Comparative Assessment of Microscopy and Rapid Diagnostic Test (RTD) In The Diagnosis of Malaria in Pregnant Women

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ABSTRACT

Malaria in pregnancy is a serious life threatening infection to the pregnant mother and the fetus. prompt diagnosis and appropriate treatment of malaria and anaemia is an integral part of the World Health Organisation malaria control strategy. This study was conducted to compare the laboratory diagnosis of malaria using microscopy and rapid diagnostic tests in pregnant women between April and June, 2017. Blood samples were collected from one hundred pregnant women and screened for malaria parasites using standard microscopy method and Rapid Diagnostic Test (RDT) procedure according to CareStartTM Malaria Pf (HRP2) Ag RDT kit manufactured by Access Bio Inc. Malaria prevalence of 100% was recorded using microscopic examination whereas 32% tested positive and 68% tested negative to RDT with a statistical difference between both methods. The sensitivity and specificity of CareStartTM were 32.0% tested and 100% respectively. The outcome of this study shows a high prevalence of malaria among pregnant women. Blood smear microscopy remains the most effective method for detection of malaria parasite.

Keywords: Computer interface, determination of optimal inclination angle, solar photovoltaic system

iSTEAMS Proceedings Reference Format
Daramola, O.O., Olatunji S. Oduleye, Unwana, E. Okon, Olugbenga O.M. & Olaoye F.A. (2019): Comparative Assessment of Microscopy and Rapid Diagnostic Test (RTD) In The Diagnosis of Malaria in Pregnant Women. Proceedings of the 17th iSTEAMS Multidisciplinary Research Nexus Conference, D.S. Adegbenro ICT Polytechnic, Itori-Ewekoro, Ogun State, Nigeria, 21st – 23rd July, 2019.Pp 77-84. www.isteams.net - DOI Affix - https://doi.org/10.22624/AIMS/iSTEAMS-2019/V17N2P9

1. INTRODUCTION

Malaria is a protozoan infection of red blood cells transmitted through bites of blood-feeding female *Anopheles* mosquitoes. It has been declared one of the most dreaded diseases of mankind as it accounts for 0.584 million deaths annually (WHO, 2014). It remains a serious human health issue and is particularly prevalent in developing countries. It is estimated that 3.3 billion people worldwide are at risk of malaria, with 90%of cases occurring in Africa south of the Sahara (WHO, 2011). The high morbidity and mortality are attributed to the development of resistance of the parasite to antimalarial drugs and of the mosquito vector to currently available insecticides. There is no malaria vaccine at present. (WHO, 2013). Recent statistics show that while it is declining in other regions of the world due to deliberate interventions, Africa seems to be an exception. Although recent estimates suggest that malaria mortality rates decreased by an impressive 47% between 2000 and 2013 globally (WHO, 2011). Malaria still remains a major public health problem in a number of countries (Abdoulaye *et al.,* 2016). Africa and India recorded almost 80% of the global malaria burden, with Nigeria leading in the sub-Saharan region with 25% (WHO, 2017).

Literature indicates that malaria in pregnancy poses a serious risk for both the mother and the unborn child. Malaria infection during pregnancy can lead to miscarriage, premature delivery, low birth weight, congenital infection, and/or perinatal death. (Olukosi *et al*., 2015). The consequences of malaria in pregnancy range from maternal anaemia, low birth weight, spontaneous abortion, stillbirth, among others (Steketee *et al*., 1996). One of the challenges in malaria management is inaccurate diagnosis of the condition (Olukosi *et al*., 2015). Clinical diagnosis of malaria in a region with many tropical infectious diseases has limited reliability since signs and symptoms are similar for many of these diseases. Clinical diagnosis of malaria without laboratory support may lead to malaria misdiagnosis and maltreatment (Oladosu and Oyibo, 2013). Though, examination of a thick blood smear by Giemsa staining technique remains the preferred method for malaria diagnosis, it is however labour-intensive and time consuming. As reliable as this technique is, a few cases may still be missed because the diagnosis of malaria during pregnancy by microscopy is often complicated by the absence of parasites in peripheral blood, due to sequestration of parasites in the placenta (Bland *et al*., 1999). There is a need for alternatives to microscopy, such as detection of antigens by rapid diagnostic tests (RDTs) that can detect antigen in the circulation, even when the parasites are sequestered. RDTs are being widely deployed, because of their ease of use and relatively low cost, but their accuracy for the diagnosis of placental malaria has not been extensively evaluated against suitable reference tests (Mayxay *et al*., 2004). In this work, the comparative analysis of microscopy and RDT in diagnosis of malaria in pregnant women was studied.

