SD])	8	[4.15]
Raven score (median [SD])	15	[4.42]
HOME score (median [SD])	27	[2.28]
Infant characteristics		
Infant sex		
Male	380	51%
Female	367	49%
Birth weight (grams)		
< 2500	60	9.6%
≥ 2500	563	90.4%
Preterm delivery (weeks)		
< 37	69	11.5%
≥ 37	528	88.5%
Estimated gestational age[a] (mean [SD])	38.4	[1.4]
Malaria at 12 months		
Yes	73	10.4%
No	628	89.6%

Figures are numbers (percentage) unless otherwise indicated [a] Estimated by Ballard score

There was no significant difference in the prevalence of elevated BLLs (χ^2; p = 0.059) or anemia (χ^2; p = 0.067) between the three cities. The difference was significant between the three cities with ID (χ^2; p = 0.029).

Table 2. BLL, anemia and ferritin levels in infants at birth and at twelve months of age

	N	Mean[a] (SD)	Percentile				
			5th	25th	50th	75th	95th
In cord blood							
Hb concentration (g/L)	682	137.5 (23.8)	100	129	142	154	174
Serum ferritin (µg/L)	433	129.1 (170.8)	15.2	82	139	202.9	472.6
At 12 months of age							
Hb concentration (g/L)	704	102.2 (14)	59	96	105	112	123
Serum ferritin (µg/L)	672	27.6 (87.5)	4.5	12.4	26.2	57.7	211.6
Blood lead level (µg/dl)	673	5.9 (6.1)	2.5	3.9	5.6	8.5	16.6

[a]Geometric means

3.3. Association between maternal and infant characteristics and BLL (Table 4)

Infants with ID at 12 months varied significantly among different quartiles of blood lead (X^2; p <0.05). BLL was negatively correlated to serum ferritin concentration at 12 months (Spearman's rho = -0.18, p <0.0001). Blood lead concentrations were higher among boys than among girls, but the difference was not significant (mean ±SD, boys, 7.4µg/dl ±6.6; girls, 7.1 µg/dl ±5.6). The geometric mean concentrations for ferritin and Hb concentration at birth and at 12 months were, respectively, for serum ferritin, 129.1µg/L27 (range, 5-1976.9) and 27.6µg/L (range, 0.4-891), and for blood hemoglobin, 137.5 g/L (range, 23-204) and 102.2 g/L (range 34-139). Of these infants, 391 (58.1%) had high BLL >5 µg/dl. BLL varied by HOME score, for every one unit increase in HOME score the mean BLL decreased by 0.23µg/dl

Table 3. Anemia and ferritin level at birth. Anemia, iron deficiency and BLL at 12 months of age

	N	%
In cord blood		
Ferritin level		
Lowest quartile	109	25%
Highest 3 quartiles	324	75%
Anemia		
No anemia	375	55%
Anemia (>140g/L)	307	45%
At 12 months of age		
Anemia		
No anemia	240	34.1%
Slight (100-109 g/L)	238	33.8%
Moderate (70-99g/L)	209	29.7%
Severe (<70g/L)	17	2.4%
Iron deficiency		
No	400	60%
Yes	264	40%
Blood lead level (µg/dl)		
< 5	282	41.9%
5-10	267	39.7%
> 10	124	18.4%

(Table 4). BLL was positively correlated to ID at one year (Spearmans's *rho* = 0.14, p < 0.0001)

