Correlation of Plasma Plasmodium Falciparum Histidine-Rich Protein-2 Levels with Disease Severity and Outcome of Malaria Patients in Sokoto Metropolis, Nigeria

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Abstract- ***Accurate diagnosis of malaria is key to proper management, control and an ideal diagnostic parameter that correlates with disease outcome is required. Hence, the study aimed to determine if plasma Plasmodium falciparum histidine-rich protein-2 (PfHRP2) levels correlate with malaria severity and whether it could be used to predict outcome in children. The study enrolled 250 volunteers, comprising 150 malaria infected, 100 healthy controls, grouped into severe malaria, uncomplicated and non-malaria(control). Clinical assessments, Biochemical and haematological parameters were determined using standard methods. There was no significant difference in the demographic characteristics of the study volunteers. The malaria status of volunteers with severe and uncomplicated malaria was confirmed by both PfHRP2 and microscopy. Levels of Lactate, Creatinine, Bilirubin, Plasmodium falciparum parasite count, and PfHRP2 were significantly($p< 0.05$) higher while levels of Bicarbonate, Glucose, PCV and Haemoglobin were significantly($p< 0.05$) lower in the severe malaria group compared to the uncomplicated and the control group. A positive correlation was observed between PfHRP2 concentration and parasite count and several outcomes of malaria disease severity. Severe malaria group show a high Area under the Curve (AUC) of 0.99 with high predictive power compared to the uncomplicated malaria group(~0.61) with predictive power and control group(~0.008) with no predictive power. Patients with very high PfHRP2 levels are most likely to manifest one or more clinical signs of severe malaria which would facilitate the identification of patients at greatest risk of progressing to complicated or severe malaria, and their isolation for prompt, effective treatment to avoid death or complications.***

Keywords: ***Plasmodium falciparum, Plasmodium falciparum histidine-rich protein 2 (PfHRP2), Severe malaria, Uncomplicated malaria, infection.***

I. INTRODUCTION

Malaria, a disease with an enormous impact on humanity for thousands of years and remains one of the most serious, life-threatening infectious diseases ranking as the third most common disease after HIV/AIDS and Tuberculosis in Africa (Cowman *et al.,* 2016), which African children bear 90% of the morbidity and mortality burden, with estimates of 1.2 million deaths every year (Murray *et al*., 2018). In 2022, there were 249 million cases of malaria worldwide with an estimated 608 000 malaria deaths, 90% (233 million cases) of which occurred in Africa, and 76% of all deaths occurred in children less than 5 years of age (Roca-Feltrer *et al.,*2008 &WHO, 2023). Four African countries accounted for approximately half (50%) of all malaria deaths worldwide: Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%) and Mozambique (4%) of which children under 5 years of age were the most vulnerable group affected by malaria accounting for 76% of all malaria deaths Worldwide (WHO, 2023). Of all the *Plasmodium* parasite species that cause malaria in humans, *Plasmodium falciparum* is the leading cause of severe malaria (WHO, 2018), but severe disease can also be caused by the other species (Barber *et al*., 2013; Rahimi *et al*., 2014 & Groger *et al*., 2017). In highly endemic areas, severe malaria affects mainly children before protective immunity is gradually acquired after repeated infections (White *et al.,*

2014). Diagnosing severe *falciparum* malaria in children living in endemic areas is problematic as delayed diagnosis and presentation at a health care facility in a region where malaria is rarely diagnosed increase the risk of mortality due to malaria (Checkley *et al*., 2012 & Lüthi & Schlagenhauf, 2015). Furthermore, the clinical diagnosis of severe malaria is unreliable, because signs and symptoms overlap with other life-threatening febrile illnesses, including pneumonia, meningitis, bacterial sepsis, and Dengue (Berkley *et al.,* 2015 & WHO, 2024). Because severe malaria kills rapidly, prompt diagnosis and management are vital (Dondorp *et al.*, 2010). On the other hand, over-diagnosis of severe malaria in African children is common and diverts attention from other infectious causes, which has been shown to contribute to mortality (Reyburn *et al.,* 2004). A rapid and reliable parasitological diagnosis of severe malaria is thus essential for proper management of patients with severe febrile illnesses.

