Blood Lead Alters The Anemia Status of Malaria Infected Children in Katanga Kampala Uganda.

Ambrose K Mukisa
  Makerere University   https://orcid.org/0000-0002-6570-1102

Denis M Kasozi
  Makerere University

Claire Aguttu
  Makerere University

Peter C. Vuzi
  Makerere University

Joseph Kyambadde (✉ joseph.kyambadde@gmail.com)
  https://orcid.org/0000-0001-9128-2063

Abstract

Background: Uganda, like other malaria endemic countries, childhood anemia due to malaria infection, malnutrition and social disadvantages remains a health challenge. Other contributing factors towards the pathogenesis of this disease like blood lead have not been fully explored. Persistent lead exposure accounts for one's blood lead levels and other many health outcomes. Recent studies report elevated environmental lead levels in urban Uganda with anthropogenic activities including leaded paints, leaded fuels spillages, lead-contaminated air, water and soils listed as major sources. Blood lead (BL) alters iron bioavailability by competitively blocking iron absorption hence anemia. BL inhibits key enzymes ferrochelatase and aminolevulinic acid dehydratase (ALAD) involved in hemoglobin (Hb) biosynthesis resulting into elevated free erythrocyte protoporphyrin (FEP). Children in this area are generally anemic due chronic exposure to malaria parasites that thrive on their red blood cells for survival. Concomitant exposure to both malaria infection and lead pollution, exerbates the anemia status of these children. This study therefore aimed at expounding the anemia status of these Ugandan children simultaneously living exposed to both malaria infection and environmental lead pollution.

Methods: Briefly venous blood samples from 198 children were microscopically assayed for malaria parasite density, hemoglobin (Hb) concentrations by the cyanmethemoglobin method, BL levels by the standard atomic absorption spectrophotometric method and FEP levels by the standard fluorimetric method.

Results: A total of 151/198 (76.3%) children were analyzed with moderate anemia (Hb <10>5 g/dL) while 8/198 (4%) had severe anemia (<5 g/dL). They had means of BL levels (9.3 µg/dL), Hb (7.5 g/dL), FEP/Hb (8.3 µg/g) and parasite density (PD) (3.21×10^3 parasites / µL). There was weak negative correlation between PD and Hb (r = -0.231, R^2= 0.15 P-value < 0.001), as compared to that between FEP/Hb and Hb (r = -0.6, R^2=0.572 P-value=0.001).

Conclusion: Basing on this study's findings, we conclude that BL is a significant contributor to anemia pathogenesis and therefore its co-existence with plasmodium malaria infection in the host exerbates the anemia status.

Trial registration: Not applicable

Introduction

Like many malaria halo endemic developing countries, Uganda is faced with several health -threatening diseases including anemia. A majority (60%) of the Uganda's urban dwellers live in slums such as Katanga [1] in Kampala capital city where social disadvantages remain a big problem [2]. Due to the poor urban planning of Kampala city, there are no gazetted residential areas so people live in or next to industries, workshops, motor garages, metal crafts yards, battery recycling plants, motor garages, mosquito breeding grounds, and landfills which predisposes them to lead intoxication. There are reports of elevated lead levels in water sources, soils, foodstuffs and air around Kampala city [3]. According to

the study done by [4], Plasmodium malaria accounts for up to 40% of all outpatient visits, 25% of all hospital admissions, and 14% of all hospital deaths [4, 5] despite the government efforts to curb its transmission [7]. 53% of the children in this area especially those under the age of 5 years have been reported to be anemic [3, 5] and this could be due to high malaria prevalence in this area.

Although, malaria infections remain the key cause of the high prevalence of anemia, there are maybe other neglected cofounders like Lead poisoning resulting from contaminated air, soils, water sources, urban-grown foodstuffs and Leaded paints [2, 6, 7, 8, 9]. Because both lead pollution and malaria geographically overlap, and produce similar hematological outcomes their co-existence in this area may affect the child's health.

Lead exposure accounts for an individual's blood lead level (BLL) and is more evident in developing fetus by directly affecting the hematopoietic system. Erythrocytes are the major sink of over 99% of the Lead present in the blood and with over 80% is bound to δ-aminolevulinic acid dehydratase (δ-ALAD) a cytosolic enzyme. This enzyme is believed to catalyze the formation of porphobilinogen from δ-aminolevulinic acid (ALA) [9, 10].

During lead intoxication, BL is believed to limit the iron transfer from endosomes toward the cytoplasm and to reduce the lifespan of circulating erythrocytes by increasing the fragility of cell membranes [11, 12].

Blood lead further triggers a reduction in red blood cells (RBC) production by specifically inhibiting Ferrochelatase a mitochondrial enzyme that catalyzes the insertion of iron into protoporphyrin during heme formation. Aminolevulinic acid synthetase (ALAS) a mitochondrial enzyme that catalyzes the formation of aminolevulinic acid (ALA) from succinyl CoA and glycine is also affected by BL [13, 14].

