 doi:10.20944/preprints202112.0371.v1

Article

Delta-Aminolevulinic acid dehydratase enzyme activity and susceptibility to Lead toxicity in Uganda's urban children

Ambrose Mukisa [1], Denis M Kasozi[1] Claire Aguttu[1] and Joseph Kyambadde[1]*

[1] Affiliation 1; Biochemistry department, Makerere University.
* Correspondence: joseph.kyambadde@gmail.com; A.M.

Abstract:

Rapid industrialisation, urbanisation and population explosion in sub-Saharan Africa escalate environmental Lead levels with subsquent elevation of blood Lead levels in children. Nutrition status, age and genetics govern one's susceptibility to Lead toxcity. This study expounded this susceptibility by relating blood Lead levels, d-aminolevelinic acid dehydratse enzyme activity (ALAD) and genetic variations of proteins that code for ALAD enzyme in urban children of Uganda. Spectrophometric analysis for blood Lead (BL), hemoglobin levels and determination d-levels aminolevelinic acid dehydratse enzyme activity of the blood samples from 198 children was performed prior to polymerase chain reaction and restriction fragment length digestion for ALAD polymorphism was done. Up to 99.5% samples coded for ALAD1 allele whereas 0.05% coded for ALAD2. Genotypes ALAD2-2 members had elevated BLL (mean 14.1 µg/dL) and reduced ALAD enzyme activity compared to others. This therefore implies that majority of children hoard BL which may affect them later in life.

Keywords: Blood Lead levels1; d-aminolevulenic acid dehydratase enzyme activity; d-aminolevulenic acid dehydratase gene polymorphism.

Introduction

Uganda like many other African countries is faced with numerous simultaneous transitions that include economic development, industrialization, population explosion and urbanization. These transitions are coming with both environmental and health changes. Population explosion is putting pressure on the environment through increased anthropogenic activities, elevated volumes of electronic wastes and this has resulted in increased volumes of toxic pollutants like Lead in both air and water bodies. Because Lead is an accumulative toxin, its increased concentration in the environment continue to pause health challenges especially to the children [1,2,3,4]. Elevated environmental Lead levels have been shown to correlate with the blood Lead levels in exposed individuals [5]. Childhood Lead exposure is associated with various health challenges that include lung, stomach, and bladder cancers, anemia, neuro cognitive disorders, intelligent quotient (IQ) lowering and stunted growth [4,6]. Although environmental Lead pollution is preventable, in many African countries including Uganda, little attention is accorded to this problem, for example recent studies conducted in different parts of Kampala slums report elevated blood Lead levels especially among children [3,7]. One's blood Levels is modulated by his age, genetics, nutritional and malaria infection status, (3,8,9]. The rate of Lead ion absorption is further shown to increase with decrease in hemoglobin levels. Following its absorption, Lead sinks in red blood cells (RBCs) where it specifically binds the delta-aminolevulinic acid dehydratase (ALAD) enzyme. This enzyme (ALAD) is a second and important enzyme in the heme biosynthetic pathway and it is involved in the condensation of glycine and succinyl CoA, decarboxylation into delta-aminolevulinic acid (ALA).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2021 doi:10.20944/preprints202112.0371.v1

It specifically catalyzes the heme formation reaction where 2 molecules of ALA are converted into monopyrrole porphobilinogen.

Enzyme ALAD is rich with thiol groups, that have high affinity for Lead ions and this renders the enzyme susceptible to inhibition [10,11]. It is a tetramer homodimer with eight identical subunits and located in the cytoplasm. In each of its subunits, it binds eight zinc atoms, where four zinc molecules act as catalysts whereas the remainder serve as tertiary structural stabilizers. In times of Lead burden, Lead ions displaces zinc from the enzyme's active site and inhibits its activity, resulting into heightened levels of ALA in circulation. Its susceptibility to Lead toxicity is dependent on one's age, nutritional status and the genetics [12]. Accumulated levels of ALA trigger the production of reactive oxygen species (ROS) which are associated with oxidative stress.

