

Erythrocyte Indices Variations In Under Five Children As A Result Of High Plasmodium Falciparum Parasitaemia

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Abstract: Nigeria accounts for 31% of the 619,000 malaria-related deaths recorded worldwide, according to the World Malaria Report 2022. Malaria caused by *Plasmodium falciparum* causes significant changes in haematological parameters and indices. The study aimed to observe erythrocyte indices and haemoglobin concentration variations in children with *Plasmodium falciparum* parasitaemia, evaluate the correlation between parasite count and indices, and assess their predictive roles. We analysed 194 children (≤ 5 years old) suspected of malaria and collected 5 ml of whole blood samples for analysis. The samples were used to prepare thin and thick blood films for qualitative and quantitative assays. A CBC was carried out to estimate HGB concentration and erythrocyte indices. The case and control group had a mean difference statistically significant at ($P < 0.001$). MCV [case group (72.26 ± 4.83), control group (85.00 ± 4.48)]; MCH [case group (23.82 ± 2.51), control group (28.85 ± 2.02)] and HGB [case group (100.78 ± 14.84), control group (135.65 ± 9.95)]. Pearson correlation showed weak negative statistical significance at ($P < 0.05$) for MCV, MCH, MCHC and HGB in the range of $(-0.38) - (-0.218)$. Regression analysis indicated that parasite count predicted MCV, MCH, MCHC and HGB at ($P < 0.05$); R values at $(0.283, 0.272, 0.326$ and 0.308 , respectively). When considering age and parasite count as predictive variables, we obtained R values of $0.285, 0.282, 0.326$, and 0.328 for MCV, MCH, MCHC, and HGB, respectively. Research results show a significant relationship between *Plasmodium falciparum* parasitaemia and variations in erythrocyte indices with haemoglobin concentration in children at significant levels.

Keywords: malaria, plasmodium falciparum, children, erythrocyte indices, haemoglobin concentration

1. Introduction

In many parts of sub-Saharan Africa and western Asia, malaria, a disease condition caused by a parasite that dwells in mosquitoes, continues to cause significant mortality among children, especially those under five (5) and pregnant women. Malaria continues to be among the primary causes of death worldwide. It is a vector-borne parasitic tropical disease in over 90 countries (WHO, 2017). Various research studies that aimed to identify the various species of *Plasmodium* have determined that over 120 *Plasmodium* species infect mammals, birds, and reptiles. However, only six are known to infect humans regularly.

The *Plasmodium falciparum* parasite remains the most prevalent species in many sub-Saharan regions and has its vector as the female anopheles mosquito (Walter & Chandy, 2022). It is still one of the top causes of mortality in most low-income nations, including many sub-Saharan and South Asian countries. Malaria is popularly referred to as a 'disease of the tropics and subtropical regions' simply because these regions account for the highest death rates, unlike in temperate regions where malaria has steadily been eradicated over the last century owing to both environmental and national intervention policies to curb and mitigate its spread (Elizabeth *et al.*, 2018).

The incidence of malaria depends on many factors, one of which is the environmental suitability for mosquitoes to thrive in terms of suitable climatic conditions, altitude, vegetation, and implementation of control measures. Besides, high malaria rates can be inextricably linked to poverty, natural disasters, and war (Elizabeth *et al.*, 2018). In rare conditions, vertical malaria transmission occurs, especially in the case of mother-to-child transmission or via blood transfusion.

Several predictions have also provided better information on how the change in climatic conditions will play a significant role in global malaria distribution in the future, and many suggestions have shown that the number of people at risk of malaria will increase, especially in tropical highlands (Caminade *et al.*, 2014). According to the epidemiological findings from the World Health Organization (2017) and several health agencies, *Plasmodium falciparum* and *Plasmodium vivax* are the two most common species worldwide, with an estimated incidence of 207 million and 8.5 million cases, respectively, in 2016. Of the two most common species of *Plasmodium*, *P. falciparum* accounts for the majority of deaths that occur in sub-Saharan Africa (approximately 190 million cases), where transmission remains intense in many locations, especially in Nigeria, although there is considerable variation in incidence within and between countries (Nkumama *et al.*, 2017; Snow *et al.*, 2017). In sub-Saharan Africa, the incidence of *Plasmodium vivax* malaria is much less common because the human population in this region is primarily Duffy antigen negative – the Duffy antigen (Fya and Fyb) makes up the Duffy blood group system.

Plasmodium falciparum is also known for producing high levels of blood-stage parasites that sequester in significant body organs and blood cells, causing severe haematological dysfunctions and anaemic conditions in African children who constitute a vast majority of malaria deaths (Elizabeth *et al.*, 2018).

There is a geographical disparity in the distribution of *Plasmodium* globally; in most parts of Africa, especially sub-Saharan Africa, *Plasmodium falciparum* malaria accounts for the highest incidence; in Asia and Oceania, malaria case numbers are generally lower, and proportions caused by *Plasmodium vivax* and *Plasmodium falciparum* are similar, whereas, in the Americas, *Plasmodium vivax* malaria cases exceed *falciparum* by more than two times (WHO, 2017).

Plasmodium malariae and the morphologically indistinguishable sympatric species like *Plasmodium ovale*, *Plasmodium curtisi* and *Plasmodium ovale wallikeri* are understudied. However, the severity of the illness is generally similar to that of uncomplicated *vivax* malaria. *Plasmodium knowlesi* is a primary zoonotic infection encountered in Southeast Asia, which can cause severe malaria (Jeyaprakasam *et al.*, 2020). Other species of *Plasmodium*, such as *Plasmodium malariae* and *Plasmodium ovale*, have a global distribution. However, *Plasmodium ovale* is predominant in Africa and Southeast Asia. *Plasmodium knowlesi*, on the other hand, has macaques as its natural hosts. In Malaysia, which has a high burden of *knowlesi* malaria, cases were initially misdiagnosed as *Plasmodium malariae* due to morphological similarities when experts examined them by light microscopy (Singh *et al.*, 2004).