In a normal heme synthesis system, the rate of iron formation and utilization is well balanced and equilibrium is altered by insufficiency of iron. High malaria burden, overproduction of protoporphyrin and decreased ferrochelatase activity due to Lead poisoning could be responsible for the high anemia scourge.

During malaria anemia, Lead becomes even more concentrated in red blood cells causing deleterious effects on several essential cellular processes, including inhibition of protein synthesis. Malaria due to Plasmodium infections becomes fatal by causing severe anemia to the host though other symptoms may occur depending on the species.

Malarial anemia is caused by massive erythrocytes lysis due to elevated parastemia resulting in parasitized red cells rupture. Because of increased parasite density in the host system, there are intrinsic and extrinsic changes to the red blood cells. This persistent situation in turn activates the splenic and other macrophages for phagocytosis [15] and resulting into anemia.

Both iron deficiency due to malaria and BL are each capable of inhibiting heme synthesis at the final step but low cellular iron concentrations during iron deficiency enhance Lead's inhibition on synthetic

reactions because of its high affinity for the side chains of some amino acids than iron.

Ferrochelatase is specifically sensitive to low iron levels in the presence of Lead poisoning [16] and zinc instead of iron is incorporated into protoporphyrin (PPN) resulting in elevated levels of zinc protoporphyrin (ZPP).

Zinc protoporphyrin or FEP concentrations have been used to measure of heme synthesis inhibition, and increased levels are an indicator of iron deficiency as a heme synthesis disorders [17].

Since red cells are at the Centre of anemia pathogenesis, elevated malaria parasite density and BLL could produce severe anemia among children. The severity of anemia status is dependent on the host's age and nutrition status [18]. The combined aftermath of these two processes in malaria-endemic regions may result in severe anemia. This work, therefore, aimed at finding the association between blood lead levels and Plasmodium parasite density and anemia status among the study group. In this study, reduced hemoglobin (Hb) concentration was considered an index of anemia while the FEB/Hb ratio as an impaired heme synthesis index due to Lead poisoning.

Methods

This was a cross-sectional study on children aged 6 -60months living in Katanga area- Kampala city. The children were first screened for Plasmodium parasite infection before recruitment using rapid diagnostic kits. 5mls of venous blood from 198 malaria positive children were collected into EDTA by Qualified nurses and technicians. The samples were then transported on ice to Makerere University Biochemistry Department laboratory and stratified according to malaria parasite density. The samples were then kept at 4^0C awaiting various analysis and determinations.

Determination of Parasite density by thick smear method

Thick smears were prepared as described by [19], air dried, stained with 10% Leishman without fixing and examined under a CX 21 Olympus microscope. Five hundred leukocytes plus the number of malaria parasites seen in the same field were recorded. The number of malaria parasites per microliter (parasite/ µL) of blood was expressed as the reciprocal of the mean counts in the three slides divided by the leukocyte counts, multiplied by a factor of 8000 i.e.

$$\text{Parasites / µL blood} = \frac{\text{Number of parasites counted} \times 8000 \text{ white blood cells/µL}}{\text{No. of white blood cells count}}$$

Determination of blood lead levels by atomic absorption spectrometry

BLL were determined using an atomic absorption spectrophotometer (Agilent 2000 series) equipped with a graphite tube atomizer and deuterium background correction facility. A hollow-cathode Lead lamp with a working current of 5mA, 283.3 nm spectral line and 0.5 nm bandwidth was used. Aliquots (500 µl) of

whole blood melted with 1.2 ml of 0.5% Triton X-100 and 1% $(NH_4)_2HPO_4$ solution were added to 1.8 ml of deionized water and 1.5 ml of 20% Trichloroacetic acid (TCA) and vortex mixed. The samples were centrifuged for 20 min at 5000 rpm and 10 µl of the supernatant injected onto the graphite tube. The calibration curve was drawn using a standard addition method as described in M-572 of INSPQ's Toxicology Laboratory method.

Fluorospectrophotometric quantification of free erythrocyte protoporphyrins

The FEP was measured following a method described by [20] using a fluorospectrophotometer set at 405nm excitation 610nm emissions. The porphyrins were extracted by adding 20 µl aliquots of whole blood to a solution containing 100 µL of 10% ammonium sulfate and 5% celite and vortex mixed for 10sec. 400 µl of 95% ethanol was then added and vortex mixed for more 20 seconds. This was followed by addition of 600 µl of acetone and further vortex mixing for 20 sec. All the samples were put on an ice bath for 20 min, vortex mixed for 20 sec. and centrifuged at 4°C for 10 min. The supernatants were harvested into small borosilicate tubes and aliquots of 300 µl mixed with 300 µl of a solution containing propylene glycol and 1.5 N HCl, (4:1) and let to stand for 20 min before reading at 405 nm excitation and 610 emissions. The FEP blood concentration was calculated using the following formulae;

$$FEP\ (\mu g/dL\ Blood) = FEP\ \mu g\ /100\ ml\ extract = \frac{F_z \times C_s \times 2.7 \times 100}{F_s \times 1.1 \times 0.2}$$

Where F_z is the sample fluorescence, C_s concentration of the standard, F_s is the fluorescence of the standard, 2.7 is the final volume of HCl phase, 100 is the conversion factor to 100ml of extract, 1.1 conversion factor for protoporphyrin measured against a coproporphyrin standard and 0.2 (20µl) is the original blood volume measured. The FEP/Hb ratio expressed as microgram Hb per gram Hb was calculated by dividing the FEP/dL RBC by gram Hb/dL.

