Asymptomatic *falciparum* Malaria and its Effects on Type 2 Diabetes Mellitus Patients in Lagos, Nigeria

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Abstract

Background: Asymptomatic malaria (ASM) constitutes a reservoir of malaria parasites that sustain transmission and threaten elimination efforts. Studies have also shown a significant relation between insulin resistance and malaria infection. However, data on the clinical effects of ASM and its patterns of carriage among adult malaria patients is limited.

Objectives: To determine the prevalence of ASM due to *Plasmodium falciparum* among adult type 2 diabetes (T2DM) patients in Lagos, Nigeria; to assess the diagnostic performance of light microscopy and histidine-rich protein 2 rapid diagnostic test (HRP-2 RDT); and to determine the effects of ASM on glycemic control and anemia.

Materials and Methods: This cross-sectional study enrolled 208 afebrile, nonobese, nonhypertensive T2DM patients, aged 40–70 years, undergoing treatment (adherence, ≥95%) at six private health facilities in Lagos, Nigeria, between March and August 2015. Sociodemographic data were obtained using a semi-structured questionnaire and clinical case files. Venous blood samples were collected and processed for fasting blood sugar estimation, packed cell volume determination and malaria parasite detection by HRP2-RDT, light microscopy and polymerase chain reaction (PCR).

Results: The mean age of the patients was 54.5 years. ASM was diagnosed in 16.8%, 7.2% and 4.3% of the patients by PCR, light microscopy and HRP2-RDT, respectively. ASM was significantly ($P < 0.05$) associated with poor glycemic control, anemia and insulin resistance. The overall parasitemia ranged from 85 to 3789 parasites/μL (median, 1580 parasites/μL). Benchmarking against the PCR results, light microscopy and rapid diagnostic tests were found to have a sensitivity (95% confidence interval) of 42.9% (26.5–59.3) and 22.9% (12.1–39), respectively, in diagnosing ASM.

Conclusion: This study revealed that T2DM patients in Lagos, Nigeria, are potential reservoirs of asymptomatic *Plasmodium falciparum*, which has a significantly negative effect on glycemic control and anemia. The study also found PCR to be the most effective diagnostic method.

Keywords: Anemia, asymptomatic malaria, falciparum, glycemic control, Nigeria, type 2 diabetes

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INTRODUCTION

Malaria remains a significant health burden in sub-Saharan Africa. However, there has been a major decline in malaria morbidity (by 42%) and mortality (by 66%) between 2000 and 2015. To further address malaria-related challenges, many African countries have adopted WHO's Global Technical Strategy for Malaria 2016-2030.[1,2] Nigeria is among the 15 African countries responsible for 80% of global malaria cases and 78% of deaths.[1] Nonetheless, the country has recently experienced a significant reduction in malaria parasite rate in children aged <5 years, from 42% in 2008 to 27% in 2015, coupled with a reduction in the overall malaria deaths from 300,000 to 122,800 in 2016.[3,4] Notably, the Nigerian government has also adopted the global malaria elimination strategy through its National Malaria Strategic Plan 2014–2020.[5]

For eliminating malaria, its silent reservoirs should be identified in high-risk populations using highly sensitive and accurate diagnostic tools, in addition to sustaining its clinical management and integrated vector control efforts in transmission-prone areas.[6,7] High-risk populations often comprise adults with asymptomatic malaria (ASM) who do not seek treatment, remain undiagnosed and serve as hotspots of malaria transmission all year round, with varying intensity.[6] Therefore, ASM plays a critical role in delaying the global elimination of malaria.[8,9] ASM is also of clinical importance because of its health impact in humans, including pregnant women.[8]

Sub-Saharan Africa is parallelly also facing a diabetes crisis, wherein >95% of the cases are of type 2 diabetes mellitus (T2DM).[10] An estimated 15.5 million Africans aged 20–79 years were reported to be suffering from diabetes in 2017.[11] The global excess mortality attributed to diabetes in adults has been estimated to be 3.8 million deaths.[10] A significant proportion of such mortality is attributed to poor glycemic control, in addition to other factors for which mechanisms of occurrence are not completely understood.[12] Recently, a study from Central Africa reported a significant relation between insulin resistance and malaria infection.[13] However, despite the high burden of malaria and T2DM in sub-Saharan Africa, limited data exist regarding their co-occurrence and its associated clinical effects.[13-16]