2. METHODS

Sampling: Five millimeters of blood was collected from 100 pregnant women by venipuncture using sterile syringe. The blood samples were transferred into a properly labeled EDTA bottles and stored in the refrigerator before analysis.

Microscopic Examination: Thick film and thin film of blood samples were prepared as described by Cheesbrough (2009). The thick blood smear was stained (without fixing) with 10% Giemsa solution. The thin smear was fixed in absolute methanol and then stained with Giemsa solution (Cheesbrough, 2009). Both the thick and thin blood films were examined under the microscope at x100 objective with the use of oil immersion, identification of species was observed and reported as described by Cheesbrough 2006.
The blood samples were also subjected to Rapid Diagnostic Test (RTD) using the Carestart™ Malaria Pf (HRP2) Ag kit manufactured by Access Bio Inc.

Estimation of Parasite Density

Parasite densities were recorded as ratio of parasites to White Blood Cell (WBC) in thick film. Plasmodium parasite was counted against 200WBC on the thick film, 200 white blood cells were counted when 10 and above parasites were found, the results was recorded in terms of number of parasites per 200 white blood cells. If after 200 white blood cells have been counted and the number of parasites was 9 or fewer counting was continued up to 500 white blood cells and the results was recorded in terms of number of parasite per 500 white blood cells (WHO, 2014).Parasite density was calculated using the formula below: The number of parasites per µl of blood is calculated using the W.H.O formula:

$$\text{Parasite/µl of blood} = \frac{\text{Number of parasites counted x individual WBCs}}{\text{Number of Leukocyte}}$$

A slide is declared negative when there is no parasite detected / counted within 500 WBCs.
Patient WBC count per ml of blood is 8000µl.

The number of trophozoites per 500 WBC were multiplied by the average WBC count of maternal blood and expressed as parasites per microlitre (µl) of blood, assuming a white blood cell count of 8000/µl of blood. Parasitaemia was classified as low (<500 parasite/µl of blood), moderate (501–5000 parasites/µl of blood) and high (>5000 parasites/µl of blood) (Allen *et al*., 1992).

Ethical Approval and Informed Consent

Ethical permission was obtained from the ethical review committee of Oba Ademola Hospital. Verbal and informed consent was obtained from all individuals before blood sample collection. They were assured of voluntary participation, confidentiality of their roles and the opportunity to withdraw at any time without prejudice.

Statistical Analysis

The data generated was analyzed for significant difference between the two methods employed during the study using chi-square test.

3. RESULTS

A total of 100 pregnant women were recruited for this study, baseline characteristics of all respondents are shown in Table 1. Their ages ranged from 18-45 years, with age group 26-30 and 34-45 having the highest percentage of 28% and 29% respectively. From the table, it is shown that 3% had primary education while majority have both secondary and tertiary education with 54% and 43% respectively. Using microscopy, 100% of participants tested positive for malaria parasite while RDT had 32% testing positive for malaria parasite. Figure 1 shows the prevalence of malaria parasite among pregnant women based on age using microscopic method with pregnant women between 34-45 years having the highest prevalence (29%) while pregnant women age18-25 (21%) having the lowest percentage. Prevalence of malaria parasite among pregnant women using RDT in pregnant women aged 31-33 and 34-45 both having the highest percentage (9%) while pregnant women age 18-25 and 26-30years both having the lowest percentage (7%) shown in Figure1. Parasite density ranged from 280-1920 parasites/µl of blood. Majority of the participant (67%) however had their parasitaemia within 501-5000 parasites/µl of blood and overall mean parasite density was 742.12 parasites/µl of blood.