The disease is caused by protozoan pathogens of the *Plasmodium spp*: *Plasmodium falciparum* and *Plasmodium malariae* are the most common species and are responsible for the largest public health burden for which humans are the exclusive mammalian hosts (Fairhurst & Wellems, 2010). The consequences of *Plasmodium* spp. infection vary in severity depending on the specie and on host factors, including the level of host immunity, which is linked to past exposure to the parasite (Moxon *et al.,* 2013; Taylor & Molyneux 2015 & Wassmer *et al*., 2015). Malaria is usually classified as asymptomatic, uncomplicated or severe (complicated) (WHO, 2014). Typical initial symptoms are low-grade fever, shaking chills, muscle aches in children. These symptoms can present suddenly (paroxysms), and then progress to drenching sweats, high fever and exhaustion (Calis *et al*., 2008). Severe *falciparum* malaria is associated with high mortality (WHO, 2016). Severe malaria complications are due to microvascular obstruction caused by the presence of red blood cell-stage parasites in capillaries (Day *et al.,* 1999 & Bernabeu & Smith 2017). While many children harbor *Plasmodium falciparum* infections, only a small proportion of those with clinical malaria progress to severe and complicated form of the disease. The determinants of disease progression and severity remain elusive. Clinical over-diagnosis of *Plasmodium falciparum* malaria in severely ill children has been an important problem in sub-Saharan Africa (Taylor *et al.,* 2004). In areas of low transmission, *falciparum* malaria is an important cause of maternal mortality (Desai *et al.,* 2007). Severe *falciparum* malaria in children presents a major diagnostic challenge in malaria-endemic countries where a high proportion of children is parasitaemic at any time (English *et al.,* 1996). Misdiagnosis of *falciparum* malaria has been reported to be associated with increased mortality in adults and children (Reyburn *et al.,* 2004 & Amexo *et al.,* 2004).

Plasmodium falciparum histidine-rich protein 2 (*Pf*HRP_2) is a water-soluble protein found in the malaria parasite and host erythrocyte (Panton *et al*., 1989) which circulates freely or bound to other proteins or antibodies in the plasma compartment, Its function is yet to be fully known, but it is produced throughout the 48-hour life cycle of the parasite (Desakorn *et al*., 2015) and when the parasite is sequestered in deep tissues (Desakorn *et al.,* 2015), it is released (schizont rupture) upon maturity from parasite-infected erythrocytes *in vivo* and *in vitro* (Kifude *et al.,* 2008where it is distributed throughout the total plasma volume. Plasma *Pf*HRP_2 can be considered a measure of total parasite burden of the preceding 48-hour (Desakorn *et al.,* 2015 & Dondorp *et al.*, 2017). Semi quantitative assessments of HRP_2 concentrations in adults show a positive correlation between HRP and malaria severity in Thailand (Dondorp *et al.*, 2017). In African, children with severe febrile illness, quantitative measures of admission of HRP_2 values predict mortality and are correlated with disease severity (Hendriksen *et al*., 2013). Quantitative measures of HRP_2 can also accurately distinguish retinopathy-positive CM from retinopathy-negative CM (Seydel *et al*, 2015). *Pf*HRP_2, used as a biomarker for *P. falciparum* infection, forms the basis of many current rapid diagnostic tests (Parra *et al.,* 1991 & Dondorp *et al.,* 2017). On postmortem analyses, *Pf*HRP_2 has been observed to line the endothelial walls of blood vessels (Hendriksen *et al.,* 2013 & Pal *et al*., 2016). Previous studies have identified *Pf*HRP_2 as a biomarker for total parasite burden, offering a more comprehensive assessment over conventional blood film analysis. While blood films only detect circulating parasites, *Pf*HRP_2 is evenly dispersed

throughout the blood plasma, reflecting the total number of parasites present in the body over the past 48 hours (Noedl *et al.*, 2002; Noedl *et al.,* 2004; Maji, 2018 & Poti *et al*., 2020).

II. MATERIALS AND METHODS

Subjects and study design

Blood samples and demographic data were obtained from subjects presenting as inpatients and out patients at Usmanu Danfodio Univeristy Teaching Hospital and Specialist Hospital, Sokoto, Nigeria, after obtaining institutional approval and signed consent form from the parents or guardians of the children who are the study participant. A total number of 250 subjects were enrolled into the study.

Sample collection and preparations

The study was conducted from May to October 2023 during the rainfall period. About 5mls of venous blood samples were collected by the attending medical officer into plain tubes with EDTA as anticoagulant. Prior to sample collection, systolic blood pressure was measured with aneroid sphygmomanometer anthropometric and clinical data were collected and recorded. Coma score was assessed by the physician. Sample for hematological and biochemical analyses were collected and analyzed for PCV (haematocrit) using microhematocrit method (1952), *Pf*HRP_2 using enzyme linked immunoassays (ELISA) by (Taylor and Voller, 1993) and Immunoassay Method (Trager and Jensen, 1979), parasitic count (microscopy of blood smear) and analyses for glucose using Glucose Oxidase Method (Barham and Trinde, 1972), lactate by Ultraviolet Method (Deutsche Gesellschaft für Klinische Chemie, 1970), haemoglobin using Colorimetric Method (Van Kampen and Zijlstra, 1961), creatinine using colorimetric Method (Bartels and Bohmer, 1972), bicarbonate using enzymatic method (Allain and Roeschlaw, 1974) and bilirubin using modified Jendrassik method (Jendrassik and Grof, 1938). Sample collection was done once and revisit was done between 3-5 days of sample collection as patients were undergoing treatment for progress of treatment and possible outcome.