Colorimetric determination of hemoglobin levels by blood cyan methemoglobin reaction method

Hemoglobin levels were determined by cyan methemoglobin reaction method, where 100 µl aliquots of samples were made to a total volume of 1000 µl 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 liter of distilled water), and incubated for 15 min at room temperature before reading at 540 nm. The blank was the reaction reagent. 500 µl of standard hemoglobin standard (0.7mg/ml) diluted with 500 µl of the same reagent and treated as above was also read. The Hb concentration in g/dl was calculated using formula;

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

Where OD =optical density or absorbance at 540nm

Results

Of the 198 children enrolled in the study, 8/198 (4%) of the children were severely anemic with Hb < 5 g/dL, mean parasite density = 7.4×10^3 parasites/ µL, mean BLL = 9.2 ± 4.3 µg/dL,

Mean FEP/Hb = 7.4 ± 2.9 µg /g. 39/198 (19.7%) of the study group were not anemic with a mean value of Hb > 10 g Hb/dL (above the WHO cut off point), mean parasite density = 1.7×10^3 parasites/ µL, mean BLL < 2 µg/dL, Mean FEP/Hb = 7.4 ± 2.9 µg /g. The details of the distribution are shown in table 1.

Table 1. Showing the mean parasite density, mean hemoglobin levels, mean BLL distribution among the 198 children that participated in the study.

Mean parasite density/ µL × 10^3	No. of samples N	Mean BLL µg/dL	Mean FEP/Hb µg /g	Mean Hb g/dL
0 .1-– 1.1	16	10.3 ± 1.9	7.9 ± 3	7.0 ± 3.2
1.2-– 2.2	39	< 2	0.6	10.4 ± 2.7
2.3– 3.3	42	10.3 ± 1.7	2.9 ± 3.2	8.5 ± 3.1
3.4–4.4	35	7.9 ± 2.1	3.9 ± 2.8	9.2 ± 3.0
4.5 – 5.5	34	7.9 ± 1.8	8.4 ± 3.2	9.3 ± 4.1
5.6–6.6	23	6.4 ± 1.8	4.9 ± 2.7	6.8 ± 3.3
6.7 – 7.7	8	9.7 ± 5.2	7.4 ± 2.0	4.9 ± 2.9
7.8 – 8.8	1	5.1	5.53	6.7

Hb = hemoglobin, BLL = blood lead levels, FEP = Free erythrocyte protoporphyrin

Table 2
Showing correlation coefficients r and p-values of different interacting variables

Interacting variables	Correlation values r	*p-value*
Parasite density and Hb	-0.231	0.035
Hb and BLL	0.552	< 0.001
Hb and FEP/Hb	-0.572	< 0.001
BLL and Parasite density	0.124	0.082
FEP/Hb, Parasite density and Hb	0.377	< 0.001

Discussion

The research results shown in Table-1 for the recruited study group portrays the silent dangers of co-existence of BL and PD in the host. We observed that only 4% of the children had Hb of < 5 g/dL and a majority (88.2%) with Hb levels greater than 5 g/dL but less than 10 g/dL (anemic) which we attribute to chronic exposure of the study children to malaria parasite infection. Because the malaria parasites depends on the hemoglobin from the host's red blood cells to reproduce, there is always stiff competition between the host and the parasites for iron and hence the observed low hemoglobin levels. We further observed that of the 198 children recruited in this study, 80.3% had a mean BL levels (9.3 µg/dL) slightly lower than those reported by Cusick et al., [7] in the same area. There was a reduced parasite densities in samples with BL than those without suggesting that lead has a direct effect on the multiplication of the parasites. We speculate that since lead has similar electronic structure and charge with iron, the host's iron homeostasis system is made to think that there is too much iron in circulation resulting into upregulation of hepcidin, a bactericidal hormone important for the regulation of iron levels. Over accumulation of hepcidin reduces the available iron in circulation hence hard times for parasite's survival. Because 60% of Kampala city habitants live in slums such as Katanga [1] where social amenities are a big problem, families in these areas live in overcrowded conditions and makeshift shelters exposed to open sewers, mosquito breeding grounds [20, 21] these make them vulnerable to malaria infections. This study found a relatively high malaria infection rate especially among children in this area as compared to one reported other parts of the city and this could as result of geographic and social disadvantages of the area [1]. A majority of the study participants are reported to have elevated blood lead levels and this can be attributed to lead-contaminated runoffs from the roadside during the rainy season and dust during the dry season to peoples' homes and yards and play areas where they come into contact with children [3, 22, 23]. Urban soils receive large inputs of Lead from various sources including depositions from the atmosphere, household wastes, and from manure and fertilizer applications [23, 24]. Since 35% of urban dwellers in Uganda are involved in urban agriculture to supplement their food resources and income, propagation of Lead concentration in urban grown foodstuff is apparent [25]. Urban grown crops accumulate Lead concentrations levels above FOA thresholds and this is reported in fruits and vegetables consumed in the country [24, 25, 26] The health burden of urban agriculture in Uganda has not been assessed, and this study hypothesizes that it could be the number one important source of dietary lead in children. Because of the similarity in environmental risk factors, both malaria and BLL in children co-cluster within specific communities and households in urban areas of Uganda. The literature on the detrimental effects of elevated blood lead levels seems not to emphasize that BLL is of danger at very low levels, especially in children. This age group is most susceptible to lead poisoning [27, 28, 29] and there is little or no known literature about the patterns for BLL and malaria infection among children living in Kampala Uganda. We can speculate that a big percentage of urban Uganda dwellers have some levels of BLL and since it is not needed anywhere in the body system, it poses a silent health threat. The BLL indicated in this study and previous studies suggest that even at its lowest levels, the d-ALAD activity is compromised and this could be due to the sensitive nature of enzymes. Results from this study shows that since enzymes are very sensitive in nature of their activity, any smallest amount of blood lead is