Table 1: Baseline Information of Pregnant Women used for the Study

DEMOGRAPHIC FACTOR		FREQUENCY	PERCENT
AGE	18-25	21	21.0
	26-30	28	28.0
	31-33	22	22.0
	34-45	29	29.0
	Total	**100**	**100.0**
LEVEL OF EDUCATION	No formal education	-	-
	Primary	3	3.0
	Secondary	54	54.0
	Tertiary	43	43.0
	Total	**100**	**100.0**

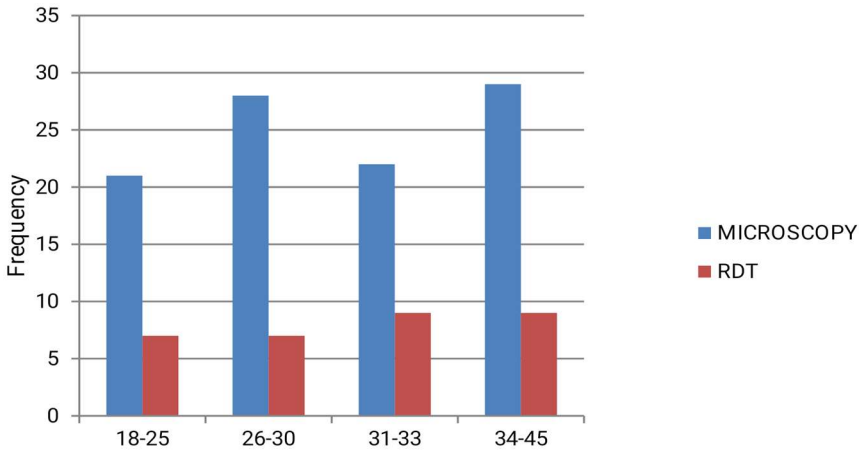

Fig 1: Comparison of Prevalence of malaria infection in pregnant women in relation to age using microscopy and RDT

The prevalence in malaria infection in pregnant women in relation to the number of IPT collected is represented in Figure 2. The participant who have collected IPT once and those that have not collected IPT have the highest prevalence of malaria infection (32%) and (36%) respectively, while those with two IPT have the lowest prevalence of malaria infection (21%) and participant who collected IPT thrice (11%). The prevalence of malaria parasite using RDT among pregnant women in relation to number of IPT collected (Figure 2).The participants who have received IPT have the highest prevalence of malaria infection (25%),while those with one IPT have the lowest prevalence of malaria infection (1%) , participant who received IPT twice (2%) and those who received thrice (4%).In addition, RDT results varied with parasite density shown in Table 2. The association between RDT results and parasitaemia level was significant (Chi-square. ($X^2(1) = 36.41^a$, p=0.000). In Table 3, the accuracy of RDT using microscopy as gold standard 68% false negative was obtained. The sensitivity and specificity of CareStart™ RDT were 32.0% and 100%

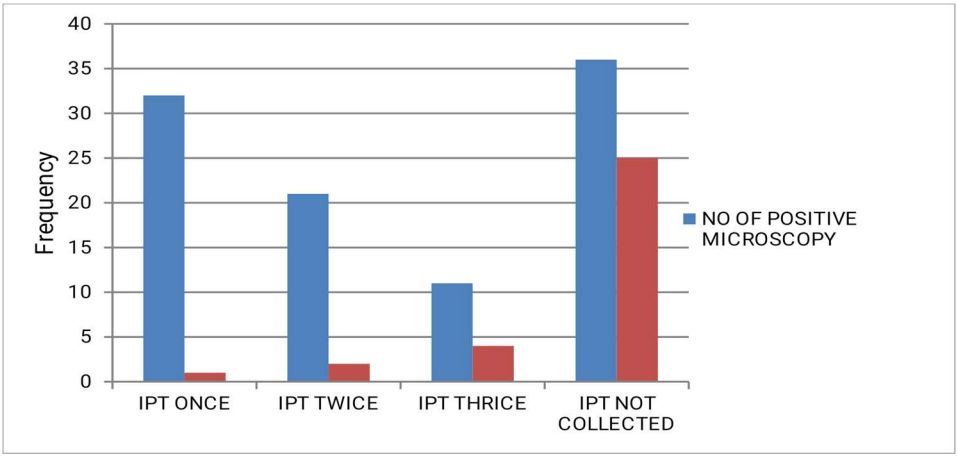

Figure 2: PT- Intermittent Preventive Treatment.

Table 2: Comparison of Rapid Diagnostic Test with parasite density grouping.
Rapid diagnostic test * Parasite density Grouping Cross tabulation

	PARASITE DENSITY			
RDT	<500 LOW	501-5000 MODERATE	>5000 HIGH	Total
Positive	0	32	0	32
Negative	33	35	0	68
Total	33	67	0	100

TABLE 3: Accuracy of RDT using microscopy as a gold standard.

