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Congenital Parasitaemia Among Neonates, the Malaria Risk Factors and Haematological Parameters Among Pregnant Women Attending Antenatal Clinic at Federal Medical Centre Makurdi, Benue State, Nigeria

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Abstract

Malaria is a major public health problem, particularly among the vulnerable population of children aged less than 5years and pregnant women. Clinical impact of malaria disease is associated with high rate of morbidity and mortality. Consequential effect of malaria disease is the congenital transmission to neonates that result in diverse clinical syndrome, ranging from neonatal sepsis to jaundice. For better understanding of malaria epidemiology in pregnant women, the study evaluate the prevalence of malaria parasitaemia, associated risk factors and congenital transmission, using polymerase chain reaction technique to determine the Plasmodium speciation and genetic diversity. Study population was pregnant women at different gestational stage attending antenatal clinic of Federal Medical Centre, Makurdi Benue State. Systemic random sampling was employed in recruiting the study subjects, and a

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well-standardized questionnaire was administered before sample (blood) collection. The samples were analyzed using Rapid Diagnostic Test (RDT), Microscopic Smear Examination and Polymerase Chain Reaction (PCR). Overall malaria parasite detection was 3.6 % by PCR, 2.0% by RDT and 4.3% by Microscopy. Comparing the demographic variables with malaria parasitaemia, high level was recorded among pregnant women within age-group <20years(16.7%, 1/5) Location had high positivity among pregnant women in rural areas (10.5%,2/17,occupation(students, 6.0%,3/50), educational background(secondary, 6.2%, 8/121), marital status(non-married, 9.1%,2/20), parity(Secundigravidae)(4.8% 6/120), Malaria preventive measures, use of mosquito coil(8.0%,2/23), intermittent preventive therapy(Yes, 5.9%,8/127) and haematinic intake(Yes, 4.3%,4/89), Drainage provided(4.3% 4/90), source of water(well, 6.7%,6/83) High malaria parasitaemia reported in second trimester (7.3%, 7/96). Evaluating the effect of malaria parasitaemia on haematological indices, high malaria parasitaemia was recorded among pregnant women with haemoglobin (<11g/dl)(4.5%, 7/150). Significant difference was observed in malaria parasitaemia and PCV(0.37-0.47, 18.2%, 2/9).3.8%(11/282) in white blood cell count within 5-11X10^9, pregnant women with normal neutrophil count(40-60)(5.7%, 4/66), 5.9%(2/32) in pregnant women with increased lymphocyte, 4.1%(7/102)in monocytopenia cases, 9.1%(2/20) in eosinophilia(>4), 12.5%(1/7) in basophilia and 5.0%(2/38) in thrombocytopenia(<150000). The diagnostic technique, RDT vs PCR shows a significant difference (Kappa=0.898). Using the MSP-1 and MSP-2 primer of amplified Plasmodium falciparum species, msp-1 amplified two clones,K1 and MAD20, MSP-2 amplified two clones, FC27 and 3D7.All families amplified at different frequencies and varied base pairs, indicative of genetic diversity. In conclusion, the prevalence of malaria parasitaemia among pregnant women was low, the genetic diversity of the various clones identified is consistent with studies conducted in Nigeria and sub-Saharan Africa, indicative of antimalarial therapy selective pressure. There was no congenital parasitaemia among the neonates.

Keywords: Parasitaemia, Haematology, Pregnant, Antenatal, Neonate

INTRODUCTION

Malaria is a public health challenge in about 90 countries mainly in tropical and sub-tropical regions (WHO, 2021). It is a life-threatening parasitic disease that is transmitted from one person to another through the bite of an infected female adult Anopheles mosquito. The causative agent is *Plasmodium* species. Five (5) known species of *Plasmodium* infect humans*:*

Plasmodium vivax, Plasmodium ovale, Plasmodium Knowlesi, Plasmodium Malariae and Plasmodium falciparum (WHO, 2021).

In Nigeria, malaria is a major public health problem, where it accounts for more cases of deaths than any other country in the world (WHO, 2020). It is transmitted all over the country; 76 % of the population lives in endemic areas where there is high transmission while 24 % of the population lives in low transmission areas. The transmission season can last all year round in the southern part of Nigeria and is about 3 months or less in the northern part of the country (WHO, 2021). According to the 2020 World Malaria Report, Nigeria had the highest number of 27% of global malaria cases in 2019 and accounted for 23% mortality in which is the highest number of deaths (WHO, 2019). In Benue State North central of Nigeria, malaria is endemic with varied prevalence; 76.9% according to a report by Houmsou *et al.*, (2012), 65.9% Amuta *et al.*, (2014) and 80.4% Adikwu *et al.*,(2017).

Congenital malaria is the presence of malaria parasites in the peripheral smear of the newborn from 24 hours to 7 days of life, it can still be defined as per Uneke (2011) definition as malaria parasitaemia in the first week of life. It is a major problem in tropical and subtropical countries and can be transmitted vertically from placenta of a pregnant woman to her fetus or perinataly during labour (Emad *et al.*, 2008). It occurs in <5% of affected pregnancies (Fischer, 2003). Symptoms of this condition are nonspecific; but may include fever, anemia and splenomegaly amongst others; some reports indicate that other signs and symptoms that may manifest are Jaundice, regurgitation, loose stools, and poor feeding. Occasionally, others such as drowsiness, restlessness, and cyanosis according to Hashemzadeh and Heydarian (2005) are usually some of the symptoms occurring 10 to 30 days after birth. This research aimed at assessing congenital parasitaemia among neonates, the malaria risk factors and haematological parameters among pregnant women attending antenatal clinic at federal medical centre Makurdi, Benue state, Nigeria.

METHODS

Study Area

The cross-sectional study was undertaken at the antenatal clinic in Federal Medical Centre Makurdi, in Benue State, Nigeria. Geographically, Makurdi is located on latitude 7°43N and longitude 8°32E within the Guinea Savannah Region of Nigeria. The mean annual

temperature ranges from a minimum of 21.7°C to a maximum of 35.2°C and an annual average rainfall of 1000 mm. There are two distinct seasonal patterns, the wet and the dry. The former lasts from April to October, while the latter lasts from November to March (Benue State, 2019). Most of the city dwellers are civil servants, military/police, traders, farmers and fishermen (Benue State, 2019). Benue State shares boundaries with five other States namely: Nasarawa State to the north, Taraba State to the east, Cross- River State to the South, Enugu State to the south-west and Kogi State to the west. Benue occupies a landmass of 34,059 square kilometres "Historical Background" (2017). Benue State is one of the North Central States in Nigeria with a population of about 4,253,641 in 2006 census (World Gazettes, 2013) in which agriculture forms the backbone of the State's economy engaging more than 70 percent of the working population. The Tiv, Idoma, Igede and Itilo people inhabit Benue, most of the Tiv people are are farmers, the popularly grown crops include: yams, guineacorn, cassava, sesame, rice, groundnut etc. The textural composition of the soil ranged from loamy sand to sandy-to-sandy loam to clay loam. While the inhabitants of the river areas engage in fishing as their primary or important secondary occupation. (Benue State, 2019)

Sample collection

Five (5) mL of venous blood were collected aseptically from each pregnant woman into EDTA bottle.They were gently mixed and analysed using Malaria HRP-II(P.f) and pLDH(Pan) Antigen Rapid Test kit (Standard Diagnostics, INC. Kyonggi-do, South Korea) Rapid Diagnostic Tests (Cheesbrough,2009). Haematological tests such as packed cell volume (PCV) were performed on the maternal sample. A thick and thin smear was made, stained with Giemsa solution, allowed to dry before examining microscopically. Parasitaemia was calculated by counting parasites against 200 white blood cells. The films were used for malaria parasite identification and density count (Cheesbrough, 2009). A 10 µl of each of the blood samples were blotted on 0.5 × 2 cm strips of 3mm Whatman filter paper (No 1) and dried in a laminar flow hood. After drying, the dried blood spots were preserved in zip-lock bag with one to three desiccants and packed in big brown envelopes for shipment to molecular laboratory at Federal University of Agriculture Makurdi for molecular analysis (Cheesbrough, 2009). Placental smears were taken from the placenta on each of the maternal surface halfway between the cord and the periphery, and were examined for the presence of parasites as adopted by Muehlenbachs *et al.,* (2012). Cord blood was collected by cannulation of the thoroughly cleaned umbilical vessels with the

cord clamped. Experienced midwives and pediatric doctors did this at the time of delivery. Each Umbilical cord blood samples were separately examined both in thick and thin blood smears.