Biology of Malaria

Several species of *Plasmodium* cause malaria; however, the most common species that accounts for the majority of severe cases, especially in sub-Saharan Africa, is *Plasmodium falciparum*, which has resulted in the death of people living within the region, especially in children under five years of age and pregnant women. The *Plasmodium* cycle in humans usually begins at the 'pre-erythrocyte liver stage'. During this stage, sporozoites are injected during a blood meal from the bite of infected female anopheline mosquitoes (Wikipedia, 2022).

Sporozoites enter the systemic circulation through the hepatic portal blood circulation via the interaction of the arterial and venous blood systems. Then, within the hepatocytes, they infect the liver cells and can result in inflammatory conditions; this usually occurs within 2 – 10 days, leading to the release of schizonts, which eventually mature and rupture, leading to the 'erythrocytic stage' (CDC, 2022). This stage has the malaria parasites infecting red blood cells, forming immature trophozoites. It is important to note that this stage contributes significantly to the variations in the erythrocyte indices assay and its clinical manifestation. Immature trophozoites can either metamorphose into mature trophozoites, forming more schizonts or gametocytes (CDC, 2022). A further explanation for this process is that a subpopulation of intraerythrocytic parasites switches to sexual transition-producing gametocytes (Josling & Llinás, 2015), which are the female and male gametocytes (Bousema & Drakeley, 2011).

When a non-infected mosquito bites an individual with these gametocytes during a blood meal, the gametocytes mature, forming flagellated microgametocytes, which exflagellate in the mosquito midgut. Male and female gametes couple to form a zygote that changes into mobile ookinetes and travels through the gut wall. These ookinetes

also mature into oocysts that eventually rupture, releasing sporozoites that migrate to the mosquito salivary gland and complete the parasite lifecycle (CDC, 2022).

Epidemiology of Malaria

Malaria occurs primarily in the tropical and subtropical regions of Africa, South and Central America, Asia, and Oceania (Cheuka *et al.*, 2019). In areas where malaria occurs, there is tremendous variation in transmission intensity and risk of infection. For example, most clinical malaria infections and mortality occur in sub-Saharan Africa, accounting for over 90% of malaria cases (CDC, 2021).

The population susceptible to malaria infection has grown during the past century despite a decrease in the proportion of persons exposed to malaria parasites. It has gradually increased from 0.8 billion in 1900 to 3.3 billion in 2010; this is a result of the exponential increase of people living in regions endemic to malaria (WHO, 2011; Hay *et al.*, 2004). However, malaria cases decreased from 244 million to 216 million due to various global interventions between 2005 and 2010. In addition, between 2000 and 2010, global malaria mortality rates decreased by 26% (Liu *et al.*, 2012).

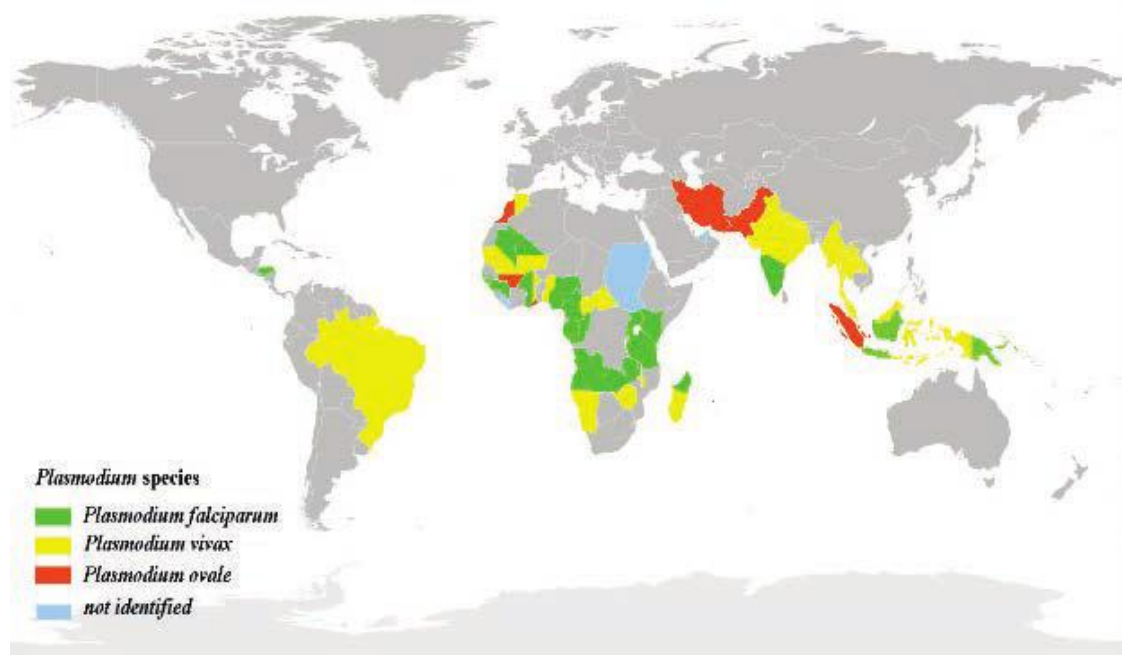


Figure 1. shows a global epidemiology distribution of *Plasmodium* species (Šubelj & Sočan, 2012).