	Microscopy		
RDT	**Negative**	**Positive**	**Total**
Negative	0	68	68
Positive	0	32	32
Total	0	100	100

4. DISCUSSION

This study revealed a malaria prevalence of 100% using microscopy among pregnant women. Women in the second trimester were more infected (58/100; 58%) than those in the third trimester. This is similar to the findings of Umeh *et al*., (2013) which reveals that women in the second trimester were more infected (60/170; 35.3%) than the rest; in addition, the younger age group (20 to 25 years) were more infected (50/140;35.7%) than the older age groups. Although 100 % infection was detected by microscopy, 68% false negative was reported for RDT. The false negative rate observed in this study is worrisome because it implies that up to 68% of the pregnant women that had malaria could have been left untreated especially if RDT is the only available diagnostic tool. It is important to point out that majority of the false negative specimen in this study had low parasitaemia. This agrees with similar studies with other rapid diagnostics tests where false negative results were reported in patients with parasitaemia of less than 50 parasites/µl of blood (Guthman *et al.,* 2002; Shiff *et al*., 1994). The false negative results obtained could also be as a result of variability in the amino acid sequence of the HRP-2 *P. falciparum* that may affect the ability of Rapid diagnostic test to detect it (WHO, 2013). In certain *P. falciparum* parasites, the HRP-2 antigen may not be detected at all due to gene deletion by individual for the production of HRP-2 and so will give a negative result with these Rapid diagnostic test (RDTs) (WHO, 2013).

It is expected that appropriate malaria RDT should have high sensitivity (95%) and specificity (97%) and ability to detect low parasite density infections (WHO, 2013). However, the WHO standard Carestart ™ RDT used in this study does not conform to the sensitivity of 95% recommended by World Health Organization value (WHO, 2013). The sensitivity could also be dependent on the quality of preparation and interpretation of the test. This low sensitivity is disadvantageous as it will impair the control intervention, while high sensitivity will improve the cost effectiveness of malaria diagnosis since the RDT is unlikely to miss-out the non-infected individuals.

Finally, it is pertinent to mention one important diagnostic advantage in microscopy which RDTs lack - the ability of the analyst to observe the morphological features of the parasite under the microscope. This makes it possible for the microscopist to identify the different parasite forms and stages commonly seen under the microscope, a feature which has a lot of implications for the diagnosis of critical parasite forms like schizonts and gametocytes. The inability of RDTs to detect such parasite forms may scale down the gravity of detection of infection especially during severity, thereby contributing directly or indirectly to morbidity and mortality. Wilson (2013) listed several other pitfalls in using RDTs for the diagnosis of malaria to include: possibility of cross reactions of the antigens in the immunochromatographic strip with rheumatoid factor, autoantibodies and certain other non-malarial infections; false positive results for *Plasmodium* species that are absent in blood when *P. falciparum* is in high concentration; the continued presence of *p*HRP-2 antigens in blood several weeks after treatment, even when parasite is already cleared from the blood and inability to fairly assess the degree of parasitaemia, among others.
Nevertheless in settings where microscopy is unavailable using RDTs can lead to significant reduction in the over prescription of anti-malarial drugs. However blood microscopy remains the standard method for diagnosing malaria since it detects all cases of Plasmodium species and allows visualization of parasite growth stages which is essential in making therapeutic decisions.

5. CONCLUSION

Stained blood film microscopy and rapid diagnostic test each with its characteristics, strength and the limitation together present the best hope for diagnosis as a key component of successful malaria control hence rapid diagnostic test does not eliminate the need for stained blood film malaria microscopy therefore all negative rapid diagnostic test must be followed with stained blood film microscopy to confirm the result. Microscopy is the more reliable method in areas where malaria is most prevalent. Carestart™ RDT cannot offer a good alternative since it has lot of limitation. Moreover, when a rapid diagnostic test is used alone it should be followed with stained blood film microscopy to ascertain in the degree of infection and to determine the malaria specie involved for proper treatment. This is because the stained blood film microscopy reveals the degree of infection as well as the species involved.

6. RECOMMENDATION

Rapid diagnostic test (RDT) has shown a proven efficacy in the diagnosis of malaria. Although Carestart™ Rapid diagnostic test had some limitation because false negative results were obtained, it can only be recommended that laboratories in developing countries should continue to employ the Giemsa quantitative analysis for routine purposes so as to achieve effective diagnosis, disease control and efficacious treatment of malaria. It is imperative that malaria microscopy, being a reliable, affordable and accessible technique be assessed regularly against emerging technologies for malaria diagnosis.

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