Several studies from different regions indicate varying blood Lead levels, biological, markers and even symptoms among people in the same locality. This observation is attributed to the polymorphic nature of delta-aminolevulinic acid dehydratase gene that encodes ALAD enzyme. Polymorphism of ALAD gene is reported to modulate one's susceptibility to Lead toxicity[13,14]. The ALAD enzyme is encoded by a single gene on chromosome 9q34 region [15]. This gene codes for two alleles i.e., ALAD-1 and ALAD-2 [16] which are codominant (Single Nucleotide Polymorphism database (dbSNP) ID: rs1800435 [17]. Their expression results into a polymorphic enzyme system consisting of three different isozymes: ALAD1-1, ALAD1-2, and ALAD2-2. Individuals dominantly expressing ALAD2, ALAD1-2 and ALAD2-2 have a higher susceptibility to Lead toxicity than those expressing the ALAD1-1 isozyme. The prevalence of the ALAD-2 allele is race specific and usually in the ranges of ranges from 0 to 20 percent [13].Therefore, the ALAD polymorphism affects and modifies Lead metabolism and delivery to target organs [18]. To date, no study regarding ALAD enzyme activity and polymorphism distribution in Ugandan population. The present study, therefore, aimed at expounding the ALAD enzyme activity and the distribution of ALAD genotypes in relation to Lead exposure susceptibility in Uganda children. To our knowledge, this is the first study to address Lead exposure susceptibility, ALAD enzyme activity and polymorphism in Ugandan children.

Methods and methodology

This study was approved by Gulu University Research Ethics Committee No. (GUREC-048) dated 31/05/2019. Intentions of the study were first clearly explained in both English and the local language to the participants' parents/guardians prior to signing the informed consent forms. Venous blood samples (n = 198) equivalent to 5mls from a cross-section of children aged 6-60months residing in Katanga slum of Kampala city Uganda (00°18′49″N 32°34′52″E) coordinates were drawn into both hepatized and EDTA tubes by qualified nurses and technicians and transported on ice to Makerere University Biochemistry Department laboratory for analysis. Visibly malnourished children were excluded from this study.

Assay for blood Lead using atomic absorption spectrophotometer

Blood Lead levels were determined on an atomic absorption spectrophotometer (Agilent 2000 series) equipped with a graphite tube atomizer, a hollow-cathode lead lamp with a working current of 5mA, 283.3 nm spectral line and 0.5 nm bandwidth following a method described by [19]. Five hundred microliter (500 µls) aliquots of blood samples were mixed with 1.2 ml of a solution containing 0.5% Triton X-100 and 1% $(NH_4)_2HPO_4$. A total volume of 1.8 ml of de-ionized water was added to each sample in the tube and this was followed by the addition of 1.5 ml of 20% Trichloro-acetic acid (TCA) prior to vortex mixing. The samples were centrifuged at 5000 rpm for 20 min and 10 µl of the supernatant from each was harvested and injected into the graphite tube. Lead standard concentrations ranged from 2

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2021 doi:10.20944/preprints202112.0371.v1

µg /dL to 50 µg /dL, the sample were analyzed in duplicate, and their mean value determined with occasional blanking with deionized/distilled water. The machine had a detection limit of 2µg/dL.

Colorimetric determination of hemoglobin levels by blood cyanmethemoglobin reaction method

Hemoglobin levels were determined following a cyanmethemoglobin reaction method described by [20]. Aliquots equivalent to 100µl of samples in duplicates were made to a total volume of 1000µl with reaction solution containing 200mg of hexacyanoferrate III, 50mg of potassium cyanide, 140mg of potassium hydrogen phosphate and 1ml of Triton X-100 in a litre of distilled water. This was then incubated for 15 min at room temperature before reading at 540 nm, with the blank being the reaction reagent. Then 500 µl of standard hemoglobin standard (0.7mg/ml) was diluted with 500 µl of the same reagent, treated as above and readings taken. The Hb concentration in g/dl was calculated using formula;

$$\text{Hb concentration (g/dL)} = \frac{\text{OD sample} \times \text{concentration of the standard (mg/dL)}}{\text{OD standard sample}}$$

Where OD =optical density or absorbance at 540nm

Determination of hematocrit levels of the study blood samples

The hematocrit of the study blood samples was assayed following a method described by [21,22]. Whole blood samples in heparinized tubes were forced into narrow diameter glass capillary tubes to two-third levels. The capillary tubes had self-sealing compound from one end. The capillaries together with the blood were loaded onto a micro-hematocrit centrifuge and ran at a relative centrifugal force of 14,000 × g for five minutes. Following centrifugation, hematocrit levels of each sample were measured within 10 minutes while the tubes were kept in a horizontal position to avoid merging of the layers. Hematocrit levels were estimated by calculating the ratio of the column of packed erythrocytes to the total length of the sample in the capillary tube.

