Original Research Article

Comparative study of the Rapid Diagnostic Test ~~performance~~ and microscopy ~~examination using~~ with PCR as a reference ~~technique~~ in ~~*Plasmodium falciparum* identification in~~ placental malaria diagnosis ~~infection~~ in Burkina Faso

ABSTRACT

Aims:The aim of this study was to assess the performance of the Rapid Diagnostic Tests and microscopy ~~identification~~ in assessing the burden of placental infection using PCR as a reference technique.

Methods: This studywasconducted ~~during one year~~ from April 2019 to March 2021in pregnant women at delivery in four health districts of Ouagadougou, the capital city of Burkina Faso. The study involved collecting placenta blood from 531 women at delivery placenta for RDTs detection of *Plasmodium falciparum*microscopy identification blood smears examination following by parasite DNA extraction for Nested PCR of *Plasmodium falciparum* detection.

Results:

The prevalence of the placental infection of *Plasmodium falciparum*identified by RDT, optical microscopy, and Nested PCR were 5.46%, 6.98% et 8.29% respectively. The estimated values of sensitivity, specificity, Positive Predictive Value and Negative Predictive Value of RDT were respectively 51.72%,95.02%, 34.10% and 97.15%while those of microscopy were 100%, 98.58%, 84.10% and 99.19% respectively.

Conclusion

The results ~~obtained from this study showed~~revealed a strong correlation between RDT ~~method~~ test and microscopy.

Keywords: Malaria; Plasmodium falciparum; diagnosis; microscopy; RDTs; PCR; Placenta; Pregnant women

1. INTRODUCTION

Malaria is a public health problem particularly in the subtropics of the world. The burden manifests more in ~~P~~pregnant women and child under five ~~bear the brunt of malaria consequences~~ [1]. According to the World Health Organization (WHO) 2020 report, 241 million cases of malaria were recorded worldwide causing 627000 deaths. The African Region has borne over 90% of the total burden of malaria disease and mortality[2].

Malaria present multiple consequences in women in pregnancy with a negative impact on the progression of pregnancy as well as on the news born at delivery. Indeed, complicationsin pregnant women infectedwith *Plasmodium falciparum*include as far as abortion, prematurity, low birth weight with hematological manifestations threatening unfortunately mothers and fetus lives [3]. In to order set an adequate malaria disease management strategy,particularly in women in pregnancy and to help controlling the disease, a correct diagnosis of malaria by microscopy and / or RDTs is needed. RDTs are normally available in all community clinics (CSPS)in Burkina Faso as an alternative to microscopy in areas where microcopy is not available. The performance of such RDTs has been studied extensively from drop of blood at fingertips and venous blood. But, even the performance of these tests using placental blood has also been evaluated, they is still have limitations in the detection of low parasitaemia.

Nowadays, the development of molecular biology techniques such as polymerase chain reaction (PCR) has greatly improved the sensitivity and specificity of malaria diagnosis [4].PCR method allows the detection of very low parasitaemias (2 to 5 parasites/µl) but unfortunately not very suitable for routine diagnosis due to the expensivecost and itscomplex technique [5].

The objective of the present study was to evaluateof the diagnostic performance of RDTin assessing the burden of malaria placental diagnosis by comparing test results withhose of microscopy, having the PCR as reference.

2. MATERIAL AND METHODS

2.1Study area and population

A cross-sectionalstudywasconducted in maternity department of four health districts of the city of Ouagadougou named, the Boulmiougou district hospital, the Paul VI hospital, the Schiphra hospital and the Noongr-Massom district hospital.

Ouagadougou the capital city is located atlat. 12°22'N and long. 1°31'W. The population was estimated to 2 684 052 in 2020. The country is subjected to tropical savanna climate with a rainy season between June and October, a cold and dry season between November and January, and a hot and dry season between February and May making malaria transmission seasonal. There are three artificial lakes located within the city intended to supply water to the population. Malaria transmission is considered to be high in Ouagadougou.

The 4 sites selected correspond to the main hospitals in Ouagadougou. Boulmiougou District Hospital is to the west of the town. The Paul VI hospital in the district of SIG-NONGHIN located in the northern part of Ouagadougou While the Schiphra Hospital and the district hospital of Noongr-Massom are in the east (Figure 1).