The patients included children aged between 1 month to 12 years of age, who were either Inpatients on admission (Severe malaria patients) and Outpatients with simple or uncomplicated malaria at Usmanu Danfodio University Teaching Hospital and Specialist Hospital Sokoto.

Patients were grouped into three groups: Group A comprised 65 patients having severe malaria disease (SMD) with asexual parasitaemia confirmed by a positive smear, anaemia (haemoglobin<5g/dl for children) and the presence of two or more indicators of severe malaria. Group B comprised of 85 patients with simple/uncomplicated malaria infection based on microscopic confirmation by a positive smear, haemoglobin levels>5g/dl for children and absence of any other indicators of severe malaria. Group C comprised of 100 patients without malaria infections (microscopic confirmation by a negative smear) which served as the control group (CG). Grouping was matched according to age and gender (mixed sampling). Parameter to define disease severity was based on WHO 2015 standard and Hien *et al*., (2010) classification which included Blantyre Coma Score<3 for children aged 6months-12 years read by a physician, Haematocrit (HCt)-<15% in children(<15years) and Jaundice with Bilirubin>42.8µmol/L and with parasitic count>100,000/µL, Hypoglycaemia with venous glucose<2.2mmol/L, Serum creatinine>265µmol/L, Peripheral asexual stage parasitaemia>10% or≥200,000 parasite/µL of blood, peripheral venous lactate>4mmol/L or peripheral venous bicarbonate<15mmol/L

Data Analyses

Data generated were entered into Excel spreadsheets and analyzed using Graph Pad Prism 5 (Instat, Sandiego, US) and R (version 4.1.2 [2021-11-01] -- "Bird Hippie"The R Foundation for Statistical Computing Platform. Significant differences between groups were assessed using ANOVA for normally distributed parameters. Logistic regression was used to determine the relationship between disease severity (independent variable) and *Pf*HRP_2 or other prognostic factors including plasma haemoglobin, creatinine, lactate, bilirubin and glucose concentrations (dependent variable). All values were considered significant at p value <0.05. ROC analysis

and survival analysis were carried out using Kaplan Meier.

III. RESULTS

Table 1: Demographic and Clinical Characteristics of Study Volunteers

S/N		No of volunteers	Severe Malaria (Group A)	Uncomplicated Malaria (Group B)	Non-Malaria (control) (Group C)
1	Age(years)	250	(0.5-12yrs)	(0.5-12yrs)	(0.5-12yrs)
2	Male	115(46%)	25(10%)	43(17.2%)	47(18.8%)
3	Female	135(54%)	40(16%)	42(16.8%)	53(21.2%)
4	Microscopy		POSITIVE	POSITIVE	NEGATIVE
5	*Pf*HRP$_2$ RDT		POSITIVE	POSITIVE	NEGATIVE

Table 2: Clinical Manifestation and Outcome for Severe Malaria Patients in the Study Subjects

S/No	Clinical Manifestation	Number of Patients Percentage (%)	Deaths
1	Prostration	7 (10.8)	-
2	Coma	6 (9.2)	3
3	Malaria	15 (23.1)	5
4	Convulsion	11 (16.9)	6
5	Severe Anaemia	8 (12.3)	-
6	Liver impairment	7 (10.8)	4
7	Renal Impairment	11 (16.9)	2

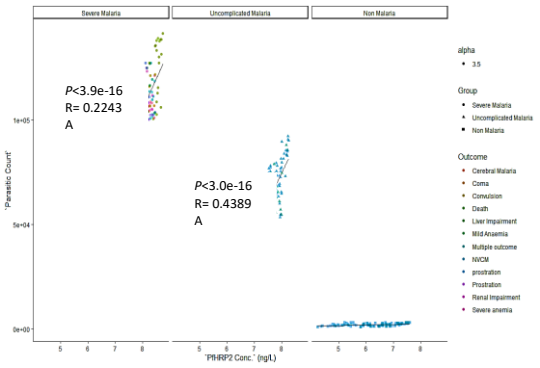

Figure 1: Correlation Analysis of *Plasmodium falciparum* count and *Plasmodium falciparum* Histidine Rich Protein 2 Levels in the Blood of Severe Malaria, Uncomplicated Malaria and Non-Malaria Study Subjects.

