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Impact of Malaria on Hematological Profiles, Iron Indices, and G6PD Activity in Pregnant Women

***Fidelis Ohiremen Oyakhire[1], Patricia Ejenawome Dele-Ochie[2], Juliana Edusola Olaniyan[3], Valentine Ikalumhe[1], Babatunde Ishola Gabriel Adejumo[4], Samson Efenarhua[5], Osamwonyi Ernest Igbinovia[6], Emmanuel Onosetale Afeikhena[7], Adebukola Adisa Ekoh-Jolly[8]**

[1]Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo State University, Iyamho, Edo State, Nigeria. [2]Department of Medical Laboratory Science, Southern Delta University, Ozoro, Delta State, Nigeria. [3]Department of Chemical Pathology, Federal Medical Center, Owo, Ondo State, Nigeria. [4]Department of Medical Laboratory Science, University of Benin, Benin City, Edo State, Nigeria. [5]Department of Natural Science, Faculty of Science and Technology, Middlesex University, London, United Kingdom. [6]Department of Laboratory Medicine, Swansea Bay University Health Board, Wales, United Kingdom. [7]Department of Pathology, Asaba Specialist Hospital, Asaba, Delta State, Nigeria. [8]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Medical Sciences, Ondo, Ondo State, Nigeria. *Email: oyakhire.fidelis@edouniversity.edu.ng
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Abstract: The global prevalence and impact of malaria on pregnancy highlight a significant public health challenge that demands enhanced preventive measures and targeted interventions. The study aimed to assess the hematological, iron, and G6PD status among pregnant women infected with *Plasmodium falciparum.* We performed a comparative cross-sectional study among two hundred (200) pregnant women infected with *Plasmodium falciparum*, who attended Edo Specialist Hospital, Benin City, to assess the hematological, iron, and G6PD status compared with fifty (50) *Plasmodium falciparum*-free pregnant women as controls. Hematological parameters, iron, TIBC (total iron binding capacity), and G6PD activity were assessed using a hematology auto analyzer and spectrophotometric methods, respectively. Data obtained were analyzed using SPSS (IBM v26). Independent t-test and one-way analysis of variance (ANOVA) were used for continuous variables, and chi-square was used for categorical variables. Pregnant women infected with *Plasmodium falciparum* had 8.70%, 9.42%, 14.44%, 8.1%, 20.17%, 16.72%, 11.31%, and 27.8% reduction in red blood cell, hemoglobin, iron, packed cell volume, G6PD, total iron binding capacity, monocyte, and lymphocyte levels, respectively, compared to *Plasmodium falciparum*-free pregnant women as controls ($p<0.05$). In addition, the levels of white blood cell, platelets, and neutrophils witness 15.58%, 10.85%, and 24.02% increase in pregnant women infected with Plasmodium falciparum compared with plasmodium falciparum free pregnant women as controls ($p<0.05$). No significant differences were observed in serum iron and G6PD levels across gestational ages ($p>0.05$). The findings underscore the need of regular monitoring of hematological and biochemical parameters in pregnant women infected with *Plasmodium falciparum* in order to enhance policy on malaria prevention and screening in endemic regions.
Keywords: Anemia; glucose-6-phosphate dehydrogenase activity; hematological profiles; iron indices malaria; serum iron.

Corresponding Author: Fidelis Ohiremen Oyakhire
Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo State University, Iyamho, Uzairue, Edo State, Nigeria
Email: oyakhire.fidelis@edouniversity.edu.ng

INTRODUCTION

The global prevalence and impact of malaria on pregnancy highlight a significant public health challenge that demands enhanced preventive measures and targeted interventions. The effect of malaria on hematological profiles, iron indices, and glucose-6-phosphate dehydrogenase (G6PD) activity in pregnant women is a critical concern due to the complex interplay between infection, maternal physiology, and fetal development (Helegbe et al., 2023).

Malaria during pregnancy adversely impacts both maternal and fetal health due to immunological changes and the accumulation of Plasmodium parasites in the placenta (Obeagu and Obeagu, 2024). This condition leads to maternal anemia and a higher rate of infant mortality (Accrombessi et al*.,* 2018). The sequestration of infected erythrocytes in the placenta disrupts the transfer of nutrients to the fetus (Obeagu and Obeagu, 2024). Collectively, studies have shown that pregnant women infected with *Plasmodium falciparum* have low hemoglobin concentration, hematocrit levels, iron and red blood cell counts, which eventually led to anemia and increase risk of adverse pregnancy outcomes such as preterm delivery, low birth weight, and perinatal mortality (Jain et al, 2022; Basu et al, 2018; Domingo et al*,* 2018).

Although extensive research has been conducted separately on malaria and pregnancy, the specific impact of malaria on hematological parameters during pregnancy remains unclear. Pregnant women experience unique physiological alterations that complicate the interpretation of hematological parameters (Rogerson & Unger, 2022). Despite its clinical importance, current knowledge remains fragmented, and existing clinical protocols may not fully address the multifaceted impact of malaria on these interconnected biochemical and hematological indices during pregnancy. Previous research has identified a correlation between malaria infection and the incidence of anemia in pregnant women (Mahamar et al., 2021). Pregnant women infected with *Plasmodium falciparum* are highly vulnerable due to pregnancy-related physiological stress and malaria-induced disruption of red blood cell integrity, iron metabolism, and immune regulation (Obeagu and Obeagu, 2024). Although malaria in pregnancy was well studied in Nigeria and sub-Saharan Africa (Goheen et al, 2017), most research has focused on clinical outcomes such as anemia and birth weight, with limited assessment of key biochemical interactions involving G6PD activity and iron status markers (serum iron and TIBC). This is an important gap because *P. falciparum* infection triggers hemolysis, inflammatory iron sequestration, and oxidative stress, all of which may influence both iron availability and erythrocyte antioxidant defense systems.