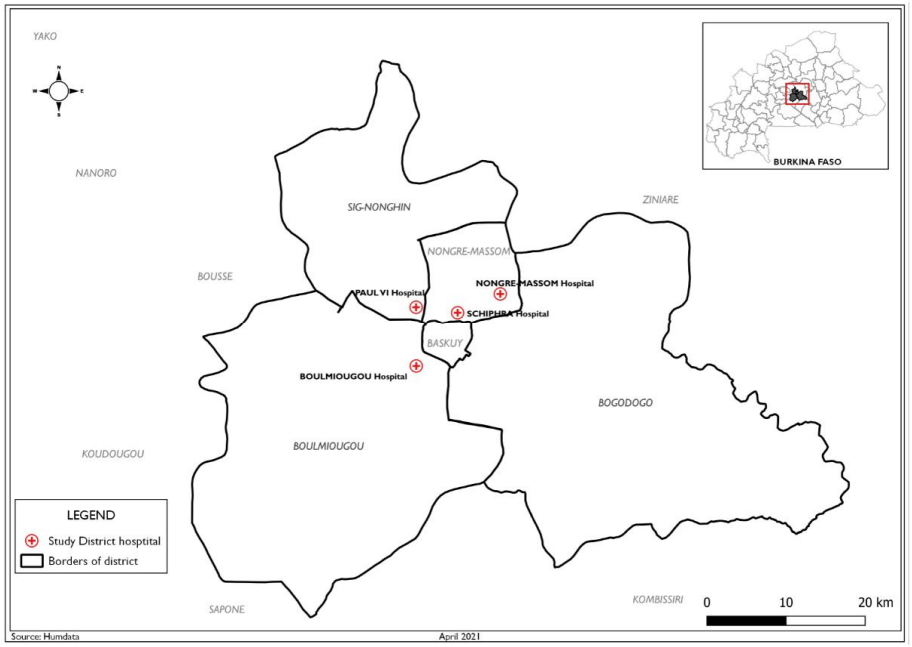

Figure 1: Map presenting the four districts hospitalsof the study areas [6].

2.2 Study population and samples

This study involved all pregnant women who accepted to participate and signed the written information consent formaccording to the inclusion and exclusion criteria. HIV positive pregnant women were excluded.

Blood sample from the placenta's maternal facewas used to prepare, thick and thin blood smears according to WHO protocol [7]. Besides,blood spotting on filter paper (Whatman N° 3) were realized for PCR in order to detect the placenta infection by *Plasmodium falciparum*.A total of 531 women were enrolledin the study. The study was carried out during one year from April 2019 to March 2021.

2.3 Demographic and clinical data collection

A structured questionnaire was used to capture demographic and clinical data from pregnant women participating to the study. Data collected included age, marital status, educational level, occupation, current and previous pregnancies, environmental and living conditions. Information about the use of Intermittent Preventing Treatment (IPT).The used or not of Insecticide Treated Nets (ITNs) was also recorded.

2.4 Placental malaria detection

2.4.1 Rapid diagnostic test (RDT) method

The commercially available RDT kit, « SD Bioline Malaria Ag-Pf » (STANDARD DIAGNOSTIC, INC. www.standardia.com) wasused for malaria detection.The batch number was 05CDDO35A. The product was manufactured in March 2019 and the expiration date listed was March 2021. Each box of 25 individually sealed strips with a desiccant was

supplied with loops, alcohols wabs and lancets. Temperature and humidity condition were monitored during transportation and storagein the hospital.Tests to detect the presence of the parasitewere performed following the manufacturer's instruction. Briefly, 5µl of placental bloodwere loaded with a transfer pipette in theindicated loop. In case the control line did not appear, the result was interpreted as invalid and the test was repeated.Test lines were interpreted as negative(no line visible) or positive (visible line). In case of visible line, its intensity varies according to the cases(Figure 2).Readings were performed 15 minutes after application of the sample and diluent

