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RESEARCH ARTICLE

Plasma Plasminogen Activator Inhibitor-2 Antigen Levels Among Plasmodium Falciparum Malaria-Infected Pregnant Women in Northern Ghana

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Abstract

Background: Pregnancy is a hypercoagulable state due to the changes that occur in the fibrinolytic and coagulation system. These changes include an increase in procoagulants and hypofibrinolysis caused by increase in Plasminogen Activator Inhibitor-2 and *Plasmodium falciparum* (*Pf*) induced coagulation through the secretion of *Plasmodium falciparum* Erythrocyte membrane protein-1(*Pf*Emp-1), exacerbating the situation in pregnant women. This study assessed plasma PAI-2 antigen levels in *Plasmodium falciparum* malaria-infected pregnant women.

Materials and Methods: This hospital-based cross-sectional study recruited 85 pregnant women, 55 (64.7%) positive for *Pf* malaria and 30 (35.3%) negatives for *Pf* malaria (control group). Venous blood was aseptically taken for thin and thick blood film malaria microscopy, complete blood count analyses and measurement of PAI-2 Ag levels using sandwich ELISA. Data were analysed with SPSS version 26.0 and $p<0.05$ was considered statistically significant.

Results: Haemoglobin, RBC, HCT, MCV, MCH, MCHC, Absolute lymphocyte count and PLT values were lower in the *Pf* malaria-infected pregnant women than in the control pregnant women group ($p<0.05$). Gestational age, gravidity, parity and inter-pregnancy intervals had no significant impact on the concentration of PAI-2 in the participants ($p=0.425$, $p=0.953$, $p=0.174$ and $p=0.826$) respectively. The PAI-2 levels were elevated in pregnant women with Pf malaria than in the control group of pregnant women ($p<0.001$). There was a significant correlation between malaria parasite density and PAI-2 Ag levels ($r=0.812$, $p<0.001$). The concentration of PAI-2 Ag was shown to increase with the progression of parasite density.

Conclusion: Plasma PAI-2 antigen levels in P. falciparum malaria-infected pregnant women is higher compared to pregnant women without malaria. PAI-2 Ag levels was shown to increase with the progression of malaria infection and increased parasite density. The pathogenesis of Pf malaria and pregnancy may contribute to the reduction in red blood cell parameters and platelets.

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Keywords: PAI-2 Antigen, *P.f* Malaria, Pregnancy.

1. Introduction

Haemostatic capacity during normal pregnancy seems to progressively increase, causing an upsurge in the plasma concentration of some coagulation factors. This could be a physiological response to ensure effective haemorrhage control after placental separation. The inhibition of the fibrinolytic system may add to this self-protective strategy, and a significant decrease in plasma fibrinolytic activity has been reported in most cases which predisposes it to hypercoagulability state (Tlamcani *et al*., 2018). There are two forms of plasminogen activators, that is the tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (Gonias, 2021) .

t-PA is found in vessel walls and is released into the blood, and it is a key fibrinolytic activator. Plasminogen Activator Inhibitor-1 (PAI-1) has been isolated from vascular endothelial cells in-vitro and is found in normal plasma. A second Plasminogen Activator Inhibitor-2 (PAI-2) is seen in pregnant plasma and is most likely from the placenta. The multifunctional protein plasminogen activator inhibitor type 2 (PAI-2) is elevated in pregnancy and a variation of other inflammatory infections. PAI-2 was initially revealed as a protease inhibitor, but recent research suggests that it may potentially function as a T-helper cell modulator and/or extracellular chaperone (Brinjikji *et al.*, 2021). Despite its recognition many years ago, the exact human significance of plasminogen activator inhibitor type 2 (PAI-2; also referred to as SERPINB2) in its biological context remains unknown. A review of the pertinent works reveals a prominent difference between the number of reports of PAI-2 in comparison with the number of reports of other members belonging to the serpin families. Undoubtedly, this protein was once thought to be functionally redundant in the serpin protein family, even though it's evident that PAI-2 blood levels are significantly higher during pregnancy, implying that it plays critical roles throughout human pregnancy. Furthermore, its overexpression in a variety of other inflammatory states supports PAI-2's involvement as an immunomodulator or general stress responder (Cater *et al*., 2022). It has also been found to be present in fibroblasts and fibroblast-like cells such as foetal lung cells, human synovial, foreskin, bone marrow stroma, etc (Westrick *et al.,* 2020)PAI-2 is also found in vascular cells, especially smooth muscle vascular cells. It is normally low in unstimulated endothelial cells but increases after an inflammatory stimulus.