According to the World Bank, malaria affects most low- and middle-income countries across the globe; there is also a shortage of effective data collection and reporting, resulting in insufficient statistical evidence. Unsteady and incomplete reports from individual health facilities may alter global malaria prevalence. Hyperendemic countries often underdiagnose malaria cases, while mild symptoms can lead to misdiagnosis. Occasionally, there is overdiagnosis (Autino *et al.*, 2012).

In areas needing more adequately trained professionals, like Medical Laboratory Scientists capable of conducting reliable rapid diagnostic tests (RDTs), malaria diagnosis is often prone to misdiagnosis. Additionally, areas with a high prevalence of febrile illnesses tend to experience misdiagnoses (Sullivan, 2010). The World Health Organization recommends microscopy as the gold standard for malaria diagnosis. However, rapid diagnostic tests (RDTs) such as test kits can also be employed in regions with limited funds and inexperienced microscopists (Autino *et al.*, 2012).

Distribution of Plasmodium Species

Various research studies investigating the different species of Plasmodium have established that although there are many species of Plasmodium, only five species cause serious harm to the human population. These species are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* (Autino *et al.*, 2012).

Plasmodium falciparum

Plasmodium falciparum is responsible for millions of deaths globally and remains the most common and dominant species. Researchers have found *Plasmodium falciparum* in countries where they occur and regions where they coexist with other species of Plasmodium. In a study aimed at determining the impact of *P. falciparum*, researchers discovered that 2.37 billion individuals worldwide face the risk of exposure. Based on geographical estimates, 26% of these individuals are in Africa, and 62% are in the Southeast Asia and Western Pacific regions (Guerra *et al.*, 2008).

According to epidemiological studies, *Plasmodium falciparum* is Africa's most common malaria-causing species. From 1998 to 2006, blood samples from nine different African countries analysed using Polymerase Chain Reaction (PCR) indicated that out of 2,588 samples collected, 1,737 were positive for Plasmodium and 1,711 (98.5%) showed positive for *Plasmodium falciparum* (Culleton *et al.*, 2008). When considering the report from other investigative studies done on the prevalence of *P. falciparum* in sub-Saharan Africa using four villages from eastern Uganda during the rainy season as a case study, it was observed that 94% of the most prevalent Plasmodium species was *Plasmodium falciparum* using thin film diagnosis (Pullan *et al.*, 2010).

Also, further research in other local areas still within eastern Africa showed a 94% prevalence of *P. falciparum*, especially during the rainy season, from July to December, using thin film diagnosis (Pullan *et al.*, 2010). A microscopic examination of pregnant women attending antenatal care clinics in Lagos, Nigeria, revealed an 88.5% prevalence of *P. falciparum* (Iriemenam *et al.*, 2011). Many reports have established that *Plasmodium falciparum* and *Plasmodium vivax* are the two main species of Plasmodium in Asia; however, it is imperative to note that the prevalence of these two species differs from one country to another.

As a result of the development of molecular techniques, there has been a significant improvement in the ability to detect specific species of Plasmodium and identify new strains that may emerge in the future. On average, the prevalence of *P. falciparum* in

many parts of Asia ranges from 52.3 – 86.4%. In Cambodia, Steenke et al. (2010) researched measuring the endemicity of malaria in Rattanakiri; they discovered that *Plasmodium falciparum* among the inhabitants of 8 villages was about 59% using the PCR technique.

Blood samples collected from Myanmar (Burma) showed that 146 patients selected for uncomplicated malaria in 2008 had a *Plasmodium falciparum* prevalence of 52.1%. The investigation was achieved using PCR analysis to investigate malaria parasites while considering patients with single and mixed infections (Jiang et al., 2010). Although the most prevalent Plasmodium species in South America is Plasmodium vivax, *P. falciparum* has the second most prevalence, approximately 25.7% (Arevalo-Herrera et al., 2011). Other sub-major species of Plasmodium include:

Plasmodium vivax

Aside from *Plasmodium falciparum* being the most common Plasmodium species globally, this is different when considering the prevalence of Plasmodium species based on regional areas. In Central and South America, *P. vivax* is the main species, constituting 71–81% of all malaria cases (Ryan et al., 2006). *P. vivax* accounts for 83.7% of all malaria infections in Brazil, according to Oliveira-Ferreira et al. (2010), and 70% and 90% of infection cases in Colombia and Ecuador, respectively (Manock et al., 2009). *Plasmodium vivax* infection is rare in many regions of Africa because the population has a high prevalence of red blood cells with Duffy negative phenotype.

As a result of this phenotypical expression of their red blood cells, it has hindered *P. vivax* merozoites' entry into erythrocytes. A large study conducted to detect the presence of *P. vivax* in nine African countries concluded with no trace of the strain in the population sampled (Culleton et al., 2008).

However, *P. vivax* transmission in West and Central Africa is still evident. For example, in a research carried out in Kenya, there was significant evidence demonstrating the presence of *P. vivax* among mosquitoes; furthermore, *P. vivax* DNA was amplified and sequenced in the blood of two Duffy-negative children (Ryan et al., 2006).

Plasmodium ovale.

In Africa, *Plasmodium ovale* prevalence is low and difficult to assess due to its difficulty in diagnosis. However, some regions of Africa and Asia have detected Plasmodium ovale using PCR technology. In Africa, *Plasmodium ovale* prevalence is low and difficult to assess due to its difficulty in diagnosis. However, some regions of Africa and Asia have detected Plasmodium ovale using PCR technology. A recent study from Mozambique observed that only 2 of 111 malaria-positive patients presented with *Plasmodium ovale* mono-infection. At the same time, 4 had both *P. ovale* and *P. falciparum* co-infections also detected (Macedo de Oliveira et al., 2011).