Table 4. Association between maternal and infant characteristics and BLL

	Blood lead level at 12 months		BLL in quartiles			
	Crude mean difference	[95% CI]	Lowest quartile (<3.9µg/dl) (n= 169)	2median quartiles (3.9 – 8.5µg/dl) (n= 336)	Highest quartile (>8.5 µg/dl) (n= 168)	*p* – value[a]
Gravidity						
Primigravidae	0	-	17.5%	52.4%	30.1%	0.12
Multigravidae	-0.33	[-1.57 ; 0.91]	27%	48.4%	24.6%	
Maternal Education						
Never schooled	0	-	25.7%	48.6%	25.7%	0.77
Primary or more	0.25	[-0.71 ; 1.20]	24.2%	51.6%	24.2%	
Maternal Occupation						
Housewives	0	-	15.1%	50.9%	34%	0.12
Employed	- 1.61	[-3.32 ; 0.12]	26%	49.8%	24.2%	
BMI (Kg/m²)						
< 18.5	0	-	25%	47.4%	27.6%	0.02
18.5-24.9	0.72	[-1.19 ; 2.63]	13.2%	55.2%	31.6%	
≥ 25	-1.61*	[-2.93 ; -0.28]	32.2%	55.2%	12.6%	
Post-partum depression						
No	0	-	25.8%	48.6%	25.6%	0.69
Yes	-0.19	[-1.16 ; 0.79]	24%	52%	24%	
Gestational age (by Ballard score)						
≥ 37 weeks	0	-				
> 37 weeks	-0.72	[-2.24 ; 0.80]				
			mean	mean	mean	
HOME score (mean)	-0.23*	[-0.43 ; -0.02]	(27.17)	(26.76)	(26.67)	0.08[b]
Raven score (mean)	0.03	[-0.08 ; 0.14]	(14.90)	(15.22)	(15.06)	0.73[b]
Family Possession (mean)	-0.15	[-0.32 ; 0.02]	(5.83)	(5.70)	(5.27)	0.15[b]

*p-value (≤ 0.05) [a]p-value of χ^2 test [b]p-value of t-test

Table 4. Association between maternal and infant characteristics and BLL (continued)

	Blood lead level at 12 months		BLL in quartiles			
	Crude mean difference	[95% CI]	Lowest quartile (<3.9µg/dl) (n= 169)	2median quartiles (3.9 – 8.5µg/dl) (n= 336)	Highest quartile (>8.5 µg/dl) (n= 168)	*p* – value[a]
In cord blood						
Anemia						
No	0	-	25%	51%	24%	0.68
Yes	-0.10	[-1.09 ; 0.89]	24.3%	48.6%	27.1%	
Ferritin in quartiles						
Lowest quartile	0	-	20.4%	51.5%	28.1%	0.83
2 median quartiles	-0.74	[-2.18 ; 0.70]	25.4%	49.2%	25.4%	
Highest quartile	-0.54	[-2.20 ; 1.13]	21.6%	48.5%	29.9%	
At 12 months of age						
Anemia						
No	0	-	26%	45.6%	28.4%	0.38
Yes	-0.23	[-1.25 ; 0.79]	24.8%	51%	24.2%	
Iron deficiency						
No	0	-	30%	44.5%	25.5%	0.00
Yes	0.85	[-0.16 ; 1.86]	18.6%	55.2%	26.2%	

[a] *p*-value of χ^2 test

Table 5. Association between potential confounding factors and mean early learning composite (ELC) and gross motor scores

Characteristics	ELC score		Gross motor score	
	Mean	*P*-value	Mean	*P*-value
Maternal characteristics				
Maternal age				
< 21	99.23	0.60	48.67	0.07
≥ 21	98.44		51.49	
Gravidity				
Primigravidae	99.8	0.32	48.2	0.02
Multigravidae	98.4		51.6	
Maternal Education				
None	96.6	0.00	50.4	0.10
Some	101.6		52.2	
Maternal Occupation				
Housewives	99.6	0.52	51.2	0.98
Employed	98.4		51.1	
BMI at recruitment (kg/m^2)				
<18.5	93.66	0.01	49.41	0.00
18.5-24.9	98.42		50.23	
≥ 25	101.76		55.55	
Family Possession score	0.11[α]	0.00	0.16[α]	0.00
RAVEN score	0.09[α]	0.02	0.12[α]	0.00
HOME score	0.19[α]	0.00	0.17[α]	0.00
Post-partum derpression (EPDS)	98.7	0.85	51.1	0.98
Yes	98.5		51.2	
No				
Marital status				
Monogamous	99.7	0.01	51.1	0.42
Polygamous	96.8		51.1	
Maternity unit				
Attogon	102	0.00	51.5	0.01
Sékou	96.7		50.3	
Allada	100.4		55.9	
Infants characteristics				
Birth weight (g)				
Low birth weight (< 2500)	97.7	0.61	45.6	0.00
Normal birth weight (≥ 2500)	98.7		51.5	
Gestational age (Ballard)				
< 37 weeks	97.00	0.35	49.58	0.35
≥ 37	98.70		51.28	
Language spoken at home				
Fon	100.50	0.00	51.85	0.01
Aïzo	96.04		49.87	
Malaria at 12 months				
Yes	94.9	0.02	46.4	0.00
No	98.9		51.7	