enough to affect their sensitivity. ALAD, a mitochondrial enzyme that catalyzes the formation of heme by insertion of iron into protoporphyrin IX is affected even at very low levels of blood lead in circulation. Lead blocks affect ALAD, preventing iron incorporation step into protoporphyrin IX hence the accumulation of the free erythrocyte protoporphyrin (FEP) i.e. the non-heme protoporphyrin.as well as FEP: Hb µg /g ratio (a lead poisoning indicator) increased exponentially with the blood Pb level in this study. Since BLL initially accumulates in erythrocytes and has a high affinity for the −SH group of enzymes, d-ALAD enzyme quickly falls victim to BLL even at very low levels. Increases in FEP levels in Lead intoxicated children even at very low BLL is indicative of the ALAD activity inhibition. Plasmodium parasites cause anemia by systematically destroying the RBCs and they seem to decrease proportionally with an increase of BLL which could be attributed to the fact that blood lead disturbs the host's iron homeostasis mechanism hence reducing the levels of RBCs important for their survival. Hepcidin (a master regulator of iron trafficking) hormone, overexpressed during lead intoxication downregulates the iron levels leading to anemia. This phenomenon is attributed to the fact that blood lead mimics iron and hence fooling hepcidin-mediated [30,]. Iron homeostasis that there is too much iron in circulation and if this persists, iron mobilization from the stores is shut down and decreased iron availability for erythropoiesis. Further to RBCs indiscriminate destruction by the malaria parasites, causing an imbalance in the cytokines levels and other mediators of inflammation such as hemozoin, a parasite-derived polymerized heme, and IL6 one of the cytokines affected, has the capacity to affect the hepcidin expression [31]. Another cytokine of key importance affected by Plasmodium parasites is interferon (IFN)-γ) which induces macrophage production of TRAIL (TNF-related apoptosis-inducing ligand) and hence an increased activation of splenic and other macrophages for phagocytosis of red cells. It seems that progression to severe anemia is multifactorial and involve both direct and indirect destruction of parasitized and non-parasitized erythrocytes, heme synthesis inhibition (ineffective erythropoiesis) and interference of hepcidin iron regulatory activity by lead intoxication. This increased clearance of uninfected erythrocytes due to extrinsic and intrinsic changes to the RBCs enhance their recognition and phagocytosis and hence anemia. Reduced iron (substrate) availability further complicates the heme synthesis mechanism by disturbing the enzyme /substrate enzymatic reaction equation. However, this study did not find a direct relationship between high blood lead concentrations with parasite density as previously reported. We attribute the decrease in parasite density to increased iron homeostasis interference to during lead intoxication and hence less available iron important for their multiplication. A negative and strong Pearson correlation value between FEP: Hb µg /g and Hb levels (Table 2 and Fig. 1) as compared to that between parasite density and hemoglobin levels (Fig. 2) seem to support our argument that blood lead is able to block heme synthesis at much lower levels. There is moderate interaction between the three variables (FEP/Hb, Parasite density and hemoglobin levels) after a multivariate analysis (Table 2) supports our argument. Children who participated in the study had no co-morbid diseases like HIV, sickle cell traits which could have attenuated the relationships between malaria and BLL. Simple correlation analysis between BLL and malaria parasites (Table 2 and Fig. 3) in the study population seems to support our argument that BLL had a little or no direct effect on parasite density levels.

To the best of our knowledge, this study represents the first reported significant association between malaria and lead poisoning during the anemia pathogenesis among the pediatric population in Uganda.