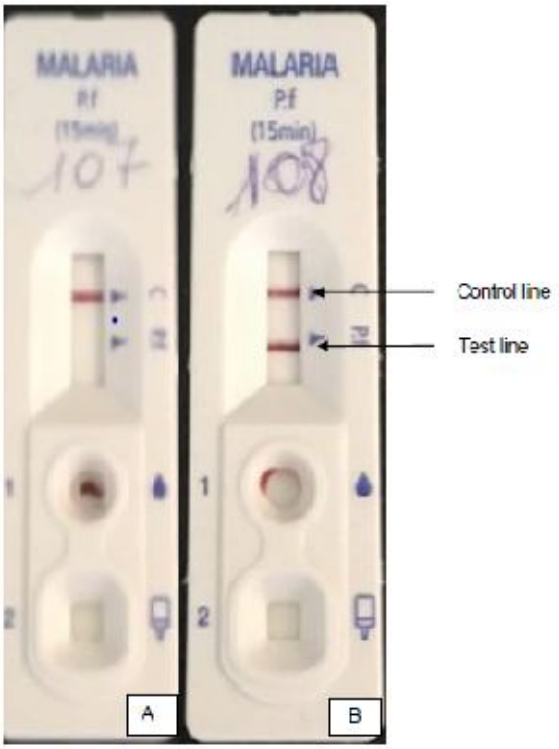

Figure 2:Malaria diagnosis with RDT. A) a negative test; B a positive test

2.4.2Microscopy

Optical Microscopy, was used according to the WHO reference method for malaria diagnostic.Thick and thin blood films were prepared on clean and grease-free glass microscope slides with the maternal face blood of theplacenta and examined by two microscopists after staining with 5% Giemsa solution for 30 minutes.Any discrepancy was read by a third reader.

The thin blood film was used to confirm the infection but mostly to identify the species of Plasmodium.The thick blood smear (Figure 3) was examined and used to calculate the parasite density of enrolled patients. Parasite density was calculated by counting the number of asexual parasites against a set number of white blood cells (WBCs) – typically 200 or 300 – in the thick blood film, using a hand tally counter. Once a field has been started, it should

alwaysbe counted to completion; the final WBC count is therefore rarely exactly to 200. If more than 500 parasites have been counted before 200 WBCs have been reached, the count is stopped after the reading of the last field has been completed. Parasite density expressed as the number of asexual parasites per microliter (µl) of blood, will be calculated by dividing the number of asexual parasite by number of WBCs counted and then multiplying by the WBC density (for patient enrolment, the WBC is assumed to be 8000 WBCs/µl, in the other assessments it will be based in the most recent WBC count). Parasite densities (parasite/µl of whole blood) were then calculated using the following formula:

$$\text{Parasite density/µl} = \frac{\text{Number of parasite counted} \times 8\,000}{\text{Number of WBC counted}}$$

When the number of asexual parasites is less than 10 per 200 WBCs, counting has been done against at least 500 WBCs (i.e. to the completion of field in which the 500th WBC is counted). A thick blood smear has been considered negative when the examination of 1000 WBCs does not reveal any asexual parasites. An external quality control of slides reading has been established with an independent laboratory.
At least 100 high power fields were examined before a thick smear was declared negative