However, there is limited evidence in Nigeria on how G6PD activity interacts with malaria-related oxidative stress and iron redistribution during pregnancy. Consequently, this study adopts an integrated biochemical approach by simultaneously evaluating G6PD activity and iron profile indices in pregnant women with *P. falciparum* infection to better understand the relationship between oxidative stress, erythrocyte protection, and iron metabolism in malaria-associated pregnancy complications.

Reduction in G6PD activity in pregnant women can increase susceptibility to hemolysis, especially under oxidative stress induced by infection or certain antimalarial treatments, thereby complicating clinical management (Helegbe et al., 2023). Understanding how malaria modulates G6PD activity is essential for optimizing therapeutic strategies and minimizing adverse effects (Helegbe et al*.,* 2023). This gap highlights the need for comprehensive evaluation and targeted diagnostic and therapeutic approaches that consider the complex interactions between malaria

infection and maternal hematological and biochemical profiles. Addressing this issue is essential to reduce adverse maternal and neonatal outcomes, improve pregnancy health, and alleviate the broader socioeconomic burden in endemic areas.

Enhancing preventive measures, such as intermittent preventive treatment in pregnancy (IPTp) with antimalarial drugs (sulfadoxine-pyrimethamine), the use of insecticide-treated bed nets (ITNs), and the prompt diagnosis and treatment of malaria episodes during pregnancy and iron supplementation policies tailored to these complex interactions, could significantly improve pregnancy outcomes in malaria-endemic regions. This study examined the impact of malaria on the hematological health of pregnant women, with a particular focus on blood counts, iron levels, and the activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD).

MATERIALS AND METHODS

We performed a comparative cross-sectional study including two hundred (200) pregnant women infected with *Plasmodium falciparum* and fifty (50) *Plasmodium falciparum*-free pregnant women as controls, to examine the impact of *Plasmodium falciparum* on hematological parameters and G6PD activity. Participants were recruited from the antenatal clinics at Edo Specialist Hospital in Benin City using a consecutive sampling technique to select confirmed *Plasmodium falciparum* cases. Age and trimester-matched pregnant women who were screened and free of *Plasmodium falciparum* using the microscopic method were used as the control group to minimize confounding factors.

Pregnant women attending antenatal clinics within the specified gestational age range who provided consent were included. Cases were confirmed with malaria through microscopy or rapid diagnostic tests, while controls were malaria-free pregnant women. Women with HIV/AIDS, chronic illnesses, or recent antimalarial treatments were excluded to avoid confounding effects.

Demographic and clinical data were collected through structured interviews and reviews of medical records to ensure comprehensive and accurate information. Data collection focused on variables such as age, gestational age, and clinical presentation relevant to the study objectives. This dual approach allowed for the verification of self-reported information against clinical documentation, enhancing data reliability.

$$n = \frac{Z^2\, p\,(1-p)}{d^2}$$

where:

Z = Z-score corresponding to the confidence level (1.96 for 95%)

p = proportion of 0.212 (Wagbatsoma and Omoike, 2008)

d = margin of error (5%)

$$n = \frac{Z^2\, p\,(1-p)}{d^2} = \frac{1.96^2 \times 0.212(1-0.212)}{0.05^2} = 257$$

The sample size for this cross-sectional study was determined using Cochran's formula for sample size estimation. The prevalence of the outcome among pregnant women infected with *Plasmodium falciparum* in the study area is 0.212, as reported by Wagbatsoma and Omoike, 2008. At a 95% confidence level and 5% margin of error, the minimum sample size calculated was 257. However, due to the limited availability of eligible pregnant women infected with *Plasmodium falciparum* within the study period and logistical constraints, a total of 250 participants were recruited using consecutive sampling of all consenting eligible pregnant women infected with

Plasmodium falciparum, which is considered adequate for generating preliminary findings.

Blood Sample Collection and Processing Procedures

Blood samples were collected from each participant using aseptic venipuncture techniques to ensure sample integrity and prevent contamination. Approximately 3 mL of venous blood was drawn into EDTA tubes for hematological analysis and 4mL into plain tubes for serum separation, respectively. The collected samples were immediately transported to the laboratory, where serum was separated by centrifugation at 3000 rpm for 10 min and stored at -20°C until further analysis.

Hematological and Biochemical Analysis Techniques

Hematological analysis was conducted using hematology auto analyzers (Biobase BK3100, China) to measure hemoglobin, hematocrit, blood cell counts, and platelets based on the impedance principle. Biochemical analysis was conducted using spectrophotometric methods to assess serum iron, total iron binding capacity (TIBC), and G6PD activity, respectively.