	Group A	Group B	Group C
Lactate	6.01±0.97mmol/L	2.06±0.78mmol/L	1.92±0.72 mmol/L
Bicarbonate	11.42±1.81 mmol/L	15.86±3.21 mmol/L	21.88±3.41 mmol/L
Creatinine	288.06±15.56µmol/L	141.27±33.04µmol/L	94.18±14.91µmol/L
Glucose	2.02±0.28 mmol/L	4.34±1.05 mmol/L	5.20±1.37 mmol/L
Bilirubin	47.06±3.95 µmol/L	16.36±5.63 µmol/L	15.27±2.84 µmol/L
PCV	13.37±2.39 %	22.92±4.23 %	group36.26 ±4.33 %

Hemoglobin	4.53±0.94g/dL	8.25±1.60g/dL	14.19±1.97 g/dL
Parasitic count	115,675±12,223/µL	69,128±10,995/µL	1,877±576/µL
*Pf*HRP 2	4362±742 (ng/L)	2529±642(ng/L)	716±512(ng/L)
AUC	0.99	0.61	0.88

Table 3: Biochemical and hematological analytical results

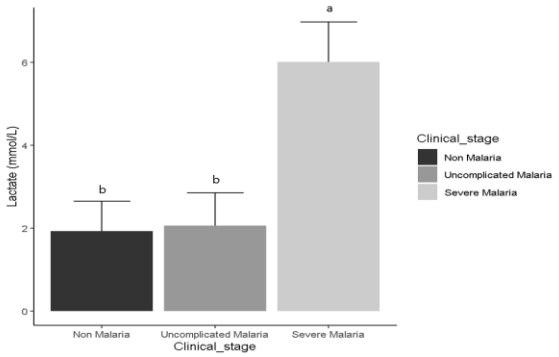

Figure 4.1: Serum Lactate Concentration of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different ($p < 0.05$), Severe malaria, Uncomplicated Malaria, Non-Malaria

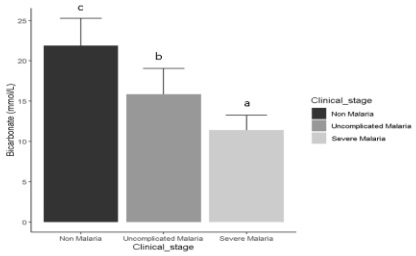

Figure 4.2: Serum Bicarbonate Concentration of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different (P<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

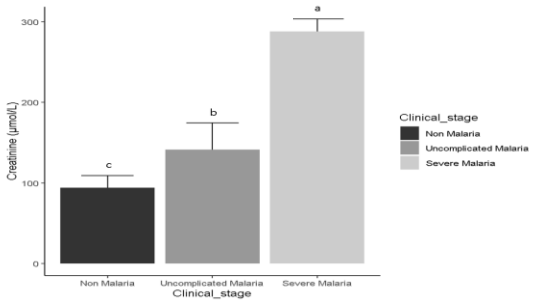

Figure 4.3: Serum Creatinine concentration of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different (P<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

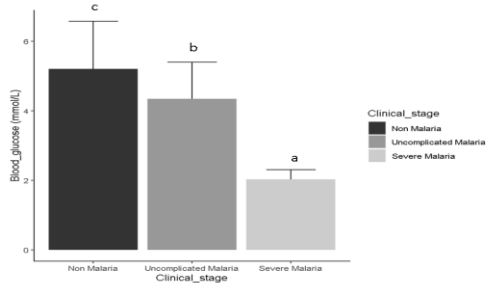

Figure 4.4: Serum Glucose Concentration of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different (P<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

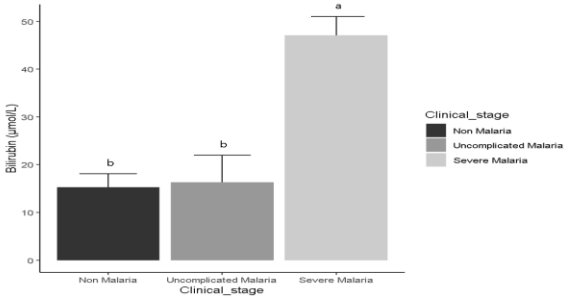

Figure 4.5: Serum Bilirubin Concentration of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different (P<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

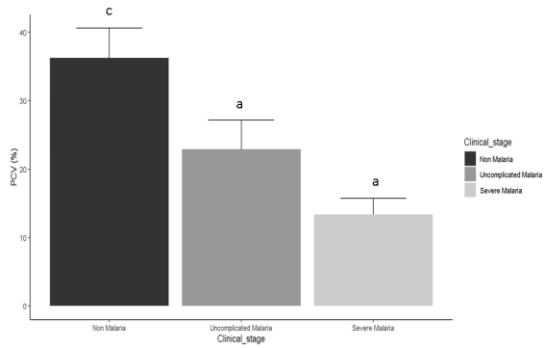

Figure 4.6: Packed cell volume (PCV) value of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different (*P*<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