Taken together, it is evident that the distribution of PAI-2 is more common than we thought initially. The most frequent form in many cell types appears to be cell-associated and of low-molecular weight, while the high-molecular weight, glycosylated form has been detected in plasma during pregnancy and gingival crevicular fluid. The presence of PAI-2 in several tissues along with t-PA proposes that it may also play a role in inhibiting t-PA (Westrick *et al.,* 2020).

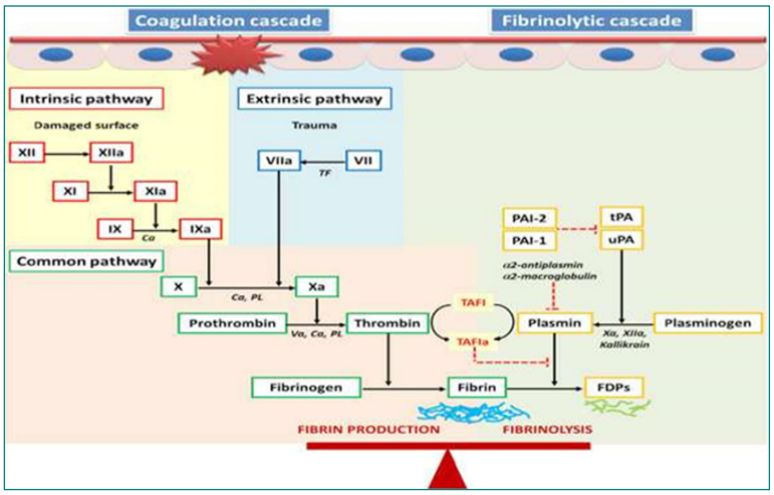

Figure 1. *fibrinolytic activity of PAI-2. ResearchGate*

Malaria is still a preventable public health concern, with considerable yearly illnesses and death. Pregnant women are twice as liable as non-pregnant women to get infected with *Plasmodium falciparum* malaria in the same condition (Harrington *et al.*, 2019).

Every year, it is projected that more than 30 million women in malaria-endemic Africa become pregnant (Cutts et al., 2020). According to a review organized in Kassena-Nankana, the total predominance of malaria parasitaemia during pregnancy is about 48%

(Harrington *et al.*, 2019). Age, multigravidity and pregnancy within the third trimester have all been linked to a lower risk of parasitaemia. The influence of malaria among pregnant women is thus increased by its asymptomatic kind in the early phases till complications arise. In areas with sporadic transmission of malaria, all pregnant women infected with *P. falciparum*, but especially primigravidae can present with clinical symptoms of coma, hypoglycaemia, pulmonary oedema, anaemia and very high mortality. In areas where malarial spread is strong and levels of acquired immunity elevated, protective immunity seems to be lowered in pregnancy, particularly in primigravidae (Bohulu, 2016).

Anaemia has multi-factorial causes in pregnancy. Folate deficiency is a known implication of pregnancy associated malaria making the structural appearances of dyserythropoiesis seem uncommon. In West Africa, haemolytic anaemia is the major clinical findings of severe malaria that occurs during pregnancy and resolves later on delivery. An unusual entity called Coomb's negative haemolytic anaemia of pregnancy may be lethal, and for an optimum maternal-foetal aftermath it desires a tireless diagnostic and appropriate treatment approach(Zulkifal et al., 2021).

There are a variety of thrombotic conditions that can occur during pregnancy. These include an increase in procoagulants, hypofibrinolysis by PAI-2, coagulation by *Plasmodium falciparum (*Semeraro *et al., 2010). Plasmodium falciparum* activates coagulation through the secretion of *Plasmodium falciparum* Erythrocyte membrane protein-1(PfEmp-1). This causes the rosetting of non-parasitized red blood cells around the endothelial walls of the blood vessels (Jensen *et al*., 2020). PAI-2, which is produced by the placenta may be implicated in pregnancy which causes hypofibrinolysis, making the situation worse in pregnant women (Erez *et al.,* 2022).

This study, thus, aimed at comparing the concentrations of plasma Plasminogen activator inhibitor-2 antigen levels, a marker of fibrinolysis amongst pregnant women with *P. falciparum* malaria and normal pregnancy, to determine the impact of malaria on the marker.

2. Materials and Methods

2.1 Study Design/Area

It was a hospital-based cross-sectional study conducted from January to September, 2022 at the ante-natal care unit of Tamale Teaching Hospital (TTH) in the Tamale metropolis. Tamale Teaching Hospital is the only tertiary referral and teaching hospital in the Northern part of Ghana (Gumanga *et al.*, 2011). The hospital is located in the eastern part of the Tamale Metropolitan district, on the main Tamale – Salaga road. The 800-bed capacity hospital serves as the referral hospital for the five (5) northern regions. TTH is affiliated with the University for Development Studies (Adam, 2018).