Estimation of Serum Iron Level

Serum iron concentration was determined using the Pointe Scientific Iron Colorimetric Assay Kit (Pointe Scientific Inc., Canton, MI, USA; Lot No: recorded per batch) based on the ferrozine-based colorimetric method. All reagents and serum samples were allowed to equilibrate to room temperature before analysis. A measured volume of serum (50 µL) was first treated with an acidic buffer to release iron from transferrin and convert all iron to the ferrous (Fe^{2+}) state. The liberated iron was then reduced and reacted with 1.0mL of ferrozine reagent to form a stable purple-colored ferrous–ferrozine complex. The reaction mixture was incubated for 10 minutes) At room temperature to ensure full color development. The intensity of the resulting chromogen was measured spectrophotometrically at 560 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) against a reagent blank. Serum iron concentration was calculated from a calibration curve generated using known iron standards provided in the kit and expressed in µg/dL or µmol/L. All analyses were performed in accordance with the manufacturer's instructions, and internal quality control sera at normal and pathological levels were included to ensure analytical accuracy and precision (Burtis and Bruns, 2015; McPherson and Pincus, 2021).

Estimation of Glucose-6-Phosphate Dehydrogenase (G6PD) Activity

Blood samples were collected into EDTA anticoagulant tubes and analyzed promptly to preserve enzyme stability. G6PD activity was determined using the Pointe Scientific Glucose-6-Phosphate Dehydrogenase Assay Kit, Lot No.: G7583-CTL-376X (Pointe Scientific Inc., Canton, MI, USA) based on a kinetic spectrophotometric method. The assay measures the rate of reduction of $NADP^+$ to NADPH catalyzed by G6PD in the presence of glucose-6-phosphate. NADPH formation was monitored by the increase in absorbance at 340 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) at 37°C. Enzyme activity was calculated from the change in absorbance per minute (ΔA/min) according to the manufacturer's instructions and expressed as U/g Hb. The reference range provided by the manufacturer is 6–18 U/g Hb (Shah et al*,* 2012)

Determination of Total Iron-Binding Capacity (TIBC)

Total iron-binding capacity (TIBC) was determined using the Pointe Scientific Iron/TIBC Colorimetric Assay Kit (Pointe Scientific Inc., Canton, MI, USA; Lot No: Lot No.: H783-CTL-306X) based on the direct iron saturation method. Venous blood samples were collected into plain tubes, allowed to clot, and serum was separated by

centrifugation at 3000 rpm for 10 minutes; hemolyzed samples were excluded. The principle of the assay is based on the saturation of transferrin with an excess of ferric ions (Fe^{3+}), followed by removal of unbound iron using an iron-adsorbing agent (basic magnesium carbonate/resin). The iron bound to transferrin is then released under acidic conditions and reacts with a chromogen reagent (ferrozine-based system) to form a colored complex, the intensity of which is directly proportional to the TIBC. All reagents, including ferric iron standard, saturating reagent, adsorbent, buffer, acid reagent, and chromogen, were prepared according to the manufacturer's instructions. In brief, 500µL of serum was incubated with 1.0mL excess ferric iron to saturate transferrin binding sites, incubated for 10minutes at room temperature, and unbound iron was removed by adsorption and centrifugation. The supernatant containing iron-bound transferrin was treated with acid to release iron, followed by the addition of chromogen reagent, and absorbance was measured at 560 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan). TIBC was calculated from a calibration curve generated using manufacturer-provided standards and expressed as µg/dL or µmol/L. Internal quality control sera (normal and pathological levels) were analyzed alongside test samples to ensure analytical accuracy. The reference range for TIBC was 250–450 µg/dL (45–72 µmol/L), as provided by the manufacturer and standard clinical chemistry guidelines (Burtis and Bruns, 2015; McPherson and Pincus, 2021).

***Plasmodium falciparum* Detection and Quantification Methods**

Plasmodium falciparum detection was performed using both rapid diagnostic tests (RDTs) and microscopic examination of Giemsa-stained blood films. For microscopy, both thick and thin blood smears were prepared on clean, grease-free glass slides using fresh EDTA-anticoagulated blood. Approximately 6 µL of blood was used to prepare the thick smear in a circular motion, while 2µL was spread to produce a thin smear with a feathered edge. The smears were air-dried, and the thin film was fixed with absolute methanol for 2 minutes, whereas the thick film was left unfixed. The slides were stained with 10% Giemsa solution prepared in buffered water (pH 7.2) for 10 minutes, after which they were gently rinsed with clean water and allowed to air-dry in a vertical position.

The stained smears were examined under a light microscope using the oil immersion objective (×100). Thick films were used for parasite detection and parasite density estimation, while thin films were used for *Plasmodium falciparum* identification and differentiation from *Plasmodium species*. A slide was considered negative only after examining at least 100 high-power fields without detecting parasites. Parasite density was determined by counting the number of *Plasmodium falciparum* against 200 white blood cells (WBCs) on the thick smear and calculated using the formula:

$$\text{Parasite Density (parasites/\u L)} = \frac{\text{Number of Parasites Counted x Patient WBC Count}}{\text{Number of WBCs Counted}}$$

For the rapid diagnostic test, the procedure was carried out according to the manufacturer's instructions. Briefly, the test cassette was labelled appropriately, and the required volume of whole blood was added to the sample well using a disposable pipette supplied with the kit. The assay buffer was then added, and the test was allowed to run for 15 minutes. Results were interpreted based on the appearance of visible colored lines in the control and test regions of the cassette. Presence of both control and test lines indicated a positive result for malaria antigen, while the

appearance of only the control line indicated a negative result. Invalid tests were repeated using a new test device (Feleke et al., 2021).