[α]Spearman's correlation

Table 6. Relationship between anemia, iron deficiency at birth and at age 1 year and mean scores of infant cognitive and gross motor function at age 1 year

	Mean difference in early learning composite scores				Mean difference in gross motor scores			
	Crude mean diff [95%CI]		Adjusted mean diff [95%CI][a+c]		Crude mean diff [95%CI]		Adjusted mean diff [95%CI][b]	
<u>In cord blood</u>								
Hb concentration								
Lowest quartile		0		0		0		0
2 median quartile	0.17	[-2.33 ; 2.67]	0.04	[-2.43 ; 2.52]	- 0.26	[-2.87 ; 2.34]	-0.65	[-3.19 ; 1.90]
Highest quartile	1.00	[-1.91 ; 3.92]	1.66	[-1.27 ; 4.59]	0.36	[-2.67 ; 3.39]	0.06	[-2.93 ; 3.05]
Serum Ferritin								
Lowest quartile		0		0		0		0
Highest 3 quartiles	-0.74	[-3.90 ; 2.41]	-0.10	[-3.11 ; 2.92]	- 0.60	[-3.71 ; 2.52]	-0.31	[-3.39 ; 2.76]
Anemia								
No anemia		0		0		0		0
Anemia (>140)	-0.35	[-2.42 ; 1.72]	-0.46	[-2.44 ; 1.53]	0.68	[-1.47 ; 2.83]	1.21	[-0.91 ; 3.33]
<u>At 12 months of age</u>								
Hb concentration								
Anemia								
No anemia		0		0		0		0
Slight (100-109 g/L)	0.81	[-1.66 ;3.27]	0.57	[-1.84 ; 2.98]	-0.86	[-3.40 ; 1.68]	-0.79	[-3.22 ; 1.63]
Moderate (70-99 g/L)	-1.59	[-4.14 ; 0.96]	-1.86	[-4.31 ; 0.59]	-3.12*	[-5.74 ; -0.49]	-2.88*	[-5.56 ; -0.20]
Severe (<70 g/L)	-6.62*	[-13.39 ; 0.14]	-6.87*	[-12.48 ; -1.26]	-12.69 *	[-19.65 ; -5.73]	-12.49*	[-16.69 ; -8.29]
Iron deficiency								
No		0		0		0		0
Yes	0.90	[-1.26 ; 3.6]	0.29	[-1.79 ; 2.37]	1.48	[-0.75 ; 3.72]	2.03	[-0.26 ; 4.32]

a the mean differences for the early composite scores adjusted for maternal education, score of family possession, maternal IQ score, HOME inventory score, language spoken at home, assistant who performed the assessment, and gestational age according to Ballard score

b the mean differences for the gross motor scores adjusted for gravidity, maternal age (categorical), score of family possession, maternal IQ score, Home inventory score, language spoken at home, birth weight (categorical), assistant who performed the assessment, and gestational age according to Ballard score

c cord blood parameters are adjusted for maternal BMI at inclusion * p-value (≤ 0.05)

3.4. Associations with the early learning composite scores

Mean scores in 747 infants assessed for their cognitive functions at the age of one year was 98.6 (SD: 13.6). The effect of anemia at 12months and its severity showed a borderline significant difference in mean early composite score between the severe anemic and non-anemic infants (-6.62 points, p = 0.055) (Table 6).The unadjusted data also showed an inverse non-significant relationship between quartiles of blood lead concentration and early composite scores (Table 7).

After adjustment for maternal education, score of family possession, maternal IQ, HOME inventory score, language spoken at home, examiner, and gestational age by Ballard score, the difference in mean scores between the severely anemic and non-anemic became more negative and statistically significant (-6.87, *p* = 0.02) (Table 6). After adjustment for maternal education, score of family possession, maternal IQ, HOME inventory score, language spoken at home, examiner, and gestational age by Ballard score, the inverse non-significant relationship between quartiles of blood lead concentration and early composite scores became more evident. The distribution of the early learning composite scores was approximately normal [annex x]. All data presented in this report are based on the original data with no transformation.

