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Searching for simpler sensitive diagnostic methods for assessing malaria prevalence during pregnancy in resource constrained setting

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Abstract

Background: Malaria infection during pregnancy causes maternal mortality with severe consequences for the foetus and infant. This research was aimed at assessing prevalence and clinical symptoms via urinalysis among pregnant women.

Method: A cross-sectional study involving 300 pregnant women purposively selected from six health care centres was conducted. Data were analysed using SPSS version 26.

Results: The overall malaria prevalence rate was 48% (n=143). Age group ≤25 years had the highest prevalence rate of 55.8% (n=68). Pregnant women in their first and second trimester had prevalence rate of 49% (n=101) and 49% (n=77) respectively, while the primigravidae recorded the highest prevalence rate of 56% (n=91). Logistic regression revealed that women younger than 30 years old had lower odds of being malaria negative OR 0.96(95%Cl 0.56-1.65), P=0.87. Women in their first trimester had higher odds of being malaria negative by 1.01 (95%Cl 0.41-2.46), P=0.99. Women who used LLINs had higher odds of being malaria negative OR 4521746.902, P=0.0000.

Conclusion: Urinalysis revealed highest prevalence rate of 27.3% (n=82) for bilirubinuria (+) samples. Bilirubinuria in pregnancy could serve as a good indicator for malaria.

Keywords: Malaria, Prevalence, Pregnancy, Urinalysis, Clinical symptoms, Isialangwa.

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Importance épidémiologique des pratiques, observations cliniques, analyse d'urine et évaluation multi-diagnostique de la parasitémie à plasmodium parmi les femmes enceintes à Isialangwa, État d'Abia, Nigéria

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Résumé

Contexte de l'étude: L'infection palustre pendant la grossesse entraîne une mortalité maternelle avec des conséquences graves pour le fœtus et le nourrisson. Cette recherche visait à évaluer la prévalence et les symptômes cliniques par analyse d'urine chez les femmes enceintes.

Méthode de l'étude : Une étude transversale portant sur 300 femmes enceintes a été menée. Les données ont été analysées à l'aide de SPSS version 26 et STATA 16.

Résultat de l'étude : Le taux global de prévalence du paludisme était de 48% (n=143). Le groupe d'âge ≤ 25 ans avait le taux de prévalence le plus élevé de 55,8 % (n = 68). Les femmes enceintes dans leur premier et deuxième trimestre avaient un taux de prévalence de 49 % (n = 101) et 49 % (n = 77) respectivement, tandis que les primigestes ont enregistré le taux de prévalence le plus élevé de 56 % (n = 91). la régression logistique a révélé que les femmes de moins de 30 ans avaient moins de chances d'être négatives pour le paludisme OR 0,96 (95 % IC 0,56-1,65), P = 0,87.

Conclusion : Les femmes au cours de leur premier trimestre avaient une probabilité plus élevée d'être négatives pour le paludisme de 1,01 (IC à 95 % 0,41-2,46), P = 0,99. Les femmes qui utilisaient des MILD avaient une probabilité plus élevée d'être négatives pour le paludisme OR 4521746,902, P=0,0000. L'analyse d'urine a révélé le taux de prévalence le plus élevé de 27,3 % (n = 82) pour les échantillons de bilirubinurie (+). La bilirubinurie pendant la grossesse pourrait être un bon indicateur du paludisme.

Mots-clés : Paludisme, Prévalence, Grossesse, Analyse d'urine, Symptômes cliniques, Isialangwa

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INTRODUCTION

Malaria remains the dreaded parasitic infectious disease that affects everyone in the tropics and subtropics with pregnant women and children under 5 years as its main target. It is caused by the protozoan parasite, *Plasmodium* which is transmitted when an infected female *Anopheles* mosquito (the vector) bites an individual. Globally, malaria burden was estimated at 229 million cases in 2019 in 87 endemic countries, but Africa had the greatest burden with 215 million cases, accounting for 94% of the total cases (1). Malaria is endemic in Nigeria. About 95% of global malaria deaths in 2019 were recorded in 32 countries and Nigeria alone accounted for 23% percent (1). Malaria in pregnancy is associated with greater risks of maternal and neonatal morbidity and mortality. In sub-Saharan Africa, 11 million pregnancies are affected by malaria infection, causing up to 100,000 infant deaths each year due to prematurity, low birth weight, neonatal Anaemia. *Plasmodium falciparum*, the dominant malaria species in most states of Nigeria (2), is able to cause infected erythrocytes to sequester in the placenta, resulting in inflammation and adverse birth outcomes (3).