In Nigeria, T2DM currently affects an estimated 4 million adults,[17] making the country one of the most burdened countries in sub-Saharan Africa. However, there is currently paucity of information on the co-occurrence of ASM and T2DM among Nigerian patients. Further, the impact of such a co-occurrence on diabetes management outcomes such as glycemic control remains unknown. Understanding patterns of interactions between malaria and T2DM in Nigeria may guide development of strategies to reduce the increasing T2DM trend and improve its case management. Accordingly, the primary objective of the present study was to determine the extent of ASM parasite reservoirs among a cohort of adult T2DM patients in Lagos, Nigeria. As secondary objectives, the effects of ASM on glycemic control and anemia and the performance of the following diagnostic tools were also evaluated: histidine-rich protein 2 rapid diagnostic test (HRP2-RDT), light microscopy and polymerase chain reaction (PCR).

MATERIALS AND METHODS

Ethical approval
The Lagos State Hospital's Ethics Committee approved this study (Ref. no.: LSHMB 027) on February 4, 2015. All study participants provided their written informed consent prior to inclusion in this study.

Study site
This study was conducted in Alimosho, a local government area of Lagos, Nigeria. The area is a malaria-endemic area with a catchment population of 1,277,714 in 2006. In the study area, similar to other parts of Lagos, malaria transmission is perennial during the rainy (April–October) and dry (November–March) seasons, with *Plasmodium falciparum* being the major causative agent and *Anopheles gambiae* the major vector.[18] T2DM is also a health problem among adults living in Alimosho, and cases of poor glycemic control among patients attending public health facilities have been reported.[19] Based on the availability of health information system to confirm diagnosis, track the duration of T2DM and provide evidence of treatment with antidiabetic therapies, six private health facilities providing T2DM care with laboratory support were purposively selected [Figure 1]. They included Talent Specialist Hospital, Egbeda; Santa Maria hospital, Igando Egan; Jamtec Clinicals, Ikotun; Crest Specialist Hospital, Isuti; Blessing Clinic, Akesan and Pakal Hospital, Obadore. The locations of these health facilities were mapped using geographical positioning system [Figure 1].

Study population
The study population comprised afebrile patients of both genders, aged ≥40 years, who had a confirmed diagnosis of T2DM and had been receiving its medications for at least 3 months before the study. Patients with T2DM were defined as those on oral hypoglycemic agents and with a homeostasis model assessment for insulin resistance (HOMA-IR) value of ≥2.5 in at least two

Figure 1: Location map showing Alimosho local government area and the study sites. ● Talent specialist hospital; ● Santa Maria hospital; ● J Jamtec Clinicals; ● Crest specialist hospital; ● Blessing Clinic; ● Pakal hospital

measurements.[20] This was above the mean HOMA-IR of 1.78 found in 10 randomly selected age-matched nondiabetic controls in the study settings. Other inclusion criteria were a ≥95% treatment adherence in the previous 2 months (i.e., not missing more than six doses during this period for twice-daily or thrice-daily regimens) and no history of malaria. These patients were enrolled by convenience on their clinic days during the study period. Patients who were obese (body mass index of ≥30 kg/m²), hypertensive (mean blood pressure of >140/90 mmHg for two measurements) and had other diabetes-related complications were not enrolled in this study.

Sample size calculation

The minimum number of T2DM patients required for this study was determined using Epi Info 7.0 (CDC, Atlanta, GA, USA) with significance (α) set at 5% and power ($1 - \alpha$) at 80%, as recently described by Sharma.[21] Assuming that an estimated 10% of the T2DM patients on oral hypoglycemic agents for at least 3 months before the study have malaria parasites in their blood with a confidence limit of 95%, the minimum sample size was calculated using the formula:

$N = z^2 pq/d^2$, where N = sample size, z = 1.96, p = 0.22, q = 0.78 and d = 0.05

Considering an attrition rate of 10%, the minimum sample size was calculated to be 153 patients. In this study, a total of 208 eligible patients who met all the inclusion criteria for this study were enrolled.