Table 7. Relationship between BLL and mean scores of infant cognitive function at 1 year of age

	Mean difference in early learning composite scores[b]			
	Crude mean diff [95%CI]		Adjusted mean diff [95%CI][a]	
Blood lead level (µg/dl)				
Lowest quartile	0		0	
2nd quartile	-0.34	[-3.18 ; 2.50]	-0.14	[-2.98 ; 2.70]
3rd quartile	-0.13	[-3.18 ; 2.93]	0.25	[-2.59 ; 3.10]
Highest quartile	-0.92	[-3.93 ; 2.08]	-1.12	[-3.97 ; 1.72]
Continuous coding	-0.02	[-0.19 ; 0.15]	-0.03	[-0.19 ; 0.14]

[a] adjusted for maternal education, score of family possession, maternal IQ, HOME score, language spoken at home, examiner, gestational age estimated by Ballard score [b] n=673 for crude analyses, n=669 for adjusted analyses

3.5. Associations with the gross motor score

The mean score for Gross Motor in 746 infants assessed for their gross motor functions at the age of one year was 51.2 (SD: 14.3). The effect of anemia at 12 moths and its severity showed a significant decrease in mean gross motor scores between the moderate and severe anemic infants compare to non-anemic infants, respectively, -3.12 points and -12.69 points (Table 6). The unadjusted data also showed a direct significant relationship between quartiles of blood lead concentration and gross motor scores. The unadjusted mean scores

of infants with values in the highest and lowest blood lead concentration quartiles increased by 4.61 points (Table 8).

After adjustment for gravidity, maternal age, score of family possession, maternal IQ, HOME inventory score, language spoken at home, examiner, low bierthweight, and gestational age by Ballard score, the significant difference in mean scores between the moderate and severe anemic infants in compare to the non-anemic infants became -2.88 points (p = 0.035), and -12.49 points (p < 0.001), respectively (Table 6).After adjustment for gravidity, maternal age, score of family possession, maternal IQ, HOME inventory score, language spoken at home, examiner, low bierthweight, and gestational age by Ballard score, the direct significant relationship between quartiles of blood lead concentration and gross motor scores became more evident. The adjusted mean scores of with values in the highest and lowest quartiles of blood lead increased by 6.96 points (p < 0.001). The multiple linear regression analyses revealed a 0.24 point increase in gross motor scores for each 1 µg/dl increase in blood lead concentration (Table 8). The distribution of the gross motor scores was not normal [annex x] and models with square-transformed did not yield results that were markedly different from the untransformed data. All data presented in this report are based on the original data with no transformation.

Table 8. Relationship between BLL and mean scores of infant gross motor function at 1 year of age

	Mean difference in gross motor scores[b]			
	Crude mean diff [95%CI]		Adjusted mean diff [95%CI][a]	
Blood lead level (µg/dl)				
Lowest quartile	0		0	
2nd quartile	3.59*	[0.57 ; 6.60]	4.69*	[1.41 ; 7.97]
3rd quartile	2.21	[-0.82 ; 5.23]	3.19*	[-0.04 ; 6.42]
Highest quartile	4.61*	[1.58 ; 7.63]	6.96*	[3.74 ; 10.19]
Continuous coding	0.17	[-0.01 ; 0.35]	0.24*	[0.04 ; 0.44]

[a] adjusted gravidity, maternal age, score of family possession, maternal IQ, HOME score, language spoken at home, examiner, low birth weight, and gestational age estimated by Ballard score [b] n=672 for crude analyses, n=558 for adjusted analyses *p-value (≤0.05)

3.6. Assessment of lead and iron/anemia interaction

Stratified analyses of the relationship between blood lead concentrations and mean scores of infant cognitive and gross motor functions at birth and at 12 months of age was performed to assess the possible interactions.