Rapid Diagnostic Test (RDT)

The detection of the malaria parasite antigen for each sample was carried out with the aid of SD BIOLINE ONE STEP Malaria HRP-II (P.f) and pLDH(Pan) Antigen Rapid Test kit (Standard Diagnostics, INC. Kyonggi-do, South Korea). The test was carried out according to manufacturer's instructions. The test device was then removed and placed on a flat dry surface. The whole blood specimen of each person was drawn with a 5µl capillary pipette provided and transferred from the capillary tube to the sample well by holding the capillary tube vertically and gently touching the sample containing end against the pad within the sample until all the blood was transferred. Four drops of the assay diluents was added into the assay diluent well. The reaction was allowed to proceed for a minimum of 15 minutes and the results were read not later than 30 minutes (Moody, 2002).

Microscopy

Thin and thick blood films were made, stained with Giemsa stain and examined using the x100 oil immersion objective of the microscope according to the method recommended by Cheesbrough (2009). Infected samples that reviewed the presence of either stages of *Plasmodium* was noted and recorded accordingly

Molecular Analysis

Extraction of the deoxyribonucleic acid (DNA)

The DNA was extracted from the dried blood spot (DBS) on the Whatman filter paper using the hole puncher of appropriate size to yield a four 2cm x 0.5cm strip.This, is according to Adam *et al*, (2015) estimated method correspond to 50µl blood spot. It was then placed into 1.5µl microcentrifuge tubes using Zymo DNA (tissue) extraction protocol of DNA miniprep Plus kit as adopted by Adam *et al.* (2015). The extraction technique used was the QUICK-DNA™ Miniprep Plus Kit, solid tissues protocol and solid tissues: 25mg. Ninety five (95) µl of water, 95µl of solid tissue buffer and 10µl of protokinase K was added to the 25mg of tissue sample, mixed thoroughly and then incubated at 55º for 3 hours. Two (2) volumes of genomic binding buffer was added to the supernatant and mixed thoroughly. The mixture was transferred to Zymo-spin™11C_XL Column in a

collection tube, centrifuged for one (1) minute at 12000g and the collection tube was discarded with the flow through. Four hundred (400) µl of DNA pre-wash buffer was added to the column in a new collection tube and centrifuged for one (1) minute and the collection tube emptied. Seven hundred (700) µl of g-DNA wash buffer was added and centrifuged for one (1) minute and the collection tube emptied, two hundred (200) µl of g-DNA wash buffer was added and centrifuged for one (1) minute and the collection tube discarded with the flow through. It was transferred to a clean microcentrifuge tube to elute the DNA. Fifty (50) µl of DNA elution buffer was added and incubated for five (5) minutes and then centrifuged for one (1) minute.

PCR Amplification

Polymerase chain reaction (PCR) method used was adopted from Snounou and Balbir, (2002). The PCR amplification was carried out in two phases;

Phase 1. Malaria species specific: Primary malaria PCR was amplified using 1.6-1.7 kilo base (kb) amplicons using rPLU1 and rPLU5, Secondary PCR was performed for malaria species specific using the genus specific primers rFAL1 and rFAL2 under the same cyclic conditions as shown in appendix 3 to yield a 206 base pair fragment.

Phase 2. Nested PCR for merozoite surface protein (msp) 1and 2. The surface antigen loci msp1 and msp2 was amplified using sequence specific primers - M1-OF, M1-OR, M2-OF and M2-OR.The DNA template was amplified using Nested PCR, with second round primers specific to allelic families.

Electrophoresis of PCR Products

PCR products were separated by gel electrophoresis on a 2.5% agarose gel stained with ethidium bromide along with 100 bp DNA size markers. Upon completion of the gel electrophoresis, gels were placed in a gel imaging cabinet digitally photographed under UV light.

Full blood count (FBC) using auto haematology analyzer BC-5300

Full blood count -PCV, HB, (WBC Total and Differential Count) was analyzed using Auto Heamatology Analyzer. The power switch at the left side of the analyzer was pressed on and the power indicator light was turned on to start the external computers which run the system software. The external computer got started and displayed on the screen. After entering the operation system, the 'BC-5300 auto-haematology analyser' icon was double

clicked to run the software and the message box popped up. The correct user name and password was shown in the Login 'message box. The Ok button was then clicked on to initialize the system. During initialization, the start-up information was displayed in the operation/ status information area at the bottom of the screen. This process lasted from 4 to 12 minutes and after the initialization process, the graph screen was entered to check the background result. At the main screen, the sample mode WB" was displayed in the next sample information area and the whole blood sample was mixed homogeniously. When the sample was ready for analysis, the analysis status icon and analyser indicator turned green showing the whole blood sample probe. The aspirator key was pressed to start the analysis and the sample probe automatically aspirated the sample. The sample tube was removed when the machine beeped. The analyzer indicator flicked green, the information area of the next sample was refreshed, at the end of the analysis, the analysis status icon returned to long-lasting green.

Data Analysis

Demographic variables and parasitological data were entered into the study database and analyzed using SPSS version 25.0. Values were expressed as descriptive statistics, frequencies and percentages. Categorical variables were analyzed using chi-square test with significant difference at $p< 0.05$.

RESULTS

A total of 305 blood specimens collected from the pregnant women were analyzed using RTD, Microscopy and confirmed with polymerase chain reaction (PCR). Table 1 shows the age related prevalence of *Plasmodium* infection among pregnant women in Federal Medical Centre, Makurdi, Benue State, Nigeria. The *Plasmodium* positivity was 3.6% among the pregnant women examined. The age group < 20 years had 16.7% (1/6) while the age group 21-30 years had the lowest with 0.0% (0/1). There was no significant difference observed between the age groups (χ^2= 5.09, p=0.165). Table 2 shows the prevalence of *Plasmodium* among pregnant women in relation to location in Makurdi, Benue State. *Plasmodium* positivity between locations showed there was high malaria among pregnant women living in the rural areas, 10.5% (2/19) while those living in urban area was 3.1% (9/286) with a significant difference between these rates of infection (χ^2= 2.791, p=0.05). Table 3 shows the prevalence of *Plasmodium* in relation to occupation of pregnant women

attending antenatal clinic at Federal Medical Centre, Makurdi, and Benue State, Nigeria. The malaria positivity was high among students with 6.0%. There was however no significant difference in the prevalence of malaria and occupation of pregnant women ($\chi 2=1.999$, $p=0.736$). Table 4 shows the prevalence of *Plasmodium* in relation to education of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, and Benue State, Nigeria. The malaria infection was highest among pregnant women with secondary education, 6.2 % (8/158) while no infection was observed among pregnant women with no formal education and primary background 0.0 % (0/9). No statistical difference was observed between malaria and the education of pregnant women attending Federal Medical Centre, Makurdi, Benue State for antenatal. ($\chi^2=4.498$, $p=0.212$).

Table 5 shows the prevalence of *Plasmodium* in relation to marital status of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria. The malaria positivity was highest in non- married pregnant women, 9.1% (2/22) than married pregnant women with 3.2% (9/283). There was however no significant difference of malarial infection between non-married and married pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria. ($\chi^2=2.051$, $p=0.152$). Table 6 shows the prevalence of *Plasmodium* in relation to gestational stage of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria. The malarial positivity was high among pregnant women in their second trimester, 7.3% (7/96) and least among pregnant women in their third trimester, 1.9% (1/51) with a significant difference statistically between the two groups ($\chi^2=5.473$, $p=0.05$). Table 7 shows the prevalence of *Plasmodium* in relation to parity of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

According to parity, Secundigravida had high malaria positivity of 4.8% (6/126) than Multiparous women 0.0% (0/65). No statistical significant difference was observed among pregnant women and their parity. ($\chi^2=3.115$, $p=0.211$).