Ethical Approval

Ethical clearance was obtained from the Edo State Ministry of Health, Nigeria (ethical code HA/732/24/D/0419267, dated 19th April 2024). The purpose of the study was explained to the participants, who provided written or verbal consent based on their literacy. Participation was voluntary and confidential in nature.

Statistical Analysis

Data management involved systematic entry and backup using secure electronic databases with restricted access to the data. Quality control included data verification to identify any inconsistencies. Data were analyzed using SPSS (IBM 26), and the results are expressed as mean± standard error. Statistical tests, including t-tests and ANOVA, were applied for continuous variables, with significance at $p<0.05$.

RESULTS AND DISCUSSION

The socio-demographic variables of the two hundred and fifty (250) subjects in the study revealed that 14.67% were between the gestation age (4-12 weeks), 56.0% were between the gestation ages of 13-28weeks, 29.33% were between the gestation age of 29-38 weeks. Among the patients, 9.3% had normocytic normochromic anemia, 87.21% had microcytic hypochromic anemia, and 3.49% had macrocytic anemia. The level of severity of anemia showed 64.29% mild anemia and 35.71% moderate anemia. Of the total study population, 80% of pregnant women were infected with *Plasmodium falciparum,* and 20% were not infected with *Plasmodium falciparum* (Figure 1-4).

Table 1 shows the levels of full blood counts among pregnant women infected with *Plasmodium falciparum* and *Plasmodium falciparum*-free pregnant women as the control group. The levels of white blood cells (WBC), neutrophils, and platelets were significantly elevated ($p<0.05$) in pregnant women infected with *Plasmodium falciparum* compared to *Plasmodium falciparum*-free pregnant women as the control group. The levels of lymphocytes, monocytes, red blood cells and hemoglobin were significantly reduced ($p<0.05$) in pregnant women infected with *Plasmodium falciparum* compared to *Plasmodium falciparum*-free pregnant women as the control group, and red cell indices (MCV, MCHC, and MCH) showed no significant difference between the groups($p>0.05$).

Table 2 shows the levels of serum iron and total iron-binding capacity (TIBC) among pregnant women infected with *Plasmodium falciparum* and *Plasmodium falciparum*-free pregnant women as the control group. Serum iron and total iron-binding capacity levels were significantly lower($p<0.05$) in pregnant women infected with *Plasmodium falciparum* compared to *Plasmodium falciparum*-free pregnant women as the control group.

Table 3 shows the levels of glucose-6-phosphate dehydrogenase among pregnant women infected with *Plasmodium falciparum* and *Plasmodium falciparum*-free pregnant women as the control group. The level of glucose-6-phosphate dehydrogenase was significantly lower ($p<0.05$) in pregnant women infected with *Plasmodium falciparum* compared to *Plasmodium falciparum*-free pregnant women as the control group.

Table 4 shows multiple comparisons of hematological parameters, serum iron, and G6PD levels in pregnant women infected with *Plasmodium falciparum*. No significant difference was observed in gestational age ($p>0.05$).

Table 1. Levels of Full Blood Counts in Pregnant Women Infected with *Plasmodium falciparum* and Pregnant Women Not Infected with *Plasmodium falciparum*

Parameters	Pregnant Women infected with *Plasmodium falciparum*	Pregnant Women not infected with *Plasmodium falciparum*	P-Value
WBC (10^9/L)	8.48±017	7.32±0.29	P=0.001
Lymphocytes (%)	30.99±0.77	42.95±1.21	P=0.001
Monocytes (%)	5.88±0.20	6.63±0.21	P=0.013
Neutrophils (%)	63.10±0.76	50.88±1.33	P=0.001
RBC (10^9/L	4.68±0.50	5.13±0.06	P=0.001
Hb(g/dl)	12.69±0.11	14.01±0.14	P=0.001
PCV (%)	38.16±0.29	41.52±0.39	P=0.001
MCV (fl)	82.06±0.51	80.19±0.96	P=0.064
MCH (pg)	27.57±0.21	27.33±0.24	P=0.450
MCHC(g/dl)	33.71±0.36	33.80±0.14	P=0.831
Platelets (10^9/L	306.27±7.39	276.34±8.81	P=0.010

Values are expressed as mean ± SEM. ** Significant (p<0.05), *Non-significant (p>0.05). WBC-white blood cells; RBC: red blood cells; Hb: hemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration.

Table 2. Level of Serum Iron and Total iron binding Capacity in Pregnant Women Infected with *Plasmodium falciparum* and Pregnant Women Not Infected with *Plasmodium falciparum*

Parameters	Pregnant Women Infected with *Plasmodium falciparum*	Pregnant Women Not Infected with *Plasmodium falciparum*	P-Value
Iron (umol/L)	18.90±0.97	22.09±1.06	P=0.032**
TIBC (umol/L)	38.81±2.02	46.60±2.53	P=0.016**

Values are expressed as mean ± SEM, **significant (p<0.05), TIBC – total iron binding capacity

Table 3. Level of Glucose 6 Phospate Dehydrogenase in Pregnant Women Infected with *Plasmodium falciparum* and Pregnant Women Not Infected with *Plasmodium falciparum*

