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Research Article

Assessing the Effectiveness of Antimalarial Drugs in Preventing Vertical Transmission of Malaria Parasites and Alleviating Fetal Oxidative Stress

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Abstract

Malaria remains a significant global health challenge, particularly in regions with high transmission rates. Vertical transmission, the transfer of malaria parasites from mother to foetus, poses serious risks to newborns, leading to various adverse health outcomes. This study aimed to investigate the prevalence of vertical transmission, evaluate the efficacy of antimalarial drugs, and assess the impact of vertical transmission on foetal development. A cross-sectional study was conducted among 260 pregnant women at Bamenda Regional Hospital and Nkwen District Hospital. Participants' medical records were reviewed to determine the type of antimalarial drug administered. Malaria diagnosis was performed using microscopic examination of thin and thick blood films. After delivery, cord blood was collected for malaria diagnosis and also centrifuged to obtain plasma for the assessment of oxidative stress biomarkers, including catalase, superoxide dismutase, lipid peroxidase, total oxidative stress, and total antioxidant defense. The overall prevalence of cord malaria was found to be 8%. Additionally, positive cord cases were associated with pre-term delivery (100%) and low birth weight (28.8%). Among the antimalarial drugs, Fansidar demonstrated the highest efficacy (99.2%) for chemoprophylaxis, while the combination of Artesunate and Amodiaquine showed 100% efficacy for chemotherapy. Analysis of oxidative stress biomarkers revealed higher activities of catalase and superoxide dismutase in malaria positive cord blood compared to negative cord blood. Positive cord samples exhibited elevated levels of total oxidative stress and reduced levels of total antioxidant defense compared to negative cord samples. These findings highlight that preventive treatments during pregnancy are not fully effective, leading to vertical transmission and increased oxidative stress in the foetus, resulting in low birth weight. Further research is needed to enhance the effectiveness of preventive interventions and mitigate the adverse consequences of vertical transmission.

Keywords: Malaria, Vertical transmission, Antimalarial drugs, Oxidative stress, Foetal development

Introduction

Vertical transmission of malaria, the transfer of malaria parasites from mother to newborn, poses a significant health risk and can lead to life-threatening consequences [1,2] *Buchwald, et al.,* (2022) Certain populations, such as children under five years of age and pregnant women, are particularly susceptible to Plasmodium infection and its associated morbidity and mortality, especially in

malaria-endemic regions *Mwaniki, et al.,* (2010). Among pregnant women, those in their first and second pregnancies (primigravidae and secundigravidae) face a higher vulnerability to placenta sequestration due to the non-recognition of Variant Surface Antigens (VSA) by the immune system. In areas of stable malaria transmission, adult women acquire immunity, resulting in asymptomatic malaria infections during pregnancy that can lead to maternal anaemia and placental and umbilical cord blood malaria infection [3,4] *Anchang Kimbi, et al.,* (2009).

For many years, Chloroquine (CQ), a 4-aminoquinoline, was considered the gold standard for malaria treatment due to its efficacy, low toxicity, and affordability [5]. However, the increasing rates of CQ and Sulphadoxine/Pyrimethamine (SP) resistance in malaria-endemic areas have led to a rise in malaria-related morbidity and mortality WHO (2020). To combat malaria, there is an urgent need for new antimalarial drugs, but standardized systems for evaluating their efficacy are currently lacking. Malaria infection, parasite feeding, immune responses, and antimalarial drugs contribute to the generation of Reactive Oxygen Species (ROS) such as hydrogen peroxide, singlet oxygen, superoxide, and reactive nitrogen species like nitric oxide, which can be detrimental to both the host and the parasite [6]. While oxidative stress can aid in parasite clearance, it can also cause cellular damage and potentially contribute to severe pathologies. Host organisms employ antioxidant defense systems, utilizing enzymes like Superoxide Dismutase (SOD) and Catalase (CAT), to combat the free radicals produced during infection [7]. In malaria patients, increased oxidative stress is often accompanied by decreased antioxidant levels [8,9]. Plasmodium possesses various antioxidant enzymes, such as lipoic acid, to evade oxidative stress and survive within the host.

Congenital malaria has a detrimental impact on newborn health, including increased susceptibility to malaria during the first month of life *Borgella, et al.,* (2013). Vertical transmission of malaria parasites from mother to child leads to severe consequences such as anaemia, congenital malaria, and heightened susceptibility to malaria infection in the early neonatal period *Schwarz, et al.,* (2008) [4]. Neonatal T cell imbalance, as well as pro-inflammatory and anti-inflammatory immune responses triggered by Plasmodium falciparum sensitization in the uterus, contribute to this susceptibility [10]. Furthermore, the emergence of antimalarial drug resistance poses a significant public health challenge, impeding malaria prevention and control. Despite the promise of future drug developments, malaria-endemic regions are facing an alarming situation where affordable treatment options are rapidly losing therapeutic efficacy. Although various studies have investigated vertical transmission of malaria and its impact on newborn health in endemic regions [11,12], only few have been investigated in the Northwest Region of Cameroon *Oumar, et al.,* (2020), there remains a critical need to investigate the efficacy of antimalarial drugs in preventing vertical transmission and address the issue of congenital malaria in this region. Thus, this study aimed to evaluate the effectiveness of antimalarial drugs in preventing vertical transmission and examine the influence of umbilical cord malaria on foetal oxidative stress development.