2.2 Study Population

The sample size for the study was calculated using Cochran's sampling formula ($N = Z^2PQ/d^2$), where Z is the value of the standard normal distribution (1.96 at 95% CI); P is the estimated prevalence rate of thrombosis in pregnant women (3.0%) (Barillari *et al.*, 2016); d is the degree of accuracy desired= 5%. From the above equation, a total of 85 pregnant women, aged ≥ 20 years old and above, were recruited during their regular ante-natal clinic visit days. For the collection of their obstetric data, a google form was created with the necessary questionnaires required for the study. Each participant was required to fill the form with their Laboratory Information Management System (LIMS) number as their identity number. Through their ID, their demographic data was retrieved.

2.3 Sample Collection and Assay

Whole blood sample of five millilitres(5mls) was collected from each participant and 2ml was dispensed into Ethylene Diamine Tetra Acetic acid (EDTA) tubes using standard venepuncture technique for the assessment of complete blood count and detection of malaria parasites. The whole blood was mixed thoroughly with the EDTA anticoagulant and the test was run on an automated 5-part haematology analyser. This test was used to assess haematological indices. Thick blood films were prepared from the EDTA anticoagulated blood. The smears were air-dried and stained with 3% Giemsa stock solution for parasite identification. A positive smear was added in every new batch of working Giemsa stain for quality control. After drying, the presence and identification of malaria parasites were performed by trained and experienced microscopists using Olympus microscopes. The remaining 3mls was put in a citrate tube and then spun at 3000rpm for 15 minutes to obtain the plasma for the assay of Plasminogen activator inhibitor-2 [PAI-2], a marker of fibrinolysis. The concentration of PAI-2 antigen was estimated using sandwich Enzyme-linked immunosorbent assay [ELISA] method. There was a strict adherence to the

reagents and their protocols as provided in the kits by the manufacturer.

2.4 Ethical Consideration and Informed Consent

Ethical approval was sought from the Review Board of the University for Development Studies (UDS). Permission was obtained from the Department of Biomedical Science and the management of the Tamale Teaching Hospital (UDS/RB/022/22). Informed consent was obtained from the participants after thorough explanation of the aims and objectives of the study.

2.5 Statistical Analysis

The data obtained was recorded and organized using Microsoft Excel Software. Data was then analysed using Statistical Package for Social Sciences (SPSS) version 26 software. p <0.05 was considered statistically significant. Categorical data were presented in frequencies with corresponding percentages in parenthesis. The numerical data were parametric and non-parametric and were presented in mean±standard deviation and in median (25^{th}-75^{th} percentiles) respectively. Student T-test and Mann-Whitney U test were used to analyze the demographic and clinical characteristics of the study participants stratified by the malaria status of the pregnant women. Student T-test and Mann-Whitney U test were used to evaluate the blood cell indices of the study participants stratified by the presence or absence of *Plasmodium falciparum* malaria. Plasma Plasminogen Activator Inhibitor-2 Antigen levels among the study participants stratified by the presence or absence of *Plasmodium falciparum* malaria were compared with Mann- Whitney U test. Association between plasma Plasminogen Activator Inhibitor-2 Antigen levels and *Plasmodium falciparum* parasite density among the pregnant women was determined using the Spearman rank correlation test. Relationship between obstetric characteristics and plasma Plasminogen Activator Inhibitor-2 Antigen Levels among *Plasmodium falciparum* infected pregnant women were generated using Mann- Whitney U test and Kruskal-Wallis test. Bivariate data were generated with Mann Whitney U-Test, and multivariate data by Kruskal-Wallis Test. p <0.05 was considered statistically significant.

3. Results

3.1 Demographic and Clinical Characteristics of the Study Participants

Table 1 shows the demographic and clinical characteristics of the study participants stratified by malaria status of the pregnant women. Of the 85 participants recruited into the study, majority (55/64.7%) were positive for *Plasmodium falciparum* malaria, whiles, 30 (35.3%) tested negative. The median age of the study participants was 29 years (25-34), with majority (32/37.6%) within the ages of 25-29 years, and only 14.2% of them were either 35 years or above. *P. falciparum* malaria was more common in participants within 25-29 years and only 10.9% of population were 35 years and had *Pf* malaria. 71.8% of population were Dagomba with only 16.5% of them being Christians. With gravidity, 15.3% were primigravida whiles 59% and 13% were multigravida and grand multigravida respectively. 22.4% of participants were of the nulliparity category with 22% and 45% of them being primiparous and multiparous respectively. The median gestational age was 24years (16.5-29.5). Demographics and clinical characteristics did not significantly differ between *Plasmodium falciparum* infected and uninfected pregnant women.