Conclusion

Basing on our study findings, low blood lead is detrimental to the child's anemia status, especially during malaria infection. Blood lead does not reduce the parasite density directly but it only achieves this by limiting the amount iron in circulation for parasite to flourish on hence their natural death. Since both blood lead and plasmodium infection have similar hematological consequences, their co –existence worsens the anemia status of the host. We propose a detailed study involving a bigger sample size to properly understand of the effects and anemia pathogenesis during lead exposure of Plasmodium malaria infection over a long period of time.

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contribution

All the authors contributed equally towards the conception and execution of this study. All the authors agreed to disseminate the findings through a journal publication

Authors' information

All the authors are from the Department of Biochemistry and sports sciences, college of Natural Sciences, Makerere University, Uganda.

Ethical considerations

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

Study participants consent considerations

Parents/guardians were given an explanation of the purpose and benefit of this study for their community. The explanation was given in the local language and parents consented on behalf of their children. They further consented on the use and storage of their children's blood samples for purposes of this research study only.

Conflict of interest

There is no conflicting interests related to this study.

Abbreviations

BL: Blood lead

BLL: Blood lead levels

PD: Parasite density

FEP: Free erythrocyte protoporphyrin

ALA: Aminolevulinic acid

ALAD: Aminolevulinic acid dehydrogenase

ZPP: Zinc protoporphyrin

PPN: Protoporphyrin

References

1. https://www.academia.edu/.../SLUMS_IN_UGANDA_The_case_of_Katanga_Slum_
2. Worrall, E., Basu, S., & Hanson, K. (2005). Is malaria a disease of poverty? A review of the literature. *Tropical Medicine & International Health*, *10*(10), 1047-1059.
3. Mghweno, L. R., Makokha, A. O., Magoha, H. S., Wekesa, J. M., & Nakajugo, A. (2008). Environmental lead pollution and food safety around Kampala City in Uganda. J Applied Biosci, 12, 642-649.
4. https://www.ubos.org/onlinefiles/uploads/ubos/.../Uganda_DHS_2016_KIR.
5. https://www.indexmundi.com/facts/indicators/SH.ANM.CHLD.ZS/compare?country=ug#country
6. https://www.jbpub.com/.../skolnik/2e/docs/.../Malaria%20in%20Uganda,%20Bauer
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. Graber, L. K., Asher, D., Anandaraja, N., Bopp, R. F., Merrill, K., Cullen, M. R. & Trasande, L. (2010). Childhood lead exposure after the phaseout of leaded gasoline: an ecological study of school-age children in Kampala, Uganda. Environmental health perspectives, 118(6), 884-889.

9. https://www.voanews.com/a/pollution-is-sil[1] ent-killer-in-uganda/2648372.html

10. Bergdahl, I. A., Grubb, A., Schütz, A., Desnick, R. J., Wetmur, J. G., Sassa, S., & Skerfving, S. (1997). Lead binding to δ-aminolevulinic acid dehydratase (ALAD) in human erythrocytes. Pharmacology & toxicology, 81(4), 153-158.

11. Apostoli, P., Romeo, L., De Matteis, M.C. et al. Effects of lead on red blood cell membrane proteins. Int. Arch Occup Environ Heath 61, 71–75 (1988). https://doi.org/10.1007/BF00381610

12. Mahaffey, K. R., & Annest, J. L. (1986). Association of erythrocyte protoporphyrin with blood lead level and iron status in the Second National Health and Nutrition Examination Survey, 1976–1980. Environmental Research, 41(1), 327-338.

13. Oppenheimer, S. J. (1989). Iron and infection: the clinical evidence. Acta Paediatrica, 78, 53-62.

14. Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: a review with recent updates. *Interdisciplinary toxicology*, *5*(2), 47-58.

15. Ghosh, K., Ghosh, K. Pathogenesis of anemia in malaria: a concise review. Parasitol Res 101, 1463–1469 (2007). https://doi.org/10.1007/s00436-007-0742-112] Goyer RA, Rhyne BC. Pathological effects of lead. Int Rev Exp Pathol 1973; 12:1–77

16. Sassa S. (1978) Toxic Effects of Lead, with Particular Reference to Porphyrin and Heme Metabolism. In: De Matteis F., Aldridge W.N. (eds) Heme and Hemoproteins. Handbuch der experimentellen Pharmakologie / Handbook of Experimental Pharmacology, vol 44. Springer, Berlin, Heidelberg

17. Marsh Jr, W. L., Nelson, D. P., & Koenig, H. M. (1983). Free erythrocyte protoporphyrin (FEP) I. Normal values for adults and evaluation of the hematofluorometer. *American journal of clinical pathology*, *79*(6), 655-660.

18. Rahman, M. S., Mushfiquee, M., Masud, M. S., & Howlader, T. (2019). Association between malnutrition and anemia in under-five children and women of reproductive age: Evidence from Bangladesh Demographic and Health Survey 2011. *PloS one*, *14*(7).

19. Trape, J. F. (1985). Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *79*(2), 181-184.

20. Piomelli, S. (1973). A micro method for free erythrocyte porphyrins: the FEP test. *The Journal of laboratory and clinical medicine*, *81*(6), 932-940.