Parameter	Pregnant Women infected with *Plasmodium falciparum*	Pregnant Women Not Infected with *Plasmodium falciparum*	P-Value
G6PD (Ug/Hb)	9.14±0.36	11.45±0.46	P=0.001**

Values are expressed as mean±SEM, ** significant (p<0.05), G6PD- glucose 6 phosphate dehydrogenase

The findings of this study imply that malaria infection during pregnancy significantly disrupts maternal hematological health by inducing inflammatory responses and anemia, altering iron metabolism, and reducing G6PD activity. These changes heighten the risk of oxidative stress and hemolytic complications, potentially compromising both maternal well-being and fetal development. Consequently,

comprehensive clinical monitoring of hematological parameters, iron status, and G6PD activity is essential in malaria-endemic regions to guide targeted interventions, including iron supplementation and antimalarial therapies. Public health strategies integrating malaria prevention, early diagnosis, and management alongside nutritional support are critical to improving pregnancy outcomes. Further longitudinal research is warranted to elucidate the progression of these hematological alterations and to optimize therapeutic protocols tailored to this vulnerable population.

The study included two hundred (200) pregnant women and fifty apparently healthy controls. It reveals that (14.67%) were early, mid (56.0%), and late (29.33%) pregnancy stages, respectively. Anemia was prevalent, with 87.21% of patients showing microcytic hypochromic anemia, 9.3% showing normocytic normochromic anemia, and 3.49% showing macrocytic anemia. Pregnant women showed elevated WBC, neutrophil, and platelet counts compared to the controls, indicating immune activation. Lymphocytes, monocytes, RBC, and hemoglobin levels were reduced, reflecting physiological adaptations. Serum iron and TIBC were lower, indicating depleted iron stores. G6PD activity is reduced, likely due to oxidative stress and metabolic demands, increasing the risk of hemolytic anemia. Iron and G6PD activity remained stable across gestational ages, possibly influenced by nutritional status, genetics, or iron supplementation rather than by gestational timing.

The anemia profile among pregnant women infected with *Plasmodium falciparum* revealed that the majority (87.21%) exhibited microcytic hypochromic anemia, followed by normocytic normochromic anemia (9.3%) and macrocytic hypochromic anemia (3.49%). Severity grading showed that 64.29% had mild anemia, while 35.71% had moderate anemia. These findings reflect the multifactorial nature of anemia in malaria-infected pregnancy, where iron deficiency often coexists with inflammation-driven iron sequestration and impaired erythropoiesis.

Table 4. Multiple Comparison of Hematological Profile in Pregnant Women Infected with *Plasmodium falciparum* and Pregnant Women Not Infected with *Plasmodium falciparum*

Parameters	4-12 weeks	12-28 weeks	29-38 weeks	P-Value
WBC (10^9/L)	8.48±017^a	7.32±0.29^a	8.40±0.33^a	P=0.951
Lymphocytes (%)	30.99±0.77^a	42.95±1.21^a	30.87±1.36^a	P=0.794
Monocytes (%)	5.88±0.20^a	6.63±0.21^b	6.57±0.53^b	P=0.027
Neutrophils (%)	63.10±0.76^a	50.88±1.33^a	61.98±1.51^a	P=0.227
RBC (10^9/L)	4.68±0.50^a	5.13±0.06^a	4.76±0.13^a	P=0.348
Hb(g/dl)	12.69±0.11^a	14.01±0.14^a	12.61±0.22^a	P=0.220
PCV (%)	38.16±0.29^a	41.52±0.39^a	38.01±0.61^a	P=0.482
MCV (fl)	82.06±0.51^a	80.19±0.96^a	81.55±1.13^a	P=0.708
MCH (pg)	27.57±0.21^a	27.33±0.24^a	27.19±0.39^a	P=0.424
MCHC (g/dl)	33.71±0.36^a	33.80±0.14^a	32.87±0.31^a	P=0.324
Platelets (10^9/L)	306.27±7.39^a	293.34±8.81^a	316.47±12.62^a	P=0.122
G6PD (Ug/Hb)	9.78±0.75^a	8.75±0.49^a	9.52±0.74^a	P=0.490
Iron (umol/L)	18.28±3.23^a	17.81±1.28^a	21.36±1.51^a	P=0.279
TIBC (umol/L)	39.05±6.77^a	36.92±2.51^a	42.35±3.47^a	P=0.508

Values are expressed as mean ± SEM,**significant ($p<0.05$), G6PD- glucose 6 phosphate dehydrogenase, TIBC- total iron binding capacity, Mean with same superscript across the groups are not significantly difference from each other ($p > 0.05$), Mean with different superscript across the groups are significantly difference from each other ($p < 0.05$)