A study was done on the indigenous people of nine African countries. Of the 1,737 analysed samples, 67 tested positive for *P. ovale*; this includes 12 cases of single infections, 51 cases of mixed infections with *Plasmodium falciparum*, and four cases

of triple infections with *Plasmodium falciparum* and *Plasmodium malariae*. Using polymerase chain reaction (PCR), malaria parasites were searched and typed. In addition, excluding samples from four countries: Rwanda, Mozambique, Angola, and Sao Tome showed that *Plasmodium ovale* infection constituted 3.9% of affected people (Culleton *et al.*, 2008).

Plasmodium malariae.

Plasmodium malariae spreads around sub-Saharan Africa, Southeast Asia, and the Amazon Basin of South America. In the western Pacific region and areas of the Amazon Basin of South America, there is a significant presence of *P. malariae* within these regions; this is also very similar to Indonesia. These regions highlighted overlap the distribution of *P. malariae* with that of *P. falciparum* (Collins & Jeffery, 2007).

According to a study conducted in nine African countries, 147 samples out of 1,737 tested positive for *Plasmodium malaria*. Of the 14 patients, 129 had mixed infections with *P. falciparum*, and 4 had triple infections with *P. ovale* and *P. falciparum* (Culleton *et al.*, 2008). This report excluded patients' samples from Rwanda, Mozambique, Angola, and Sao Tome, *P. malariae* infections that represented 8.5% of all malaria infections (Culleton *et al.*, 2008). Furthermore, a report conducted by Iriemenam *et al.* (2011) supports the presence of *Plasmodium malariae*. From November 2001 to October 2002, in Nigeria, the researchers randomly recruited 350 pregnant women attending antenatal clinics and collected blood samples. Out of these, 96 (27.4%) tested positive for the malaria parasite, and 11 (11.5%) were positive for *P. malariae* when tested by microscopy.

Plasmodium knowlesi.

The recently identified *Plasmodium* species is the *Plasmodium knowlesi* infection, mainly locally in the Southeast Asia Region and affecting both monkeys. The infection was reported first in monkeys and then in humans. People who go or live around forests are most likely to be affected. From February to November 2010, 63 out of 253 patient samples collected in the Sabah region tested positive for *Plasmodium falciparum* using Polymerase Chain Reaction (PCR) for suspected malaria (Jooven-Neoh *et al.*, 2011).

Malaria Infection among Children under Five years.

The most significant casualties of malaria infection, especially in the sub-Saharan region, are children under five years old because of their vulnerability. The most common feature in children infected with malaria is severe anaemia, as children and adults also experience hypoglycaemia and cerebral malaria, especially in severe cases [34]. Furthermore, when children experience repeated malaria, especially in regions endemic to malaria, the affected children are susceptible to diarrhoea, respiratory infections, and other illnesses (Greenwood, 1997).

According to a report by Murphy and Breman (2001) on the burden of malaria in children, an estimated 2% of children who survive cerebral malaria end up with learning impairments and disabilities, including epilepsy and spasticity. This condition

is a result of the malarial parasites causing brain damage. Children below five years of age constitute the highest mortality cases owing to several factors. First, children are usually born with reduced acquired immunity, which develops as they mature through environmental exposure and immune response to antigens. Low levels of acquired immunity make children highly vulnerable to malaria parasitaemia, resulting in severe complications. In addition, children are highly vulnerable to malaria, considering the number of malarial parasites produced in the red blood cell volume; this is because repeated malaria can result in severe anaemia.

Another significant cause of the high mortality rate of malaria among children is low birth weight, especially during pregnancy, which increases the risk of death during the early stages of life (PMIE, 2019). In addition, when we consider social determinants, such as access to healthcare facilities, children in sub-Saharan Africa fall victim to a lack of quality healthcare. Most children reside in rural regions with limited quality healthcare, leading to numerous preventable deaths.

The global prevalence of malaria among children under five years of age is 16%, which remains a serious global challenge (WHO, 2016). However, tremendous efforts have been made to reduce this prevalence through a periodic supply of mosquito-treated nets, access to quality, and rapid diagnostic procedures and treatment. As previously highlighted, several steps have been implemented to address the challenges of malaria burden in children under five years of age through various initiatives from global leaders, private individuals, non-governmental organisations, and agencies. Nevertheless, recent trends indicate that progress in malaria control is slowing in the highest-burden countries (WHO, 2017).

According to most global statistics, Nigeria bears approximately 25% of global morbidity due to population increases and inadequate healthcare facilities. Recent reports have estimated the number of casualties from malaria annually, and it has been estimated that malaria is accountable for 60% of outpatient cases and those who come for hospital visits, 50 million cases nationwide, and 100,000 deaths (WHO, 2020; Onwujekwe *et al.*, 2013; Beargie *et al.*, 2019).

It has been found that children under five in Nigeria are the most vulnerable, in tandem with other regional and global reports. Children under five experience an average of 2–4 episodes per year, accounting for as much as 90% of the national malaria mortality (Edelu *et al.*, 2018). In a report by the United States Embassy in Nigeria, the impact of malaria on those under five resulted in an estimated 36% mortality rate (USEN, 2020).

When it comes to practical ways of reducing the mortality burden of under-five children suffering from the impact of malaria, the use of cost-effective treatment plans remains the best available option; if there is no treatment, a delay in treatment, or if treatment is not practical in cases where treatment is absent, delayed, or ineffective, this can potentially lead to life-threatening complications (Edelu *et al.*, 2018). Studies indicate that a family's financial status dramatically affects their decision to seek medical care in Nigeria when their child is suspected of having malaria. Only about

20% of children under five with a fever are taken to healthcare centres for clinical assessment and testing for parasites (WHO, 2019).