Study design

This was a cross-sectional, prospective study of ASM infection during March–August 2015, among T2DM patients receiving care at selected private health facilities in Alimosho, Lagos, Nigeria. After providing their written informed consent, the patients were asked to

fast, not consume alcohol, smoke tobacco or engage in strenuous work activity/exercise after 10 pm the day before examination. On the examination day, between 8 and 10 am, a pretested semi-structured questionnaire was administered to the patients for obtaining the sociodemographic information, including the number of children aged <5 years in their household and frequency of fruits and vegetable servings per week. The patients were also clinically examined, underwent anthropometric measurements and provided venous blood samples for laboratory measurement of packed cell volume (PCV), fasting blood glucose (FBG) estimation and malaria diagnosis. Two different trained microscopists computed the results of light microscopy and HRP-2 RDT, with the results of each being blinded from the other.

Anthropometric measurements

All patients were directed to be in light clothing and barefooted for their body weight and height measurement using an electronic weighing and a meter rule. The body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Normal BMI was defined as 18.5–24.9 kg/m², underweight as <18.5 kg/m² and overweight as 25–29.9 kg/m².

Blood glucose estimation

FBG was estimated from plasma recovered by centrifugation of whole blood samples collected into fluoride oxalate using a calibrated glucometer (Roche Diagnostics, Indianapolis, USA). Each assay was done in duplicates by a single trained medical laboratory scientist and the average of the two was computed. Patients with a FBG >126 mg/dL in two consecutive measurements were regarded as having poor glycemic control.

Packed cell volume determination

To determine PCV (%), heparinized tubes filled with whole blood were centrifuged at 10,000 rpm for 5 min in a microhematocrit centrifuge, followed by PCV measurement using a microhematocrit reader (Hawsley and Sons, London, UK). This was done by the same trained medical laboratory scientist. Participants with PCV values of <36% were defined as anemic, assuming that 3% PCV is equivalent to 1 g/dL hemoglobin concentration in whole blood.[22] In this study, mild, moderate and severe anemia is defined as PCV values of 33–36%, 30–33% and <30%.

Insulin resistance measurement

In a fasted state, plasma insulin concentration was measured by a solid-phase sandwich ELISA using the Insulin Human ELISA Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. HOMA-IR was calculated by

multiplying insulin (U/mL) by FBG (mg/dL) and dividing by 405.[23]

Detection, identification and quantitation of malaria parasites

Rapid diagnostic test

SD-Bioline Malaria-Ag-Pf/Pan™ (Batch no. 05CDD006A, Standard Diagnostic Inc. Suwon City, South Korea) test kit targeting the HRP2 of *P. falciparum* was used to detect its presence in the ethylenediaminetetraacetic acid (EDTA) blood samples of the patients. Same as light microscopy, this test was also carried out within 30 min of sample collection, according to the manufacturer's protocol. Results were interpreted following World Health Organization guidelines.[24]

Light microscopy

Within 30 min of blood collection in EDTA tubes, thick and thin blood films were prepared on two grease-free slides per sample, followed by staining with 10% Giemsa stain (Capitol Scientific Inc. Austin Texas, USA). Slides were examined under oil immersion by two trained microscopists blinded to HRP2-RDT results by counting the number of parasites against 500 leukocytes in the thick film and quantifying parasitemia as parasites per microliter of whole blood. This quantification assumed that there are 8000 leukocytes per microliter of blood. A slide was considered negative after examining 100 high-power fields without finding a malaria parasite.[25] The thin film was examined for malaria parasite speciation. All malaria parasites were identified as *P. falciparum*, and no discordance of >15% count were recorded to warrant examination by a third expert microscopist. For every positive slide, an average count of two readings was computed and recorded by the single trained microscopist.