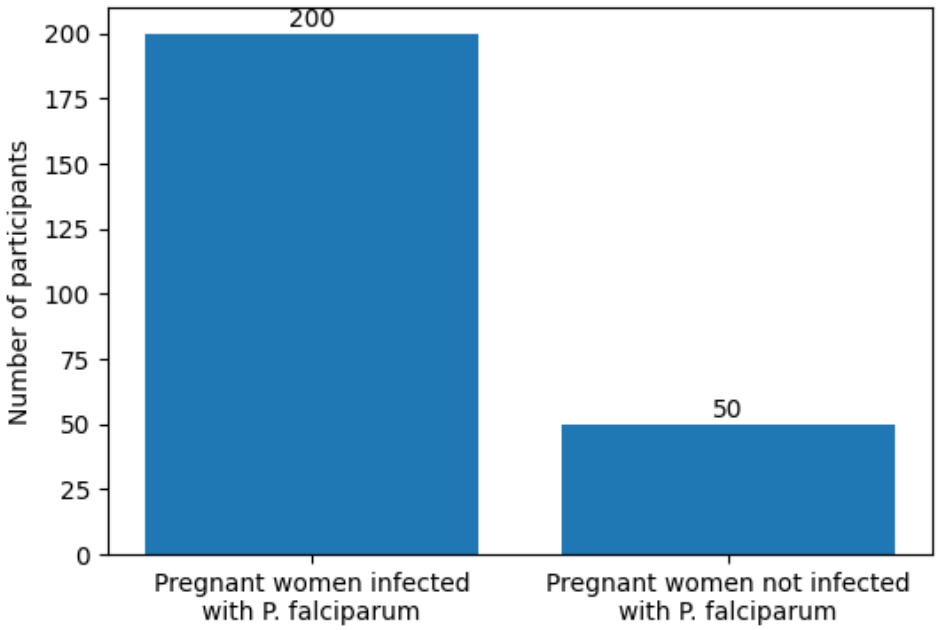

Figure 1. Distribution of study participants by *Plasmodium falciparum* infection status (n=250). The study included 250 pregnant women, comprising 200 (80%) pregnant women infected with *Plasmodium falciparum* and 50 (20%) pregnant women not infected with *Plasmodium falciparum*.

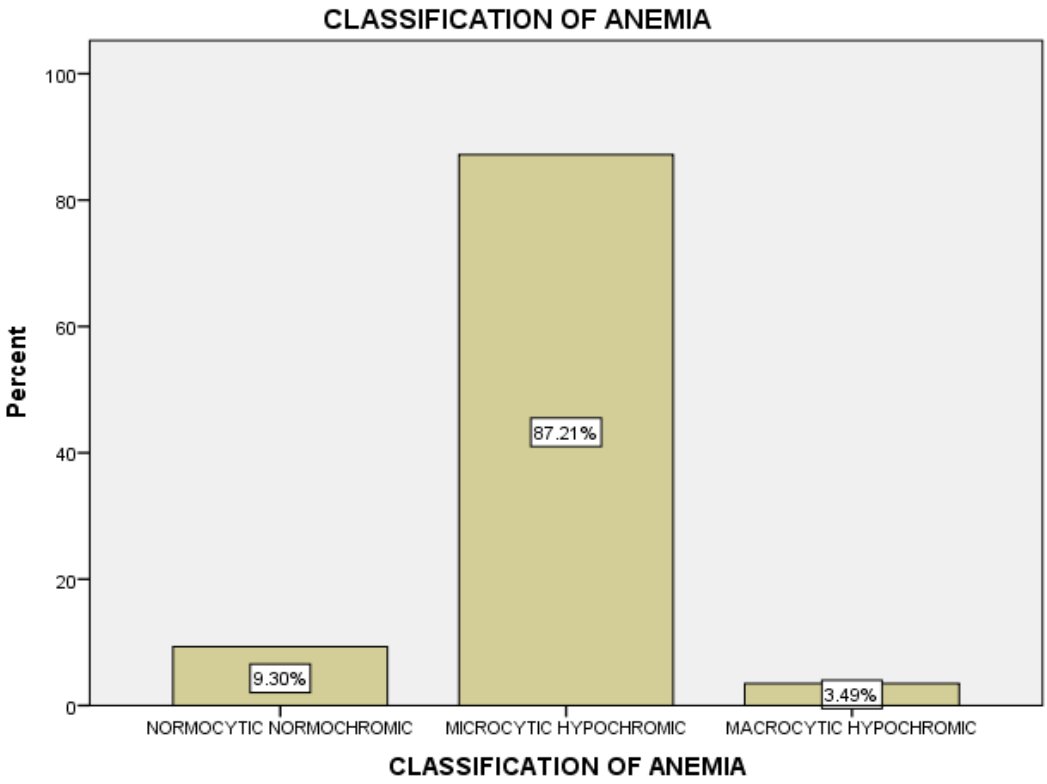

Figure 2. Classification of anemia among Pregnant Women Infected with *Plasmodium falciparum* in Edo Specialist Hospital