3.6.1. **At birth** (Table 9)

The stratified analyses on anemia status at birth indicated that infants who were anemic at birth showed significantly higher adjusted mean differences in gross motor scores than the

Table 9. Relationship between BLL and mean scores of infant cognitive and gross motor function at age 1 year stratified by anemia and ferritin at birth

		Mean difference in early learning composite scores		Mean difference in gross motor scores	
	N	Crude mean diff [95%CI]	Adjusted mean diff [95% CI][a]	Crude mean diff [95%CI]	Adjusted mean diff [95% CI][b]
In new born in lowest quartile of ferritin					
BLL (µg/dl)					
Lowest quartile		0	0	0	0
Quartile 2		-7.67 [-15.94 ; 0.60]	-5.80 [-14.07;2.47]	3.62 [-5.33 ; 12.57]	2.71 [-5.78;11.19]
Quartile 3		1.11 [-6.42 ; 8.63]	1.48 [-5.93;8.90]	1.92 [-6.22 ; 10.10]	-0.11 [-7.75;7.53]
Higher quartile		-3.80 [-11.48 ; 3.088]	-3.43 [-11.13;4.27]	3.15 [-5.12 ; 11.42]	1.20 [-6.97;9.37]
In new born in highest 3 quartiles Of ferritin					
BLL (µg/dl)					
Lowest quartile		0	0	0	0
Quartile 2		0.48 [-4.56 ; 5.51]	-0.08 [-4.95;4.79]	4.04 [-0.65 ; 8.74]	3.97 [-0.67;8.61]
Quartile 3		-2.06 [-7.06 ; 2.94]	-1.71 [-6.51;3.08]	-0.11 [-4.77 ; 4.56]	-0.12 [-4.73;4.49]
Higher quartile		-0.78 [-5.68 ; 4.13]	-1.41 [-6.14;3.32]	5.60* [1.02 ; 10.19]	5.73* [1.20;10.27]
In infants with anemia at birth					
BLL (µg/dl)					
Lowest quartile		0	0	0	0
Quartile 2		0.90 [-3.95 ; 5.74]	1.80 [-2.92;6.52]	6.36* [1.61 ; 11.12]	7.11* [2.48;11.73]
Quartile 3		-1.62 [-6.29 ; 3.06]	-0.45 [-4.99;4.10]	5.00* [0.41 ; 9.58]	6.24* [1.77;10.70]
Higher quartile		-1.00 [-5.63 ; 3.63]	-0.55 [-4.98;3.88]	8.05* [3.50 ; 12.61]	8.85* [4.44;13.26]
In infants without anemia at birth					
BLL (µg/dl)					
Lowest quartile		0	0	0	0
Quartile 2		-1.00 [-5.06 ; 3.10]	-1.69 [-5.57;2.19]	2.63 [-1.66 ; 6.92]	2.86 [-1.75;7.48]
Quartile 3		1.40 [-2.73 ; 5.53]	0.97 [-2.94;4.88]	0.51 [-3.86 ; 4.88]	0.60 [-4.05;5.24]
Higher quartile		-0.55 [-4.71 ; 3.61]	-1.24 [-5.21;2.72]	2.67 [-1.73 ; 7.07]	4.10 [-0.64;8.84]

a the mean differences for the early composite scores adjusted for maternal education, score of family possession, maternal IQ score, HOME inventory score, language spoken at home, assistant who performed the assessment, and gestational age according to Ballard score

b the mean differences for the gross motor scores adjusted for gravidity, maternal age (categorical), score of family possession, maternal IQ score, Home inventory score, language spoken at home, birth weight (categorical), assistant who performed the assessment, and gestational age according to Ballard score * p-value (≤ 0.05)

Table 10. Relationship between BLL and mean scores of infant cognitive and gross motor function at age 1 year stratified by anemia and ID at 12 months of age