21. Lowassa, A., Mazigo, H. D., Mahande, A. M., Mwang'onde, B. J., Msangi, S., Mahande, M. J., ... & Kweka, E. J. (2012). Social economic factors and malaria transmission in Lower Moshi, northern Tanzania. *Parasites & vectors*, *5*(1), 129.

22. Mwangi, T. W., Bethony, J. M., & Brooker, S. (2006). Malaria and helminth interactions in humans: an epidemiological viewpoint. *Annals of Tropical Medicine & Parasitology*, *100*(7), 551-570.

23. Kakulu, S. E. (2003). Trace metal concentration in roadside surface soil and tree back: A measurement of local atmospheric pollution in Abuja, Nigeria. Environmental Monitoring and Assessment, 89(3), 233-242.

24. Nabulo, G., Oryem-Origa, H., & Diamond, M. (2006). Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. Environmental Research, 101(1), 42-52.

25. Eriyamremu, G. E., Asagba, S. O., Akpoborie, I. A., & Ojeaburu, S. I. (2005). 1962361. Evaluation of lead and cadmium levels in some commonly consumed vegetables in the Niger-Delta oil area of Nigeria. *Bulletin of environmental contamination and toxicology*, *75*(2), 278-283.

26. Maduabuchi, J. M., Nzegwu, C. N., Adigba, E. O., Aloke, R. U., Ezomike, C. N., Okocha, C. E., ... & Orisakwe, O. E. (2006). Lead and cadmium exposures from canned and non-canned beverages in Nigeria: a public health concern. *Science of the Total Environment*, *366*(2-3), 621-626.

27. Mielke, H. W., Gonzales, C. R., Smith, M. K., & Mielke, P. W. (1999). The urban environment and children's health: soils as an integrator of lead, zinc, and cadmium in New Orleans, Louisiana, USA. *Environmental research*, *81*(2), [9] 117-129.

28. https://wonder.cdc.gov/wonder/prevguid/p0000029/p0000029.asp

29. Nriagu, J., Oleru, N. T., Cudjoe, C., & Chine, A. (1997). Lead poisoning of children in Africa, III. Kaduna, Nigeria. *Science of the Total Environment*, *197*(1-3), 13-19.

30. Cheria[1] n, S., Forbes, D. A., Cook, A. G., Sanfilippo, F. M., Kemna, E. H., Swinkels, D. W., & Burgner, D. P. (2008). An insight into the relationships between hepcidin, anemia, infections and inflammatory cytokines in pediatric refugees: a cross-sectional study. *PloS one*, *3*(12), e4030.

31. Wang, L., Johnson, E. E., Shi, H. N., Walker, W. A., Wessling-Resnick, M., & Cherayil, B. J. (2008). Attenuated inflammatory responses in hemochromatosis reveal a role for iron in the regulation of macrophage cytokine translation. *The Journal of Immunology*, *181*(4), 2723-2731
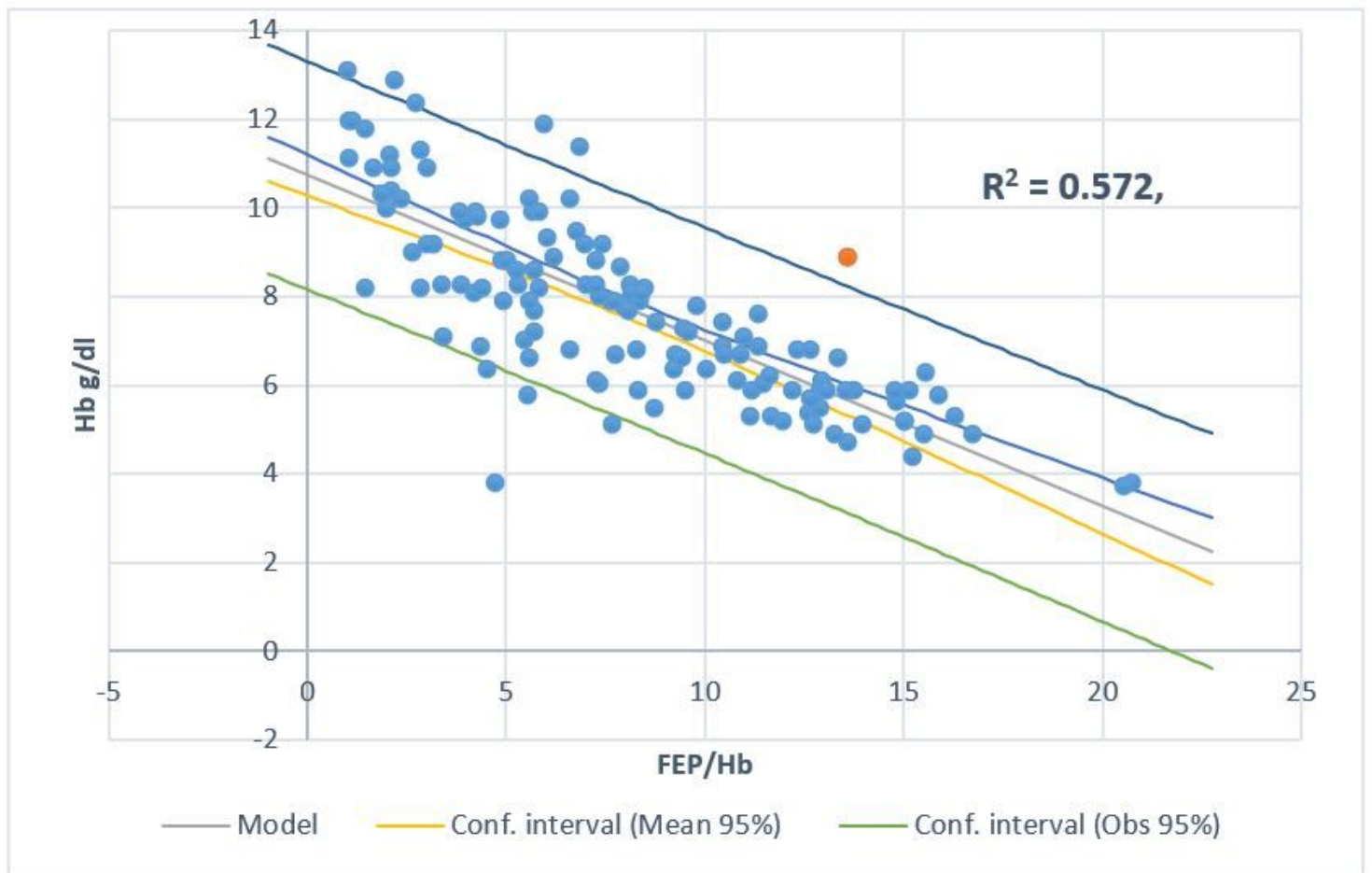
Figures