There has been serious effort through global partnership to mobilise resources for the use of cost effective tools to significantly reduce malaria morbidity and mortality, leading to a decrease in malaria cases of 238 million to 229 million cases and malaria mortality from 680,000 in 2000 to 409,000 in 2019 globally (1).These reductions are mainly due to increased use of long lasting insecticide treated nets (LLINs), increased availability of highly effective Artemisinin –based Combination Treatment (ACTs), regulated intermittent malaria treatment in pregnancy and improved diagnosis of malaria. While the use of LLINs and ACTs have significantly increased towards universal coverage levels in most countries, improvement in malaria diagnosis has been much slower (4) due to problems associated with laboratory diagnosis. Until the diagnosis of malaria is given the desired priority, the battle of malaria control in developing countries may continue.

WHO and the Roll Back Malaria initiative stressed the need for prompt and accurate diagnosis before commencement of treatment as the key to effective management of malaria. Most of the time, laboratory diagnosis is out of reach and clinical diagnosis which is not usually accurate becomes the basis for therapeutic care for most patients with fever in malaria endemic areas. Malaria diagnosis, thus, is the most neglected aspect of malaria research, yet, sensible management of malaria needs a diagnostic tool with high level of sensitivity and specificity values. This will go a long way to prevent wastage of treatment cost, stop the acceleration of development of artemisinin resistance, increase the impact of interventions and reveal the true picture of malaria incidence or prevalence, especially in pregnant women.

Microscopic examination of Giemsa stained blood smear has always been the gold standard for malaria diagnosis and control because it is not expensive to perform, it can differentiate between the malaria species and quantify parasite density yet, it has many draw backs including scarce expert microscopists (5).Malaria random diagnostic test (MRDT) kit, a device that promptly detects malaria antigen in a small amount of blood by immunochromatographic assay with monoclonal antibodies directed against the parasite antigen and impregnated on a test strip (6) is another diagnostic tool for malaria infection. It needs simple training, no manpower or electricity, thus, it can be used both in urban and rural areas, especially where expert microscopist is scarce. These notwithstanding, many healthcare providers are not too confident with the sensitivity of the MRDT kits.

Urinalysis (urine analysis) is the first of all laboratory tests and still remains the most valuable and highly important means of diagnosis in clinical medicine. It is a significant indicator of several health conditions like renal disease, nephropathy, glomerulonephritis, hyperglycemia, schistosomiasis, to mention a few. This to a large extent, suggests that malaria may have significant effect on urine composition (7).Among the population of malaria endemic areas, self-diagnosis and treatment of malaria has partly been based on yellow colouration of urine and other clinical signs like fever, loss of appetite, headache, cough/catarrh, leading to over diagnosis. Studies that documented urinary abnormalities/clinical features in malaria infection are few and the extent to which malaria species and the degree of parasitaemia affects urinary composition in patients with malaria infection especially among pregnant women remain largely unknown. Thus, the study to check clinical symptoms, urinalysis and multi-diagnostic evaluation of *Plasmodium* parasitaemia among pregnant women with different perceptions on malaria control in Isialangwa.

We live in a resource-limited malaria endemic setting. Malaria microscopy requires competent microscopist, rigorous maintenance, effective quality control/assurance and regular power supply. These requirements are not easy to come by, yet prompt and accurate diagnosis of malaria before treatment is highly solicited for. Health seeking behavior of greater percentage of the populace is considerably poor. People prefer self-diagnosis/treatment to seeking medical attention from health care personnel. Some find it difficult, submitting themselves to painful needle pricks for blood collection. Delay in malaria diagnosis/treatment, especially among pregnant women can be dangerous; there have been reported cases of abortion, anaemia, stillbirth, poor pregnancy outcome due to malaria infection. Urine analysis possesses untapped potentials for easy and quick detection of malaria infection (7). Studies on urinalysis abnormalities in malaria patients are limited and very scarce among pregnant women. The findings of this work will document the urinary abnormalities in malaria parasitized pregnant women.

This research work is aimed at studying clinical symptoms, urine peculiarities and multi diagnostic evaluation of *Plasmodium* parasitaemia among pregnant women with different control practices on malaria control in Isialangwa, Abia state. The specific objectives included:

1) Determining malaria parasites prevalence
2) Determine malaria control practices among pregnant women of Isialangwa, Abia state
3) Investigating urine peculiarities, clinical symptoms among the infected subjects and asymptomatic malaria infection
4) Evaluating the sensitivity of four WHO certified malaria random test kits.