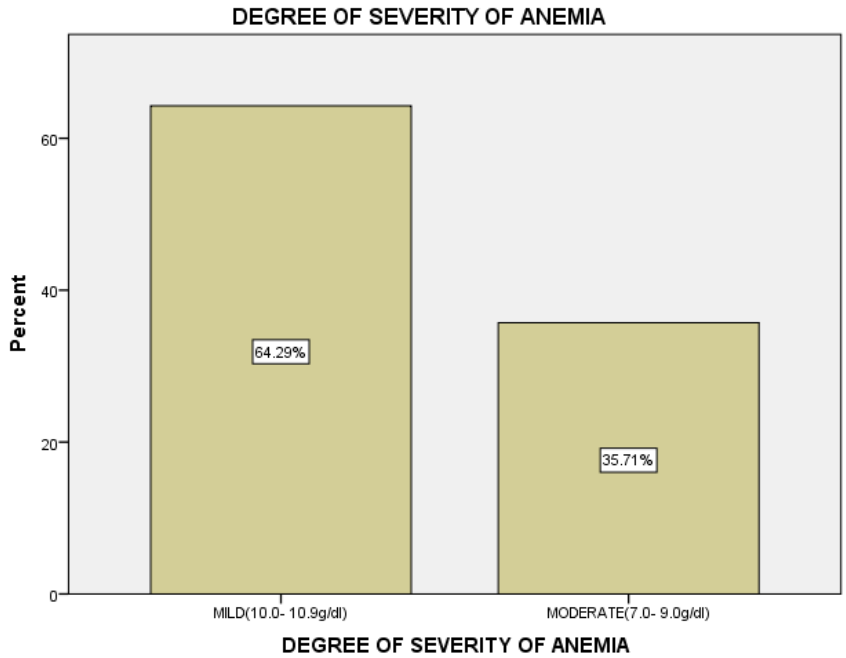

Figure 3. Degree of severity of anemia among Pregnant Women Infected with *Plasmodium falciparum* in Edo Specialist Hospital

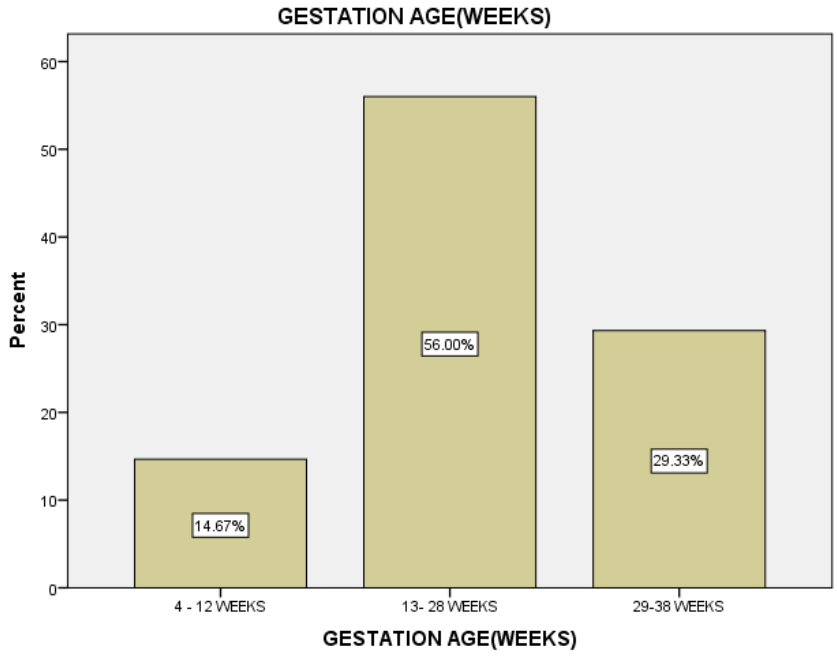

Figure 4. Gestational Age of Pregnant Women Infected with *Plasmodium falciparum* in Edo Specialist Hospital

The predominance of microcytic hypochromic anemia is consistent with iron-restricted erythropoiesis and true iron deficiency, which is frequently exacerbated in *P. falciparum* infection due to increased iron demand in pregnancy, hemolysis of parasitized red blood cells, and hepcidin-mediated iron trapping within macrophages (Spottiswoode et al., 2014; Goheen et al., 2017). The progression of pregnancy further

worsens anemia due to physiological hemodilution, expanded plasma volume, and increased fetal–maternal iron transfer requirements, thereby amplifying susceptibility to malaria-associated anemia (Guyatt and Snow, 2001).

The observed severity pattern, where most cases were classified as mild anemia, aligns with reports indicating that malaria-associated anemia in pregnancy is often predominantly mild to moderate rather than severe, particularly in areas of stable transmission where partial immunity exists (Menendez et al., 2010; Desai et al., 2007). Overall, these findings underscore the synergistic contribution of *P. falciparum* infection and pregnancy-related physiological changes to altered hematological indices and anemia severity distribution. Normocytic normochromic anemia's lower prevalence suggests less contribution from chronic disease or acute blood loss in this cohort, while the small percentage of macrocytic anemia may indicate folate or vitamin B12 deficiencies, though less common (Akbarpour et al*,* 2022).

Table 1 shows elevated white blood cell, neutrophil, and platelet counts in pregnant women versus controls ($p<0.05$), reflecting pregnancy immune adaptations (Bakrim et al*,* 2018). and a hypercoagulable state (Barakauskas et al*,* 2023). Decreased lymphocyte, monocyte, RBC, and hemoglobin levels ($p<0.05$) indicate fetal immune tolerance (Magatti et al*,* 2019), and hemodilution from plasma expansion (Costantine, 2014).