Erythrocyte Indices Response to Malaria

Malaria dramatically affects the structure and integrity of red blood cells, which play a significant role in oxygen transportation to body cells by combining with haemoglobin to form oxyhaemoglobin. During red blood cell formation, erythroid maturation involves the gradual condensation of nuclear chromatin and its removal. The removal is followed by the synthesis of haemoglobin and a concomitant reduction in cell size due to division and water loss (Sarma, 1990).

Red blood cell indices play a massive role in understanding red blood cell maturation and defects; they further measure erythrocytes' size, shape, and physical characteristics. In clinical settings, erythrocyte Indices, or Red Blood Cell (RBC) indices, are components of routine blood tests, including Full Blood Counts (FBC) and Complete Blood Counts (CBC). This test helps understand the features and characteristics of different blood cells, such as erythrocytes, leucocytes, and thrombocytes (platelets) (George-Gay & Parker, 2003).

The three primary red indices are MCH, MCV, and MCHC. In some cases, the red cell distribution width (RDW) is also included. Erythrocyte indices, also called red blood cell indices, are commonly used to detect and classify anaemia, which can vary during anaemia conditions (George-Gay & Parker, 2003).

Mean corpuscular volume (MCV) is the most practical value of the erythrocyte indices; it measures the size of red blood cells as some red blood cells can be macrocytic, microcytic or normocytic – with an average size (80 – 100fL). Furthermore, various conditions can lead to variations in size, also known as anisocytosis (Sarma, 1990). Mean corpuscular haemoglobin concentration (MCHC) measures the relative haemoglobin concentration per red blood cell. As previously mentioned, haemoglobin plays a role in gaseous exchange; however, it is crucial to note that changes can occur in mean corpuscular haemoglobin concentration in diseased conditions, leading to low or high values in the case of hyperchromasia or hyperchromasia. MCHC can be elevated in hereditary spherocytosis or sickle cell disease and reduced in conditions such as iron deficiency, chronic diseases, thalassemia, and lead poisoning. For example, in spherocytosis, the MCHC is increased due to the loss of the red cell membrane and other structural components and the resulting spherical shape assumed by the cell (Sarma, 1990). Mean Corpuscular Haemoglobin quantifies the amount of haemoglobin per red blood cell. The average values for MCH are 27.0 – 31.0 pg per cell.

The classification of anaemia remains one of the most practical relevant erythrocyte indices. When it comes to the effect of malaria on the red blood cells, it has been observed that children and pregnant women frequently experience normochromic and normocytic anaemic conditions in severe malaria, according to Haldar and Mohandas (Haldar & Mohandas, 2009). In areas endemic to malaria, this anaemia accounts for over 50% of malaria-associated mortality. Malaria causes significant changes in

erythrocyte morphology and haemoglobin levels during the erythrocytic phase of the *Plasmodium falciparum* life cycle. Trophozoites release and degrade haemoglobin from erythrocytes in the blood circulation, causing an immune response to infected red blood cells (Moore *et al.*, 2006). In the pathogenesis of severe anaemia, haemoglobin concentration is defined as (Hb <5g/dL); this is in contrast to the normal haemoglobin concentration range of 136 -180 g/L for males and 120 – 150 g/L for females using reference values from clinical laboratories in Nigeria.

Nonetheless, there is further destruction of infected red cells by the reticuloendothelial system and a decreased production of red cells in response to anaemia due to dyserythropoietic processes, which appears to play a role in the severe anaemia experienced (Mohandas & An, 2012). Similar to other studies, some reports highlight the destruction of uninfected red blood cells; however, the mechanism of how this occurs is yet to be fully understood (Mohandas & An, 2012).

Red Cell Volume Regulation and Parasite Invasion

As previously mentioned, children under five usually experience normocytic and normochromic anaemia. Children often experience hypochromic conditions; however, it is essential to note that red blood cells regulate red cell volume. In addition, red blood cells undergo cell hydration, enabling them to maintain a cell haemoglobin concentration between narrow limits of 29–37 g/dL with a mean cell haemoglobin concentration (MCHC) of 33 g/dL (Mohandas & An, 2012).

The hydration process of red blood cells allows for the invasion of malarial parasites; however, red blood cells effectively inhibit malaria invasion by dehydrating red cells. Dehydration often results in a concentration of >37 g/dL, leading to decreased invasion efficiency and a cell haemoglobin concentration of >41 g/Dl (Mohandas & An, 2012). Red blood cell dehydration is observed in most hemoglobinopathies, including HbAS, HbSS, HbAC, HbSC, and HbCC, highly prevalent in Africa, and hereditary xerocytosis, a less prevalent red cell membrane disorder. People with dominant or recessive genes for sickle cells resulting in different haemoglobinopathies are less likely to be severely affected by *Plasmodium falciparum* parasitaemia. Mohandas and An. (2012) both explain that in events of haemoglobinopathies, 5 - 30 % of the red cells have cell haemoglobin concentration > 37 g/dL; however, in normal individuals, this accounts for less than 1% of circulating red cells, having red cell haemoglobin concentration > 37 g/dL.

Conclusively, dehydration in red blood cells markedly reduces the risk of acquiring a high degree of parasitaemia, decreasing the disease's severity. During the erythrocyte stage, when schizonts infect red blood cells, the malaria parasite residing within the vacuole increases in size and, throughout its 48-hour life cycle, digests 70% of haemoglobin obtained from the red cell cytoplasm, leading to the generation of amino acids needed for protein synthesis (Mohandas & An, 2012). This process reduces the haemoglobin concentration, reducing mean corpuscular haemoglobin concentration (MCHC).