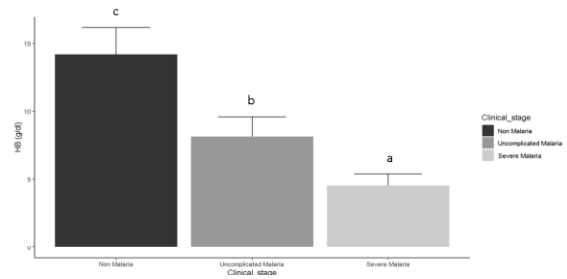

Figure 4.7: Hemoglobin levels of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different (*P*<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

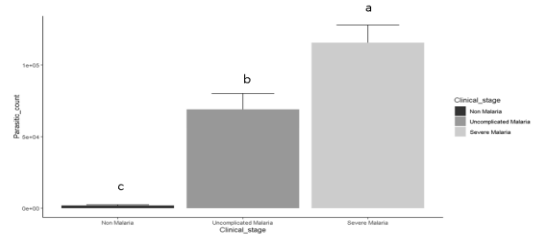

Figure 4.8: *Plasmodium falciparum* Parasite Count in the Blood of Severe Malaria, Uncomplicated Malaria and Non-Malaria Subjects

Groups with different alphabets are significantly different (*P*<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

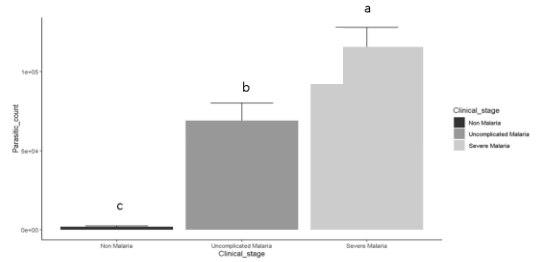

Figure 4.9: *Plasmodium falciparum* Histidine Rich Protein 2 levels in the Blood of Severe Malaria, Uncomplicated Malaria subjects and Controls

Groups with different alphabets are significantly different (*P*<0.05), Severe malaria, Uncomplicated Malaria, Non-Malaria

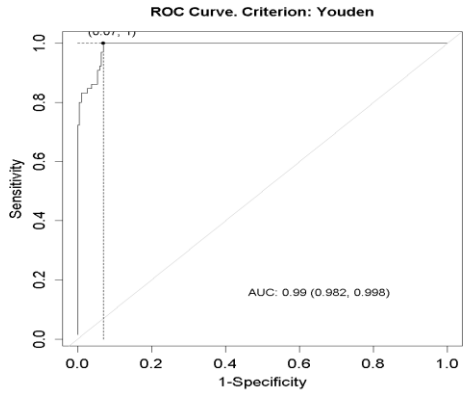

Figure 2.1: ROC curves of *Pf*HRP$_2$ for Optimal Cut-off in Severe Malaria detection.

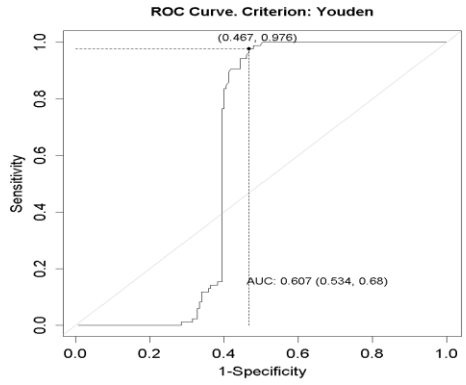

Figure 2.2: ROC curves of *Pf*HRP$_2$ for Optimal Cut-off in Uncomplicated Malaria detection.

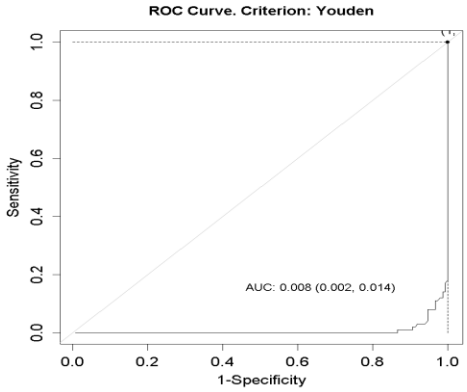

Figure 2.3: ROC curves of *Pf*HRP$_2$ for Optimal Cut-off in Non-Malaria detection.