Malaria diagnosis by polymerase chain reaction

a. Parasite genomic DNA extraction: For extracting the parasite genomic DNA, blood spot samples in 3 mM Whatman filter paper were hemolyzed by soaking in a 1.5-mL Eppendorf tube containing 0.5% saponin in phosphate-buffered saline (PBS) (pH 7.2), followed by incubation for 10 min at room temperature. After centrifugation at 14,000 rpm for 10 min, the supernatant was discarded and blood spots were washed with 1 mL of PBS. Then, 150 µL of 2% Chelex-100 (BioRad, Hercules, CA, USA) and 50 µL of sterile water were added to the blood spot, followed by incubation at 100°C for 10 min. After centrifugation at 10,000 rpm for 1 min, the supernatant was collected into a fresh Eppendorf tube and stored at 4°C prior to PCR assay within 24 h of preparation.

b. PCR: The nested PCR protocol of Fuehrer *et al*.[26] and Snounou *et al*.[27] was adopted to amplify the 18s rRNA gene of the malaria parasite using species-specific primers; cycling conditions and annealing temperatures are shown in Table 1. All PCR amplifications (Techne TC-312 thermocycler; Techne, Burlington, NJ, USA) were performed in 20 µL volume containing 1.5 mM $MgCl_2$, 200 nM of each of the dNTPs, 20 picomole of each primer and 1. 25 U of *Taq* polymerase (Invitrogen®, Carlsbad, CA, USA). Electrophoresis was performed on 2.5% agarose gel to analyze the PCR products. A sample was considered positive if a a 500-, 125- or 105-bp product for *P. falciparum*, *Plasmodium malariae* or *Plasmodium ovale* was detected. In this study, all the ASM cases detected were caused by *P. falciparum* [Figure 2].

Data management and analysis

Data were entered into Microsoft Excel, checked for entry errors, edited and transferred to SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA) for analysis. Categorical variables were expressed as frequency and percentages and continuous variables as mean ± standard error of mean and median. Medications taken by the patients were categorized into two groups: single therapy, if treatment involved only one hypoglycemic agent, or

Table 1: Primers, sequences, cycling conditions and annealing temperatures for the amplification of 18s rRNA genes of *Plasmodium* species

| Gene	PCR round	Primer name	Primer sequence (5′-3′)	Cycling conditions	Annealing condition (°C)	Positive control
18s rRNA	Primary	rPLU1 rPLU5	TTAAAATTGTGCAGTTAAAAGGA CCTGTTGTTGCCTTAAACTTC	Initial denaturation: 95°C for 5 min; PCR: 25 cycles of 94°C for 1 min, 58°C for 2 min, 72°C for 2 min; final elongation: 72°C for 5 min	58	3D7
18s rRNA	Secondary	rFALf rFALr rMALf rMALr rVIVf rVIVr rOVAf rOVAr	TTAAACTGGTTTGGGAAAACCAAATATATT ACACAATGAACTCAATCATGACTACCCGTC ATAACATAGTTGTACGTTAAGAATAACCGC AAAATTCCCATGCATAAAAAATTATACAAA CGCTTCTAGCTTAATCCACATAACTGATAC ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA ATCTCTTTTGCTATTTTTTAGTATTGGAGA GGAAAAGGACACATTAATTGTATCCTAGTG	Initial denaturation: 95°C for 5 min; PCR: 30 cycles of 94°C for 1 min, 65°C for 2 min, 72°C for 2 min; final elongation: 72°C for 5 min	65	3D7

PCR – Polymerase chain reaction

combination therapy, if treatment involved two or more oral hypoglycemic agents. Data of T2DM with and without ASM (as frequency and percentages) were compared using chi-square test or Fisher's exact test. The measures of performance of HRP2-RDT and light microscopy, such as sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV), were benchmarked against that of PCR and expressed as percentages (95% confidence interval [CI]).