Determination of delta-aminolevulinic acid dehydratase (ALAD) enzyme activity

The blood δ−ALAD enzyme activity in all the samples collected was measured following a method described by [23]. The ALAD enzyme activity of each sample in duplicate was determined by having 0.20mls incubated with 1.30mls of Triton X-100 reagent in disposable plastic tubes and there after adding 1 ml of buffered ALA substrate (0.01M). The buffered ALA substrate was prepared by dissolving 0.1676g of ALA-HCL in 100mls of phosphate-citrate buffer pH 6.65. The buffer was previously prepared by dissolving Na_2HPO_4 6.703g/dL (0.25M) and citric acid 5.25g/dL (0.25M). Aliquots equivalent to 1ml from each sample and blanks (distilled water) were added to tubes containing 1ml of TCA reagent.

To both test and blank aliquots, 1.0mls of the modified Ehrich's reagent previously prepared by dissolving 10g of p-dimethylaminobenzaldehyde (DMBA), in 420mls of acetic acid and diluted to 1L with distilled water before storing at 4^0C from which a working solution was made by mixing 50mls of DMBA-acetic acid with 8mls of 70% perchloric acid. Following the addition of the modified Ehrich's working reagent, the mixtures were allowed to stand for 13min for color to develop before measuring at 555 nm on a spectrophotometer.

The corrected absorbance A = (Test absorbance – the blank absorbance) were used to calculate the activity of the enzyme.

Corrected Absorbance:

$$\frac{A \times 1250}{\quad} = \text{units of ALAD enzyme activity,}$$

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2021 doi:10.20944/preprints202112.0371.v1

Hematocrit

Where 12500 is the blood dilution factor.

Delta-aminolevulinic acid dehydratase (ALAD) Genotyping

Blood samples were analyzed following a method described by [24,25], the genomic DNA from blood samples was extracted using a Qiagen genomic DNA purification kit following the manufacturers instruction. The resultant DNA products were purified prior to polymerase chain rection (PCR) amplification. The PCR reaction mixture equivalent to 50-µL contained 1× buffer (10 mM Tris-HCl, pH 8.8; 50 mM KCl), 2 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmol each primers;

(Forward, 5'-AGACAGACATTAGCTCAGTA-3',

and reverse, 5'GGCAAAGACCACGTCCATTC-3') and 3U Taq DNA polymerase.

The running conditions were; pre-denaturation at 94 °C for five min, followed by thirty-five cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, synthesis at 72 °C for 1 minutes and final extension at 72 °C for five min. The amplified products (916-bp region of genomic DNA) in volumes of 10 µL were digested overnight with MspI restriction enzyme (2.5 units) in a 20 µL reaction mixture containing 50 mM sodium chloride, 10 mM Tris-HCl, 10 mM magnesium chloride, 1mM dithiothreitol (pH 7.9) at 37 °C. The fragments were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV illumination system. ALAD1-2 samples had both a 583- and a 512-bp fragment, whereas ALAD1-1 individuals had a single 583-bp fragment.

Data analysis;

Results were expressed as means, and the statistical significance evaluated by one way analysis of variance (ANOVA) using GraphPad Prism eight version.

Results

Following genotyping the samples for ALAD alleles, the outcome is shown in the table 7.1 below with corresponding BLL, Hb levels, hematocrit and ALAD enzyme activities. The results indicate that ALAD1-1 allele is the most predominant with moderately high hemoglobin levels and seemingly normally functioning ALAD enzyme. The frequency of ALAD 2 allele is shown to be the least predominant as compared to ALAD1 allele. Comparing the hemoglobin levels across all the groups, it is apparent that ALD2 allele members have lower Hb levels.