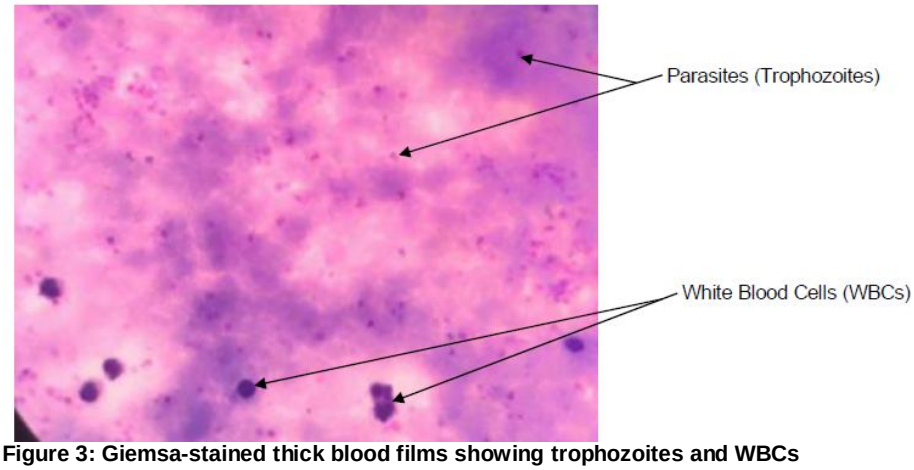

Figure 3: Giemsa-stained thick blood films showing trophozoites and WBCs

2.4.3 Nested-PCR assay

2.4.3.1*DNA* extractionmethods

Plasmodium falciparum DNA wasextractedfromdriedblood spots usingQIAamp® DNA Mini Kit (Qiagen,51304) according to the manufacture'srecommendation. Eluted DNA wasimmediatelyused in amplification reactions or stored at – 20 □C until PCR was performed.

2.4.3.2 Nested-PCR analysis

The DNA amplification methodwasdescribedelsewhere by nestedpolymerasechain reaction.The primerssequences for first (nested) amplification were: rPLU5 5-CCT GTT GTT GCC TTA AAC TTC-3 (forward) rPLU6 5-TTA AAA TTG TTG CAG TTA AAA CG-3 (reverse). For the second (nested) amplification wereused rFAL1 5-TTA ACC TGG TTT GGG AAA ACC AAA TAT ATT-3 (forward) rFAL2 5-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3 (reverse).

Amplification was performedanApplied Biosystem 2720 Thermal cycler (Part n°4369669 Serial N° 27250192363).The finalvolume of the preparationwas 20µl containing 1µl of genomic DNA, 2µl of polymerase chain reaction (PCR) buffer 10X, 0.5µl of 10µM of each primer, 1.25µl of 1 mM of dNTP, 0,8µl of 50mM of $MgCl_2$and 0,1of 5UI Taq polymerase. The system was programmed to 5 min for initial denaturationat 95°C, to 1min of denaturation at 94°C and this was followed by 24 cycles, each consisting of 1 min of denaturation at 94°C, 2 min of annealing at 58°C, 2 min of extension at 72°C. At the final cycle, an additional 5 min of incubation at 72°C wasperformed to complete the extension. For second amplification the cycle condition outer PCR 30 cycles. The amplified PCR productswereeitherstoredat + 4°C or analyzedimmediately by electrophoresis on anagarose gel at 1.5%.
DNA fragments from the second amplification electrophoresis were evaluated and the data interpreted as positive for *Plasmodium falciparum* when the DNA size is approximately 205 bp.

2.5 Statistical analysis

Data were analyzed using STATA software. The *p value*< 0.05 wasretained as the significative level for all these tests.For the calculation of sensitivity, specificity, negative and positive predictive value, the following formulashave been used:
The Sensitivitydefined as the probability that a test result being positive when the causation agent is present. It is estimated by the proportion of true positive in sick subjects. As a percentage, it represents the actual rate of positivity. It is calculated as follow:

$$\text{Sensitivity (\%)} = \frac{TP}{TP + FN} X\ 100$$

With: TP= number of true positive results and FN= number false negative results
False Negative rate: is probability that test result will be negative when the causation agent of disease is present.
The*Specificity* is defined as the probability that the result of a test being negative when causative agent of the disease is absent. It is estimated by the proportion of true negative in non-sick subjects. As a percentage, it represents the real rate of negativity. It is calculated as follow:

$$\text{Speci icity (\%)} = \frac{TN}{TN + FP} X\ 100$$

With: TN= number of truenegative results, FP= Number of false positive results
False positivity rate: this is the probability that result of a test will be positive when the causative agent of the disease is absent.
The positive predictive value(PPV) is the probability that the causation agent of a disease is present when the test is positive. It is estimated by the proportion of true negative (TN) among the subjects declared positive.

$$PPV = \frac{TN}{TN + FP} X\ 100$$

3. RESULTS AND DISCUSSION

3.1 *Study population* Sociodemographiccharacteristics

A total of 531 pregnantwomenwereincluded in thisstudy. The study participants average age was 26.9 years. Most of the participants were enrolled at the Paul VI district Hospital and at

the Noongr-Massom district hospital:36% (191/531) and 38% (201/531) respectively.The number enrolled at Boulmiougou district hospitaland Schiphradistrict hospitalare quite similar 12% (64/531) and, 14% (75/531) respectively (figure 4)