	Mean difference in early learning composite scores				Mean difference in gross motor scores			
	Crude mean diff [95%CI]		Adjusted mean diff [95%CI][a]		Crude mean diff [95%CI]		Adjusted mean diff [95%CI][b]	
In infants with ID at 12 months								
BLL (µg/dl)								
Lowest quartile		0		0		0		0
Quartile 2	-0.68	[-6.17; 4.82]	-1.52	[-6.93;3.89]	2.99	[-2.61 ; 8.59]	3.06	[-2.55;8.66]
Quartile 3	-1.26	[-6.35 ; 3.84]	-2.10	[-7.09;2.89]	-1.20	[-6.40 ; 4.00]	-1.00	[-6.21;4.21]
Higher quartile	-3.41	[-8.73 ; 1.91]	-5.18*	[-10.36;0.00]	3.75	[-1.68 ; 9.17]	4.71	[-0.69;10.11]
In infants without ID at 12 months								
BLL (µg/dl)								
Lowest quartile		0		0		0		0
Quartile 2	-1.40	[-5.26 ; 2.46]	-1.23	[-5.04;2.57]	3.56	[-0.35 ; 7.47]	3.72	[-0.17;7.60]
Quartile 3	-0.49	[-4.73 ; 3.75]	0.66	[-3.43;4.76]	4.64*	[0.35 ; 8.93]	5.37*	[1.10;9.65]
Higher quartile	0.06	[-3.81 ; 3.94]	-0.06	[-3.78;3.67]	5.96 *	[2.03 ; 9.91]	6.25*	[2.35;10.14]
In infants with anemia at 12 months								
BLL (µg/dl)								
Lowest quartile		0		0		0		0
Quartile 2	1.36	[-2.34 ; 6.07]	1.30	[-2.33;4.93]	4.47*	[0.72 ; 8.23]	5.06*	[1.00;9.12]
Quartile 3	1.39	[-2.28 ; 5.06]	1.20	[-2.34;4.75]	3.60	[-0.12 ;7.32]	3.72	[-0.23;7.66]
Higher quartile	0.37	[-3.37 ; 4.11]	-0.78	[-4.38;2.82]	6.22*	[2.42 ; 10.02]	7.62*	[3.60;11.63]
In infants without anemia at 12 months								
BLL (µg/dl)								
Lowest quartile		0		0		0		0
Quartile 2	-4.08	[-9.37 ; 1.21]	-3.16	[-8.40;2.09]	2.45	[-2.97 ; 7.88]	2.72	[-3.13;8.57]
Quartile 3	-2.99	[-8.34 ; 2.35]	-1.79	[-7.19;3.61]	0.64	[-4.85 ; 6.12]	1.61	[-4.16;7.39]
Higher quartile	-3.20	[-8.23 ; 1.83]	-2.44	[-7.48;2.60]	2.29	[-2.87 ; 7.45]	4.62	[-0.84;10.07]

a the mean differences for the early composite scores adjusted for maternal education, score of family possession, maternal IQ score, HOME inventory score, language spoken at home, assistant who performed the assessment, and gestational age according to Ballard score

b the mean differences for the gross motor scores adjusted for gravidity, maternal age (categorical), score of family possession, maternal IQ score, Home inventory score, language spoken at home, birth weight (categorical), assistant who performed the assessment, and gestational age according to Ballard score

* p-value (≤ 0.05)

non-anemic infants. Among anemic infants at birth, gross motor scores increased by 8.85 points ($p < 0.001$) in the highest quartile of blood lead compared to infants in the lowest quartile. Among non-anemic infants at birth gross motor scores increased by only 4.10 points ($p = 0.09$). The infants in the highest 3 quartiles of ferritin at birth, gross motor scores was significantly higher (5.73, $p = 0.01$) in the highest quartile of blood lead compared to the infants in the lowest quartile. There was no interaction at birth for the early learning composite scores.

3.6.2. At twelve months of age (Table 10)

Among infants with ID at twelve months of age, there was a decrement by -5.18 points ($p = 0.05$) in early learning composite scores between the highest quartile of BLL compared to infants in the lowest quartile. A reverse interaction between with ID status was observed, among iron replete infants at 12 months, gross motor scores were significantly higher with infants in 3rd and highest quartile of BLL (5.37 points; $p = 0.01$ and 6.25 points; $p = 0.002$) respectively, compared to infants with iron deficiency at the same age. An interaction between lead in blood and anemia at one year was also observed. Among anemic infants at 12 months of age, gross motor scores were significantly higher in infants in the 2nd quartile and highest quartile of BLL. The gross motor scores increased by 5.06 ($p = 0.02$) in 2nd quartile and increased by 7.62 ($p < 0.001$) in highest quartile, in compare to infants in lowest quartile of BLL. The differences were non- significant among the non-anemic infants at 12 months of age.