Table 1: Age related prevalence of *Plasmodium* infection among pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria. (Figures in brackets are %)

	Plasmodium		
Age groups	Negative (%)	Positive (%)	Total
< 20	5(83.3)	1(16.7)	6
21-30	229(97.4)	6(2.6)	235
31-40	59(93.7)	4(6.3)	63
41-50	1(100.0)	0(0.0)	1
Total	294(96.3)	11(3.6)	305

χ^2= 5.09, p=0.165

Table 2: Prevalence of *Plasmodium* infection among pregnant women in relation to their location in Makurdi, Benue State, Nigeria.

	Plasmodium		
Location	Negative (%)	Positive (%)	Total
Urban	227(96.9)	9(3.1)	286
Rural	17(89.5)	2(10.5)	19
Total	294(96.3)	11(3.6)	305

χ^2=2.791, p=0.05.

Table 3: Prevalence of *Plasmodium* in relation to occupation of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

	Plasmodium		
Occupation	Negative (%)	Positive (%)	Total
Student	47(94.0)	3(6.0)	50
Civil servant	36(97.3)	1(2.7)	37
Trader	141(97.3)	4(2.7)	145
Others	70(95.9)	3(4.1)	73
Total	294(96.4)	11(3.6)	305

χ^2=1.999, p=0.736.

Table 4: Prevalence of *Plasmodium* in relation to education of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria

	Plasmodium		
Education	Negative (%)	Positive (%)	Total
No Formal and Primary Education	9(100.0)	0(0.0)	9
Secondary	121(93.8)	8(6.2)	129
Tertiary	155(98.1)	3(1.9)	158
Total	294(96.4)	11(3.6)	305

χ^2=4.498, p=0.212.

Table 5: Prevalence of *Plasmodium* in relation to marital status of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

	Plasmodium		
Marital Status	Negative (%)	Positive (%)	Total
Married	274(96.8)	9(3.2)	283
Non-Married	20(90.9)	2(9.1)	22
Total	294(96.4)	11(3.6)	305

χ^2 =2.051, p=0.05.

Table 6: Prevalence of *Plasmodium* in relation to gestational stage of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

	Plasmodium		
Gestational Stage	Negative (%)	Positive (%)	Total
First Trimester	50(98.0)	1(2.0)	51
Second Trimester	89(92.7)	7(7.3)	96
Third Trimester	155(98.1)	3(1.9)	158
Total	294(96.4)	11(3.6)	305

(χ^2=5.473, p=0.05).

Table 7: Prevalence of *Plasmodium* in relation to parity of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

	Plasmodium		
Parity	Negative (%)	Positive (%)	Total
Primigravida	109(95.6)	5(4.4)	114
Secundigravida	120(95.2)	6(4.8)	126
Multiparous	65(100.3)	0(0.0)	65
Total	294(96.4)	11(3.6)	305

(χ^2=3.115, p=0.05).

The prevalence of *Plasmodium* in relation to taking preventive therapy intermittently in pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria is shown in Table 10. The malarial positivity was high among pregnant women that were administered with intermittent preventive therapy 5.9% (8/135) than pregnant women that had no intermittent preventive therapy 1.8% (3/170). There was a significant difference in malarial infection between pregnant women on intermittent preventive therapy and pregnant women not on intermittent preventive therapy (χ^2=3.748, p=0.053).

Figure 1 Show the prevalence of *Plasmodium* in relation to preventive measures used by the pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria. Pregnant women that used mosquito coils had the highest infection of 8.0 % (2/25), followed by those women that used long lasting insecticide treated nets (LLTNs), 3.4%(7/207) while those women using insecticide spray recorded low infection of 2.7 % (2/73).

Table 8: Prevalence of *Plasmodium* in relation to intermittent preventive therapy of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

	Plasmodium		
Intermittent Preventive Therapy	Negative (%)	Positive (%)	Total
Yes	127(94.1)	8(5.9)	135
No	167(98.2)	3(1.8)	170
Total	294(96.4)	11(3.6)	305

(χ^2=3.748, p = 0.053)

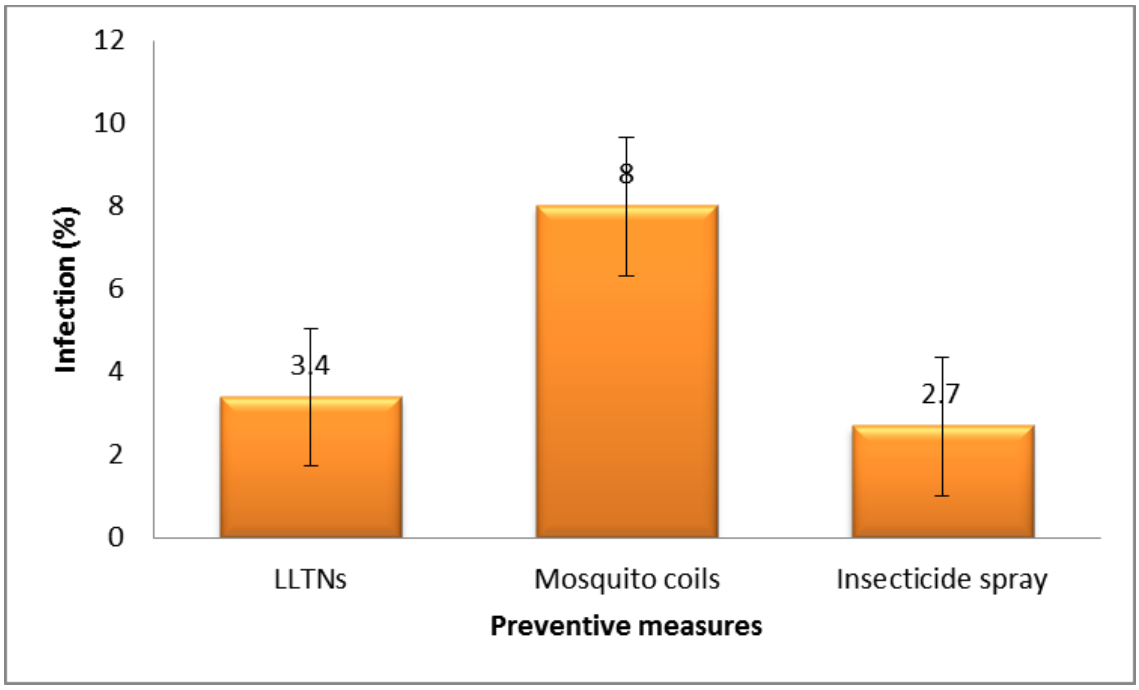


Fig 1:Prevalence of *Plasmodium* in relation to preventive measures used by pregnant women attending antenatal clinic at the Federal Medical Centre, Makurdi, Benue State, Nigeria

Table 9: Prevalence of *Plasmodium* in relation to haematinic intake of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

	Plasmodium		
Haematinic Intake	Negative (%)	Positive (%)	Total
No	205(96.7)	7(3.3)	135
Yes	89(95.7)	4(4.3)	93
Total	294(96.4)	11(3.6)	305

(χ^2=0.186, p=0.667).