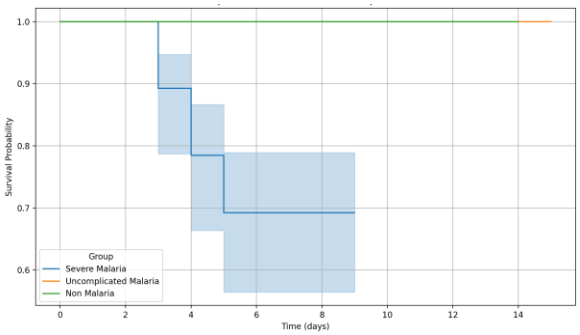

Figure 3: Kaplan-Meier Survival analysis curve for group A

The mean lactate level was found to be significantly higher in the severe malaria group compared to the uncomplicated and non-malaria group which may explain the observed metabolic acidosis that manifest in the group. Taylor *et al.* (1993) and English *et al.* (1997) reported that metabolic acidosis is a common finding (43–46% prevalence) in African children admitted to hospital with malaria. Hyperlactatemia has been reported to be strongly associated with reduced plasma bicarbonate and is a major contributor to the acidaemic state in malaria (Herdman *et al.,* 2015) and the observed significant increase in lactate may be thought to occur due to a combination of microvascular obstruction by sequestered parasites, vascular endothelial dysfunction, anaemia, and inflammation driving increased glycolysis. Metabolic acidosis is strongly associated with an increased risk of death (Newton *et al.,*2005). This report is in agreement with the bicarbonate levels of the severe malaria groups in the present study. Prognosis has been shown to be worse in patients whose lactate levels do not normalize within a few hours of admission and the levels of lactate in fatal cases were nearly double those of survivors (Krishna *et al.,* 1994). The principal cause of acidosis is thought to be tissue hypoxia and resultant anaerobic glycolysis generating lactic acid, and additional accumulation and impairment of the metabolism of lactic acid and ketone bodies (English *et al.,* 1997, Planche *et al.,* 2003). Renal dysfunction is one of the complications found in the study subjects with severe malaria. Renal dysfunction associated with malaria is due to acute tubular necrosis and less commonly interstitial nephritis and glomerulonephritis; multiple mechanisms including parasitized erythrocytes causing tissue hypoperfusion and immune complex deposition have been proposed (Elsheikha & Sheashaa, 2007; Silva da *et al.,*2017). The optimal assessment of renal function requires serial biochemical measurements of blood and/or urine using a patient's baseline renal functions as a reference (Makris & Spanou, 2016). In this study, serum creatinine level was assessed and it showed that in the severe malaria group creatinine level was significantly higher when compared to other groups and may explain the observed renal impairment amongst patients in the severe malaria group. Assessment of creatinine levels, however, is not routinely done in most malaria endemic settings. However, the presence of acute renal impairment has not only been shown to be a risk factor for the development of chronic kidney disease but also for long-term neurological impairment in cerebral malaria (Conroy *et al.,* 2016). Significantly low level of blood glucose (hypoglycaemia) was observed in the severe malaria groups. Similarly, hypoglycaemia has been reported previously in 1–32 % of children admitted to Sokoto hospitals with malaria, (Bassat 2008; Jallow *et al.,* 2012; Jiya & Sani, 2016), with a greater prevalence in children below 3 years of age, those with convulsions or coma, hyperparasitaemia, and those receiving quinine treatment (Dondorp *et al.,* 2017). The pathophysiology of hypoglycemia is multifactorial and increased glucose utilization, impaired gluconeogenesis, and hyperinsulinemia secondary to quinine therapy may all contribute to the observed lower glucose level (Madrid *et al.,* 2014) in the severe malaria group. Many studies have demonstrated that hypoglycemia is an important risk factor for death (Molyneux *et al.,* 1989, Kremsner *et al.,* 2009 & Kendjo *et al.,* 2013). Similarly, severe malaria which

is the most common complication of *P. falciparum* infections, and accounts for 12.3 % in the above study, affecting the youngest children in areas of high transmission (Calis *et al.,*2008, Snow *et al.,* 1994), has been reported to manifest clinically with pallor, lethargy, and poor feeding. This study further demonstrates the association of severe malaria anaemia with the severe malaria group and it is consistent with the findings of Jiya & Sani (2016) who reported that children with severe anaemia were significantly associated with fatal malaria outcomes, hence, should be managed with utmost care and urgency. Comparison of all the three study groups packed cell volume (PCV) and haemoglobin content confirms that children with low levels of PCV and haemoglobin in Sokoto state Nigeria suffer from complications of severe malaria. This finding agrees with previous study in the same study region (Jiya *et al*., 2020). The plasma concentration of *Pf*HRP$_2$, a measure of total parasite biomass (Dondorp *et al.,* 2015), was associated with malaria severity, depth of coma and mortality in the study subjects of the present study. Because microscopy is the gold standard for the diagnosis of malaria (Wongsrichanalai *et al.,* 2007), the correlation between parasite density by microscopy and the parasite load by *Pf*HRP$_2$ measured by Spearman's correlation analysis supports similar findings in adults from areas of relatively unstable transmission in Asia and Indonesian Papua (Dondorp *et al*., 2015). However, it is particularly significant because it extends the association between parasite biomass and disease severity to African children (Ochola *et al*., 2005), the demographic sub-group with the greatest burden of severe malaria morbidity and mortality. *Pf*HRP$_2$ levels correlate with parasitemia based on peripheral blood microscopy in children with severe malaria. This study shows a clear increase in plasma *Pf*HRP$_2$ concentrations according to disease severity from uncomplicated malaria to severe malaria. Complications of severe malaria have been seen to be prevalent in patients of severe malaria group which includes hypoglycaemia, severe anaemia, convulsion, coma, prostration, impaired organ function, unconsciousness, and acidosis. A previous study (Hendrisken *et al.,* 2012) reported that African children with severe malaria found that plasma *Pf*HRP$_2$ levels are robust predictor of mortality, and modelling revealed the proportion of fatal cases attributable to this factor. *Pf*HRP$_2$ levels have mainly been used for outpatient management of uncomplicated malaria, but the challenges of microscopy in sub-Saharan Africa are likely to extend their use to inpatient settings (McMorrow *et al*., 2008).