METHODOLOGY

Study Area

The study was conducted in six Primary Health Care centres (PHCs) in two rural local Government Areas(LGAs) {Isialangwa south and Isialangwa North}of Abia state, namely: PHC I (Isialangwa South, PHC II (Isialangwa South, PHC III (Isialangwa South, PHC IV (Isialangwa North, PHC V (Isialangwa North and PHC VI (Isialangwa North).Abia state is located between longitudes 07^0 00' and 08^0 10' east and latitudes 04^0 45' and 06^0 07' north. The state covers a total land area of 2440 square mile (6,320 km^2) and is divided into three senatorial districts and 17 Local Government areas (LGAs). By 2006 census, the population of Abia state was 2,845,380 people (8). There are two main climatic regimes in Abia state: a dry season and a wet season; November – March and April-October respectively. The mean annual rainfall is 2400mm per year. The maximum and minimum temperature are 31.9^0c and 22.5^0c while the daily sunshine rate is about 4.4 hours. Average relative humidity is about 74%, occurring mostly during the wet season. Some parts of the state are characterized by indiscriminate waste disposal system in an ever-increasing population and pools of stagnant water in the streets especially during the rains. It is also common to find farms with broad-leaf plants around living houses. In addition, households have water storing containers where mosquitoes can breed. All these factors favour both the breeding of malaria vectors and the disease transmission; thus, malaria is predominantly hyper endemic in the state and transmission is all year round but more intense between April and October (9).

Study Design and Population

The study was a cross-sectional descriptive design, done in six health care centres in the study areas. The study lasted for six months (February to August, 2019). Urinalysis and malaria diagnosis (using four different WHO certified MRDT kits, namely: Paracheck, careStart, Global and LabAcon confirmed by microscopy) were conducted on 300 pregnant women (150 from each of the two study LGAs).

Three health centres from each of the two local governments (Isialangwa South and Isialangwa North) LGA were purposively selected for the study due to accessibility and proximity. Due to the limited number of pregnant women in the various health care centres, all the pregnant women that attended the respective health care centres were invited to participate in the study. However only those who consented to participate in this study and met the inclusion criteria were enrolled. The following groups of pregnant women were excluded from the study: the HIV infected, those with renal/liver disease, those presently on malaria treatment, those with sickle cell Anaemia syndrome and women below 16 years and above 40 years. Overall a total of 50 participants were enrolled from each health care centre over the six months study period. A case was established with a positive malaria parasite test, confirmed by microscopy while the pregnant women with negative malaria test served as control. The abnormalities in the urine detected

by urinalysis were compared and correlated with the clinical symptoms in both study groups.

Ethical Considerations: Ethical clearance for the study was obtained from Abia State Ministry of Health, Umuahia (Ethical clearance certificate no. AB/MH/AD/904/T and Abia State Primary Health Care Development Agency (ABIA SPHCDA), Umuahia (Ethical clearance certificate number: AB/PHCDA/157/XXX. With these clearance letters, permissions were sought and received from the Local Government councils and the primary healthcare centres identified for the study. The objectives and details of the study were highlighted, and their permission sought to enable access to their patients. The consent of the study participant was also sought and only those who consented were enrolled in the study. The registered nurses and midwives assisted in sample collection.

Collection of Demographic Data

Pre-tested structured questionnaires were used to collect socio-demographic data (age, gestation stage, parity, level of Education, marital status), malaria control measures used at home and possible clinical malaria symptoms (fever, cough, vomiting, loss of appetite, weakness, headache, etc).

Samples Collection

Venipuncture blood sample (4 mls) was collected from the arm of each of the pregnant women by the nurses in the respective centres, using syringe. Samples were collected after cleaning the surface with cotton wool moistened with methylated spirit. The blood samples were collected into sterile EDTA (ethylene diamine tetra acetic acid) containers and taken to the laboratory for analysis (10).Furthermore, 10mls of clean-catched mid-stream urine samples were collected in clean specimen bottles from each of the pregnant women, duly labeled and examined with medi-test combi 9 urine test strip, within one hour of collection (10).

Blood Smear and Microscopy Procedure

Thick and thin blood smears were prepared immediately upon blood collection in different slides. For thick films, 12µL of blood was spread in a diameter of 15mm, while 2µL of blood was used for thin films as described by Cheesbrough, 2006. The films were first allowed to dry for at least, 45 minutes (thin) and 12 hours (thick) (10).The thin films were fixed in absolute methanol for 2 seconds and air- dried. The blood films were stained with 3% Giemsa stain solution. The stained slides were taken to Abia State University Teaching Hospital where the slides were examined microscopically under oil immersion(100X) objectives independently by two laboratory technologists with discrepancies resolved by a third reader. Thick film was used for malaria parasite detection and density. The level of parasitaemia was graded as: 1-10 parasites per 100 thick film fields (+), 11- 100 parasites per thick film fields (++) and above 100 parasites per 100 thick film fields (+++). A negative result was recorded after thorough examination of 100 fields without any parasite (11).