Materials and Methods

Study Site

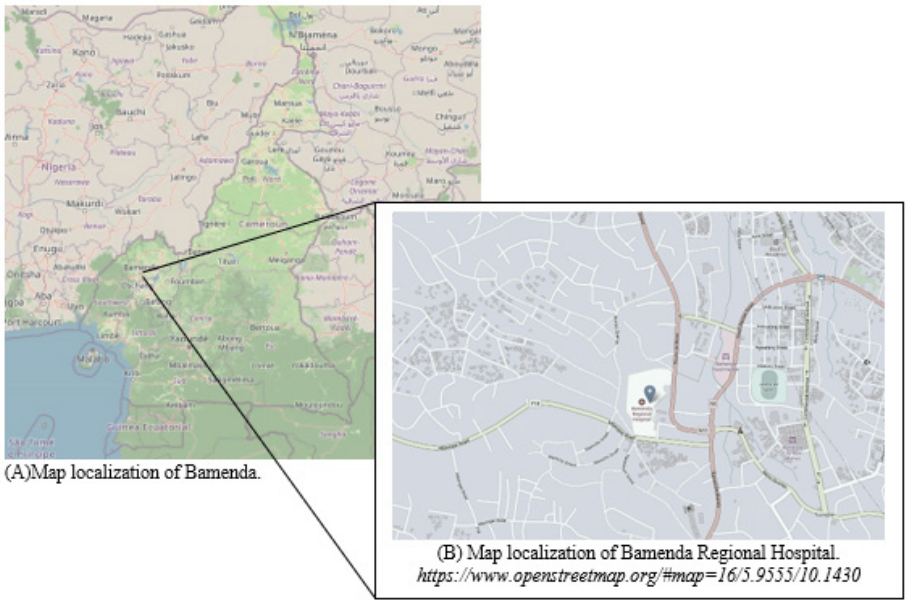

Figure 1: (A) Map localization of Bamenda, the regional capital of the Northwest Region of Cameroon. (B)Localization of the Bamenda Regional Hospital.

The study was conducted between May 1st and July 31st at two hospitals in the Northwest Region of Cameroon, namely the Bamenda Regional Hospital and the Nkwen District Hospital Figure 1. Bamenda, the regional capital, is located within the coordinates of latitude 5.940N to 5.980N and longitude 10.150E to 10.180E *Acho Chi* (1998). The city is situated along the Cameroon Volcanic line, characterized by the presence of two prominent features: the High Lava plateau and a lower plateau, with altitudes of 1400m and 1100m above sea level, respectively. These hospitals serve as major healthcare facilities for patients not only from the immediate region but also from surrounding areas. The hospitals are well-equipped with adequate infrastructure, medical equipment, trained medical personnel, accessible road networks, and sufficient security measures. The Northwest Region experiences a tropical climate with two distinct seasons: a prolonged rainy season spanning from March to October (eight months) and a short dry season with sunny weather from November to February (four months). The month of August typically records the highest rainfall, while September to October marks a period of reduced precipitation. Malaria transmission in this region follows a seasonal pattern, with high transmission rates during the rainy season. *Plasmodium falciparum* is the predominant malaria parasite species, responsible for over 90% of malaria infections in the region [13] (Figure 1).

Study Population

The study targeted a specific population of pregnant women with uncomplicated falciparum malaria who were seeking care at the Bamenda Regional Hospital and willingly participated in the study. In order to be included, each pregnant woman needed to meet certain criteria, such as being in the third trimester of pregnancy and providing informed consent by signing the consent form. It should be noted that a minority of women, primarily residing in remote villages, lacked knowledge about malaria control measures and had not sought antenatal care during the earlier stages of their pregnancy. On the other hand, the majority of women were well-informed about malaria control measures, and most of them had received intermittent preventive treatment with Fansidar. A total of 260 women met the inclusion criteria and provided their consent to participate in the study.

Study Design

The study design diagram consists of the following components (Figure 2).