The absence of differences in red cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) ($p>0.05$), indicates that despite reduced RBCs and hemoglobin levels, cell morphology remained stable. This aligns with the findings that pregnancy affects cell quantity rather than quality (Li et al*,* 2017). These changes reflect immune adaptation and oxygen demand during pregnancy (Bohn and Adeji, 2021). Lower serum iron and total iron-binding capacity (TIBC) in pregnant women ($p<0.05$) indicated pregnancy-related changes. Serum iron levels decrease during pregnancy due to increased iron demand for fetal growth and maternal red blood cell mass, consistent with iron deficiency anemia during pregnancy [Van Santen et al, 2012]. Decreased TIBC may indicate altered iron transport during pregnancy, although some studies have reported increased TIBC for iron needs, while others have found decreased TIBC linked to transferrin changes (Georgieff, 2023). These findings confirm that pregnancy impacts maternal iron reserves, affecting iron levels and iron-binding capacity (Georgieff, 2023). Lower levels of glucose 6 phosphate dehydrogenase (G6PD) in pregnant women versus controls ($p<0.05$) align with previous studies showing altered G6PD activity during pregnancy (Kindzelskii et al., 2002). G6PD protects red blood cells from oxidative damage by maintaining reduced glutathione levels (Nicol et al., 2000). Decreased G6PD activity in pregnant women results from increased NADPH and glutathione consumption due to oxidative stress, potentially causing hemolytic anemia, particularly in G6PD-deficient populations. This decline may be related to hormonal changes during pregnancy that affect enzyme stability, indicating increased vulnerability to oxidative damage (Nicol et al., 2000). These findings emphasize the importance of G6PD monitoring in pregnant populations to prevent adverse outcomes (Shah et al*,* 2012).

The findings in Table 4 show no significant difference in serum iron and glucose-6-phosphate dehydrogenase (G6PD) levels across gestational ages ($p>0.05$) among pregnant women, aligning with previous studies (Chen, 2006). Serum iron levels during pregnancy are influenced by the progressive increase in maternal and fetal iron requirements, including expansion of maternal erythrocyte mass, placental development, and fetal growth. Although iron demand increases throughout gestation,

serum iron concentrations do not always decline uniformly across trimesters because they are affected by dietary iron intake, supplementation, and physiological adaptations that enhance intestinal iron absorption (Bothwell, 2000; Fisher and Nemeth, 2017). In pregnancies complicated by *Plasmodium falciparum* infection, iron metabolism is further altered by malaria-induced hemolysis and inflammatory responses. The release of pro-inflammatory cytokines stimulates hepcidin production, which reduces intestinal iron absorption and inhibits iron release from macrophages, thereby decreasing circulating serum iron despite the presence of adequate or increased body iron stores (Spottiswoode et al., 2014; Drakesmith & Prentice, 2012). Consequently, serum iron levels in malaria-infected pregnant women may reflect a state of functional iron deficiency arising from iron sequestration rather than absolute iron depletion. This phenomenon may obscure the expected gestational changes in serum iron concentration and contribute to the development of anemia during pregnancy. Therefore, serum iron should be interpreted alongside other iron status markers such as total iron-binding capacity (TIBC) to provide a comprehensive assessment of iron metabolism in pregnant women with malaria (Goheen et al., 2017; Fisher and Nemeth, 2017). G6PD activity, crucial for redox balance, may not fluctuate significantly across gestational ages, suggesting that pregnancy-induced oxidative stress does not differentially affect enzyme levels or that compensatory mechanisms maintain enzyme activity (Maurya et al.,2016). This stability contrasts with reports of progressive changes but aligns with studies where iron status and enzymatic activity are influenced more by nutritional status, genetic factors, or comorbidities than by gestational timing (McLimore et al., 2013; Tuncalp et al., 2020). The lack of variation may indicate that interventions like iron supplementation help maintain these parameters during pregnancy (McLimore et al., 2013; Tuncalp et al., 2020)

This study had some limitations that should be considered when interpreting the findings. The comparative cross-sectional design was effective for identifying associations between malaria infection and hematological changes in pregnancy; however, it limited the ability to establish a causal relationship between the variables and may have introduced selection bias. In addition, recruitment of participants from selected healthcare facilities may restrict the generalizability of the findings to the wider population of pregnant women in different settings. The cross-sectional nature of data collection also limited the assessment of longitudinal hematological changes throughout the course of pregnancy and malaria infection. Furthermore, variations in laboratory assay performance, sample handling procedures, and timing of specimen collection could have influenced the accuracy and consistency of the measured hematological parameters. Future studies employing prospective cohort designs are therefore recommended to provide better insight into the temporal relationship and progression of hematological alterations among pregnant women with and without malaria infection.

CONCLUSION

This study showed that malaria infection in pregnant women disrupts their hematological profiles, iron metabolism, and G6PD activity by increasing white blood cells, neutrophils, and platelets while reducing lymphocytes, monocytes, red blood cells, and hemoglobin, indicating inflammation and anemia. Serum iron and iron-binding capacity decrease, affecting iron storage. Reduced G6PD activity increases the vulnerability to oxidative stress in regions with G6PD deficiency. These changes may affect maternal health and fetal development. These findings support the practice of monitoring hematological parameters and G6PD activity alongside iron

supplementation and antimalarial therapy, and also enhance policy on integral malaria prevention and screening in endemic regions. Further studies should explore the longitudinal impact of malaria on G6PD activity throughout pregnancy and its implications for fetal development. Additionally, research into the effectiveness of targeted nutritional and pharmacological interventions in mitigating these effects is warranted.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRİBUTİONS

FOO conceptualized this study. The methodology was developed by BIGA, PED, and SE. Data curation was performed by FOO, VI, AA and EOA. The original draft was prepared by FOO, AAE and JEO, and OEI and FOO reviewed and edited the manuscript. FOO supervised the study. Funding acquisition was managed by FOO. All authors have read and approved the final manuscript.

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