RESULTS

Occurrence and sociodemographic determinants of ASM, anemia and poor glycemic control are summarized in Table 2. Of the 208 T2DM patients included in this study,
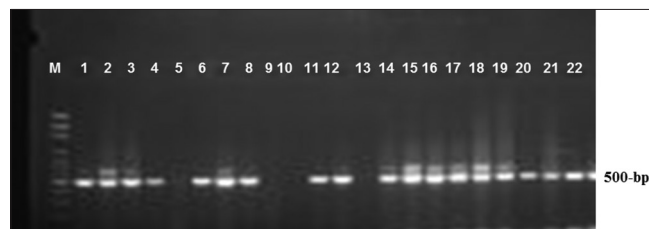
Figure 2: Agarose gel electrophoresis of the polymerase chain reaction amplified 18s rRNA gene of *Plasmodium falciparum* from the clinical samples of the type 2 diabetes mellitus patients. M = 100 base pair DNA ladder markers; Lane 1 = Positive control, 3D7 *Plasmodium falciparum*; Lanes 2–4, 6–8, 11, 12, 14–22 = *Plasmodium falciparum*-infected blood samples; Lanes 5, 9, 10 and 13 = non-*Plasmodium falciparum*-infected blood samples. Faint bands on lanes 2, 3, 7, 14–19 were artifacts

ASM was diagnosed in 35 (16.8%), 15 (7.2%) and 9 (4.3%) patients by PCR, light microscopy and HRP2-RDT, respectively. The PCR-positive patients also comprised all patients diagnosed by light microscopy and HRP2-RDT, thereby validating the use of PCR for benchmarking.

Forty-two (20.2%) patients had mild to moderate anemia and 138 (66.3%) had poor glycemic control, and ASM was significantly associated with both ($P < 0.04$). In addition, ASM was found to be significantly ($P < 0.05$) associated with age, male gender and living with 1–2 children aged <5 years [Tables 2 and 3]. Further, the overall parasitemia ranged from 85 to 3789 parasites/μL. The median of parasitemia was 1580 parasites/μL, FBG was 134.3 mg/dL and PCV was 37.8%.

Of all the clinical and behavioral factors analyzed, only HOMA-IR was found to be associated with ASM ($P < 0.05$). However, three of these factors, including a T2DM duration of >5 years and HOMA-IR >2.5, were significantly ($P < 0.05$) associated with poor glycemic control [Table 4], ruling out the influence of cofounders such as overweight and obesity.

Compared to PCR, the number of false negatives by light microscopy and HRP2-RDT were 20 (57.1%) and 27 (77.1%), respectively; interestingly, HRP2-RDT detected one ASM case not identified by PCR [Table 5].

Table 2: Sociodemographic determinants of asymptomatic malaria, anemia and poor glycemic control among the type 2 diabetes patients

Characteristics	n	ASM*, n (%)	P	Anemia, n (%)	P	PGC, n (%)	P	P^
Age (years)								
≤50	87	25 (28.7)	0.001	17 (19.5)	0.84	47 (54)	**0.001**	**<0.001**
>50	121	10 (10.7)		25 (20.7)		94 (78.5)		
Sex								
Male	93	21 (22.6)	0.046	20 (21.5)	0.67	55 (59.1)	**0.047**	**<0.001**
Female	115	14 (12.2)		22 (19.1)		83 (72.2)		
Education								
No education	31	5 (16.1)	0.78	7 (22.6)	0.95	10 (32.2)	**<0.001**	**<0.0001**
Primary	103	18 (17.5)	0.92	20 (19.4)	0.78	73 (70.9)	0.33	0.3
≥Secondary	74	12 (16.9)	Reference	15 (21.1)	Reference	55 (77.5)	Reference	Reference
Marital status								
Married	112	18 (16.1)	0.75	19 (17)	0.21	64 (57.1)	**0.02**	**<0.001**
Not married	96	17 (17.7)		23 (24)		74 (77.1)		
Occupation								
Working	125	20 (16)	0.69	20 (16)	0.06	75 (60)	**0.017**	**0.017**
Not working	83	15 (18.1)		22 (26.5)		63 (75.9)		
Household, number of children aged <5 years								
0	132	18 (13.6)	Reference	17 (12.9)	Reference	88 (66.7)	Reference	
1-2	58	15 (25.9)	**0.04**	16 (27.6)	**0.014**	42 (72.4)	0.43	0.9
≥3	18	5 (27.8)	0.11	9 (50)	**<0.01**	8 (44.4)	0.065	**0.007**
Use of LLIN the previous night among children aged <5 years living with type 2 diabetes patients (*n*=76)								
Yes	30	7 (23.3)	0.78	9 (30)	0.56	19 (63.3)	0.44	0.29
No	46	12 (25)		11 (25.9)		25 (54.3)		