The results further indicate that members with genotype ALAD1-1 had their ALAD enzyme activity functioning moderately as compared to the rest. The results were statistically analyzed with Minitab 19 statistical software for correlation and significance and the results are shown in table 7.2 below. From the results, ALAD enzyme activity and hemoglobin levels strongly correlated with blood Lead levels across all the genotypes.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2021 doi:10.20944/preprints202112.0371.v1

Table 7.1: The gene distribution of ALAD isozymes among the study participants. Their corresponding levels of blood Lead, ALAD enzyme activity, hemoglobin and hematocrit.

Genotype	Frequency of ALAD iso-zymes	Blood lead levels (mean) µg/dL	ALAD enzyme activity (mean) Units/L	Hemoglobin levels (mean) g/ dL	Hematocrit volume (%) (mean)
ALAD 1-1	0.889	8.8	39.6	8.9	27.6
ALAD 1-2	0.106	12.3	34.7	6.8	29.2
ALAD 2-2	.005	14.1	33.8	6.1	32.9

3.2: Correlations (Pearson) r and p-values between different ALAD isozymes and blood Lead levels, ALAD enzyme activity, hemoglobin levels and hematocrit volumes.

	Blood Lead levels µg/dL	ALAD enzyme activity Units/L	Hemoglobin levels (g/ dL)	Hematocrit volume (%)
ALAD 1-1	r = 0.42, p-value 0.02	r = 0.66, p-value = <0.001	r = 0.51, p-value = <0.001	r = 0.11, p-value = <0.07
ALAD 1-2	r = 0.62, p-value = <0.001	r = 0.71, p-value = <0.001	r = 0.69, p-value = <0.001	r = 0.16, p-value = 0.06
ALAD 2-2	r = 0.67, p-value = <0.001	r = 0.71, p-value = <0.001	r = 0.64, p-value = <0.001	r = 0.12, p-value = 0.11

Discussion

Various factors including duration (time) of exposure [26] levels of environmental Lead pollutant in the area, nutritional status, age and the genetics accounts for one's Lead poisoning susceptibility [27,28]. Even with similar environmental settings and confounding factors, variations in susceptibility to Lead poisoning among individuals exist [29,30].

This study therefore aimed at expounding the relationship between genetic variations of proteins that code for ALAD enzyme and Lead susceptibility among individuals (children aged 6-60 months) living in the same geographical area (Katanga Uganda). Based on the available rich literature about the stoichiometric inhibitory effect of blood Lead ions on ALAD activity [31,32], we hypothesized that ALAD allele frequency distribution account for one's blood Lead levels. The extent of this ALAD enzyme inhibition is dependent on one's ALAD protein genetics [13]. From the fact that this enzyme (ALAD) is polymorphic [33] with a G-to-C transversion at position 177 (db SNP ID: rs1800435) and two alleles (ALAD1 and ALAD2) with three isozymes; ALAD 1-1, ALAD 1-2, and ALAD 2-2, dominating ALAD allele accounts for his/her Lead toxicity susceptibility. Delta-aminolevulinic dehydratase enzyme polymorphism differ by race, and geographical location. Form the current study findings, we report significant correlations between ALAD genotype and Hb level, ALAD genotype and ALAD enzyme activity, and blood Lead levels (table 8.2). Blood Lead levels were elevated in carriers of ALAD 2-2 isozyme than those of both ALAD 1-2 and ALAD 1-1 isozyme carriers (table 8.1). The variations in Lead burden among these three groups observed in table 8.1 are attributed to the difference in the electronegativity of the amino-acids lysine and asparagine that code for these isozymes. From this study findings, we concur with reports of various previous studies from different regions that indicate low prevalence of ALAD 1-2 and ALA2-2 as compared to ALAD 1-1 genotype. Based on the study results in table 8.1, it is acceptable that having ALAD 1-2 and ALAD 2-2 alleles as the less predominant phenotypes delay Lead poisoning symptoms like ALAD enzyme