Antigen-Based Malaria Rapid Diagnostic Tests

Each of the blood samples was tested for the detection of malaria parasite antigen using each of the four brands of random diagnostic test (RDT) kits selected for the study (Global device, CareStart, LabAcon and Paracheck). These tests were done same day of sample collection and according to the manufacturers' guidelines and procedures. Quality of test kits was assured by keeping the test kits within temperature ranges recommended by the manufacturers during the study period.

Urine Analysis

10mls of clean-catched mid-stream urine samples collected from each of the women were examined with medi-test combi 9 urine test strip, within one hour of collection, following Cheesbrough, 2006.

Data Analysis

In this study, continuous variables were expressed as mean ± standard deviation or medians (interquartile range {IQR}) and compared using the student's t-test. Categorical variables such as marital status were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Chi-square test was used to study associations between demographic profile of patients and knowledge and usage of LLINs. Odds ratios (OR) were used to compare the relative odds of the malaria infection, given patients' knowledge, possession and usage of LLINs. All analyses were performed using **SPSS version 26** (*JBM Corp. Released 2019. IBM SPSS Statistics for Windows, version 26.0. Armonk: NY: IBM Corp).* The level of significance was set at ($P<0.05$).

RESULTS

Table 1 shows the demographic characteristics of the study participants. A total of 300 pregnant women participated in this study. The age of the participants ranged from 16 – 40 years, with a mean age of 29.12 ± 4.60 years with the majority 140 (46.7%), aged between 26 – 30 years. More than 89.3% (n=268) of the mothers had formal education and 97% (n=291) were married. Majority of women were multigravidae and primigravidae 40.7% (n=122) and 33.7% (n=101) respectively. Almost half, 44.3% (n=133) of the study participants were in their third trimester of pregnancy.

Table 2 shows the prevalence rate of malaria infection in the study population. The malaria prevalence rate recorded in Isialangwa North (54%) was higher than that of Isialangwa South (41%) Overall, the prevalence of malaria was found to be 48% (95% CI: 42% - 53%) in the current study. Of the six centres studied, PHC IV (Isialangwa North) had the highest prevalence of 70% (95% CI: 56% - 81%) of malaria while PHC I (Isialangwa South) had the least prevalence of 34% (95% CI: 22% - 48%). Women in 1st and 2nd trimesters had prevalence rates of malaria 49% (n=77) while women in the 3rdtrimester had a prevalence rate of 46% (n=122) (Table 2). 'As shown in Table 2, the highest proportion of malaria cases were observed among the primigravidae 56% (n=51), followed by the multigravidae 46% (n=61), with the least proportion among the secundigravidae 41% (n=31). The difference in malaria cases between levels of parity was not statistically significant, as follows; primigravidae vs secundigravidae (z=1.9, P=0.054), secundigravidae vs multigravidae (z=0.7, P=0.48).The study also showed that the prevalence rates of malaria among pregnant women with no formal education, primary, secondary and tertiary education were 38% (n=12), 44% (n=7), 53% (n=83) and 43% (n=41), respectively. It is therefore worthy of further investigations to possibly understand the reason or factors behind the very low prevalence rate recorded by the women without formal education.. However, the difference in malaria prevalence between different levels of education was not statistically significant, No formal education vs Primary education (z=0.4, P=0.69), Primary vs Secondary (z=0.7, P=0.49), Secondary vs Tertiary (z=1.9, 0.06) and No formal vs Tertiary (z=1.5, P=0.12). Of the 75% (n=224) of the participants possessing and using LLINs, only 45% (n=101) were infected with malaria. However, the possession and use of LLINs did not result in a statistically significant difference in the number of malaria infections, (z=1.4, P=.17) and z=1.5, P=0.13 respectively.

Figure 2 shows the results of malaria test using different diagnostic methods. A comparative assessment of the different diagnostic tools used to test for malaria infection showed differences in sensitivity with thick blood film microscopy being the most sensitive 143(47.67%) and the gold standard whereas CareStart random kit was the least sensitive diagnostic tool with 5(1.67%).

Table 3 shows the urinalysis pattern of the pregnant women. The women with amber and clear (AC) urine colour showed the highest prevalence rate of 25% (n=75). Those with urine pH of 6.0 had the highest prevalence rate of 22% (n=66). The women with proteinuria (++), bilirubinuria (+), ascorbic acid (negative), blood (trace) and urobilinogenuria (negative) showed malaria prevalence rates of 20% (n=60), 27.3% (n=(82), 23.3% (n=70), 21% (n=63) and 20.3% (n=61) respectively. This means bilirubinuria has the highest correlation with malaria prevalence.

Table 4 is a logistic regression analysis showing the Odds Ratios (OR) of malaria infection among the pregnant women in this study. Factors that were investigated in relation to the risks of malaria infection were parity, educational level, gestational period and use of LLINs. However, only parity was found to have a statistically significant association with the risk of malaria infection (p=0.0193).