3.2 Blood Cell Indices of the Study Participants Stratified by the Presence or Absence of *Plasmodium Falciparum* Malaria

Table 2 shows the blood cell indices of the study participants stratified by status of *P. falciparum*. The median blood cell indices of the eighty-five participants in the study were Hb, g/dl [11.6 (10.7-11.9)], RBC x 10^{12}/L [3.7 (3.5-3.9)], HCT% [32.5 (30.0-33.8)], MCV, fL (80.2±6.9), MCH, pg [28.9 (27.6-31.3)], MCHC, g/dl [36.0 (35.0-37.0)], RDW-CV% [11.3 (10.5-12.1)]. TWBC x 10^9/L (8.0±1.9), Absolute Lymphocyte Count x 10^9/L [2.2 (1.8-2.9), Absolute Neutrophil Count x 10^9/L (5.0±1.5), Platelet x 10^9/L [197.0 (136.5-306.0)], MPV, fL (6.0±0.7) and PDW% [6.8 (6.3-7.6)], Haemoglobin [11.4 (9.9-11.7) vs 11.9 (11.7-12.5), *p<0.001*], RBC x 10^{12}/L [3.6 (3.3-3.8) vs 3.9 (3.7-4.1), *p<0.001*], HCT% [32.2 (28.1-33.5) vs 32.9 (31.6-35.0), *p=0.006*], MCV, fL [(78.5±7.4) vs (83.3±4.4), *p=0.002*], MCH, pg [28.6(27.2-30.1) vs 31.1(28.8-32.2),*p=0.001*], MCHC, g/dl [36.0(35.0-37.0) vs 37.0(36.0-38.0),*p=0.023*], and platelet×10^9/L [151.0 (125-194) vs 341 (287.5-372.5), *p<0.001*] were significantly lower in the *P. falciparum* malaria infected participants compared to those without *Pf* infection respectively. However, severe *Pf* infected participants had significantly higher Absolute Lymphocyte Count x 10^9/L [2.0 (1.7-2.7) vs 2.6 (2.0-3.1), *p<0.007*] than their counterparts uninfected with *Pf*. But RDW-CV, TWBC, Absolute Neutrophil Count, MPV, did not differ between the two groups.

Table 1 *Demographic and Clinical Characteristics of the Study Participants*

Variables	Total (n=85)	Pregnant Women		*p-value*
		With Pf. Malaria (n=55, 64.7%)	Without Pf. Malaria (n=30, 35.3%)	
Age (Years)	29.3±4.8	29.1±4.6	29.7±5.3	0.593
Age Category				0.694
20-24 Years	15 (17.6)	9 (16.4)	6 (20.0)	
25-29 Years	32 (37.6)	22 (40.0)	10 (33.3)	
30-34 Years	26 (30.6)	18 (32.7)	8 (26.7)	
≥35 Years	12 (14.2)	6 (10.9)	6 (20.0)	
Religion				0.517
Islam	71 (83.5)	47 (85.5)	24 (80.0)	
Christianity	14 (16.5)	8 (14.5)	6 (20.0)	
Ethnicity				0.441
Dagomba	61 (71.8)	41 (74.5)	20 (66.7)	
Others	24 (28.2)	14 (25.5)	10 (33.3)	
Gravidity	3 (2-4)	3 (2-4)	2.5 (2-4)	0.142
Gravidity Category				0.223
Primigravida	13 (15.3)	7 (12.7)	6 (20.0)	
Multigravida	59 (69.4)	38 (69.1)	21 (70.0)	
Grand Multigravida	13 (15.3)	10 (18.2)	3 (10.0)	
Parity	2 (1-3)	2 (1-3)	1 (0-2)	0.080
Parity Category				0.057
Nulliparity	19 (22.4)	10 (18.2)	9 (30.0)	
Primaparity	22 (25.9)	12 (21.8)	10 (33.3)	
Multiparity	45 (51.7)	33 (60.0)	11 (36.7)	
Gestational Age	24.0 (16.5-29.5)	24.0 (18.0-28.0)	21.5 (15.8-32.0)	0.890
Gestational Age Category				0.970
1st Trimester	11 (12.9)	7 (12.7)	4 (13.3)	
2nd Trimester	35 (41.2)	23 (41.8)	12 (40.0)	
3rd Trimester	39 (45.9)	25 (45.5)	14 (46.7)	
BMI	27.2±5.7	26.5±5.2	28.4±6.5	0.156
IPI (69)				0.936
<2 years	16 (23.2)	11 (22.9)	5 (23.8)	
>2 years	53 (76.8)	37 (77.1)	16 (76.2)	
IFA Supplement				0.154
Yes	68 (80.0)	41 (74.5)	27 (90.0)	
No	17 (20)	14 (25.5)	3 (10)	