P^ – P value adjusted by parasitemia for PGC. Significant *P* or *P^* values are in bold. PCR – Polymerase chain reaction; ASM – Asymptomatic malaria by PCR; PGC – Poor glycemic control; LLIN – Long-lasting insecticidal net. *$P<0.05$ was considered significant

Table 3: Assessment of asymptomatic malaria by glycemic control and packed cell volume among the type 2 diabetes patients

Parasite indices	*n*	PGC, *n* (%)	FBG (mg/dL), mean ± SEM	Anemia, *n* (%)	PCV (%), mean ± SEM
ASM^					
Yes	35	29 (82.9)	139.8 ± 5.9	13 (37.1)	35.3 ± 0.6
No	173	109 (63)	124.7 ± 7.2	29 (16.8)	39.5 ± 1.1
P		**<0.05**	**<0.05**	**<0.05**	**<0.05**
Parasitemia* (parasites/μL)					
<100	2	1 (50)	126 ± 4.4	1 (50)	35.8 ± 0.7
100-1000	5	2 (40)	134.3 ± 5.1	2 (50)	35.1 ± 0.5
>1000	8	6 (75)	145.2 ± 5.9	6 (66.7)	34.2 ± 0.4
P (ANOVA)		>0.05	**<0.05**	>0.05	>0.05
Parasitemia, range (median)		**Range^@ (median) mg/dL**		**Range^@ (median), %**	
85-3780 (1580)		120-158 (134.3)		32-41.3 (37.8)	

^ASM – ASM cases detected by PCR; *Parasitemia based on light microscopy; @Range (median) of ASM cases only. PGC – Poor glycemic control; Significant *P* values were bolded. PCR – Polymerase chain reaction; ASM – Asymptomatic malaria by PCR; FBG – Fasting blood glucose; PCV – Packed cell volume; SEM – Standard error of mean

Table 4: Clinical and self-management behavioral characteristics of the type 2 diabetes patients with asymptomatic malaria and poor glycemic control

Characteristics	N	ASM^, *n* (%)	P	Poor glycemic control, *n* (%)	P
Duration of diabetes, years					
≤5	116	19 (16.4)	0.84	63 (54.3)	**<0.001**
>5	92	16 (17.4)		75 (81.5)	
HOMA-IR ≥2.5					
No	77	7 (9.1)	**0.02**	38 (40.3)	**<0.0001**
Yes	131	28 (21.8)		100 (81.7)	
Medication					
Monotherapy	103	19 (18.4)	0.46	62 (60.2)	0.06
Combination therapy	105	16 (15.2)		76 (72.4)	
Frequency of exercise in the previous week, days					
<3	135	20 (14.8)	0.29	91 (67.4)	0.66
≥3	73	15 (20.5)		47 (64.4)	
Frequency of fruit and vegetable servings per day					
<3	139	27 (19.4)	0.16	95 (68.3)	0.39
≥3	69	8 (11.6)		43 (62.3)	
Frequency of self-blood glucose monitoring per week, days					
≤5	142	23 (16.2)	0.72	88 (62)	**0.05**
>5	66	12 (33.3)		50 (75.8)	
Cigarette intake					
Yes	23	5 (21.7)	0.5	19 (82.6)	0.08
No	185	30 (16.2)		119 (64.3)	

^ASM – ASM cases detected by PCR; Significant *P* values are in bold. HOMA-IR – Homeostasis model assessment for insulin resistance; PCR – Polymerase chain reaction; ASM – Asymptomatic malaria by PCR