Using logistic regression, the study observed that women younger than 30 years old had lower odds of being malaria negative OR 0.96(95%Cl 0.56-1.65), P=0.87. Women in their first trimester had higher odds of being malaria negative by 1.01 (95%Cl 0.41-2.46), P=0.99. Primigravids had lower odds of being malaria negative OR 0.56 (95%Cl 0.29-1.08), P=0.08, although the OR were not statistically significant. Unmarried (single) women had higher odds of being malaria negative OR 1.51 (95%Cl 0.32-7.18), P=0.61 and women with no formal education exhibited higher odds of being malaria negative OR 2.06 (95% Cl 0.77-5.50) P=0.15. Women that possessed Long Lasting Insecticidal Nets (LLINs) had lower odds of being malaria positive OR 3.57×10-7 (95% Cl 1.717×10-7(P=0.000), in the same vein women who used LLINs had lower odds of being malaria positive OR 4521746.902, P=0.0000.

DISCUSSION

Malaria parasitaemia in the studied population was found to be moderately high at

48% of studied population. This compares well with the prevalence rate of 41.7% in a previous study byImakwu *et al*., 2020) (12) conducted among pregnant women at Ebonyi state. The findings of Amala and Wokem within Port Harcourt, in Nigeria in 2018 revealed a lower prevalence rate of 34.5% (13), whereas Amadi and Nwankwo in 2012 observed a malaria prevalence rate of 54.0% in Umuahia metropolis (14).Solomon *et al*., 2020 found a malaria prevalence rate of 15.2% (n=35) in their study conducted in Southern Ethiopia among asymptomatic pregnant women (15). Trans*et al*., 2020 also found a placental malaria prevalence of 44.3% (n=101) in a study conducted in Tororo, Uganda among pregnant women (16). Omer *et al.*, in 2021found a maternal malaria prevalence of 49.7% (n=92) among pregnant women in Blue Nile State, Sudan (17). The moderately high prevalence rate of malaria in the study area can be attributed to the exposure of these pregnant women in the rural communities to infected mosquito bites due to the favourable climatic conditions and mosquito breeding sites in the areas; presence of bushes, farms with broad leaves like plantains, cocoyams, etc. around the living houses; even the uncovered water storing containers where malaria vectors breed in various houses in the area. These and more, encourage the multiplication of the malaria vector.

Previous studies have observed four important factors that determine the spread/epidemiology of malaria in pregnancy which include: environmental, vectoral, parasite and host factors (12).Although the pregnant women indicated use of protective measures, they may not have adequately and consistently used them, thus the significantly high malaria prevalence rate. Bello and Ayede (2019) in their study in Ibadan also recorded no significant association between malaria prevalence and the use of protective measures (18). The lower prevalence rates found with the random kits when compared to the prevalence rate found with thick film microscopy further supports the thick blood film microscopy as the gold standard for malaria diagnosis (19). RDTs sensitivity have been found to decrease with low parasitaemia (<100 parasites/µL), genetic variability and prozone effect (19).However, Paracheck showed higher sensitivity than all other random kits with CareStart as the least sensitive brand. This is in line with the findings of Kavanaugh *et al.*, (2021)(19).

Age groups of ≤25 years had the highest prevalence rate of 55.8%. This is in line with the findings of Enoch and Gloria in 2017 and alsoImakwu *et al*., 2020(12) who found the age group of ≤25 to be the most susceptible to malaria but contradicts with the findings of Amadi and Nwankwo in 2012, who observed higher prevalence rates among older pregnant women of age groups 28-32 and 43-47 years (14).

The highest malaria prevalence rate of 49% was observed among the pregnant women in their 1st trimester as well as those in their second trimester and lowest among the pregnant women in their third trimester 46%. This finding is in line with the findings of other researchers Frank *et al*., 2016) (20) who also observed highest malaria prevalence rates among pregnant women in their first trimester and with the findings of Udoma *et al*., 2015) who observed highest prevalence rates at the second trimesters (21). The pregnant women possibly had lowered immunity from the sudden onset of pregnancy, the baseline immunity acquired in endemic areas notwithstanding. With respect to parity, malaria prevalence was highest among the primigravidae 91(56%). This could be due to the development of new utero-placental vasculature during the 1st pregnancy which has no pre-exposure to malaria infection and therefore immunologically naïve and susceptible (22). Moreso, early onset of efficient antibody response in multigravidae and the delayed production of antibodies in primigravidae account for the gravidity dependent and differences in prevalence rates of malaria infection among pregnant women (14).This finding is in agreement with the findings of several other researchers (14, 22). Only nine women out of the 300 examined were single mothers of which 3(33%) were infected with malaria while 140(48%) out of 291 married women were infected.