Table 10 shows the prevalence of *Plasmodium* in relation to drainage system around the residence of the selected pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria. The *Plasmodium* positivity was high among pregnant women with drainage, which were contaminated with refuse accounting for 4.3% (4/94) while the least was among those that live around stagnant water, which were mainly spread, with crude oil accounting for 2.5% (2/79). There was no significant difference observed between malaria and drainage system (χ^2=0.389, p=0.823). Figure 3 shows the prevalence

of *Plasmodium* in relation to source of water used by pregnant women that attended antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria. The highest malaria positivity was recorded among pregnant women that used well as source of water, 6.7 % while those that used borehole water had, 1.9%. Table 11 shows *Plasmodium* parasitaemia among pregnant women in relation to their White Blood Cell (WBC) Count. The prevalence of *Plasmodium* parasitaemia of 3.8 % (11/293) was recorded among pregnant women with normal WBC count of 5-11 X 10^9, while the pregnant women with leucopenia (<4.1 X 10^9/L) and leukocytosis (> 11 X 10^9 /L) had no malaria parasitaemia, 0.0% (0/6). There was no statistical significant difference observed between malaria and white blood cells ($\chi 2^{=}0.67$, p=0.792). Table 12 shows *Plasmodium* parasitaemia among pregnant women in relation to neutrophil count. The prevalence of malaria parasitaemia of 5.7 % (4/70) was recorded among pregnant women with normal neutrophil count 40-60% while those with neutropenia <40 had 0.0% (0/5). There was no statistical significant difference between malaria and Neutrophil count ($\chi^{2=}1.291$, p= 0.524).

Table 10: Prevalence of *Plasmodium* in relation to drainage system around the residence of pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria

	Plasmodium		
Drainage System	Negative (%)	Positive (%)	Total
Drainage not provided	132	127(96.2)	5(3.8)
Drainage Provided	94	90(95.7)	4(4.3)
Stagnant Water	79	77(97.5)	2(2.5)
Total	305	294(96.4)	11(3.6)

(χ^2=0.389, p=0.823).

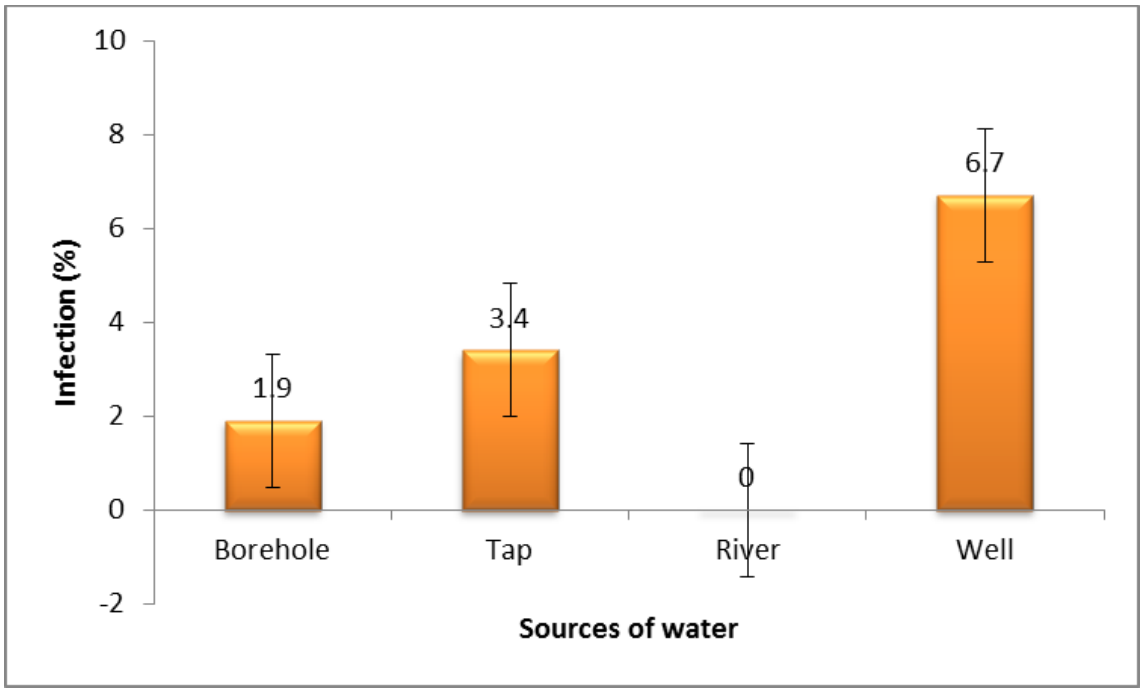


Fig 2: Prevalence of *Plasmodium* in relation to source of water used by pregnant women attending antenatal clinic at Federal Medical Centre Makurdi.

Tables 11: *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their White Blood Cell (WBC) Count.

	Plasmodium		
WBC Count (10^9)	Negative (%)	Positive (%)	Total
Leukopenia (<4)	6(100.0)	0(0.0)	6
Normal (4-11)	282(96.2)	11(3.8)	293
Leukocytosis (>11)	6(100.0)	0(0.0)	6
Total	294(96.4)	11(3.6)	305

χ^2=0.67, p=0.792.

Table 12: *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their neutrophil count

	Plasmodium		
Neutrophil	Negative (%)	Positive (%)	Total
Neutropenia (<40)	5(100.0)	0(0.0)	5
Normal (40-60)	66(94.3)	4(5.7)	70
Neutrophilia (>60)	223(97.0)	7(3.0)	230
Total	294(96.4)	11(3.6)	305

χ^2=1.291, *p*=0.524.

Table 13 shows *Plasmodium* parasitaemia among pregnant women in relation to lymphocyte count. The *Plasmodium* parasitaemia was recorded among pregnant women with lymphocytosis of >40, 5.9% (2/34) while those with lymphocytopenia of <20 had 0.0% (0/24). There was no statistical difference between malaria and lymphocyte count (χ^2=1.405, p=0.495). Table 14 Shows *Plasmodium* parasitaemia among pregnant women in relation to their monocyte count. The *Plasmodium* parasitaemia was 4.1% (7/169) among pregnant women with monocytopenia of <2, while those with monocytosis of >8 had no malaria parasitaemia, (0.0%). There was no statistical significant difference between malarial infection and monocyte count of women and those with monocytopenia (χ^2=0.389, p=0.823). Table 15 shows *Plasmodium* parasitaemia among pregnant women in relation to their eosinophil count.The *Plasmodium* parasitaemia of 9.1% (2/22) was observed among pregnant women with eosinophilia of >4, while the least was found in pregnant women with normal eosinophil of 1-4, 3.0% (4/132). There was however no statistical significant difference between malaria parasitaemia and eosinophil count of the women (χ^2=2.067, p=0.356). Table 16 shows the *Plasmodium* parasitaemia among pregnant women in relation to their basophil count.The *Plasmodium* parasitaemia was 12.5 % (1/8) among pregnant women with basophilia of <1 while those with normal basophil of 0.5-1 had 3.1 % (1/32). There was no significant difference statistically between malaria infection and their basophil count (χ^2= 1.875, p=0.392).

Table 13: *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their lymphocyte count

	Plasmodium		
Lymphocyte	Negative (%)	Positive (%)	Total
Lymphocytopenia (<20)	24(100.0)	0(0.0)	24
Normal (20-24)	238(96.4)	9(3.6)	247
Lymphocytosis (>40)	32(94.1)	2(5.9)	34
Total	294(96.4)	11(3.6)	305

χ^2=1.405, p=0.495.

Table 14: shows *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their monocyte count.

	Plasmodium		
Monocyte	Negative (%)	Positive (%)	Total
Monocytopenia (<2)	102(95.9)	7(4.1)	169
Normal (2-8)	129(97.0)	4(3.0)	133
Monocytosis (>8)	3(100.0)	0(0.0)	3
Total	294(96.4)	11(3.6)	305

χ^2=0.389, p=0.823.

Table 15: shows *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their eosinophil count

	Plasmodium		
Eosinophil	Negative (%)	Positive (%)	Total
Eosinopenia (<1)	146(96.7)	5(3.3)	151
Normal (1-4)	128(97.0)	4(3.0)	132
Eosinophilia (>4)	20(90.9)	2(9.1)	22
Total	294(96.4)	11(3.6)	305

χ^2=2.067, p=0.356.