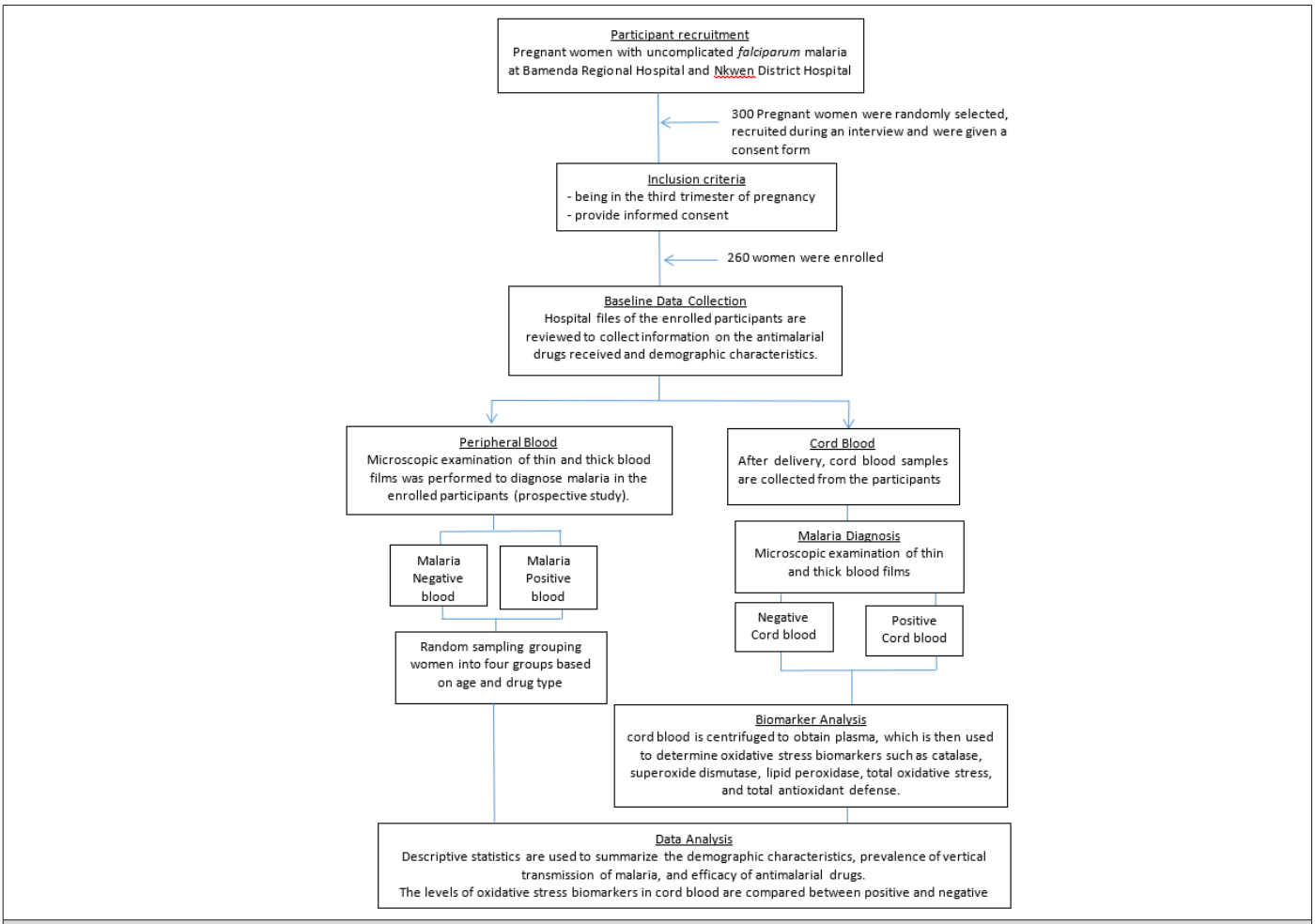

Figure 2: Experimental study design.

Inclusion and Exclusion Criteria

Inclusion Criteria: The study included all pregnant women within the age range of 15-40 years who were currently residing in the Northwest Region of Cameroon. Specifically, it focused on pregnant women in their third trimester of pregnancy who were attending antenatal care at the Bamenda Regional Hospital and Nkwen District Hospital. Eligible participants were required to demonstrate their willingness to comply with the study protocol throughout the duration of the study and provided voluntary informed consent by signing the informed consent form.

Exclusion Criteria: The study excluded pregnant women who were not currently residing in the Northwest Region and did not fall within the age range of 15-40 years. Pregnant women who were not in their third trimester of pregnancy and were attending the Bamenda Regional Hospital and Nkwen District Hospital were not included. Also, participants who were unable to comply with the study protocol throughout the duration of the study were also excluded. Furthermore, pregnant women who refused to sign the informed consent were not included in the study.

Sample Size Determination

The sample size for the study was determined based on the prevalence of malaria parasite in cord blood at the Bamenda Regional Hospital as reported by *Oumar, et al.,* in (2020). The formula N=p(1-p) z^2/d^2 [14] was used to calculate the sample size, where N represents the sample size, p denotes the prevalence of malaria parasite in the cord blood (standard deviation), z represents the statistic for the desired level of confidence (Z-score or Z-value) (1.96 for a 95% confidence level), and d represents the accepted margin of error (5%). Considering the prevalence of malaria parasite in the cord blood at the Bamenda Regional Hospital as 21.3%, the minimum sample size was determined to be 255. To account for potential loss to follow-up or withdrawals, an additional 2% of the minimum sample size was considered. Consequently, a total of 260 placenta and cord blood samples were collected from pregnant women at the Bamenda Regional Hospital and the Nkwen District Hospital (PMI) from April 1st to May 2nd, 2022.