In this study, the highest prevalence of malaria with respect to educational status was observed among those with secondary education 83(53%), followed by the pregnant women with primary education 7(44%) while women with tertiary education recorded 41(43%). Suprisingly, women with no formal education recorded the least infection level (12(38%). It is therefore worthy of further investigations to possibly understand the reason or factors behind the very low prevalence rate recorded by the women without formal education. The lower infection rate observed among pregnant women with tertiary education could be attributed to their higher living standards, awareness and better use of preventive measures against malaria infection. This study also revealed that the use of malaria preventive measures conferred some levels of protection to the users but did not eliminate the

infection entirely. This is in line with the findings of other researchers like (23, 24). Thus, use of integrated control measures are recommended. 80(26.67%) malaria prevalence rate seen among 220 asymptomatic women is really worrisome. Several studies have also shown prevalence of asymptomatic malaria among pregnant women and association with Anaemia, still birth, poor pregnancy outcome and other consequences of malaria infection in pregnancy (25).This study documented significantly higher urinary protein, bilirubin and traces of blood in malaria infected pregnant women, indicating a high suspicion for malaria infection in pregnancy, even in the face of negative blood film. Haemolysis of malaria parasitized and non-parasitized red blood cells is considered as an important factor causing mild jaundice, hence the appearance of bilirubin in urine. This finding is in tandem with the discoveries of some other researchers who found significantly higher proteins and bilirubin in malaria infected patients (7). Urinalysis, though not an alternative diagnostic tool for malaria infection, may be an aid to clinicians working in malaria endemic resource- limited countries as the first line care for timely recognition of patients at risk, allowing prompt care to reduce the dangerous consequences of malaria in pregnancy. Urinary abnormalities like proteinuria, bilirubinuria as well as amber and clear observations may assist in identifying patients with severe malaria infection as it detects pathological changes in malaria patients. However, the limitations in terms of the number of pregnant women accessible in the various health care centres could be attributed to the inability to access the health care centres due to lack of access roads and financial constraints. However, the six centres which participated in the study are relatively busy centres as they enjoy better patronage. Another limitation is the possibility that certain physiological conditions could stimulate similar urinary parameters as seen in the malaria positive pregnant women.

CONCLUSION

Malaria prevalence in Isialangwa is significantly high among pregnant women. Thick blood film microscopy remains the standard for malaria diagnosis. Paracheck malaria random kit was the most sensitive of the four malaria random kit brands used and CareStart random kit was the least sensitive. Integrated protective measures conferred appreciable level of protection on the pregnant women against malaria. Urinary abnormalities like bilirubinuria and proteinuria as well as amber and clear urine appearance in pregnancy should be further investigated for malaria infection even in asymptomatic situation to avoid the adverse effects of malaria in pregnancy.

Conflicts of Interest: The authors declare that they have no competing interests regarding the manuscript.

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Table 1: demographic characteristics of study participants

Variable		Total	Age-group (Years)			
			=25 (n=68)	26-30 (n=140)	31-35 (n=64)	36 (n=
Mean Age(SD)		29.12 (4.6)	23.1 (1.8)	28.4 (1.4)	33.1 (1.5)	38
Median age (IQR)		29 (26-32)	24 (22-25)	28 (27-30)	33 (32-35)	38
Area (LGA)	Isialangwa South	150 (50%)	32 (47.1%)	68 (48.6%)	33 (51.6%)	17
	Isialangwa North	150 (50%)	36 (52.9%)	2 (51.4%)	31 (48.4%)	11
Centre	PHC I (Isialangwa South)	50 (16.7%)	24 (35.3%)	18 (12.9%)	6 (9.4%)	2 (
	PHC II (Isialangwa South)	50 (16.7%)	4 (5.9%)	27 (19.3%)	12 (18.8%)	7 (
	PHC III(Isialangwa South	50 (16.7%)	1 (1.5%)	26 (18.6%)	12 (18.8%)	11
	PHC IV (Isialangwa North)	50 (16.7%)	19 (27.9%)	21 (15%)	8 (12.5%)	2 (
	PHC V (Isialangwa North)	50 (16.7%)	13 (19.1%)	24 (17.1%)	11 (17.2%)	2 (
	PHC VI (Isialangwa North)	50 (16.7%)	7 (10.3%)	24 (17.1%)	15 (23.4%)	4 (
Gestation	First Trimester	101 (33.7%)	45 (66.2%)	36 (25.7%)	15 (23.4%)	5 (
	Second Trimester	77 (25.7%)	11 (16.2%)	42 (30%)	14 (21.9%)	10
	Third Trimester	122 (40.7%)	12 (17.6%)	62 (44.3%)	35 (54.7%)	13
Parity	Primigravidae	91 (30.3%)	48 (70.6%)	34 (24.3%)	8 (12.5%)	1 (
	Secundigravidae	76 (25.7%)	14 (16.2%)	52 (37.1%)	5 (7.8%)	5 (
	Multigravidae	133 (44.3%)	6 (8.8%)	54 (38.6%)	51 (79.7%)	22
Marital status	Single	9 (3%)	5 (7.4%)	3 (2.1%)	0(0%)	1 (
	Married	291 (97%)	63 (92.6%)	13 (97.9%)	64(100%)	27
Education	No formal education	32 (10.7%)	15 (22.1%)	12 (8.6%)	4 (6.3%)	
	Primary	16 (5.3%)	4 (5.9%)	6 (4.3%)	4 (6.3%)	
	Secondary	156 (52%)	37 (54.4%)	72 (51.4%)	30 (46.9%)	
	Tertiary	96 (32%)	12 (17.6%)	50 (35.7%)	26 (40.6%)	