Table 16: *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their basophil count

	Plasmodium		
Basophil	Negative (%)	Positive (%)	Total
Basopenia (0.5)	256(96.6)	9(3.4)	265
Normal (0.5-1)	32(96.9)	1(3.1)	32
Basophilia (>1)	7(87.5)	1(12.5)	8
Total	294(96.4)	11(3.6)	305

χ^2=1.875, p=0.392.

Table 17 shows the *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their Platelet (Thrombocyte) count. The *Plasmodium* parasitaemia of 5.0 % (2/40) was found among pregnant women with Thrombocytopenia (<150.000), while those within the normal range of 150.000-450.000 had prevalence of 3.4 % (9/265). There was no statistical significant difference between malaria and the Platelet counts (χ^2=0.257, p=0.612). Table 18 shows the *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their Haemoglobin (Hb) count. The *Plasmodium* parasitaemia of 4.5% (7/157) was found among pregnant women with haemoglobinaemia of <11g/dl, while those with normal haemoglobin of 12-16 g/dl had parasitaemia of 2.7% (4/148). There was no significant difference between malaria infection of the women and haemoglobin counts (χ^2=0.676, p=0.411). Table 19: *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their Packed Cell Volume (PCV) counts.The *Plasmodium* parasitaemia of 18.2 % (2/11) was found among pregnant women with erythrocytosis > 0.37-0.47 while those on normal PCV range of <0.37-0.47 had 2.9 %(2/70). There was however, a significant difference between malaria and Packed cell volume (PCV) count (χ^2=6.984, p=0.030).

Table 17: *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their Platelet (Thrombocyte) counts

	Plasmodium		
Platelet (Thrombocyte) Count	Negative (%)	Positive (%)	Total
Thrombocytopenia (<150.000)	38(95.0)	2(5.0)	40
Normal (150.000-450.000)	256(96.6)	9(3.4)	265
Total	294(96.4)	11(3.6)	305

χ^2=0.257, P=0.612

Table 18: Levels of *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their Haemoglobin (Hb) count

	Plasmodium		
Haemoglobin (g/dl)	Negative (%)	Positive (%)	**Total**
Haemoglobinaemia	150(95.5)	7(4.5)	157
Normal	144(97.3)	4(2.7)	148
Total	294(96.4)	11(3.6)	305

χ^2=0.676, P=0.411.

Table 19: *Plasmodium* parasitaemia among pregnant women attending antenatal clinic at the Federal Medical Centre Makurdi in relation to their Packed Cell Volume (PCV)

		Plasmodium	
Packed Cell Volume (PCV)	Total	Negative (%)	Positive (%)
Normal (0.37-0.47)	70	68(97.1)	2(2.9)
Anaemic (< 0.37-0.47)	224	217(96.9)	7(3.1)
Erythrocytosis (>0.37-0.47)	11	9(81.8)	2(18.2)
Total	305	294(96.4)	11(3.6)

χ^2=6.984, p=0.030.

Table 20: compares the sensitivity of the diagnostic test of RDT (SD Malaria Ag P.f/Pan) with that of polymerase chain reaction (PCR). The number of women that tested negative using the RDT test trip was 1.7%, (5/299), while those that tested positive were 100.0 % (6/6). Table 21 shows the performance of RDT (SD Malaria Ag P.f/Pan) and PCR. The sensitivity of RDT was 45.5% at 95% level of confidence while specificity was 100% at 95% level of confidence. The positive predictive value (PPV) was 100% at 95% level of confidence and the negative predictive value (NPV) was 64.7% at 95% level of confidence. Table 22: shows primers used to amplify primary malaria PCR using rPLU1 and rPLU5 at 1.6-1.7 kilo base (kb) and Secondary PCR was performed for malaria species specific using the genus specific primers rFAL1 and rFAL2 under the same cyclic conditions to yield a 206 base pair fragment.

Table 20: Comparism of the diagnostic sensitivity test of RDT (SD *Plasmodium* Ag P.f/Pan) and polymerase chain reaction (PCR)

RDT	PCR		Total
	Negative (%)	Positive (%)	
Negative	294 (98.3)	5 (1.7)	299 (100.0)
Positive	0 (0.0)	6 (100.0)	6 (100.0)
Total	294 (96.4)	11 (3.6)	305 (100.0)

Kappa = 0.698

Table 21: Shows the performance of RDT (SD *Plasmodium* Ag P.f/Pan) and PCR.

Statistic	Value	95% CL
Sensitivity	45.5%	21.4 -71.9%
Specificity	100%	98.4% -100%
PPV	100%	100 % - 100%
NPV	64.7%	59.3% -70.1%

Table 22: Primers used for *Plasmodium Species* detections in women attending antenatal clinic at the Federal Medical Centre Makurdi.

Primer name	Sequence 5'-3'	Amplicon size (base pair)
rPLU1	TCA AAG ATT AAG CCA TGC AAG TGA	Primary reaction detects
rPLU5	CCT GTT GTT GCC TTA AAC TCC	*Plasmodium* species 1.6 – 1.7 kb
rFAL1	TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT	Secondary reaction detects *P. falciparum* 206 bp
rFAL2	ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC	

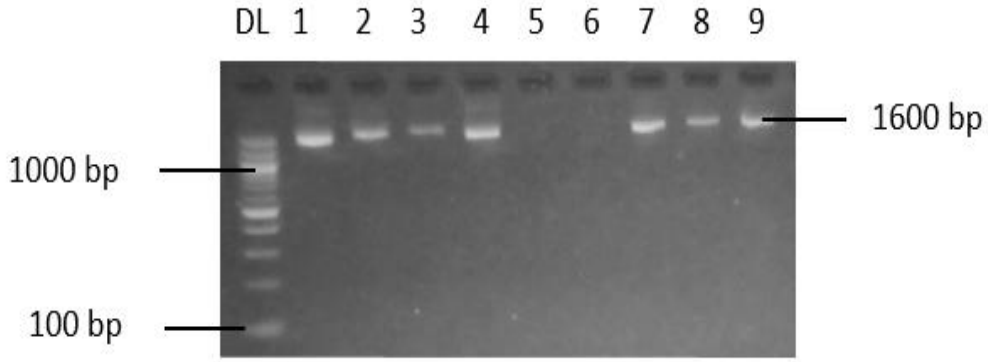


Plate 1: Agarose gel showing 1600 base pair (bp) fragment of malaria species from primary reaction of rPLU1 and rPLU5 primers DL = 100 bp DNA ladder, lanes 1, 2, 3, 4, 7, 8 and 9 = positive samples

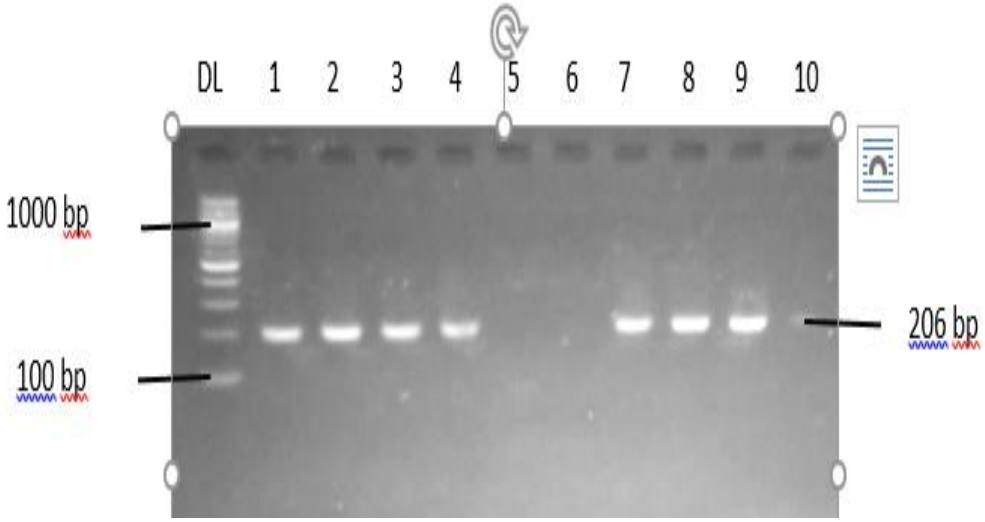


Plate 2: Agarose gel showing 206 bp fragment of *P. falciparum* from secondary reaction of rFAL1 and rFAL2 primers DL=100 bp DNA ladder, lanes 1, 2, 3, 4, 7, 8, 9 = positive samples; lanes 5, 6, and 10 = negative samples.