Figure 4: Proportion of enrolled pregnant women per study site

The present study highlights the possible setbacks in correctly identifying placental malaria infection by RDT and microscopy in pregnant women. This social layer was chosen because it is particularly vulnerable. Pregnancy weaker her immunity and make her more susceptible to malaria infection, increasing the risk of disease, severe anemia and death.
The nested PCR technique used in our study is an analytical technique that improves the PCR product. The nested PCR technique requires reagent it requires expensive reagents and apparatus. As a result, in more developing countries like Burkina Faso, PCR is not a common technique in biomedical analysis laboratories but it is an adequate molecular technique recommended in therapeutic efficacy studies and in epidemiological research.

3.2 Results of biological analyzes
3.2.1 Placental infection

We determined the prevalence of the placental infection of *Plasmodium falciparum*based the threemethodsusing RDT, microscopy, and PCR (Figure 5).

Figure 5: Placental prevalence of the three different diagnostic methods

The aim of this study was to determined prevalence of placental infection of *Plasmodium falciparum* in pregnant women receiving intermittent preventive treatment in Burkina Faso. 531 pregnant women was included in this study,the use of RDT, microscopy and PCR considered as the reference method respectively gave the prevalence of placental infection with*Plasmodiumfalciparum*5.46%, 6.98%, 8.29%. The prevalence found in this study is lower than those revealed by Liu *et al* 2016 who found respectively 12.6%, 0.9% and 10.6% [8]. In addition, it is possible that our study presents lower prevalence of placental malaria because pregnant use of intermittent preventive treatment and treated mosquito net. Use of IPT and ITNs reduces the risk of malaria and placental malaria infection.

Out of 37 positives by microscopy only 14 are positive by RDT and PCR however out of 494 negatives by microscopy 15 are positive by RDT and one by PCR and six positives by PCR (Figure 6)

Figure 6: Flow diagram showing malaria diagnosis using microscopy, RDT and PCR

3.2.2 Comparison of *P.falciparum* positive cases detected by the RDT compared to PCR

The table I resume results of RDT compared with PCR 15 subjects were both RDT and PCR positive while 491 were negative. In fact, out of 29 RDT positive, 14 were declared negative by PCR (48.27%). On the other hand, out of 502 results declared negative with RDT, 25 were revealed positive by PCR (4.98%).

Table I: RDT value compared to PCR value

RDT		PCR	
	Positive	Negative	Total
Positive	15	14	29
Negative	25	477	502
Total	40	491	531

A false negative rate of 4.98% was found for RDT. However, Alemu *et al.* 2014 also used nested PCR as a reference technique and found a false negative rate of 13.1% in their study.

False negative are a big public health problem because there is a part of the population that returns home without a correct diagnosis and treatment, not complying with the rule fast and correct diagnosis, and treatment with confirmed presence of the parasite.

3.2.3 Comparison of *P.falciparum*positive cases detected by Microscopy to PCR

The table IIrepresent microscopy results compared to PCR results. These two tests show that 33 parturients were both microscopy and PCR positive while 498parturients were negative. In fact, out of 37 positives by optical microscopy, 4 were declared negative by PCR. On the other hand, out of 494 negatives, 7 were PCR positive.

Table II:Results of microscopy compared with PCR

Microscopy	PCR		
	Positive	Negative	Total
Positive	33	4	37
Negative	07	487	494
Total	40	491	531

3.2.4 Comparison of the performance of RDT with microscopy

In the table III are presented the results of RDT compared with microscopyUsing PCR as the reference. RDT and microscopy had comparable sensitivities (p=0.17), comparable specificities (p=0.68) and negative predictive values comparable (p=0.82) but these two techniques didn't have comparable positives predictive values (p=0.03). These comparisons show that RDT and microscopy do not have the same diagnostic value.