Blood Sample Collection, Processing and Storage

Prior to delivery, approximately 1ml of peripheral blood was collected from pregnant women using sterile syringes. The collected blood was then dispensed into Ethylene Diamine Tetraacetate (EDTA) wet tubes, which were labeled anonymously. Subsequently, the tubes were transported to the laboratory, where a clean, grease-free microscopic slide was used to prepare thin and thick blood smears from the collected samples. These smears were subjected to microscopic examination to detect parasite species and determine parasite density. If immediate analysis was not required, the samples were stored in a refrigerator at -18°C.

After complete delivery of the placenta and umbilical cord, approximately 2-3ml of umbilical cord and placenta blood were collected separately using sterile syringes. The collected blood samples were dispensed into two EDTA tubes and labeled anonymously with a study number. The tubes were then transported from the collection site (labor room) to the laboratory. In the laboratory, thin and thick blood films were prepared from the umbilical cord and placenta blood samples using a clean, grease-free slide. These films were examined to determine the parasite species and parasite density, or alternatively, stored in a refrigerator at -18°C if immediate analysis was not required. Finally, the umbilical cord blood sample in the EDTA tube was centrifuged at 3000rpm for 3minutes to obtain plasma. Using a dry test tube (without anticoagulant) and a reliable micropipette, 200µl of the plasma was collected and dispensed into the dry test tubes for subsequent biochemical analysis or stored in the refrigerator for future use.

Procedure For Peripheral Blood Collection (Venous Blood Collection): The necessary materials for blood collection, including cotton, 70% alcohol, syringes, tourniquets, test tubes, test tube holders, and gloves, were gathered. A test tube was labeled with the unique patient identification number. A tourniquet was applied to the upper arm of the pregnant woman, and a suitable prominent vein was selected. The chosen vein was cleaned with 70% alcohol and allowed to air dry. A syringe was then extracted from its case and inserted at the base of the vein to draw 1ml of blood. Subsequently, the tourniquet was released, and the needle on the syringe was carefully removed. Dry cotton gauze was applied to the puncture site. The used needle was discarded in a safety box. The collected blood was transferred into a labeled test tube, and the blood was mixed with an anticoagulant by gently inverting the tube six times, following the protocol established by the University of Florida in 2022.

Procedure For Placenta and Umbilical Cord Blood Collection: The necessary materials for placenta and cord blood collection, including test tubes, syringes, and gloves, were assembled. Placenta blood was collected upon complete delivery of the placenta. Given the abundant presence of veins on the placental surface, a syringe was utilized to puncture one of the veins, and 2ml of blood was withdrawn. The collected blood was then transferred into a properly labeled test tube containing Ethylene Diamine Tetraacetate (EDTA) as the anticoagulant. Umbilical cord blood was collected immediately after its detachment from the fetus. A syringe was inserted into the detachment area to withdraw blood from the cord, which was subsequently transferred into a well-labeled EDTA test tube.

Procedure to Take Baby's Weight and Gestational Age: Immediately following delivery, the weight of the baby was measured using an electronic digital scale with an accuracy of ±10 grams, following the methodology described by *Oumar, et al.,* in 2020. The recorded weight of the baby was then categorized into three groups: low birth weight (<2.5kg), normal birth weight (2.5-4.5kg), and high birth weight (>4.5kg), based on the classification provided by *Oumar, et al*., in 2020. Additionally, the gestational age of the wom-

en was obtained from the delivery record booklet in the labor room immediately after delivery, and the corresponding gestational age was recorded.

Quantification Of Plasmodium Parasitemia in Placenta and Cord Blood

Thick Blood Smear: Thick blood smears were prepared by aspirating 5µl of placenta blood and cord blood samples onto separate labeled slides. After air drying, the smears were stained with 10% Giemsa solution. Slides were examined under a 100X magnification oil immersion objective lens, and parasite density was determined by counting parasites and White Blood Cells (WBCs). Parasitemia was calculated using the formula: Number of parasites ×8000 WBCs/µl divided by the number of WBCs counted. Two microscopists independently read the slides, with discordant results re-examined by a third microscopist. Parasite density was averaged from the closest counts obtained.

Thin Blood Smear: This study presents a standardized protocol for preparing and examining thin blood smears to detect and calculate parasite density in placenta and cord blood samples. The protocol involves collecting 5µl of each sample using a 100µl micropipette and placing them on clean slides labeled with identification numbers. A spreader slide is then used to evenly spread the blood, creating a feathering edge. The smears are air-dried, fixed with methanol to preserve red blood cell morphology and enable parasite visualization, and air-dried again. Staining is performed with 10% Giemsa for 8-15minutes, followed by washing and air-drying. Examination is conducted using an Olympus CX-21 microscope at 20X or 40X magnification or oil immersion at 100X objectives, ensuring the lenses and eyepiece are properly cleaned. Parasite density is determined by counting parasitized and unparasitized red cells and white cells in one field, with density calculated using a specific formula. Asexual forms of the parasite are counted, while sexual forms are noted but not included. This standardized protocol provides an accurate method for detecting parasites and calculating density in malaria diagnosis and research.