 doi:10.20944/preprints202112.0371.v1

inhibition [34,35], than individual who have ALAD 1-1 as the dominating allele. We statistically analyzed data groups (ALAD 1-1 vs ALAD 1-2 and 2-2 individuals) using one way analysis of variance (ANOVA). Step-wise regression and multiple analyses of variance were used to assess the contribution effect of different ALAD genotypes towards blood Lead levels, ALAD enzyme activity and hemoglobin levels of the study participants (table 8.1). Compared to other isozymes, ALAD 1 -1 genotype which is encoded by the less electronegative protein (lysine) was the most dominant and because of this it binds less Pb ions hence more susceptibility to Lead poisoning. This is owed to the fact that Lead ions bind ALAD with high electronegative charge tightly hence reducing the amount of bioavailable Lead ions in circulation [36]. The, unbound Lead ions circulating freely in the body systems end up affecting many vital organs. However, in times of oxidative and nutritional challenges, this tightly bond Lead is released back into circulation resulting manifestations like anemia, impaired intelligent quotient (IQ) etc.

These findings therefore, reveal that ALAD-2 allele variant modifies Lead kinetics by making it more available in circulation while lowering its uptake into cortical bones.

This study therefore, concludes that ALAD polymorphism is of great importance during the toxicokinetics of Lead poisoning during exposure and therefore recommend a further ALAD genotyping involving a bigger study population.

Ethics approval and consent to participate

This study was approved by Gulu University Research Ethics Committee No. (GUREC-048).

Consent for publication

Guardians and parents were clearly explained the study objectives to which they consented.

Availability of data and materials

All data generated or analysed during this study are available on request.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Mukisa Ambrose, Denis Kasozi, Claire Aguttu and Joseph Kyambadde]. The first draft of the manuscript was written by [Mukisa Ambrose and Claire Aguttu edited by Denis Kasozi and Joseph Kyambadde]. All authors read and approved the final manuscript.

Funding Statement

Not applicable

REFERENCES

1. Shen, X. M., Yan, C. H., Guo, D., Wu, S. M., Li, R. Q., Huang, H., Ao, L. M., Zhou, J. D., Hong, Z. Y., Xu, J. D., Jin, X. M., and Tang, J. M. (1998). Low-level prenatal lead exposure and neurobehavioral development in children in the 7rst year of life: A prospective study in Shanghai. Environ. Res. 79, 18}27.
2. Shen, X. M., Yan, C. H., Guo, D., Wu, S. M., Li, R. Q., Huang, H., Ao, L. M., Zhou, J. D., Hong, Z. Y., Xu, J. D., Jin, X. M., and Tang, J. M. (1997). Prevalence of elevated blood lead levels of children in Shanghai. Chinese J. Preventive Med. 31, 9}12. [Chinese]
3. Shen, X. M., Guo, D., Wu, S. M., and Xu, J. D. (1990). Resilience of children to lead poisoning: A pilot study. J. Clin. Pediatr. 8, 105}106. [Chinese]
4. Centers for Disease Control and Prevention (CDC). (1991). ‘‘Preventing Lead Poisoning in Young Children.’’ U. S. Department of Health and Human Services, Public Health Service, CDC.
5. Mukisa, A., Kasozi, D., Aguttu, C., Vuzi, P. C., & Kyambadde, J. (2020). Relationship between blood Lead status and anemia in Ugandan children with malaria infection. *BMC pediatrics*, *20*(1), 1-7.
6. Steenland, K., & Boffetta, P. (2000). Lead and cancer in humans: where are we now? American journal of industrial medicine, 38(3), 295-299.
7. Cusick, S. E., Jaramillo, E. G., Moody, E. C., Ssemata, A. S., Bitwayi, D., Lund, T. C., & Mupere, E. (2018). Assessment of blood levels of heavy metals including Lead and manganese in healthy children living in the Katanga settlement of Kampala, Uganda. BMC public health, 18(1), 717.
8. Desai, Meghna R., Dianne J. Terlouw, Arthur M. Kwena, Penelope A. Phillips-Howard, Simon K. Kariuki, Kathleen A. Wannemuehler, Amos Odhacha et al. "Factors associated with hemoglobin concentrations in pre-school children in Western Kenya: cross-sectional studies." The American journal of tropical medicine and hygiene 72, no. 1 (2005): 47-59.
9. Kwena, A. M., Terlouw, D. J., De Vlas, S. J., Phillips-Howard, P. A., Hawley, W. A., Friedman, J. F., ... & ter Kuile, F. O. (2003). Prevalence and severity of malnutrition in pre-school children in a rural area of western Kenya. The American journal of tropical medicine and hygiene, 68(4_suppl), 94-99.
10. Papanikolaou, N. C., Hatzidaki, E. G., Belivanis, S., Tzanakakis, G. N., & Tsatsakis, A. M. (2005). Lead toxicity update. A brief review. Medical science monitor, 11(10), RA329-RA336.
11. Chia, S. E., Yap, E., & Chia, K. S. (2004). δ-Aminolevulinic acid dehydratase (ALAD) polymorphism and susceptibility of workers exposed to inorganic lead and its effects on neurobehavioral functions. Neurotoxicology, 25(6), 1041-1047.
12. Yang, Y., Wu, J., & Sun, P. (2012). Effects of delta-aminolevulinic acid dehydratase polymorphisms on susceptibility to lead in Han subjects from southwestern China. International journal of environmental research and public health, 9(7), 2326-2338.
13. Kelada, S. N., Shelton, E., Kaufmann, R. B., & Khoury, M. J. (2001). δ-Aminolevulinic acid dehydratase genotype and lead toxicity: a HuGE review. American journal of epidemiology, 154(1), 1-13.
14. Ziemsen, B., Angerer, J., Lehnert, G., Beckman, H. G., and Goedde, H. W. (1986). Polymorphism of delta-aminolevulinic acid dehydratase in lead exposed workers. Int. Arch. Occup. Environ. Health 58, 245}247.
15. Potluri, V. R., Astrin, K. H., Wetmur, J. G., Bishop, D. F., & Desnick, R. J. (1987). Human δ-aminolevulinate dehydratase: Chromosomal localization to 9q34 by in situ hybridization. Human genetics, 76(3), 236-239.
16. Battistuzzi, G., Petrucci, R., Silvagni, L., Urbani, F. R., and Caiola, S. (1981). Delta aminolevulinate dehydrase: A new genetic polymorphism in man. Ann. Human Genet. 45, 223}229.