Some malaria parasites have derived proteases that digest haemoglobin inside the erythrocytes; some characterised proteases include the cysteine-protease falciparum and the aspartic-protease plasmepsins (Rosenthal, 2004). Members of these protease families also play a crucial role in the rupture of the red cells, leading to anisocytosis and allowing the release of merozoites (trophozoites) (Rosenthal, 2004). Furthermore, the development of the malaria parasite induces significant structural and morphological changes in the infected red cell, including alterations in the cell's rheological and adhesion characteristics (Cooke *et al.*, 2004).

2. Materials and Method

2.1. Materials

Materials used for the study include Biorapid microscope slides, methanol, distilled water, Mindray BC-2800 haematological analyser, Olympus CX22 light microscope, Giemsa Stain stock solution GK1826.0250 and 5ml K2E(EDTA) vacutainer sample bottles.

2.2. Aim and Objectives

This study aimed to evaluate and estimate the relationship between *Plasmodium falciparum* parasitaemia and erythrocyte indices in children aged (5) and below living in a malaria-endemic region of Port Harcourt, Rivers State, Nigeria. The objectives of this research are centred towards i.) Estimate the correlation and level of significance between *Plasmodium falciparum* parasitaemia and haemoglobin concentration in children aged five (5) and below. ii.) Estimate the correlation and level of significance between *Plasmodium falciparum* parasitaemia and the major erythrocyte indices – Mean Cell Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and Mean Cell Volume (MCV) in children aged five (5) and below.

2.3. Place and location of the study

The researchers conducted the research at the Medical Laboratory Department, Military Hospital Port Harcourt, Port Harcourt, Nigeria, from November 2022 to March 2023.

2.4. Experimental design

A total of 194 samples were collected, of which 100 were confirmed positive for *Plasmodium falciparum* infection at different parasitic stages using Giemsa stain microscopy. Twenty (20) samples out of the total samples collected served as controls. Of the 100 confirmed positive for *Plasmodium falciparum* infection, the whole blood was assayed for Complete Blood Count (CBC), which provided results for the erythrocyte indices and haemoglobin concentration. A similar assay was carried out on the twenty (20) control samples that were confirmed to be malaria-negative using Giemsa stain microscopy. Afterwards, the results were statistically analysed.

2.5. Test Groups

The researchers examined 100 medical treatment patients' *Plasmodium falciparum* positive test samples collected at Military Hospital Port Harcourt, Rivers, Nigeria, using Giemsa stain microscopy.

Inclusion Criteria

Age: children of any sex ≤ 5 years of age

Children who had a clinical diagnosis of suspicion of malaria and were confirmed positive for malaria

Exclusion Criteria

The research did not include children six years or older, including children diagnosed with other underlying health complications that could affect erythrocyte indices, such as sickle cell anaemia, thalassemia, tuberculosis, and other inflammatory conditions.

2.6. Control groups

The research study included 20 control groups confirmed malaria-negative through microscopic analysis using Giemsa stain.

Inclusion criteria

The research study included control samples from healthy children aged five (5) and below, including children who were not reported of any disease condition that could interfere with their erythrocyte or red blood cell indices.

Exclusion criteria

Children above six (6) years of age with signs and symptoms of underlying infections or diseases were excluded from the study.

2.7. Sample collection

Each subject provided 5 mL of venous whole blood, collected in a vacutainer sample bottle containing K2E (EDTA) for malaria testing and complete blood count (CBC). The whole blood samples were promptly transported to the haematological laboratory unit for analysis within 30 minutes.

2.8. Haematological assay

A complete blood count test was used to measure the following parameters:

- Mean Corpuscular Haemoglobin Concentration (MCHC)
- Mean Cell Volume (MCV)
- Mean Corpuscular Haemoglobin (MCH)
- Haemoglobin concentration (HGB)

In addition, a malaria test involving qualitative and quantitative analyses was performed on all 194 and control samples.

2.9. Assay Method and Procedure

The healthcare professional used a 5 ml vacutainer sample bottle to collect whole blood for analysing malaria. They prepared thick and thin blood films on a single glass slide marked with an identification number. After air-drying the films, they fixed the thin films in methanol and stained them for 10 minutes with a 1:10 dilution factor. Finally, they viewed the slides under $\times 100$ oil immersion lenses using a microscope and determined haemoglobin concentration and erythrocyte indices (MCV, MCH, and MCHC) using the Mindray BC-2800 haematology analyser.

3. Results

Statistical analysis was done using IBM SPSS for Windows (version 25). A normal distribution was assumed based on red blood cell parameters. A few outliers were removed from the data parasite counts to ensure normality in the data distribution. The mean difference between the groups (case and control) of erythrocyte indices was analysed using an independent sample t-test. The association between the parasite count and the red blood cell indices was performed using Pearson correlation, adjusted R² was reported as the coefficient of determination, and the predictability of the red blood cell parameter by parasite count was assessed by linear regression. $P < 0.05$ was considered statistically significant for the association between variables or the difference < 0.05 .

Table 1.