Preparation of Plasma Sample for Biochemical Assay

Preparation of Sample: After the umbilical cord blood of pregnant women were collected in an EDTA test tube, the blood was centrifuge at 3000rpm for 3minutes. A good micropipette was used to aspirate 300µl of the plasma (without the buffy coat) into a clean dry test tube which was later use for biochemical analysis on the same day or stored at -180°C for a maximum of 30days.

Biochemical Assay

Catalase Test: The reaction between catalase and hydrogen peroxide yields water and oxygen, which can be quantified by stopping the reaction with a dichromate and acetic acid solution. The resulting chromic acetate is then measured calorimetrically at 610nm. Notably, the presence of dichromate does not interfere with the accurate determination of chromic acetate, as it exhibits no absorbance in the specific wavelength range. By controlling the reaction time and measuring the remaining hydrogen peroxide, this assay enables the reliable assessment of catalase activity. This colorimetric approach offers a convenient and effective means of evaluating catalase activity in various biological samples [15].

Total Oxidative Stress (TOS) Assay: The Total Oxidative Stress (TOS) assay is performed to assess the oxidative status of a sample. The principle of the test involves the iron catalytic degradation of hydrogen peroxide, Generating Alkoxyl (RO) and Peroxyl (ROO) radicals. These radicals react with a chromogen (N-N-dimethyl-phenylenediamine sulphate), resulting in the formation of a colored compound whose absorbance is measured at 505nm. The intensity of the color is directly proportional to the number of radical compounds produced, following the Beer's-Lambert Law. This assay's procedure involves aspirating 1ml of acetate buffer (pH 5.2) into test tubes corresponding to the number of samples. Then, 100µl of a 20-fold diluted plasma sample in phosphate buffer and 25µl of chromogen are added to each test tube. The absorbance is measured at 505nm against the blank using a spectrophotometer (*Dogan, et al.,* 2021).

Total Antioxidant Defense (TAD) Assay: The principle of the assay involves the development of a stable and colored cation through the reaction between iron trichloride (FeCl3) and a chromogen (N-N-dimethyl-phenylenediamine sulphate) in an acidic medium (pH 5.2). The antioxidant compounds present in the sample reduce the chromogen's radical cation, resulting in a decolorization of the solution, which is proportional to a specific concentration. The absorbance values obtained from the samples are compared to a standard curve for quantification purposes *Wu, et al.,* (2013). The procedure entails aspirating 1ml of acetate buffer (pH 5.2) into test tubes corresponding to the number of samples. Then, 25µl of chromogen and 10µl of FeCl3 are added to each test tube. Following this, 10µl of a 20-fold diluted plasma sample in phosphate buffer is added to the mixtures. The absorbance is measured at 505nm against the blank using a spectrophotometer.

Superoxide Dismutase: The Superoxide Dismutase (SOD) assay is based on the inhibition of the oxidation of adrenaline to adrenochrome by SOD. The formation of adrenochrome is directly proportional to the activity of SOD and can be measured at a wavelength of 480nm [16]. to perform the assay, cuvettes corresponding to the number of samples were filled with 1600µl of carbonate buffer (30mM, pH 10.2). Then, 200µl of the sample (plasma) and 200µl of adrenaline were added to initiate the reaction. The optical density was measured at 480nm. The activity of SOD was calculated in units of SOD per mg of protein, with one unit defined as the quantity of SOD required to inhibit 50% of the oxidation of adrenaline to adrenochrome within 1 minute. The specific activity of SOD (SOD/mg of protein) was determined by dividing the number of SOD units by the mg of protein in the sample. Additionally, the percentage inhibition (%I) was calculated using the formula ODsample/ODblank x 100.

Thiobarbituric Acid Reactive Substance (TBARS): The Thiobarbituric Acid Reactive Substance (TBARS) test is used to analyze secondary oxidative products, particularly malondialdehyde, which is generated from polyunsaturated fatty acids. Malondialdehyde reacts with thiobarbituric acid, producing a red-colored compound that can be measured photometrically at a wavelength of 532nm [17].

For the procedure, individual test tubes corresponding to the number of samples were filled with 0.5ml of phosphate buffer (50mM, pH 7.4) using a micropipette. Then, 0.5ml of the sample (plasma) was added to each tube, followed by 0.5ml of 20% Trichloroacetic Acid (TCA) for protein precipitation, and 1ml of 0.67% thiobarbituric acid. A separate tube with phosphate buffer and thiobarbituric acid served as the blank. The tubes were incubated in a water bath at 90°C for 10minutes and then rapidly cooled in ice water. The contents of each tube were transferred to centrifugal tubes and centrifuged at 3000rpm for 15minutes at 4°C. The supernatant was collected, and its optical density was measured at 530nm against the blank using a spectrophotometer. The TBARS level was expressed in nanomoles/mg of protein.