4. Discussion

In a mother-child study that was conducted in southern Benin to examine the relation between BLL and psychomotor functions of one year infants, high BLL level was associated with higher gross motor scores. This association was particularly strong in anemic infants at one year of age. This study was also interested in assessing the interaction between lead and ID/anemia on the psychomotor functions at one year where the research is not well developed. Within the iron deficient infants, the deficit in cognitive function in relation to high BLL is significant in comparison to iron-replete infants.

4.1. Strengths

To our knowledge, this study is the first study to assess the effects of BLL on psychomotor development in Benin, and one of the few studies that examined the effect of lead on cognitive and motor functions in one year old infants. Furthermore, we stratified results by anemia and ID to explore interaction with BLL and child development in an area where the literature is relatively poor. One of the main strengths of our study is to adjust for many know risk factors. We used several indicators to assess for the socioeconomic status including score of family possession, HOME inventory score, and maternal education. Studies looking at risk factors for poor child

development are relatively rare in sub-Saharan Africa. Another strength of our study is the relatively large sample size.

4.2. Limitations

The main limitation is the importance of missing data for biological factors at birth (hemoglobin in cord blood, ferritin in cord blood, gestational age, and birth weight), because cord blood was not sufficient to perform biological assessments for all births. However, this selection should be non-differential according to BLL and child development and should only result in a loss of power. There was no data collected about the BLLs at birth for mothers and for newborns because exposure to lead in this population was unknown before assessments in our one year old infants. Differences in scores between examiners may reflect some information bias. However, to account for this, we first adjusted for the examiner and second further conducted some sensitivity analyses. Despite we used a well-known method to assess iron status in our study by taking adjusting serum ferritin by CRP to define ID's cut-off, this assessment in the context of infection and inflammation may not be accurate as this approach may be susceptible to residual misclassification if the high CRP levels resolve before the ferritin response.

4.3. BLL and malaria

Malaria is a parasitic infection which represents a risk for more than 40% of the world's population, with the huge burden affecting young children under five years in sub-Saharan Africa. Some studies suggest that BLL is associated with malaria infection [33]. Although, we had information about malaria status of the infants at birth and at 12 months of age, we decided not to include it in our analysis. We hypothesized that malaria was a mediator (intermediate factor) in the association between BLL and psychomotor development of the infants at 12 months of age, and as such we decided not to adjust for it.

4.4. ID/anemia and psychomotor development

The results indicate a delay in the cognitive functions and gross motor skills in infants at age of one year in relation to the anemia status at birth and at one year. The results were statistically significant for moderate and severe anemia at one year with gross motor scores, and for severe anemia at one year with cognitive function. These observations are consistent with previous observations in different studies. In a study conducted in Chile, among anemic infants, the level of hemoglobin was correlated with the performance [36]. The timing, duration, and severity of ID/anemia are essential in determining the type of delay that will be manifested [7,36]. Many parts of the brain are becoming myelinated during the first two years of life [36]. Recent evidence shows that brain iron is crucial for normal myelination, in addition to the role of iron in central nervous system neurotransmitter function. Iron is also required for the enzyme ribonucleotide

reductase that regulates central nervous system cell division [37]. Unexpectedly, we did not find this deficit with ID. May be the effect is not clear at this young age, and it will be more evident later. Animal studies indicated a lasting deficit in brain when iron deficiency anemia occurs early in development [36].Obtaining evidence of similar effects in human studies has faced many methodological challenges. The research on the effects of iron effects on infant development has depended mainly on standardized tests of infant development, which have serious limitations and afford unknown relations to the developing brain [36].

4.5. Lead and child development

Unexpectedly, in our study higher blood lead level at 12 months of age was more consistently associated with higher gross motor scores even after adjusting for covariates such as birth weight, gestational age, parity, maternal age, score of family possession, maternal IQ, HOME score, language, and the examiner. Of course, this observation must be interpreted cautiously, even if this observation was consistent with results by Ruiz-Castell et al. (2012) [38].Tellez-Rojo et al. (2006) found that BLL at 12 months of age was inversely associated with Psychomotor Development Index at 24 months of age, but not with Mental or Psychomotor Development Indexes at 12 months of age [39].