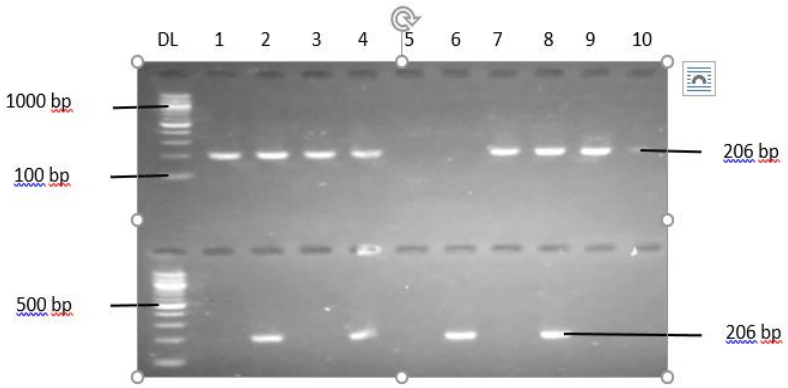


Plate 3: 100 bp DNA ladder: lanes 1, 2. 3, 4, 7, 8 and 9 = positive samples ; Lower gel: lanes 2, 4, 6, and 8 = positive samples; bringing all positive samples to eleven (11).

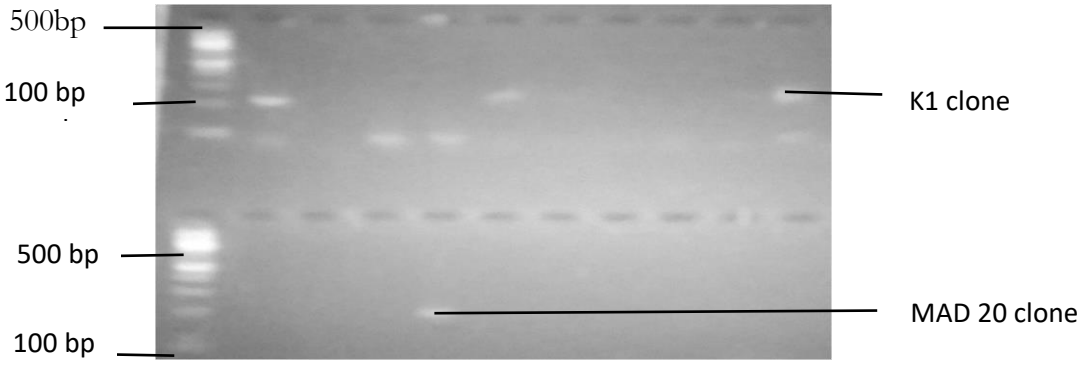


Plate 4: Agarose gel of variable sizes of *P. falciparum msp*-1 parasite clones from secondary reactions of msp-1, M1-KF and M1-KR primers showing K1 parasite clones (Upper gel) and MSP-1 M1-MF and M1-MR primers showing a single MAD20 parasite clone at lane 4 (lower gel).

DL=100 bp DNA ladder.

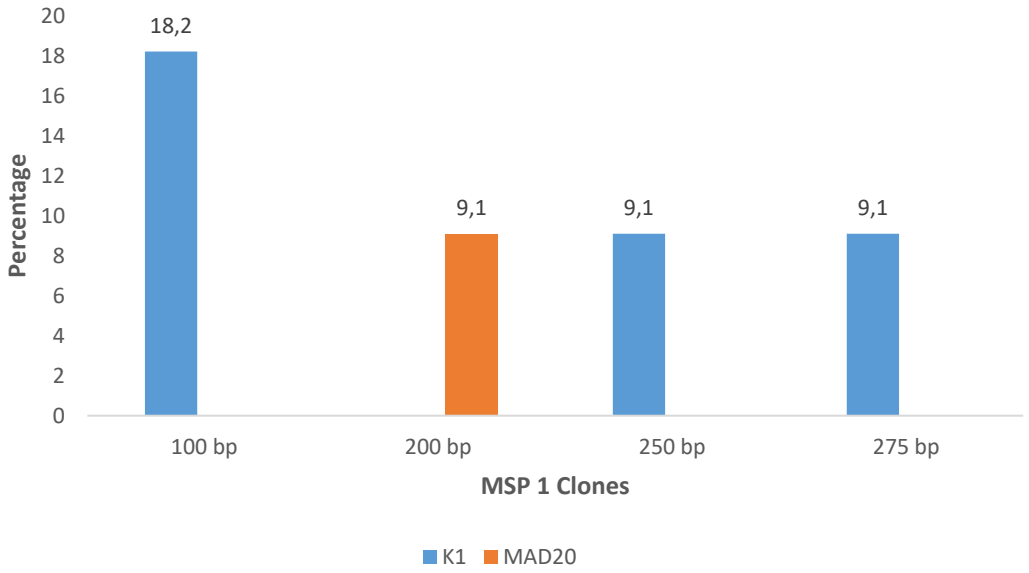


Fig 3: Shows the diversity of *P. falciparum* infection using *msp* -1. Two families were seen, K1 and MAD20, K1 had three clones that occurred at varied frequencies and varied base pairs, the most prevalent were the 100bp clone while MAD20 was one, the RO33 family were not amplified.

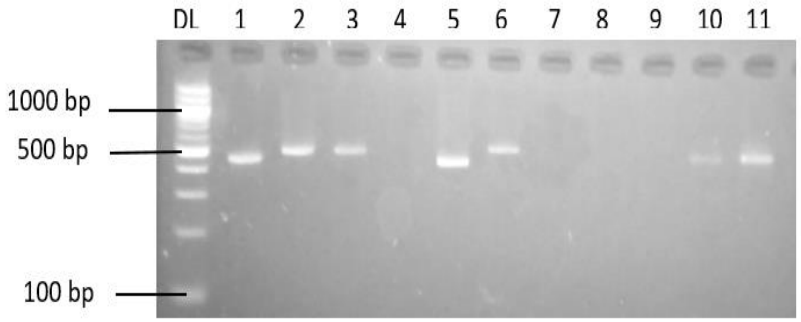


Plate 5: Agarose gel of variable sizes of *P. f*alciparum 3D7 parasite clones from secondary reaction of MSP-2 M2-ICF and M2-ICR primers showing diversity of parasite population in *P. falciparum* infections,DL=100 bp DNA ladder, positive samples are: lanes 1 = 450 bp, 2= 510 bp, 3= 510 bp, 5=430, 6=510 bp, 10=450 bp, 11=

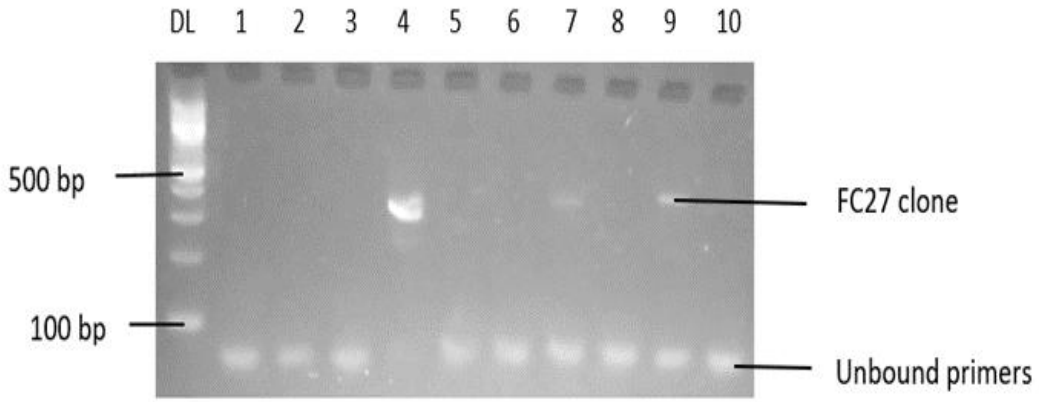


Plate 6: Agarose gel of variable sizes of *P. falciparum* FC27 parasite clones from secondary reaction of MSP-2 M2-FCF and M2-FCR primers showing diversity of parasite population in *P. falciparum* infections DNA ladder (DL) 100bp positive samples are: lanes 4=320bp, 7=350bp, 9=350bp