Statistical Analysis

All data obtained from pregnant women were entered into Excel spreadsheet, validated and analyzed using SPSS Statistics Version 20 (SPSS Inc., Chicago, IL) to determine the prevalence of malaria in pregnancy. The significance of differences in proportions were explored using the Pearson's Chi square test, whereas the differences in group means were assessed using the t test, Analysis of Variance (ANOVA). Statistical results were considered significant when the two-sided P value was ≤ 0.05.

Ethical Consideration

The study obtained ethical clearance from the Faculty of Health Sciences at the University of Bamenda under reference number 2022/0695H/UBa/IRB prior to commencing the research. Additionally, administrative clearance was acquired from the Regional Delegation of Public Health with reference number 127/ATT/NWR/RDPH/BRIGAD. Prior to the initiation of the study, authorization was obtained from both the Bamenda Regional Hospital and P.M.I Nkwen Hospital. Informed consent was obtained from the women after thoroughly explaining the consent document to the best of their comprehension.

Results

Demographic and Clinical Information of the Study Population

A total of 260 pregnant women in their third trimester of pregnancy participated fully in the study. Among these women, 21 (8.1%) were below the age of 20 years, 231 (88.8%) were between the ages of 20 to 45 years, and 8 (3.1%) were above the age of 45 years, with an average mean age of 32 years. Out of the 260 women, 130 (50%) took Sulphadoxine Pyrimethamine, 58 (22.3%) took Artesunate Amodiaquine, 39 (15%) took Artemether Lumefantrine, 8 (3.1%) took Chloroquine, and 25 (9.6%) did not take any of the aforementioned drugs. The majority of these drugs were administered during the second trimester (58.9%), followed by the third trimester (29.2%), and lastly the first trimester with a percentage of 10.2%, as indicated in (Table 1).

Table 1: Demographic and Clinical Information of the study population.

Variables	Category	Frequency	Percentage
	<20	21	8.1
	20-45	231	88.8
	>45	8	3.1
	Total	**260**	**100**
Age Group (years)	**Mean± SD**	**32.5±0.33**	
	Artesunate. Q	58	22.3
	Artemether L	39	15
	Sulphadoxine	130	50
	Chloroquine	8	3.1
	No drug used	25	9.6
Chemotherapy	Total	260	100
	First trimester	24	10.2
	Second trimester	139	58.9
	Third trimester	69	29.2
Trimester of Drug Administration	2 and 3 trimesters	4	1.7

Prevalence of Malaria Parasite in the Cord Blood

The result on Table 2 shows that out of 260 cord samples examined from both positive (104) and negative (156) mothers, the overall umbilical cord prevalence was 8% (Table 2).

Table 2: Prevalence of cord malaria status in relation to mother peripheral status.

Cord Malaria Status				
Mother Peripheral Status	**Negative N (%)**	**Positive N (%)**	**Total**	***p*-value**
Negative	149	7	156	0.207
-156	-95.5	-4.5		
Positive	96	8	104	0.207
-104	-92	-8		
Total	245	15	260	
p-value	0.209	0.209		

Prevalence of Cord Malaria Status in Relation to Mother Peripheral Status and Drug Type

The results presented in Table 3 demonstrate that among the 156 women who tested negative after peripheral blood examination and received antimalarial drugs, both artemether lumefantrine and artesunate amodiaquine exhibited the highest efficacy, achieving

a 100% effectiveness rate each. Sulfadoxine Pyrimethamine also demonstrated a high level of effectiveness, with a percentage effectiveness of 99.2%. However, among the 104 women who tested positive after peripheral blood examination and underwent treatment with antimalarial drugs, Artesunate amodiaquine and Artemether lumefantrine were found to be highly effective, both demonstrating a 100% effectiveness rate. Chloroquine showed a moderate effectiveness, with a percentage of 50% (Table 3).

Table 3: Prevalence of cord malaria status in relation to mother status and drug type.

Mother Status	Drug Types	Cord Malaria Status		*P* Value
		Negative	Positive	
Negative (156)	Artemether lumefantrine	1(100%)	-	0.257
	Artesunate amodiaquine	3(100%)	-	
	Chloroquine	-	2(100%)	
	Sulphadoxine pyrimethamine	128(99.2%)	1(0.8%)	
	No drug	17(81%)	4(19%)	
	Total	**149(95.5%)**	**7(4.5%)**	
Positive (104)	Artemether lumefantrine	38(100%)	_	0.002
	Artesunate amodiaquine	55(100%)	_	
	Chloroquine	3(50%)	3(50%)	
	Sulphadoxine pyrimethamine	_	1(100%)	
	No drug	_	4(100%)	
	Total	**96(92.3%)**	**8(7.7%)**	

Gestational Age and Birth Weight Following the Umbilical Cord Blood Malaria Status

Table 4: Gestational age and birth weight following the umbilical cord blood malaria status.