Table 5: Performance evaluation of light microscopy and histidine-rich protein 2 rapid diagnostic test using polymerase chain reaction as a reference for the diagnosis of asymptomatic malaria among the type-2 diabetes patients

Diagnostic test	PCR results		Total	Performances, % (95% CI)			
	Positive	Negative		Sensitivity	Specificity	PPV	NPV
Light microscopy							
Positive	15	0	15	42.9 (26.9-59.3)	100	100	89.6 (82.3-93.9)
Negative	20	173	193				
Rapid diagnostic test							
Positive	8	1	9	22.9 (12.1-39)	99.4 (96.8-99.9)	88.9 (56.5-98)	86.4 (81-90.5)
Negative	27	172	199				

P<0.05 was considered significant. PCR – Polymerase chain reaction; CI – Confidence interval; PPV – Positive predictive value; NPV – Negative predictive value

Benchmarking against PCR, light microscopy was found to have a sensitivity (95%CI) of 42.9% (26.5–59.3), specificity of 100%, NPV of 89.6% (82.3–93.9) and PPV of 100% for diagnosing ASM, while those for HRP2-RDT were 22.9%(12.1–39), 99.4% (96.8–99.9), 86.4% (81–90.5) and 88.9% (56.5–98), respectively. The proportion of cases that were correctly diagnosed by HRP2-RDT and light microscopy were 86.5% and 90.4%, respectively, indicating that light microscopy is more accurate in diagnosing cases of ASM [Table 6].

Table 6: Diagnostic effectiveness analysis between light microscopy and histidine-rich protein 2 rapid diagnostic test for asymptomatic malaria in the studied patients

Indicator	Rapid diagnostic test	Microscopy
Number of cases correctly diagnosed	180	188
Proportion correctly diagnosed (%)	86.50	90.40*

*P (χ^2)=0.1

DISCUSSION

Nigeria's adoption of the global malaria elimination target by 2030 through its National Malaria Strategic Plan 2014–2020 has necessitated the need for new surveillance approaches aimed at identifying potential reservoirs of malaria parasites and development of novel interventions to interrupt transmission. In this study, 35 of the 208 afebrile T2DM patients were found to harbor *P. falciparum* by PCR. This finding agrees with a previous study conducted in Ghana by Danquah *et al*.,[16] who found that T2DM patients harbored *P. falciparum* asymptomatically. Therefore, this collective evidence suggests that adult T2DM patients are potential reservoirs of *P. falciparum*.

In malaria-endemic settings, it is well-known that adults are better protected from uncomplicated and severe malaria compared with children aged <5 years, as they are likely to have acquired antimalarial immunity over time from constant exposure to malaria parasites.[28] However, it is now important to investigate high-risk adult populations for asymptomatic carriage of malaria parasites in a malaria-endemic setting like Nigeria for ensuring malaria elimination. The ASM diagnosed among the adult T2DM cohort studied may also be attributed to antiparasitic immunity, which prevents parasitemia from reaching a clinical level called pyrogenic threshold and premunition for preventing secondary infections in adults with primary asymptomatic infection.[28-32]

In this study, the rate of ASM in T2DM patients according to PCR was 16.8%, higher than that found by light microscopy (7.2%) and HRP-2 RDT (4.3%). Danquah *et al*.,[16] in their study of 675 T2DM Ghanaians patients, also found higher ASM rate with PCR (14.1%) than light microscopy (0.9%). Therefore, our findings further add evidence that PCR is more sensitive in identifying *P. falciparum* reservoirs than light microscopy and HRP2-RDT, and thus its use would ensure that even cases of low parasitemia are detected. It also implies that in malaria endemic areas, if T2DM patients have poor glycemic control despite adherence to medication, PCR should be carried out despite negative malaria test by HRP-2 RDT or light microscopy to completely rule out ASM.[31]

The single positive case detected by HRP2-RDT for which PCR was negative suggests a false positive result due to persistent antigenemia because light microscopy was also negative. It has been shown that *P. falciparum* HP2 antigen may persist for weeks after successful clearance of the parasites by an effective antimalarial therapy.[33] Our findings also support previous findings of ASM being common in malaria-endemic settings and that light microscopy detects only a small proportion of these ASM cases.[34] In the current study, the median parasitemia level was 1580/μL (range: 85–3780/μL), which differs from the 880/μL (range: 80–4950/μL) found by Danquah *et al*.[16] in a Ghanaian population. However, such differences can be attributed to different sample size, geographical location and level of malaria elimination activities between the studied settings.