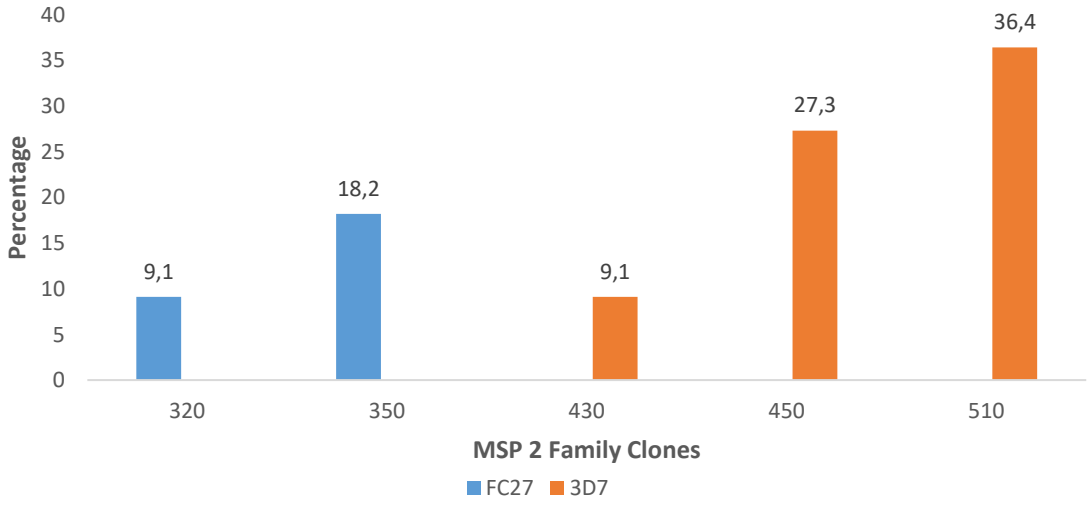


Fig 4: Shows the diversity of *P. falciparum* infection using MSP-2. Two families were seen, FC27 and 3D7, In FC27, two different parasite clones were detected. Each occurred at different frequencies and varied base pairs. In 3D7 family, three different clones were detected with varied frequencies. The most prevalent clone was the 510 bp clone.

DISCUSSION

Malaria in pregnancy is a major public health problem with attendant clinical and societal implications in maternal and child health. It becomes important because of the vulnerability of the pregnant woman and her child. These consequences are evident in anaemic status and negative impact on the foetus that could result in miscarriages and stillbirth. The molecular characterization of malaria parasite in this study is the first report in the study area found *P. falciparum* as the cause of malaria among pregnant women in Makurdi, Benue State. However, there was no congenital parasitaemia observed in the neonates. The study recorded a low prevalence of 3.6% malaria parasitaemia was observed. This is similar to observations made in other studies conducted in Lagos 2.0% and in Bauchi 2.1% by Oluwagbemiga *et al.* (2018) and Kadas *et al.* (2019) respectively. Similar low prevalence were also reported in other ccountries; 2.3% in Bangladesh (Wasif *et al.,* 2013) and 4.7% in Colombia (Vasquez *et al.,* 2018). This low prevalence could be due to the fact that women attending the antenatal clinic were educated on the need to prevent exposure bites from the infected vectors of the disease. They adhered to the use of long lasting treated nets (LLITNs) and other malaria control measures such as Sulfadoxine-Pyrimethamine (SP). The malaria parasitaemia with regards to the pregnancy period showed that women in their second trimester had the highest prevalence (7.3%) when compared to the women in their first and third trimesters. The findings of Kadas *et al.,* (2020) in Bauchi, 37.7% and Amuta *et al.,* (2014) in Benue, 65.9% reported higher malaria parasitaemia in pregnant women at their second trimester. This could be attributed to the fact that puerperal blood parasitaemia in pregnant women occurs at the beginning of the 2nd trimester between 13 and 20 weeks gestation (Zhou *et al.,* 2002). The *Plasmodium* parasitaemia in relation to location showed that those living in the rural areas recorded high parasitaemia. This could be due to the fact that they do not have the knowledge on how to use protective clothes to prevent themselves from bites of the infected vectors as some of them are involved in farming crops along the banks of river Benue. These are swampy areas that encourage the breeding of mosquitoes, which bite these women as they go to their farms.

The level of education was considered for pregnant women. The highest rate of parasitaemia was seen in women that had secondary education, 6.2%. This agrees with the findings of Eisele *et al.* (2012) who reported women with secondary education as having the highest malaria parasitaemia of 38.0%, these women are literate, they find it hard to comply with the practice of sleeping under the long lasting insecticide treated nets as well

as using insecticide spray as many of them complained of not breathing well while using long lasting treated nets. With regards to occupation, students were seen to have a high parasitaemia of 6.0%. This may be due to the fact that these students exposed themselves to the bite of the infected vectors when they go out to study at night most especially when they cannot use good protective clothing. It was observed that younger women aged < 20 years were more prone to malaria with 16.7%. It could be the fact that these young women lack awareness of the use of preventive measures taken against the malarial disease at the onset of pregnancy and the general immune suppression as well as loss of acquired immunity to malaria. A similar finding was done by Beeson and Dutty, (2005) in related studies as reported in the literature.

With regards to preventive measures used by the pregnant women in this study, it was observed that pregnant women that used mosquito coil had the highest malaria parasitaemia 8.0%,this shows that the use of mosquito coil is not effective enough to prevent mosquito from biting the women, the pregnant women that used long lasting insecticide treated nets had a lower malaria parasitaemia 3.4%,the use of long lasting insecticide treated nets reduces human contact with the mosquitoes, thus leading to a significant reduction in the incidence of malaria, this confirms the report of WHO (2019), on the use of insecticide treated nets as an effective means in reducing the lethal impact of malaria. The *Plasmodium* parasitaemia in respect to parity showed that prim and Secundigravida women were more infected than their multigravida counterparts.. This could be due to poor knowledge on the effect of exposure to mosquito's bites during their pregnancy period in contrast to multigravidae who are already knowledgeable on preventives and control measures against the infection in their previous pregnancies as well as having developed immunity against the infection. This is in agreement with those of other studies by Amuta *et al. (*2014), Azab *et al.* (2017) and Kadas *et al.* (2020) that reported prim gravida to be more exposed than multigravida