Characteristics		Malaria Diagnosis in Umbilical Cord Blood	
		Positive N (%)	Negative N (%)
Gestational	Preterm	10 (100)	-
Age	Mid-term	-	5 (100)
	Post term	5 (2)	240 (98)
Weight	Low Birth weight	15 (28.8)	37 (71.2)
	Normal Birth weight	-	201(100)
	High Birth weight	-	7 (100)

The findings from Table 4 reveal important information regarding the delivery status and birth weight of pregnant women with positive and negative umbilical cord blood. Among the positive women, all 10 individuals gave birth prematurely, accounting for 100% of the cases, while the remaining 5 positive women had post-term deliveries, representing 2% of the total. In contrast, the majority of negative women (240) had post-term deliveries, accounting for 98%, while the remaining 5 negative women had mid-term deliveries, constituting 100%. Furthermore, the data in Table 4 indicate that out of the 15 umbilical cord samples confirmed positive through microscopy, all corresponding babies had low birth weight, amounting to 28.8%. Conversely, out of the 245 umbilical cord blood samples confirmed negative through microscopy, 37 babies had low birth weight (71.2%), 201 babies had normal birth weight (100%), and 7 babies had high birth weight (100%) (Table 4).

Catalase, Superoxide Dismutase and Total Antioxidant Activities on Umbilical Cord Blood

Table 5: Catalase, Superoxide dismutase and Total antioxidant activities on umbilical cord blood.

CAT Normal Value (19.5-21.5 U/ml)	Umbilical Cord Malaria Status		
Drug taken by mother	**Positive**	**Negative**	***p* value**
Yes	23.24 ± 0.96(7)	19.30±1.28(10)	P < 0.05
No	22.60±0.45(8)	17.73±4.53(10)	P < 0.001
***p* value**	P > 0.05	P > 0.05	
SOD (105-110U/ml)			
Yes	112.73±31.89(7)	41.63±21.14(10)	P < 0.001
No	106.30±23.46(8)	30.26±23.10(10)	P < 0.001
***p* value**	P > 0.05	P > 0.05	
TAD (12.5-15.5U/ml)			
Yes	10.22±3.04(7)	21.67±4.22(10)	P < 0.001
No	12.00±1.07(8)	16.48±3.00(10)	P < 0.05
***p* value**	P > 0.05	P < 0.01	

Note*: Values represent mean ± SD values. Values in bracket are number of patients per group and normal values of oxidative stress biomarkers.

The results presented in Table 5 demonstrate the differences in catalase activity and superoxide dismutase (SOD) activity in umbilical cord blood among women who took antimalarial drugs and those who did not and were diagnosed either positive or negative for umbilical cord malaria. Women who took the drug and tested positive for umbilical cord malaria showed higher catalase activity (23.24±0.96 and 22.60±0.45) and SOD activity (112.73±31.89 and 106.30±23.46) compared to women who did not take the drug. Conversely, women who took the drug and tested negative for umbilical cord malaria exhibited lower catalase activity (19.30±1.28 and 17.73±4.53) and SOD activity (41.63±21.14 and 30.26±23.10) in umbilical cord blood. The differences in catalase activity (p=0.001) and SOD activity (p=0.0001) were found to be significant between women who took the drug and were positive for umbilical cord malaria, and those who did not take the drug and were negative for umbilical cord malaria, and vice versa. Additionally, the Total

Antioxidant Defense (TAD) level in umbilical cord blood was lower (10.22±3.04 and 12.00±1.07) in women who took the drug and were positive for cord malaria, while it was higher (21.67±4.22 and 16.48±3.00) in women who took the drug and were negative. A significant difference (p=0.0001) was observed between women who took the drug and were positive for umbilical cord malaria, and those who did not take the drug and were negative. However, there was no significant difference (p=0.0164) between women who did not take the drug and were positive, and women who took the drug and were negative for umbilical cord malaria (Table 5).

Total Oxidative Stress and Thiobarbituric Acid Reactive Substance on Umbilical Cord Blood

The results presented in Table 6 indicate notable differences in the levels of TOS and TBARS in umbilical cord blood among women with positive and negative cord malaria tests, with and without antimalarial drug intake. For women who tested positive, both those who took the drug and those who did not had lower TOS levels (6.57±1.45 and 8.51±1.09, respectively) compared to women who tested negative, whose TOS levels were higher (21.40±13.84 and 21.01±9.26). There was no significant difference (p=0.0014) between women who took the drug and tested positive and women who did not take the drug and tested negative for cord malaria. However, a significant difference (p=0.0001) was observed between women who did not take the drug and tested positive for cord malaria and those who took the drug and tested negative. On the other hand, the TBARS levels were higher in umbilical cord blood for women who tested positive, regardless of drug intake (3.84±0.57 and 4.18±1.02), compared to women who tested negative (2.15±0.45 and 2.00±0.58). There was a significant difference (p=0.0001) between women who received the drug and tested positive for cord malaria and those who did not receive the drug and tested negative. However, no significant difference (p=0.1294) was observed between women who did not receive the drug and tested positive and those who received the drug and tested negative for cord malaria (Table 6).