n=number of participants, BMI=body mass index, IPI= inter-pregnancy interval, IFA=iron/folic acid. Parametric (presented in mean±standard deviation) by Student T-Test; Non-parametric (presented in median (25^{th}-75^{th} percentiles)) by Mann Whitney U-Test. Categorical data presented in frequencies with corresponding percentages in parenthesis. $p<0.05$ was considered significant.

Table 2. *Blood Cell Indices of the Study Participants Stratified by the Presence or Absence of Plasmodium falciparum Malaria*

Variables	Total (n=85)	Pregnant Women		*p-value*
		With Pf. Malaria (n=55, 64.7%)	Without Pf. Malaria (n=30, 35.3%)	
Hb (g/dl)	11.6 (10.7-11.9)	11.4 (9.9-11.7)	11.9 (11.7-12.5)	**<0.001**
RBC x10^{12}/L	3.7 (3.5-3.9)	3.6 (3.3-3.8)	3.9 (3.7-4.1)	**<0.001**
HCT%	32.5 (30.0-33.8)	32.2 (28.1-33.5)	32.9 (31.6-35.0)	**0.006**
MCV (fL)	80.2±6.9	78.5±7.4	83.3±4.4	**0.002**
MCH (pg)	28.9 (27.6-31.3)	28.6 (27.2-30.1)	31.1 (28.8-32.2)	**0.001**
MCHC (g/dl)	36.0 (35.0-37.0)	36.0 (35.0-37.0)	37.0 (36.0-38.0)	**0.023**
RDW-CV%	11.3 (10.5-12.1)	11.4 (10.5-12.2)	11.2 (10.3-11.7)	0.193
TWBC x10^9/L	8.0±1.9	7.9±2.2	8.0±1.4	0.843
Neut. # x10^9/L	5.0±1.5	5.1±1.7	4.9±1.3	0.502
Lymph. # x10^9/L	2.2 (1.8-2.9)	2.0 (1.7-2.7)	2.6 (2.0-3.1)	**0.007**
Mon. # x10^9/L	0.4 (0.3-0.6)	0.5 (0.3-0.6)	0.4 (0.3-0.6)	0.432
Eos. # x10^9/L	0.09 (0.06-0.14)	0.08 (0.06-0.13)	0.10 (0.07-0.15)	0.334
Baso. # x10^9/L	0.01 (0.01-0.02)	0.01 (0.01-0.03)	0.01 (0.01-0.03	0.522
Platelet x10^9/L	197.0 (136.5-306.0)	151.0 (125.0-194.0)	341.0 (287.50-372.5)	**<0.001**
MPV	6.0±0.7	5.9±0.6	6.2±0.7	**0.073**
PDW%	6.8 (6.3-7.6)	6.8 (6.1-7.6)	7.4 (6.5-7.9)	**0.034**

Hb=Haemoglobin, RBC=Red Blood Cell, HCT=Haematocrit, MCV=Mean Cell Volume MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, RDW-CV=Red Cell Distribution Width-Coefficient of Variation, TWBC= Total White Blood Cell, Lymph#=Absolute Lymphocyte count, Neut#= Absolute Neutrophil count MPV=Mean Platelet Volume, PDW= Platelet Distribution Width, PCT=Plateletcrit, g/dl=Grams per decilitre, fL=Femtolitre, pg=Picogram. Parametric data presented as mean±standard deviation were compared by Student T-Test and Non-parametric data presented as median (25^{th}-75) were compared by Mann Whitney U-Test. p<0.05 was considered significant.

3.3 Plasma Plasminogen Activator Inhibitor-2 Antigen Levels among the Study Participants Stratified by the Presence or Absence of *Plasmodium falciparum* Malaria

Figure 2 shows the plasma plasminogen activator inhibitor-2 (PAI-2) antigen (Ag) levels stratified by the presence or absence of *Pf* malaria. The median PAI-2 Ag level among the *Pf* malaria infected participants in the study was 81.5 pg/ml (73.9-90.7), *p<0.001*. The median PAI-2 level was relatively higher in the participants with *Pf* malaria infection than those without the infection [81.5 (73.9-90.7) vs 41.5 (32.8-47.3), *p<0.001*].