Although HRP2-RDT is considered an essential tool for enhancing the diagnosis of malaria in sub-Saharan Africa, especially in areas where access to light microscopy and PCR are limited or lacking, its performance in this study was not encouraging. In fact, of the three methods used, HRP2-RDT had the lowest sensitivity (only 22.9% compared with PCR). Benchmarking against PCR results, HRP2-RDT made correct diagnosis in only 86.5% of the cases compared with 90.4% by light microscopy. This can be attributed to our study having a number of cases with <100 parasites/μL, which is below the detection threshold of RDT.[34] In terms of light microscopy, its low sensitivity in this study may be attributed to the number of patients with submicroscopic ASM infections ($n = 20$). It should be noted that other studies have also recently reported submicroscopic malaria infection in T2DM patients and other carriage populations from sub-Saharan Africa.[16,34,35]

In this study, although HRP2-RDT diagnosed a lower proportion of ASM cases in T2DM patients than light microscopy, these results should be interpreted with caution. This is because previous studies have found inconsistent results while comparing these methods in different populations such as pregnant women, apparently healthy blood donors and other geographical locations in sub-Saharan Africa.[35-37] Therefore, to validate the findings of this study, the authors recommend large-scale studies from other parts of Nigeria where the efficiency of other diagnostic methods such as loop-mediated isothermal PCR may also be evaluated.

In this study, ASM (both microscopic and submicroscopic) was found to be significantly associated with poor glycemic control. As ASM was also significantly associated with HOMA-IR >2.5, insulin resistance may also be

an interlinking factor between ASM and poor glycemic control. Further, two recent studies on T2DM patients from Central Africa and Sweden reported a significant relation between insulin resistance and malaria infection.[13,38] The finding of the current study could connote serious clinical implications for Nigerian patients, as it suggests that ASM can potentially compromise the management of T2DM and may cause anemia of unknown etiology. However, the mild anaemia cases seen in this study may also be due to chronic hydrolysis of erythrocytes by the malaria parasites in the infected patients despite low parasitemia. Therefore, the authors recommend integration of ASM screening into the care of Nigerian patients with T2DM, especially among those with anemia and poor glycemic control despite >95% treatment adherence.

The present study also confirmed previously established factors for poor glycemic control in T2DM patients such as age, level of education, duration of disease and frequency of self-blood glucose monitoring.[39,40] Therefore, intensified and sustained awareness development through education, training and engagement of diabetes patients is also needed as a measure to control the rising trend of T2DM in the study area and Nigeria.

It is important to note that the present study is limited by its cross-sectional design and its sampling coverage. These factors hindered the determination of protective immunity against clinical malaria offered by ASM in the infected patients. This also prevents generalization of the findings to all T2DM patients in the country. Therefore, future longitudinal studies would not only increase sampling coverage but also characterize the genetic diversity and complexity of ASM infection among T2DM patients.

CONCLUSION

This study found that in Lagos, Nigeria, T2DM patients are potential reservoirs of asymptomatic *P. falciparum* and that ASM has a significantly negative effect on glycemic control. The authors recommend that in T2DM patients with poor glycemic control despite optimal treatment adherence, malaria screening should be carried out using PCR to detect submicroscopic ASM.

Ethical considerations
This study was approved by the Ethics Committee of Lagos State Hospital (Ref. no.: LSHMB 027) on February 4, 2015, and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki, as revised in 2013. All the participants provided written informed consent before their inclusion in this study.

Peer review
This article was peer reviewed by three independent and anonymous reviewers.

Conflicts of interest
There are no conflicts of interest.

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