Table 6: TOS and TBARS levels on umbilical cord blood following the umbilical cord malaria status.

TOS (10.5-16.5 U/ml)	Umbilical Cord Malaria Status		
Drug taken by mother	**Positive**	**Negative**	***p* value**
Yes	6.57±1.45(7)	21.40±13.84(10)	P < 0.05
No	8.51±1.09(8)	21.01±9.26(10)	P < 0.05
***p* value**	P > 0.05	P > 0.05	
TBARS (2.45-3U/ml)			
Yes	3.84±0.57(7)	2.15±0.45(10)	P < 0.001
No	4.18±1.02(8)	2.00±0.58(10)	P < 0.001
***p* value**	P > 0.05	P > 0.05	

Note*: Values represent mean ± SD values. Values in bracket are number of patients per group and normal values of oxidative stress biomarkers.

Discussion

The results of this current research provide valuable insights into the prevalence of umbilical cord malaria, its impact on birth outcomes, and the effectiveness of antimalarial drugs. The prevalence of umbilical cord malaria in this study was found to be 12.5%, which is lower than the prevalence reported by *Oumar, et al.,* (2020) in a similar study conducted at the Bamenda Regional Hospital. The lower prevalence observed in this study may be attributed to increased awareness and education among pregnant women regarding the dangers of malaria and the implementation of preventive measures. Additionally, the administration of antimalarial drugs such as Artesunate amodiaquine, Artemether lumefantrine, and Sulfadoxine/Pyrimethamine (Fansidar) to pregnant women may have contributed to the reduction in prevalence. These findings align with previous research conducted by *Anchang Kimbi, et al.,* (2020) which demonstrated the efficacy of Fansidar in preventing vertical transmission of malaria.

The study also revealed a higher incidence of pre-term delivery and low birth weight in women with umbilical cord malaria, indicating the impact of vertical transmission on delivery dates and infant weight. Similar findings have been reported by *Nkwabong, et al.,* (2020) and *Oumar, et al.,* (2020), [18] highlighting the association between malaria infection during pregnancy and adverse birth outcomes. The presence of umbilical cord malaria contributes to a deficiency of red blood cells, leading to restricted nutrient and oxygen supply to the developing foetus, resulting in low birth weight. Moreover, the analysis of oxidative stress markers demonstrated higher catalase and superoxide dismutase activities in positive umbilical cord blood samples compared to negative samples. This increase in antioxidant activity can be attributed to the body's response to the excessive production of Reactive Oxygen Species (ROS) induced by the inflammatory nature of malaria parasites. Conversely, total antioxidant defense levels were found to be lower in positive cord blood, indicating a decrease in the body's ability to counteract oxidative stress. These findings are consistent with *Oumar, et al.,* (2020) and suggest that malaria infection in the cord blood leads to reduced antioxidant defense mechanisms.

In terms of lipid peroxidation, Thiobarbituric Acid Reactive Substance (TBARS) levels were higher in positive cord blood, indicating increased oxidative damage. This finding aligns with studies conducted by *Nsiah, et al.,* and *Ayodele, et al.,* (2020), [19] which demonstrated elevated levels of malondialdehyde, a marker of lipid peroxidation, in malaria-infected individuals. The higher TBARS levels in positive cord blood can be attributed to the inflammatory response induced by malaria parasites, leading to increased production of reactive oxygen species. On the other hand, Total Oxidative Stress (TOS) levels were lower in positive cord blood, indicating a decrease in overall oxidative stress. This result is consistent with the previous studies conducted by *Ebrahim, et al.,* (2019) and *Oumar, et al.,* (2020), [20] which reported lower TOS levels in malaria-infected individuals compared to non-infected individuals. The lower TOS levels in positive cord blood may be attributed

to the effect of antimalarial drugs, which reduce parasitemia and consequently decrease the production of reactive oxygen species. In conclusion, this study provides valuable insights into the prevalence of umbilical cord malaria, its impact on birth outcomes, and the efficacy of antimalarial drugs. The findings highlight the importance of implementing preventive measures and administering appropriate antimalarial treatment to pregnant women to reduce the prevalence and adverse effects of umbilical cord malaria. Further research is warranted to explore the underlying mechanisms of vertical transmission and oxidative stress in cord malaria and to evaluate the long-term effects on maternal and child health [21-30].

Conclusion

The study showed that the prevalence of umbilical cord malaria was low among pregnant women attending the Bamenda Regional Hospital. The presence of malaria parasite at the umbilical cord results in preterm delivery and low birth weight. Moreover, the drugs given to pregnant women for prophylaxis are not 100% effective and the presence of malaria parasite at the umbilical cord induces oxidative stress in the foetus.

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Conflict of Interest

No conflict of interest.

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