Table 2: prevalence rate of malaria infection

All Infections		N	Number of positive cases (%)	Proportion of positive cases (95%CI)
		300	143(48)	0.48 (0.42-0.53)
Age-Group (years)	=25	68	38(56)	0.56 (0.44-0.67)
	26-30	138	56(41)	0.41 (0.33-0.49)
	31-35	65	33(51)	0.51 (0.39-0.63)
	36-40	29	16(55)	0.55 (0.38-0.72)
Centre	PHC I (Isialangwa South)	50	17(34)	0.34 (0.22-0.48)
	PHC II (Isialangwa South)	50	19(38)	0.38 (0.26-0.52)
	PHC III (Isialangwa South)	50	26(52)	0.52 (0.39-0.65)
	PHC IV (Isialangwa North)	50	35(70)	0.70 (0.56-0.81)
	PHC V (Isialangwa North)	50	23(46)	0.46 (0.33-0.60)
	PHC VI (Isialangwa North)	50	23(46)	0.46 (0.33-0.60)
Area (LGA)	Isialangwa South	150	62(41)	0.41 (0.36-0.52)
	Isialangwa North	150	81(54)	0.54 (0.43-0.59)
Gestation(Trimester)	First Trimester	101	49(49)	0.49 (0.39-0.58)
	Second Trimester	77	38(49)	0.49 (0.38-0.60)
	Third Trimester	122	56(46)	0.46 (0.37-0.55)
Parity	Primigravidae	91	51(56)	0.56 (0.46-0.66)
	Secundigravidae	76	31(41)	0.41 (0.30-0.52)
	Multigravidae	133	61(46)	0.46 (0.38-0.54)
Marital Status	Single	9	3(33)	0.33 (0.12-0.65)
	Married	291	140(48)	0.48 (0.42-0.54)
Education	No formal education	32	12(38)	0.38 (0.23-0.55)
	Primary	16	7(44)	0.44 (0.23-0.67)
	Secondary	156	83(53)	0.53 (0.45-0.61)
	Tertiary	96	41(43)	0.43 (0.33-0.53)
Control Measures	Non	1	1(100)	1 (0.21-1.0)
	Closed Windows	9	6(67)	0.67 (0.35-0.88)
	Window Nets	11	5(45)	0.45 (0.21-0.72)
	LLINs	14	6(43)	0.43 (0.21-0.67)
	Insecticides	23	12(52)	0.52 (0.33-0.71)
	LLINs & others	208	94(45)	0.45 (0.39-0.52)
	Multiple	34	19(56)	0.56 (0.39-0.71)
Possession of LLINs	Yes	224	101(45)	0.45 (0.39-0.52)
	No	76	41(54)	0.54 (0.43-0.65)
Usage of LLINS	Yes	224	101(45)	0.45 (0.39-0.52)
	No	76	42(55)	0.55 (0.44-0.66)

TABLE 3: urinalysis pattern of the pregnant women

Urine appearance	**Number infected**	**Percentage %**
PAC	43	14.3
AC	75	25.0
PACL	25	8.33
TOTAL	143	48
pH		
5.00	12	4.00
6.00	66	22
7.00	56	18.67
8.00	9	3.00
TOTAL	143	48
PROTEINURIA		
NEGATIVE	29	9.67
+	54	18
++	60	20
TOTAL	143	48
BILIRUBINURIA		
NEGATIVE	61	20.3
+	82	27.3
++	0	0
TOTAL	143	48
ASCORBIC ACID		
NEGATIVE	70	23.3
+	59	19.7
++	14	4.66
TOTAL	143	48
BLOOD		
NEGATIVE	58	19.3
TRACE	63	21
+	22	7.33
TOTAL	143	48
UROBILINOGENURIA		
NEGATIVE	61	20.33
+	54	18
++	28	9.33
TOTAL	143	48