R² = 0.572,

Legend: —— Model —— Conf. interval (Mean 95%) —— Conf. interval (Obs 95%)

Figure 1

Scatter graph showing how FEP/Hb levels relates with hemoglobin levels of malaria infected children
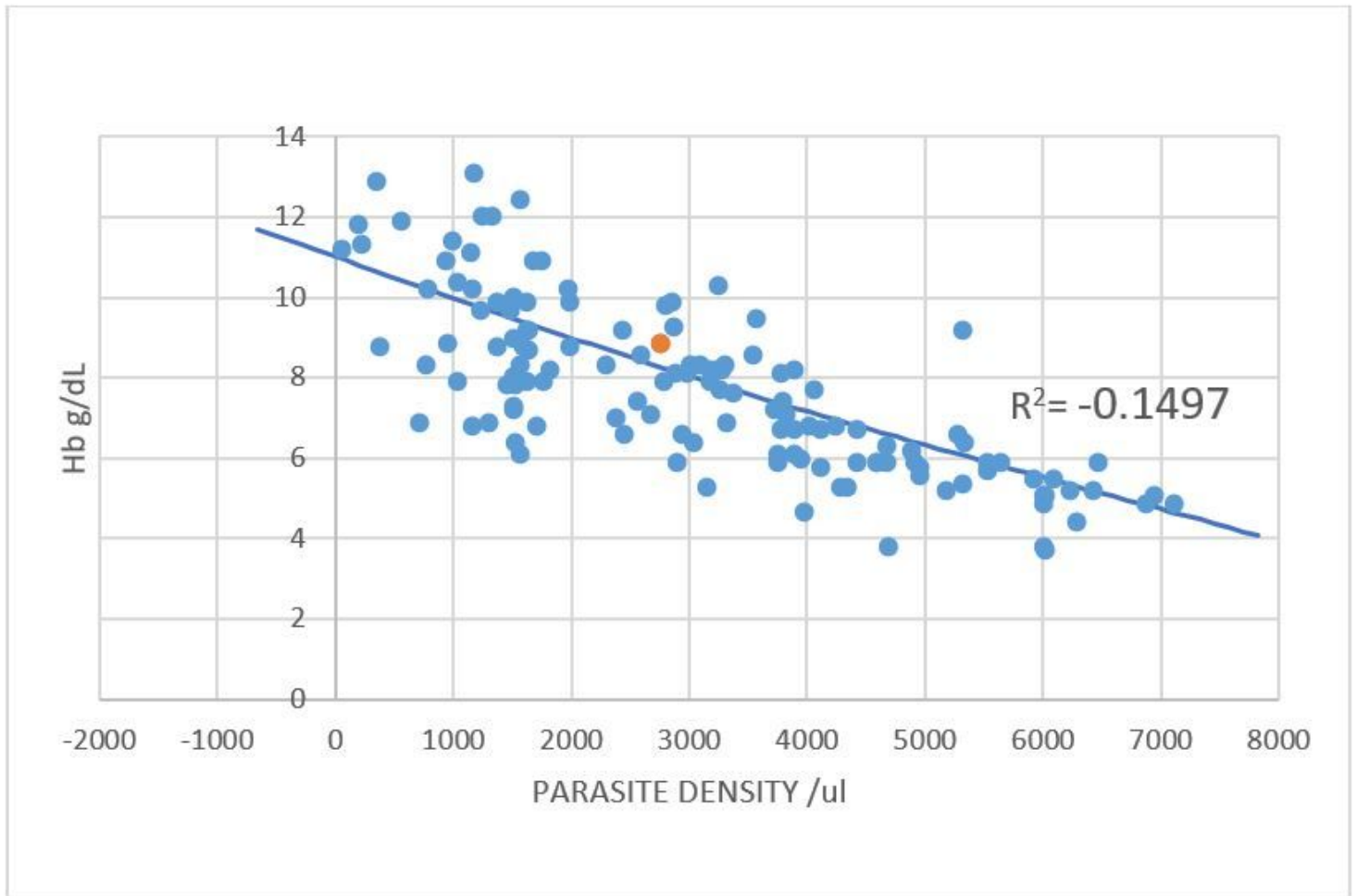
Figure 2