Variables	Case	Control	t-value	p-value
MCV	72.26 $\pm$ 4.83	85.00 $\pm$ 4.48	10.88	<0.001
MCH	23.82 $\pm$ 2.51	28.85 $\pm$ 2.02	8.41	<0.001
MCHC	31.67 $\pm$ 1.76	33.16 $\pm$ 1.56	3.5	0.001
HBG	100.78 $\pm$ 14.84	135.65 $\pm$ 9.95	1005	<0.001

Table 1 shows the mean comparison between the study population's case and control groups of the red blood cell indices (MVC, MCH, MCHC and HBG). From standard descriptive analysis, the result showed that the red blood cell parameter of the control group was significantly higher than the case group. The mean MVC level of the control subject (85.00 $\pm$ 4.48) was higher than the case subject (72.26 $\pm$ 4.83). The mean difference test by t-test showed that the mean difference was statistically significant (p-value <.001). The mean MCH level of the control subject (28.85 $\pm$ 2.02) was higher than the case subject (23.82 $\pm$ 2.51). A similar pattern was also seen in other indices such as MCHC [control (33.16 $\pm$ 1.56); case (31.67 $\pm$ 1.76)] and HGB [control

(135.65±9.95); case (100.78±14.84)]. There was a statistical significance in haemoglobin concentration between case and control groups $P<.001$.

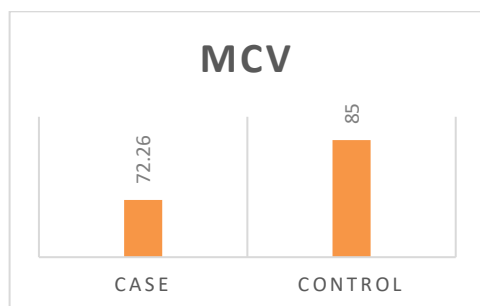


Figure 2. MCV Mean difference

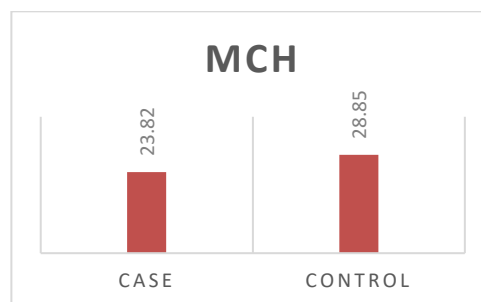


Figure 3. MCH Mean difference

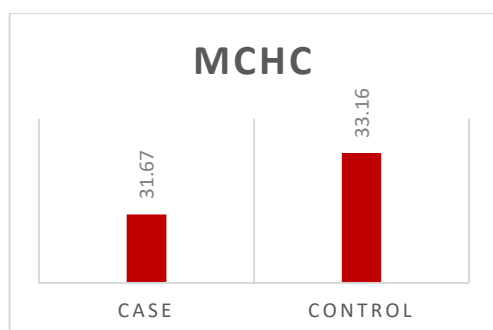


Figure 4. MCHC Mean difference

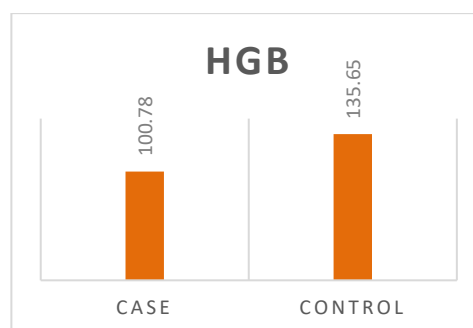


Figure 5. HGB Mean difference

Table 2.

Variables	R	p-value
MCV	-0.283	0.004
MCH	-0.272	0.006
MCHC	-0.326	0.001
HGB	-0.309	0.002

Table 2 shows a Pearson correlation between parasite count and red blood cell indices (MCV, MCH, MCHC and HBG). There was a low to very low negative significant correlation at ($P<0.05$) between parasite count and MCV as well as MCH ($r=-0.283$, $p<0.05$) and ($r=-0.272$, $p<0.05$), respectively. The table also showed a low negative significant correlation between parasite count and MCHC as well as HBG at ($P<0.05$) ($r=-0.326$, $p<0.05$) and ($r=-0.309$, $p<0.05$), respectively.

Table 3

Variables	R	R ²	Adj R ²	F	p-value
MCV	0.282	0.080	0.070	8.552	0.004
MCH	0.272	0.070	0.064	7.834	0.006
MCHC	0.326	0.106	0.097	11.667	0.001
HGB	0.308	0.094	0.085	10.271	0.002

Table 3 shows a linear regression analysis on red blood cell indices (MCV, MCH, MCHC and HGB) regressed on predictive variable parasite count. The result shows parasite counts significantly predicted MCV=0.28, MCH=0.27, MCHC=0.32 and HGB=0.31 at $P<0.05$. The regression variance was valued at (MCV=0.080, MCH=0.07, MCHC=0.11 and HGB=0.09). This model explains parasite count regression variance of MCV=8%, MCH=7%, MCHC=10.6% and HGB=9.4%.

Table 4

Variables	R	R ²	Adj R ²	F	P-value
MCV	0.285	0.081	0.062	4.272	0.017 ^b
MCH	0.282	0.079	0.060	4.179	0.018 ^b
MCHC	0.326	0.106	0.088	5.776	0.004 ^b
HGB	0.328	0.108	0.089	5.844	0.004 ^b

Table 4 shows a linear regression analysis on red blood cell indices (MCV, MCH, MCHC and HGB) regressed on predictive variables age and parasite count. The result shows that parasite count and age significantly predicted MCV=0.29, MCH=0.28, MCHC=0.33 and HGB=0.33 at $P<0.05$. The regression variance was valued at (MCV=0.08, MCH=0.08, MCHC=0.11 and HGB=0.11). This model explains age and parasite count explains regression variance of MCV=8.1%, MCH=7.9%, MCHC=10.6% and HGB=10.8%.

4. Discussion

The results showed a significance level when the mean of the case group was compared with that of the control group. Previous studies on the response of children ≤ 5 years to malaria, especially malaria caused by *Plasmodium falciparum*, have identified that the severity ranges from haematological disorders to severe impacts on vital organs of the body.