The current WHO guideline leaves uncertainty about the best method for the parasitological diagnosis of severe malaria in young children. The findings of our study suggest that a *Pf*HRP$_2$ -based RDT is considerably better than routine microscopy. To optimize the diagnosis of severe malaria and severe illness, a diagnostic algorithm could be employed in which only negative RDT results should be confirmed by reliable microscopy. A reduction in the workload of the hospital laboratory could improve microscopy quality. A negative RDT result should trigger contemplation of an alternative diagnosis. A positive RDT result does not exclude co-existing bacterial infections, and antimicrobial treatment is recommended. The prognostic usefulness of plasma *Pf*HRP$_2$ concentration is in line with previous reports involving African children. The findings were supported by 2 studies involving African children, (Rubach *et al.,* 2012; Seydel *et al.,* 2015). Parasite densities that can be tolerated without causing symptoms vary substantially between individuals of different age groups, transmission intensities, and seasons (Vounatsou *et al.,* 2000, Benjon *et al.,* 2007). In moderate-to-high transmission settings, children aged<5years represent a heterogeneous group with regard to levels of immunity. This is reflected by the younger age of children with severe malaria and by the older age of asymptomatic children had parasite densities of >10 000 parasites/μL. The accuracy of *Pf*HRP$_2$ concentration thresholds for defining malaria-attributable disease will vary with the level of acquired immunity in the population (Plucinski *et al.,* 2019), because this factor determines the relative sizes of populations with asymptomatic parasitemia (Mouatcho & Goldring, 2013) compared with populations with uncomplicated or severe malaria, and thus determines the corresponding overlap of plasma *Pf*HRP$_2$ distributions (Plucinski *et al.,* 2020). In many infectious diseases, pathogen load is thought to be linked to outcome (Cunnington *et al.,* 2013). In malaria, high parasitemia can indicate a high parasite replication rate, insufficient clearance of parasites by the host response, and or a longer duration of infection (Georgiaddou *et al.,* 2019). Peripheral

hyperparasitaemia is considered an important indicator of severe malaria (Molyneux *et al.,* 1989; WHO, 2014). The definition of hyperparasitaemia is dependent on the setting: in low prevalence settings and returning travelers the threshold defining severe malaria is often set at 4–5% of red blood cells (WHO, 2015), whereas in areas of stable endemicity the WHO recommends >10% (WHO, 2014, Lallo *et al.,* 2016). This is consistent with the result of this study and therefore may classify the study region as a a stable endemic region. Hyperparasitaemia has been associated with an increased likelihood of symptomatic malaria infections as well as malaria-associated mortality (Kendjo *et al.,* 2013). Circulating parasitemia is a poor indicator of total body parasite load in *falciparum* malaria because the late asexual blood stage parasites sequester in the microcirculation and are not usually sampled in the blood used to estimate parasitemia (Cunnington *et al.,* 2013). This suggests that the effect of hyperparasitaemia on outcome may only be apparent at lower transmission intensities.