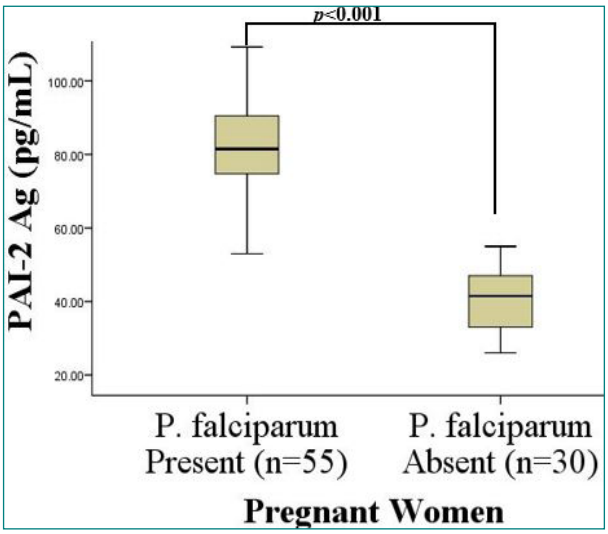

Figure 2. *Plasma Plasminogen Activator Inhibitor-2 Antigen Levels among the Study Participants Stratified by the Presence or Absence of Plasmodium falciparum Malaria. P= Plasmodium, PAI-2 Ag= Plasminogen Activator Inhibitor-2 Antigen, n=number of participants, pg/mL=Picogram per Milliliter. Data were compared using Mann Whitney U-Test Test. p<0.05 was considered significant.*

3.4 Association Between Plasma Plasminogen Activator Inhibitor-2 Antigen Levels and *Plasmodium Falciparum* Parasite Density Among the Pregnant Women

Figure 3 shows a spearmann rank correlation was used to describe the correlation between PAI-2 levels and malaria parasite density. A correlation coefficient of *r= 0.812* was obtained and a *p-value =<0.001* was obtained depicting a significant correlation between PAI-2 and malaria parasite density.

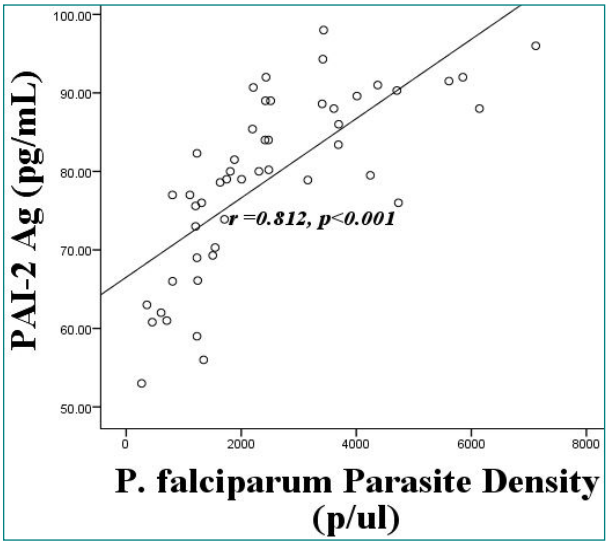

Figure 3. *Association between plasma Plasminogen Activator Inhibitor-2 Antigen Levels and Plasmodium falciparum Parasite Density among the Pregnant Women. PAI-2 Ag= Plasminogen Activator Inhibitor-2 Antigen, pg/mL=Picogram per Milliliter, p/ul= Parasites per microlitre, r=correlation coefficient. Association was determined using Spearman Correlation Test. p<0.05 was considered significant.*

Table 3. *Relationship between Obstetric Characteristics and Plasma Plasminogen Activator Inhibitor-2 Antigen Levels among Plasmodium falciparum Infected Pregnant Women*