As a result of maternal immunity loss and poor development of adaptive and innate immunity, children are vulnerable to malaria (Schumacher & Spinella, 2012). Due to this and several other factors, the presence and level of parasite density with parasite count give rise to variations in erythrocyte indices and haemoglobin concentration. Our analysis showed a significant difference in mean difference for MCV; the case group had a mean $\pm$ S.D of 72.26 $\pm$ 4.63, which explains the microcytic condition. This change concerns the observation made by Mohandas and An (2012) regarding the impact of specific proteases on erythrocytes, resulting in reduced erythrocytic indices.

However, the findings of Kotepui et al. (2014) on the effect of malarial infection on haematological parameters contrast our findings, which suggest an increase in the concentration of MCV in people infected with malaria (Kotepui et al., 2014). Malarial parasitaemia in children causes severe destruction of erythrocytes and a decrease in the production of red blood cells (Chang & Stevenson, 2004). In severe infections, erythrocytes tend to have a larger central pallor when viewed microscopically. Besides severe infections in children, iron deficiency anaemia (IDA) and hemoglobinopathies are the most frequent causes of microcytosis (Irwin & Kirchner, 2001).

A statistically significant difference in haemoglobin concentration and MCH was also found between the case and control groups. The case group with a mean $\pm$ S.D of 23.82 $\pm$ 2.51 and 100.78 $\pm$ 14.84 for MCH and HGB, respectively, showed a reduction in the haemoglobin concentration in children. In addition, during the intra-erythrocyte cycle, malaria trophozoites digest haemoglobin and reduce its levels (Moore *et al.*, 2006).

As a result of the severe impact of plasmodium parasitaemia in children, there is an impact on mean corpuscular haemoglobin concentration (MCHC) and a significant negative correlation between malaria parasite count and changes in MCHC. Furthermore, regression analysis showed that parasite count could predict significant changes in mean corpuscular haemoglobin concentration ($P<.05$). Correlation analysis suggests that with an increase in parasite count, there is a decline in the concentration of MCHC and HGB levels, as reported in several other studies (Moore *et al.*, 2006; Starck *et al.*, 2021). Kotepui et al. (2015) suggested an increase in MCH and MCHC in *Plasmodium falciparum* parasitaemia due to the release of immature erythrocytes into the blood circulation (Kotepui *et al.*, 2015)

Predictive analysis carried out by Starck et al. (2021) showed that malaria density predicted the prevalence of anaemia based on the level of severity, and this is also concerning our findings, which suggest a regression of 28.3% for MCV at a variance of 8%. A moderate level of regression was observed between parasite count and MCHC and haemoglobin concentration at 32.6% and 30.8%, respectively, and at a variance of 10.6% and 9.4%, respectively. From previous literature on the predictive impact of malaria density on haemoglobin concentration, much emphasis has been placed on *Plasmodium falciparum*'s role in reducing haemoglobin levels by damaging the integrity of red blood cells, thereby releasing haemoglobin into blood circulation. At the same time, other mechanisms involve stimulating the immune system to destroy affected erythrocytes and interfering with erythropoietic processes.

5. Conclusion

Plasmodium falciparum parasitaemia remains a major global issue in many regions, particularly in many geographical regions in sub-Saharan Africa and numerous regions of Asia, resulting in high mortality rates in children five years and below. Concerted efforts have been made to reduce the high mortality rate through governmental and non-governmental interventions, focusing on malaria eradication in the coming years.

Malaria often shares similar signs and symptoms with many tropical diseases, which can lead to misdiagnosis when diagnosed medically. Red blood cells are the primary target in the *Plasmodium* spp life cycle, especially during the 'erythrocyte cycle. As a result, trophozoites cause a significant level of damage to the morphology and content of red blood cells. Erythrocyte indices play a helpful role in determining the size and haemoglobin levels of red blood cells, particularly in the classification of anaemia, a common feature of malaria in children.

Plasmodium parasitaemia in children affects the stability of erythrocyte indices, which form part of the Complete Blood Count (CBC). Several investigations to determine the correlation between malaria density and variations in red blood cell indices support our observations of a significant correlation between parasite count and changes in erythrocyte indices and haemoglobin concentrations.

Findings such as this further buttress the need for clinicians to pay careful attention to haemoglobin and erythrocytic levels when performing haematological assays for malaria. In addition to physicians requesting malaria tests during the preliminary investigation, other supportive tests, such as CBC, should be adopted to help provide a holistic examination for patients.

6. Limitations

The limitations of this study, as observed by the researchers, were associated with factors that could limit the accuracy of diagnosis and the results generated. We observed that few children had co-infections, such as bacterial and viral infections, which interfered with the erythrocyte indices and haemoglobin concentrations; however, concerted efforts were made to resolve this. In addition, some patients had already started antimalarial treatment before hospital visits, reducing parasite density.

While the study acknowledges that other factors, such as nutrition and illnesses, may affect erythrocyte indices in children, it carefully controlled for these variables by only including children who were healthy and free of known illnesses or malnutrition. Also, the study did not address the potential impact of Giemsa stain on the analysis results. Further investigation into this matter could be helpful. The study notes the possibility of false positives or negatives in the blood samples, which could affect the reported correlation between parasite count and erythrocyte indices. However, the study minimised this risk by adhering to a standardised laboratory protocol and having multiple trained technicians analyse the blood samples.

We employed microscopy for both qualitative and quantitative detection of malaria, the clinical gold standard; however, some patients reported as unfavourable could have been reported as positive through Polymerase Chain Reaction.

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