17. http://www.ncbi.nlm.nih.gov/SNP/index.html
18. Fleming, D. E., Chettle, D. R., Wetmur, J. G., Desnick, R. J., Robin, J. P., Boulay, D., Richard, N. S., Gordon, C. L., and Webber, C. E. (1998). Effect of the delta-aminolevulinate dehydratase polymorphism on the accumulation of lead in bone and blood in lead smelter workers. Environ. Res. 77, 49}61.
19. Navarro, J. A., Granadillo, V. A., Parra, O. E., & Romero, R. A. (1989). Determination of lead in whole blood by graphite furnace atomic absorption spectrometry with matrix modification. Journal of Analytical Atomic Spectrometry, 4(5), 401-406.
20. Balasubramaniam, P., & Malathi, A. (1992). Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. Journal of postgraduate medicine, 38(1), 8.
21. Adeleye, Q. A., Oniyangi, O., Audu, L. I., & Pettifor, J. (2021). The optimal time for haematocrit check after packed red blood cell transfusion among children with anaemia. South African Journal of Child Health, 15(3).
22. McGovern, J. J., Jones, A. R., & Steinberg, A. G. (1955). The hematocrit of capillary blood. *New England journal of medicine*, *253*(8), 308-312.
23. Burch, H. B., & Siegel, A. L. (1971). Improved method for measurement of delta-aminolevulinic acid dehydratase activity of human erythrocytes. *Clinical Chemistry*, *17*(10), 1038-1041.
24. Wetmur, J. G., Kaya, A. H., Plewinska, M., and Desnick, R. J. (1991). Molecular characterization of the human deltaaminolevulinate dehydratase 2 (ALAD) allele: Implications for molecular screening of individuals for genetic susceptibility to lead poisoning. Am. J. Human Genet. 49, 757}763.
25. Wetmur, J. G., Bishop, D. F., Cantelmo, C., and Desnick, R. J. (1986). Human delta-aminolevulinate dehydratase: Nucleotide sequence of a full-length cDNA clone. Proc. Natl. Acad. Sci. USA 83, 7703}7707.
26. Schwartz, B. B., Lee, B. K., Stewart, W., Ahn, K. D., Springer, K., and Kelsey, K. (1995). Associations of ALAD genotype with plant, exposure duration, and blood lead and zinc protoporphyrin levels in Korean lead workers. Am. J. Epidemiol. 142, 738}745.
27. Mahaffey, K. R. (1974). Nutritional factors and susceptibility to lead toxicity. *Environmental health perspectives*, *7*, 107-112.
28. Goyer, R. A., & Mahaffey, K. R. (1972). Susceptibility to lead toxicity. *Environmental health perspectives*, *2*, 73-80.
29. Needleman, H. L., & Bellinger, D. (1991). The health effects of low-level exposure to lead. *Annual review of public health*, *12*(1), 111-140.
30. Benkmann, H. G., Bogdanski, P., and Goedde, H. W. (1983). Polymorphism of delta-aminolevulinate acid dehydratase in various populations. Human Hered. 33, 62}64
31. Warren, M. J., Cooper, J. B., Wood, S. P., & Shoolingin-Jordan, P. M. (1998). Lead poisoning, haem synthesis and 5-aminolaevulinic acid dehydratase. Trends in Biochemical sciences, 23(6), 217-221.
32. Rogan, W. J., Reigart, J. R., & Gladen, B. C. (1986). Association of amino levulinate dehydratase levels and ferrochelatase inhibition in childhood lead exposure. The Journal of pediatrics, 109(1), 60-64.
33. Wetmur, J.G., Lehnert, G., Desnick, R.J., 1991b. The delta-aminolevulinate dehydratase polymorphism: higher blood lead levels in lead workers and environmentally exposed children with the 1-2 and 2-2 isozymes. Environ. Res.56, 109–119
34. Astrin, K. H., Bishop, D. F., Wetmur, J. G., Kaul, B. C., Davidow, B., and Desnick, R. J. (1987). Aminolevulinic acid dehydratase isozymes and lead toxicity. Ann. N. Y. Acad. Sci. 514, 23}29.
35. DeBruin, A. (1968). Effect of lead exposure on the level of delta aminolevulinic acid dehydratase activity. Med. Lav. 59, 411}428