Variables	PAI-2 Ag Levels (pg/mL)	95%CI	S.E.	*p-value*
Age Category				0.920
20-24 years	56.0 (48.0-88.5)	49.2-86.2	8.0	
25-29 years	71.6 (56.2-88.5)	61.0-81.0	4.3	
30-34 years	76.5 (56.0-86.0)	61.6-78.6	4.1	
>35 years	62.5 (37.5-88.1)	45.1-78.6	7.6	
Gestational Age				0.425
1st Trimester	66.0 (51.0-88.5)	51.8-88.5	4.0	
2nd Trimester	76.0 (47.0-88.5)	60.3-78.5	4.4	
3rd Trimester	76.0 (50.0-88.4)	59.2-75.7	4.0	
Gravidity				0.953
Primagravida	66.0 (44.8-93.2)	27.8-108.2	12.6	
Multigravida	75.8 (48.5-88.0)	62.5-74.9	3.1	
Grand Multigravida	70.3 (46.3-88.8)	53.0-84.3	7.2	
Parity				0.174
Nulliparity	78.8 (59.4-85.7)	50.7-98.6	7.5	
Primaparity	58.4 (45.0-79.1)	52.1-70.7	4.5	
Multiparity	77.0 (48.0-89.6)	64.5-79.1	3.6	
IFA Supplement				0.022
Yes (n=35)	71.7 (43.5-84.4)	59.6-72.0	3.1	
No (n=13)	84.0 (59.0-96.0)	67.3-90.7	5.4	
IPI				0.826
<2 years	68.5 (52.5-87.5)	59.4-82.5	5.4	
>2 years	75.6 (45.0-88.3)	61.5-74.4	3.2	

n=number of participants, PAI-2 Ag= Plasminogen Activator Inhibitor-2 Antigen, pg/mL=Picogram per Milliliter, CI=Confidence Interval, S.E.= Standard Error. Bivariate data were generated with Mann Whitney U-Test, Multivariate by Kruskal-Wallis Test. p<0.05 was considered significant.

3.5 Relationship between Obstetric Characteristics and Plasma Plasminogen Activator Inhibitor-2 Antigen Levels among *Plasmodium falciparum* Infected Pregnant Women

A Mann-Whitney U test and Kruskal- Wallis was used to generate the results of the obstetric characteristics association with PAI-2. No significant value was gained for any of the obstetric characteristics' association with PAI-2 Ag levels.

4. Discussion

85 voluntary pregnant women were recruited for the study. The median age of the study participants was 29 years (25-34), with majority (32/37.6%) within the ages of 25-29 years, and only 14.2% of them were either 35 years or above. *P. falciparum* malaria was more common in participants within 25-29 years and only 10.9% of population were 35 years and had *Pf* malaria. In a similar study in Central Sudan (Mostafa *et al*., 2015), 163 pregnant women between the ages of 18-60 were recruited whereas 260 participants were used for a similar study in River State, Nigeria (Ken-Ezihuo *et al.*). Also, since the study's focus was on *P. falciparum* malaria-infected pregnant women, the prevalence of malaria among the pregnant women was 64.7%. This is in contrast with separate studies in Lagos, South-West Nigeria and Bobo-Dioulasso, Burkina Faso which recorded a prevalence rate of 7.7% in a research with 1084 participants, and 18.1% in a population of 579 participants respectively(Agomo *et al.*, 2009; Cisse *et al.*, 2014).

The study showed that the haemoglobin, RBC, HCT, MCV, MCH and MCHC were significantly decreased in pregnant women infected with *Plasmodium falciparum* malaria [11.4(9.9-11.7)] g/dl, [3.6(3.3-3.8) $x10^{12}$/L], [32.2 (28.1-33.5)] %, (78.5±7.4) fL, [28.6 (27.2-30.1)] pg and [36.0 (35.0-37.0)] g/dl compared to pregnant women without malaria [(11.9 (11.7-12.5)] g/dl, [3.9 (3.7-4.1))] $x10^{12}$/L, [32.9 (31.6-35.0)] %, (83.3±4.4) fL, [31.1 (28.8-32.2)] pg and [37.0 (36.0-38.0)] g/dl respectively. The *p-values* were *<0.001, <0.001, 0.006, 0.002, 0.001* and *0.023* respectively.

The pathophysiology of anaemia in malaria is complicated and multifaceted. Even though not fully known, it is sometimes assumed to occur from a combination of haemolysis of parasitized erythrocytes, enhanced eradication of both parasitized and non-parasitized erythrocytes, as well as ineffective erythropoiesis. Other causative factors may involve reduced red blood cell deformability and splenic sequestration resulting in an increased rate of clearance from the circulation. This phenomenon vividly explains the findings of decreased red cell indices from this study (White, 2018).

The leukocytic changes observed in the pregnant women was the lymphocyte count associated with malaria infection. Lymphocytopaenia was found in pregnant women positive for *Plasmodium falciparum* [2.0 (1.7-2.7)] $x10^9$/L against pregnant women negative for *Plasmodium falciparum* [2.6 (2.0-3.1)] $x10^9$/L with a *p*-value of *0.007*. The decrease in lymphocyte counts in pregnant women with malaria detected in this study may be due to the redeployment of lymphocytes and associated splenic sequestration (Kotepui *et al*., 2014).