It is not clear whether infants with better gross motor ingested more lead or whether lead influenced gross motor. Increased gross motor scores in relation to BLL may reflect that infants with better gross motor are more likely to crawl earlier and to move more easily than children with poor gross motor, thus allowing them to eat paint chips or soil for example that may be sources of lead in this setting. These children should be further followed to study the BLL effects on the longer term. Children with good gross motor in infancy may be those at highest risk of neurotoxicity of lead later in childhood. The few studies looking at this association either found an association [39], or no association [40,41] or an inverse association [38]. We did not found an association between BLL and the early composite score at 12 months of age. However, under the hypothesis that children with best gross motor may be more likely to have high levels of BLL, the association between the early composite score and BLL may be biased and the association likely to be underestimated. Indeed, gross motor and cognitive scores are likely to be associated. In a study conducted in Nigeria in Sub-Saharan Africa [42], early walkers were likely to be asked by their caregivers to complete errands that take them outside of the house, getting the opportunities to use and develop language, memory, and problem-solving skills. Those children had better cognitive test scores than children who were not given such responsibilities. In our study, children in highest quartiles of BLL may be those with best gross motor, and thus with relatively good cognitive function. Another possibility is that the toxic effect of lead on cognitive functions is more evident in older age. Wesserman et al.(1994) found that

BLLs after 18 months of age were more strongly related to cognitive development than BLLs before 18 months [43,44]

4.6. Association between ID/anemia and BLL

Many authors suggested an association between iron deficiency and high BLLs in children. One study in particular suggested that ID may predispose children to high BLLs [7]. At two consecutive lead screening visits, children with ID at first visit were four times more likely to have high BLL (≥10 μg/dl) at second visit than were iron-replete children (Wright et al. 2003) [45]. Accordingly, we found that ID at 12 months was associated with higher levels of BLL, but ferritin, anemia at birth and anemia at 12 months of age were not.

4.7. Interaction between lead and ID/anemia

In our study we had many infants with ID and they may be more susceptible to lead toxicity. This susceptibility could be explained by the higher lead absorption among the iron deficient infants, and promotion of lead excretion among infants with better iron status [7].

We found an association between the highest quartile of BLL and the early composite score in ID children at 12 months of age, but not in non-ID children. This suggests an interaction between ID at 12 months, BLL and cognitive function in infants.

Because gross motor scores in our study may reflect more the ability of the child to ingest BLL, it is somewhat difficult to interpret interactions between ID/anemia, BLL and gross motor scores.

4.8. Hyperactivity

David et al. (1977) found that lead levels were not increased in hyperactive children with a known organic etiology (e.g., birth trauma, head injury), bur were higher in other hyperactive children [46,48]. These observations could lead us to reject the proposition that hyperactivity in infants predisposes them to the unnecessary intake of lead. Further studies are needed to address this hypothesis especially in young infants at one year of age.

An alternative hypothesis for the positive association between BLL and gross motor scores may be that gross motor scores may be improved in children with modified behavior due to lead exposure. Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental behavioral disorder. This multifactorial condition occurs in 3% to 9% of school aged children [47]. Research has been consistent in linking exposure to lead during childhood with ADHD symptoms [47-50]. ADHD is typically not diagnosed before school age. However, little is known on the association between behavior and gross motor in infants exposed to lead in early infancy [18]. No information was available in our study on behavior. Long-term follow-up of these children at school-age may allow studying further the association between gross motor at one year of age and subsequent hyperactivity disorders at 5 or 6 years.

4.9. Recommendations

Timing and duration are important factors in determining the types of delays that will be manifested. Effects of lead on the brain may depend on age and the peak of exposure [48] .The peak BLL, which occurs approximately at 2 years old in the United States [50], is unknown in our study, so we recommend to follow up the children, repeat the assessment at age of 5 or 6 years, and examine the effects of lead on the psychomotor functions of the children which we expect to be more evident in older age groups.

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