Scatter graph showing how parasite density relates with hemoglobin levels of malaria infected children.
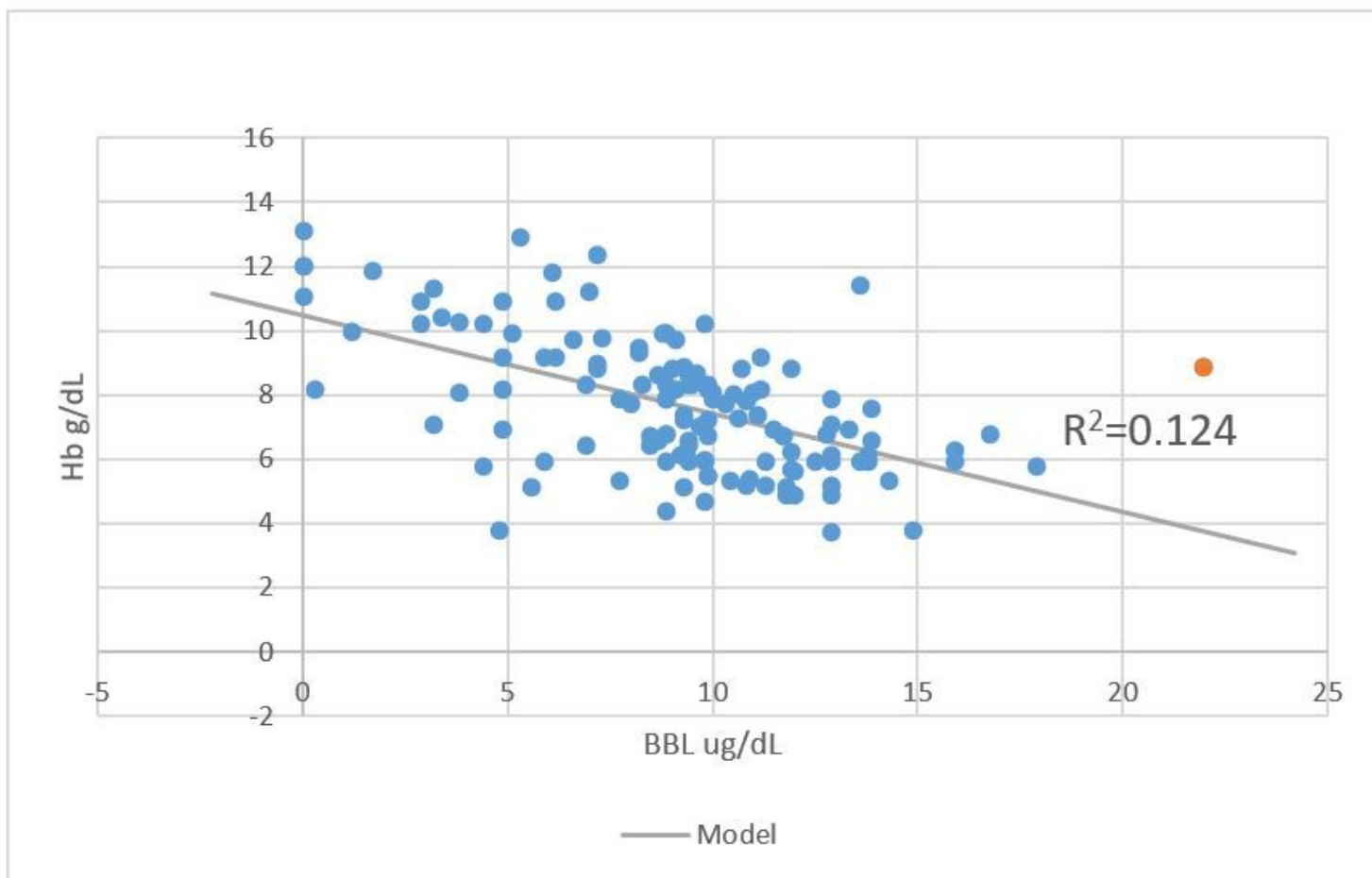
Figure 3

Scatter graph showing how BBL levels relates with hemoglobin levels of malaria infected children.