High malaria parasitaemia (4.3%) was observed in women that were taking haematinics. This agrees with the study of Kadas *et al.* (2020) who also had high *Plasmodium* parasitaemia among pregnant women that were taking haematinics. The use of haematinics does not actually prevent malaria but help to boost the level of the red cells of these women in order to prevent them from becoming anaemic during the pregnancy as a result of the malarial disease. Married pregnant women had a low *Plasmodium* parasitaemia 3.2/% than the non-married women who had 9.1%.This could probably be due to the fact that most married

women are being cared for by their husbands and as such are not exposed to doing menial jobs or engaging in extensive farming or night trading that can put them at risk of being bitten by these infected mosquitoes that transmit the malarial parasites. The reverse is the case for the unmarried women who have to fend for themselves and their children by engaging in menial jobs, farming extensively or being engaged in night hawking without being protected from the bites of these mosquitoes as they cannot afford the appropriate clothings to shield themselves from these mosquitoes. There is however no significant difference observed between malaria and this type of relationship, a finding that contradicts the earlier work of Kadas *et al.* (2020) in Bauchi who recorded high parasitaemia among the married women in their own study. High *Plasmodium* parasitaemia (5.9%) was seen in pregnant women using intermittent preventive therapy against those that were not using preventive therapy. This could be a result of resistance to the antimalarial drug (SP) been taken by these women as previously reported by Fagbemi *et al.* (2020). It could also be due noncompliance by these women not taking their drugs the way they were prescribed by their physician Yaya *et al.,* (2018). There is the possibility of these women buying or consuming sub-standard or fake antimalarial drugs. Some of them even while taking the drugs were still not protecting themselves from mosquito bites, leading to the problem of relapse infection from latent dormant phase of the *Plasmodium parasites* (CDC, 2015). The present study recorded malaria parasitaemia of 4.3% in those that had drainages. , These drainages were not well evacuated as people dump refuses into them giving a very good breeding site for mosquitoes which transmits malaria. It is pertinent to note that mosquitoes breed freely in open gutters and drainages as observed by Uneke *et al* (2007). Pregnant women that make use of well water as their source of water had the highest rate of parasitaemia of 6.7%. This is due to the fact that mosquitoes hover on the surfaces of these wells and on the stagnant water puddles around the well. They are good breeding sites for these mosquitoes which eventually bite these pregnant women and transmit the malaria parasites.

The haematological changes occur in pregnancy in order to meet the demands of the developing foetus with alteration in blood volume (Grebreweld *et al.,* 2018). This study observed some changes as seen in the Packed Cell Volume (PCV) or haematocrit concentration in which 18.2%. *Plasmodium* parasitaemia was found among women that had PCV > 0.47. This may be due to increased maternal erythropoiesis as a result of iron demand by the foetus during growth as pregnancy progresses and increases iron

supplement intake. This is in agreement with the study of Kadas *et al.* (2020) who also recorded an increase in haematocrit level in the pregnant women in Bauchi area. Haemoglobin value in this study was observed to decrease with those that have haemoglobin level of < 11g/dL having high parasitaemia of 4.5%. Though the difference was not significant, this agrees with the study of Adam *et al.*, (2005) and Osaro *et al.*,(2019) who reported haemoglobin level of < 11g/dL . This reflects a state of anaemia which is mainly due to parasitized red blood cells, nutritional status of the infected pregnant women as well as splenic clearance of parasitized and defected red cells. Thrombocytopenia or decrease in platelet count of < 150,000 is also consistent with other findings in previous studies of Erhart *et al.,* (2004) and Ahmed *et al.,* (2014).They agreed that thrombocytopenia is a major complication of malaria which occurs as a result of release of Adenosine diphosphate (ADP) following the hemolysis of parasitized red blood cells. White blood cells count (WBC) in this study was normal since they are responsible for the body defense during pregnancy as a result of building immunity for the foetus and immune response against malaria infection.Majority of patients with uncomplicated *P.falciparum* malaria usually have their total value of leucocytes count within the normal range. This agrees with the findings of Tangpukdee *et al.,* (2008), Nnaemeka *et al.,* (2014) and Osaro *et al.,* (2019). They all reported that the WBC counts in pregnant women of the studies range from being low to normal range. The eosinophilia (9.0%) observed in this study is usually expected in malarial infection which is parasitic. Eosinophils are produced to phagocytize these parasites. This according to Sahoo *et al.,* (2015) is also observed in cases of allergies and autoimmune diseases.

Polymerase chain reaction (PCR) as a tool for diagnosis of malaria is highly sensitive and consistent in the detection of parasites as it has the ability to detect malaria infections even where parasites count are as low as 5 / µl of blood. In this study, *P. falciparum* was the species that was detected as seen in Plate I. This agrees with the study carried out by Osaro *et al* (2019) who found *P. falciparum* as the predominant species responsible for malaria in pregnant women in Sokoto, Sokoto State. The finding is consistent with the report of Jeremiah *et al.* (2010) who also found *P. falciparum* as the predominant strain responsible for malaria in Nigeria. Polymorphic markers in isolates *P. falciparum* were used to examine the genetic diversity in this study. The Merozoites Surface Protein- 1 (*msp*-1) and Merozoites Surface Protein-2 (*msp*-2) are highly polymorphic marker;, *msp*-1 markers were able to amplify two families, K1 and MAD20 families as found, while *msp*-2 markers were able to

amplify 2 families, FC27 and 3D7. In FC27, two different alleles were detected, each occurred at different frequencies. The 3D7 had 3 alleles also at different frequencies. The most frequent 3D7 clone was the 510 bp clone. This shows the genetic diversity of *P. falciparum* species, a finding that agrees with previous study by Olasehinde *et al.* (2012) who also reported families FC27 and 3D7 among the isolates in *msp*-2 with different frequencies. This study also observed that the *msp*-2 marker was a more variable genetic marker to evaluate diversity and complexity of *P. falciparum* infections than the *msp*-1 marker.

Comparing the diagnostic tool PCR, RDT (SD Malaria Ag P.f/Pan) RDTs are lateral-flow devices that use antibodies to capture and detect parasite proteins by immunochromatography, This involves antigen- antibody interactions on a nitrocellulose test strip (WHO, 2017). Many proteins released by malaria parasites during blood stage infection including PfHRP2 are immunogenic and generate an antibody response, such antibodies could bind circulating antigens and form immune complexes whilst in circulation or when a blood sample is lysed on an RDT thereby interfering with the binding of antigen to antibodies on the RDT test lines, this explains poor performance of RDTs particularly in detecting moderate and low parasite densities. There is a possible reduction in sensitivity for the diagnosis of *P.falciparum* malaria using PfHRP2-detecting RDTs among pregnant women with high levels of specific antibodies and low density infection, as well as possible interference with tests configured to detect soluble PfHRP2. Possible explanations for imperfect sensitivity at high parasite density include deletion of the pfhrp2 gene, according to WHO, (2017), varying quantities of proteins produced by different parasites(Baker *et al.,* 2011), the prozone effect (Luchavez *et al.,* 2011), the performance characteristics of the capture and detection of antibodies in the kit, including their thermal stability(Lee *et al*., 2012), as well as manufacture quality.

In this study, the sensitivity of RDT is low (45.5%, 95% CI, 21.4-71.9%) This may probably be that, the pregnant women infected with *P.falciparum* parasites might have developed antibodies against PfHRP2 antibody. This agrees with the findings of (Ho *et al.,* 2014). The low sensitivity of the RDTs could be as a result of their inability to detect parasites at low densities as similarly reported in a study by (Orish *et al.,* 2018). RDTs are best used as screening test for malaria and should not be used alone, especially given the fact that they are not always sensitive as a result of the above phenomena's associated with

them. PCR is more sensitive and specific for diagnosing malaria. Its only problem is however the cost, since the reagents are highly expensive and not often affordable.

CONCLUSION

In conclusion, the study found a low prevalence of malaria parasitaemia among pregnant women attending antenatal clinic at Federal Medical Centre, Makurdi, Benue State. Haematological parameters in this study were also affected by the malaria parasites, as there was a significant increase in the haematocrit (PCV) level. There was significant difference statisticallly between *Plasmodium* parasitaemia and the area of residence of the pregnant women as the women in the rural areas were more prone to *Plasmodium* parasitaemia than those in urban areas. There was a significant difference of malarial infection between pregnant women that were not on intermittent preventive therapy and pregnant women with no intermittent preventive therapy. There was statistical difference between *Plasmodium* parasitaemia and gestational stage of the pregnancies as women in their second trimester were more prone to *Plasmodium* infection than those in first or third trimesters. Comparing the diagnostic tools; PCR and RDT (SD Malaria Ag P.f/Pan) used in this study, PCR was more sensitive and specific as a *Plasmodium falciparum* diagnostic tool than RDT. The different family clones of *P.falciparum* using *msp* -1 and *msp*-2 marker is indicative of genetic diversity of *P.falciparum.*

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