In the current study, 10.8% prostration in the severe malaria group was recorded. Prostration is a sign of weakness and may also include mild cerebral impairment, resulting in the inability to sit unassisted or to breastfeed in those under the age of 6 months (WHO, 2014). It can progress to a coma or be present in the post-ictal phase following a seizure (Pottkämper *et al*., 2020). While prostration is commonly reported in severe malaria (Helbok *et al.,* 2009), it can be a subjective observation (WHO, 2014). The prognostic value of *Pf*HRP$_2$ levels in African children (Mabeza *et al.,*1995) is likely due to its association with a moderate in mortality risk, which is significantly lower compared to children presenting with other severe malaria features (Kendjo *et al.,*2013). This is consistent with the WHO assessment that prostration has a low prognostic value. The neurological abnormalities in severe malaria include seizures and impaired consciousness (Jiya *et al,* 2006). The severity of impaired consciousness is often measured using the Blantyre coma scale. This is a modified version of the Pediatric Glasgow Coma Scale, specifically constructed to allow rapid and straight forward assessment of the severity of malaria-induced coma (Sternbach, 2000). Impaired consciousness and seizures are associated with a higher risk of mortality in pediatric malaria patients (Anand & Puri 2005; Georgiadou *et al.,* 2019). It is important to note that the definition of coma varies between studies (Jain and Iverson, 2021) and that some studies (Sternbach, 2000) do not state a formal definition. This has resulted in some variability in the effect size estimation of impaired consciousness (Mattei & Teasdale, 2020). Cerebral Malaria is one of the most feared clinical complications of malaria (Idro *et al.*, 2020). The pathogenesis is still debated (Gay *et al.,* 2012; Cespedes *et al.,* 2018), but in *P. falciparum* malaria, the sequestration of parasites in the microvasculature of the brain appears to be a necessary feature, and cerebral oedema (brain swelling) appears to be the final common pathway leading to death (Jha *et al*., 2019). Mortality rate is variable depending on the setting and presence of other severe malaria features however, even when these are accounted for it can still be as high as 15-25 % in tertiary care centers (Birbeck *et al.,* 2010, Seydel *et al.,* 2015).

The area under receiving operating characteristic (AUROC) curves of *Pf*HRP$_2$ in diagnosing severe malaria indicates that *Pf*HRP$_2$ (AUC = 0.99) could identify patients with and without severe malaria at an optimal cutoff *Pf*HRP$_2$ concentration of 3240.00 mmol/L and above, having a sensitivity of 100% and specificity of 93%. Similarly, the area under receiving operating characteristic (AUROC) curves of *Pf*HRP$_2$ in diagnosing uncomplicated malaria subjects, gave an overall performance of 0.61 ($p < 0.05$) from the ROC curve analysis, indicating that *Pf*HRP$_2$ (AUC = 0.61) was able to identify patients with and without uncomplicated malaria at an optimal cutoff *Pf*HRP$_2$ concentration within the range of 1437 mmol/L to the cutoff value of severe malaria set at 3240 mmol/L, with a sensitivity of 97% and specificity of 53%. Meanwhile, the area under receiving operating characteristic (AUROC) curves of *Pf*HRP$_2$ in diagnosing non-malaria gave an overall performance, to be 0.008 ($p>0.05$) from the ROC curve indicating that *Pf*HRP$_2$ (AUC = 0.008) was unable to identify patients without malaria disease at an optimal cutoff *Pf*HRP$_2$ concentration of 69 mmol/L having a sensitivity of 100 % and specificity of 0 %. Therefore, the AUC values provide insight into the predictive performance of *Pf*HRP$_2$ levels for diagnosing malaria in different groups. Group A shows strong predictive

power, Group B shows moderate predictive power while Group C shows very weak predictive power for malaria diagnosis using these markers. From our findings, the survival curve for severe malaria shows the most rapid decline, meaning individuals in this group experience the event (e.g., severe illness or death) much sooner than those in other groups. Survival probability at specific time points shows that on day 10, the survival probability for severe malaria might be significantly lower, by 50%, meaning half of the patients have experienced an event by this time. Our data confirms that severe malaria has significantly worse survival outcomes compared to other groups.

Accurate diagnosis of malaria is key to effective treatment and control, and delayed diagnosis increases morbidity and mortality. Our results show that patients with very high *Pf*HRP$_2$ concentration are most likely to be manifesting one or more clinical signs of severe malaria. The current format of this test, plasma measured in a 96-well ELISA plate, is not conducive to use in a remote, low-tech clinical setting where rapid results are required. Clinicians in these settings are often required to make disposition decisions with a limited amount of objective data. A rapid quantitative *Pf*HRP$_2$ diagnostic test would facilitate the identification of patients at greatest risk for progressing to complicated or manifesting severe malaria. These patients could be referred to a center with capacity for higher acuity care, while those with minimal risk could be managed with oral or intramuscular antimalarials on an outpatient basis. This study shows that the plasma *Pf*HRP$_2$ concentration, as a measure of the total parasite burden determining disease severity defines malaria-attributable disease and can be used to estimate the proportion of malaria-attributable disease in children in moderate-to-high transmission settings and can distinguish severe malaria from severe febrile illness with coincidental peripheral blood parasitemia. Based on the research findings, future research could focus on developing and validating rapid, quantitative tools for assessing plasma *Pf*HRP$_2$ concentrations in low-resource settings. Such tools would be invaluable for clinicians, enabling them to quickly identify patients at high risk of severe malaria and make informed decision about patient's care. Additionally, further studies could investigate the molecular and immunological mechanism underlying the correlation between *Pf*HRP$_2$ levels and disease severity, potentially identifying new therapeutic targets. Exploring the role of other biomarkers alongside *Pf*HRP$_2$ in predicting clinical outcomes in malaria could also enhance diagnostic accuracy and improve patient management strategies in endemic regions.

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