Table III: Comparison of parameter of RDT with microscopy

Diagnostics performence	RDT	Microscopy	*P*
Sensitivity	51.72% (15/29)	100% (37/37)	0.09
Specificity	95.02% (477/502)	98.58% (487/494)	0.68
Positive predictivevalue	34.10% (15/44)	82.10% (37/44)	**0.01**
Negativepredictive value	97.15% (477/491)	99.19% (487/491)	0.82

Several studies have been carried out on the comparison of diagnostic tests for malaria in Burkina Faso, but the particularity of the present study is that it is focused on maternal malaria infection due to the complexity of malaria in pregnancy

The RDT and microscopy had a sensitivity revealed a placental infection of 51.72%, 100% withspecificity 95.02%, 98.58%, .Our results are lower than Kattenberg et al., 2012 who found 47% and 30% respectively for RDT and microscopy [1]. Our study therefore shows decrease in the prevalence of malaria in pregnant women in Burkina Faso. This decrease can be explained by improved prevention of malaria in women through chemoprophylaxis and the use of treated mosquito net.

The use of microscopy and PCR revealeda *Plasmodium* infection of 6.97% and 7.53%, respectively. Microscopy had a sensitivity of 89.19% withspecificity 98.58%. Our results are below than those of Yavo et al in 2002 in Côte d'Ivoire [9] which showed sensitivity of 100% and specificity of 88.1% and those of Munier et al 2009 in Senegal with of 96% and specificity 87% [10]. There was no significant difference between RDTs and microscopy in detecting placental malaria (*P*= 0.82)

The use of RDT and PCR revealeda *Plasmodium* infection of 5.46% and 7.53% respectively. RDT had 51.72% with specificity 95.02%.

The positive predictive value was respectively 34.10% and 82.10%for RDT and microscopy. These values are due to the presence of detected false positive. Our results are below than those presented by Mfuh et al 2019 in USA [11] which had found respectively 94%, 99% and 54%.

The negative predictive value probability showed that the disease was absent when the test is negative. In our study for RDT, microscopy it was respectively 97.15% and 99.19%. These values are due to the presence of false negative. This result looks higher than those presented by Daniel et al 2007 in Bangladesh who found 72.7% [12]. This negative predictive value gives confidence that if RDTs are to diagnose malaria in pregnancy very few infected women will be missed.

The present study highlights the possible setbacks in correctly identifying placental malaria infection by RDT and microscopy in pregnant women. This social layer was chosen because it is particularly vulnerable. Pregnancy weaker her immunity and make her more susceptible to malaria infection, increasing the risk of disease, severe anemia and death.

The nested PCR technique used in our study is an analytical technique that improves the PCR product. The nested PCR technique requires reagent it requires expensive reagents and apparatus. As a result, in more developing countries like Burkina Faso, PCR is not a common technique in biomedical analysis laboratories but it is an adequate molecular technique recommended in therapeutic efficacy studies and in epidemiological reserch.

Most of the accross-sectionals urveys use microscopy as a comparator to check the diagnostic accuracy of RDTs, due to non-availability of resources and expertises in the field condition to deploy molecular tools.

4. CONCLUSION

In the context of the burden of malaria on pregnant women in Sub-Saharan Africa and in Burkina Faso, it is very important that good and effective diagnosis methods be used to allow prompt malaria management and limiting the know consequences of malaria pregnancy.Microscopy and RDT remain the preferable method in field settingin addition, microscopy and RDT were unable to detect high number of *P. falciparum* cases that were identified by PCR. Our study determined the sensitivity, specificity, negative predictive value and positive predictive value of rapid diagnostic test, microscopy and polymerase chain reaction in the diagnosis of pregnant women. The results obtained from this study showed a strong correlation between RDT method and microscopy.

CONSENT

Written informed consent was obtained from all study participants.

ETHICAL APPROVAL

Approval for this study was granted by the National Ethics Committee for Health Research of Burkina Faso (deliberation N° 2019-4-056). The study received administrative approval from the district medical officer, the mayor, and the head of each local health centerwhere the study took place. Pregnant women were approached when reporting delivery. Only pregnant women volunteer who signed an inform consent form for their participation were enrolled

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

WHO: World Health Organization, DNA: Deoxyribonucleic acid, *Pf:Plasmodium falciparum*, RDT: Rapid diagnosis test,PCR: Polymerase chain reaction, IPT: Intermittent Intermittent, Preventing Treatment, ITNs: Insecticide Treated Nets, Ag: Antigen, UV: Ultra violet WP: *WP : positive witness, WN : negative witnes,* bp: base pair, TP= true positive, FN= false negative