 doi:10.20944/preprints202112.0371.v1

36. Miyaki, K., Lwin, H., Masaki, K., Song, Y., Takahashi, Y., Muramatsu, M., & Nakayama, T. (2009). Association between a polymorphism of aminolevulinate dehydrogenase (ALAD) gene and blood lead levels in Japanese subjects. International journal of environmental research and public health, 6(3), 999-1009.

Conset form

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 December 2021 doi:10.20944/preprints202112.0371.v1

Parental Consent to Participate in a Research Study
College of Natural Sciences • Makerere University, Uganda

Title of Study: Role of blood lead during the anemia pathogenesis among Katanga children aged under five years with *Plasmodium* parasite infection

Investigators:

Name: Mukisa Ambrose **Dept:** Biochemistry **Phone:** 0790222111

Name: Dr. Kyambadde Joseph **Dept:** Biochemistry **Phone:** 0772510824

Name: Dr. Denis Kasozi **Dept:** Biochemistry **Phone:** 0785939611

Parent/Guardian Name: _____

Sample; Blood

Introduction

- Your child is being asked to be in a research study on the *Role of blood lead on anemia pathogenesis in children with malaria* She /he was selected as a possible participant because *she/he has malaria infection and is the resident of lead polluted area*
- We ask that you read this form and ask any questions that you may have before allowing your child to participate in this study.

Purpose of Study

- The purpose of the study is to *investigate the interaction between blood lead levels and Plasmodium parasite infection during anemia pathogenesis in children under five years of age*
- Ultimately, this research may be published in journals

Description of the Study Procedures

- If you decide to allow your child to participate in this study, s/he will be asked to do the following things: blood sample provision collected by venipuncture once

Risks/Discomforts of Being in this Study

There may be unknown risks.