Key:
Pac = Pale Amber and Clear; Ac = Amber and Clear; Pacl = Pale Amber and Cloudy; Prevalence is Relative to the Total Number Examined (N=300)

Table 4: relative risk of malaria based on demographic characteristics and prevention methods

RISK RELATIVE TO DEMOGRAPHIC CHARACTERISTICS		Proportion with malaria	Odd ratio (95%CI)	P-value
Age=30	Yes	94/208 (45.2%)	0.85 (0.67-1.08)	0.19
	No	49/92 (53.3%)		
First Birth (Primiparous)	Yes	51/91 (56%)	1.39 (1.05-1.08)	0.02
	No	55/136 (40.4%)		
Low Education	Yes	19/48 (39.6%)	0.80 (0.55-1.66)	0.25
	No	124/252 (49.2%)		
Gestation (1st Trimester)	Yes	49/101 (48.5%)	1.03 (0.80-1.32)	0.83
	No	94/199 (47.2%)		
RISK RELATIVE TO MALARIA PREVENTION METHODS				
Possession of LLINS	Yes	102/224 (45.5%)	0.82 (0.64-1.06)	0.13
	No	41/76 (53.9%)		
Usage of LLINS	Yes	101/224 (45.1%)	0.82 (0.64-1.05)	0.11
	No	42/76 (55.3%)		

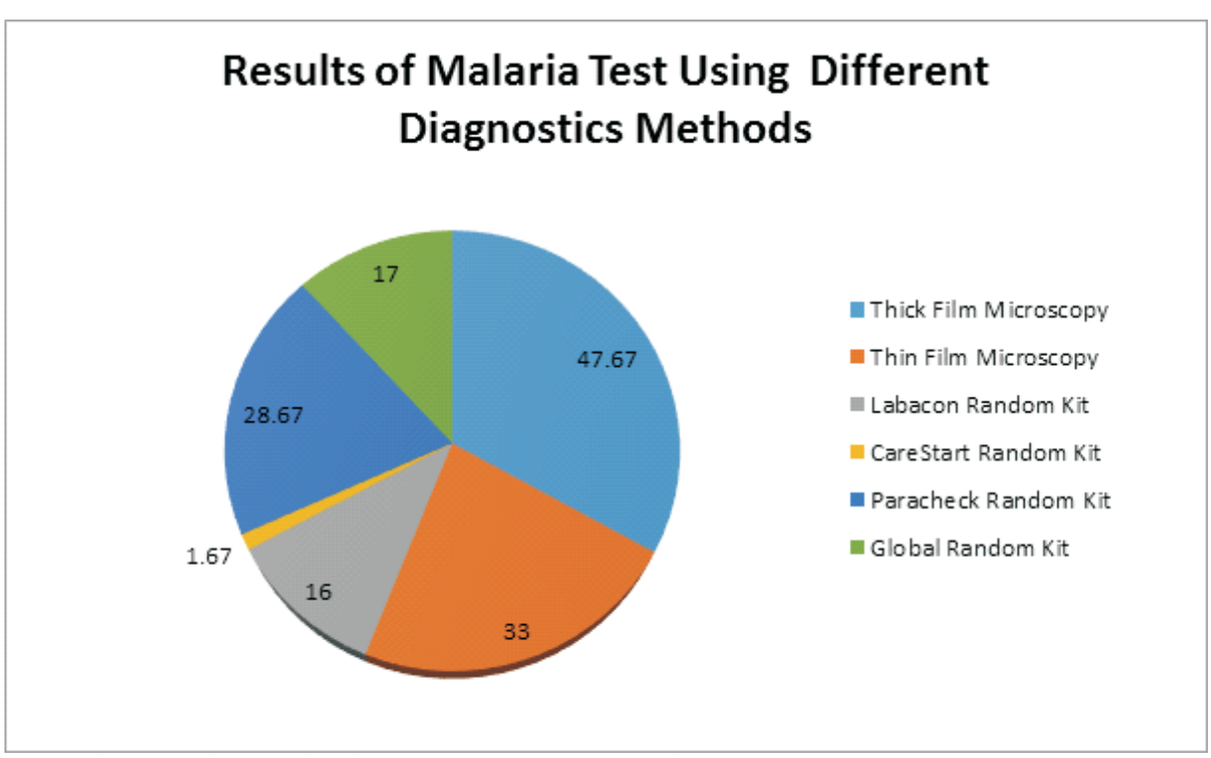

Figure 2: Results of Malaria Test Using Different Diagnostics Methods