This finding also revealed that the platelet count was decreased in the pregnant women positive for *Plasmodium falciparum* [151.0 (125.0-194.0)] $x10^9$/L as compared to the control group of pregnant women negative for *Plasmodium falciparum* [341.0 (287.50-372.5)] $x10^9$/L. The *p*-value was statistically significant (*p-value*=<0.001). The proposed action of the reduced platelet count may result from peripheral destruction (Martina *et al*., 2024) intense splenic sequestration of platelets as well as platelet utilization by the process of disseminated intravascular coagulation (DIC). Another factor may also be due to the reduced life span of the platelets (Haile *et al.*, 2022).

According to the study, the concentration of participants who are positive for *P. falciparum* malaria was significantly higher than the concentration of those without *P. falciparum* malaria. Thus, PAI-2 Ag levels were elevated in pregnant women with malaria *(p<0.001)*.This confirms a study by (Ken-Ezihuo et al.) in Rivers State, Nigeria, in which PAI-2 levels were elevated in all the pregnant women though more elevated in malaria-infected pregnant women (568.00±12.51 ng/mL) than the other experimental groups (456.31±5.94 for pregnant women negative for malaria, 427.86±6.95 for non-pregnant women, all in ng/mL). The observed elevation in all the pregnant women aligns with previous reports that plasminogen activators are elevated for the good of pregnant women and in gestational tissues as they are required for normal placentation especially the PAI-2(Csutak *et al*., 2017). This result also agrees with what has been reported that increase in the concentration of PAI-1 and PAI-2 infected women could also be as result of large numbers of proinflammatory cytokines being released due to malaria infection in pregnancy (Tadu *et al.*, 2023) .

The results from the study showed a statistically significant value, *p=<0.001* and a correlation coefficient, *r= 0.812* between malaria parasite density and PAI-2 Ag levels. This reveals a significant correlation between malaria parasite density and PAI-2 Ag levels. The concentration of PAI-2 Ag was shown to increase with progression of parasite density.

Though no previous studies have been done on the impact of malaria parasite density on PAI-2 Ag levels, similar studies done in Central Sudan which looked at malaria parasite density revealed that suppressed fibrinolysis is evident at the placental level in association with malaria infection (Mostafa *et al.*, 2015).

The study aimed at assessing the impact of the obstetric data; gestational age, parity, gravidity, inter-pregnancy intervals and iron/folic acid supplementation on the levels of PAI-2 in pregnant women. Gestational age was categorised into first trimester, second trimester and third trimester. The PAI-2 Ag concentrations were [66.0(51.0-88.5)] pg/ml, [75.8 (48.5-88.0)] pg/ml and [70.3 (46.3-88.8)] pg/ml respectively with a *p-value* of 0.425. Gravidity, that's the number of pregnancies was categorised into primigravida, multigravida and grand multigravida. The PAI-2 Ag concentrations were [66.0(44.8-93.2)]pg/ml, [75.8(48.5-88.0)]pg/ml and [70.3(46.3-88.8)]pg/ml respectively with a *p-value* of 0.953. Parity, which is the number of births, was also categorised into nulliparity, primiparity and multiparity. The PAI-2 Ag concentrations were [78.8(59.4-85.7)] pg/ml, [58.4(45.0-79.1)] pg/ml and [77.0(48.0-89.6)] pg/ml respectively with a *p-value* of 0.174.

These results denote that gestational age, gravidity and parity had no impact on the concentration of PAI-2 in the participants. In all, the median of the parameters was used.

In consonance with a study by (Bouwland-Both *et al.*, 2013) in the Netherlands, which revealed that PAI-2 results which was significant with a *p-value= <0.001* was independent of parity. A similar study by (Yanyi *et al.*, 2022) also suggested that PAI-2 concentrations increased throughout pregnancy (preconception versus 32 weeks of gestation; 38.73 versus 102.23 ng/ml, and 0.024 versus 151.06 ng/ml, respectively). So, gravidity and parity's effect on the levels of PAI-2 in any term of pregnancy is not significant.

5. Conclusion

PAI-2 levels in *P. falciparum* malaria-infected pregnant women were higher compared to pregnant women without malaria. There was a significant correlation between malaria parasite density and PAI-2 Ag levels. The concentration of PAI-2 Ag was shown to increase with the progression of parasite density. *Plasmodium falciparum* malaria infection poses a very high risk on the levels of PAI-2 Ag levels of a pregnant woman causing more thrombotic effects.

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Data Availability

All relevant data are available in this article and its supplementary file.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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