Benefits of Being in the Study

- The benefits of participation are [*explain benefits of participation that will be gained by the participants and/or other. If a benefit is not likely to occur to each participant do not include*.
- [*If there are no expected benefits, state as such.*]

Confidentiality

- This study is anonymous. We will not be collecting or retaining any information about your child's identity.

Payments

There won't be any payments of the participants.

Right to Refuse or Withdraw

- The decision to participate in this study is entirely up to you and your child. You are welcome to observe the interview if you wish. Your child may refuse to take part in the study *at any time* without affecting your relationship with the investigators of this study or Smith College or losing benefits to which you are otherwise entitled. Your child has the right not to answer any single question, as well as to withdraw completely from the interview at any point during the process; additionally, you have the right to request that the interviewer not use any of the interview material.

Right to Ask Questions and Report Concerns

- You have the right to ask questions about this research study and to have those questions answered by me before, during or after the research. If you have any further questions about the study, at any time feel free to contact me, ***Mukisa Ambrose***] at ***amukisa@cns.mak.ac.ug]*** or by telephone at **0790222111**. If you like, a summary of the results of the study will be sent to you. If you have any other concerns about your rights as a research participant that have not been answered by the investigators, you may contact Makerere University Institutional Review Board.
- If you have any problems or concerns that occur as a result of your participation, you can report them to **Dr. Kyambadde Joseph (supervisor**)

Consent

- Your signature below indicates that you have decided to allow your child participate as a research subject for this study, and that you have read and understood the information provided above. You will be given a signed and dated copy of this form to keep, along with any other printed materials deemed necessary by the study investigators.

Parent/Guardian Name: _____

Parent/Guardian Signature: _____ Date: _____

Investigator's Signature: _____ Date: _____

GULU UNIVERSITY

P.O. Box 166 Gulu Uganda
Website: www.gu.ac
Email: guluuniversity.rec@gmail.com

Tel: +256-4714-32096
Fax: +256-4714-32913
Mob: +256772305621/776812147

RESEARCH ETHICS COMMITTEE

31st May 2019

APPROVAL NOTICE

Mukisa Ambrose
Makerere University

Re: **Application No. GUREC-048-19**

Type of review:
[X] Initial review
[ ] Amendment
[ ] Continuing review
[ ] Termination of study
[ ] SAEs
[ ] Other, Specify:_____

Title of proposal: "**Assessing social programs for refugees in northern Uganda: a mixed methods study comparing refugee and host communities"**

I am pleased to inform you that Gulu University Research Ethics Committee **(GUREC)** approved the above referenced application.

Approval of the research is for the period of **22nd April 2019 to 21st April 2020**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the **GUREC** for re-review and approval prior to the activation of the changes. The **GUREC** application number assigned to the research should be cited in any correspondence.

GULU UNIVERSITY
INSTITUTIONAL REVIEW COMMITTEE
APPROVED
31 MAY 2019
FACULTY OF MEDICINE
P. O. Box 166, Gulu

3. Any unanticipated problems involving risks to participants must be promptly reported to the **GUREC**. New information that becomes available which could change the risk: benefit ratio must be submitted promptly for the **GUREC** review.

4. Only approved and stamped consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The **GUREC** may conduct audits of all study records, and consent documentation may be part of such audits.

5. Regulations require review of an approved study not less than once per 12-month period. Therefore, a continuing review application must be submitted to the **GUREC** eight (**8**) weeks prior to the above expiration **date of 21st April 2020** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely manner may result in suspension or termination of the study, at which point new participants may not be enrolled and currently enrolled participants must be taken off the study.

6. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following documents have been approved in this application by the **GUREC:**

	Document	**Language**	**Version**	**Version Date**
1	Protocol	English	Version 2.0	30th May 2019
2	Data Collection Tools	English	Version 2.0	30th May 2019
3	Informed consent Document	English/Luganda	Version 2.0	30th May 2019

GULU UNIVERSITY
INSTITUTIONAL REVIEW COMMITTEE
APPROVED
31 MAY 2019
FACULTY OF MEDICINE
P.O. Box 166, Gulu

Signed,

Mr. Robert Kiduma
For; Chairperson
Gulu University Research Ethics Committee