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Research Article

Detection of Asymptomatic Malaria Parasites by Microscopy and CareStart™ Malaria HRP2/pLDH(*Pf*) among Newly Admitted Students on a University Campus, Southeast Nigeria

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Abstract

Background and Objective: Different types of malaria parasites affect human red blood cells. It exists in tropical regions and is transmitted by female *Anopheles* mosquito. Healthy subjects recorded wide prevalence of asymptomatic malaria parasites wherever malaria disease is endemic. This study determined the occurrence of asymptomatic malaria parasites among the newly admitted students on a University Campus, Southeast Nigeria. **Materials and Methods:** Blood samples of 210 students were investigated using microscopy and CareStart™ malaria HRP2/pLDH(*Pf*) rapid diagnostic test. Dipstick antigen-capture assay was done according to manufacturer's instructions. Blood smears were prepared and examined following standard procedure. Data were analyzed using Statistical Package for the Social Sciences and Chi-square. Accuracy of CareStart™ Malaria HRP2/pLDH(*Pf*) were also determined. **Results:** Forty-six (21.9%) students were identified as being infected with malaria parasites by microscopy. CareStart™ Malaria HRP2/pLDH(*Pf*) showed that out of 210 students, 40 (19.0%) were infected. Only *Plasmodium falciparum* was identified. The accuracy, sensitivity, specificity, positive and negative predictive values of CareStart™ Malaria HRP2/pLDH(*Pf*) were 97.1, 87, 100, 100 and 96.5%, respectively, well correlated with microscopy. The Likelihood ratio for positive and negative results were -87.9 and -86%, respectively. Both diagnostic tools showed that ages 24-27 years' age range had the highest (27.3%) percentage parasite positive respectively ($p > 0.05$) and female gender recorded higher percentage parasite positive with both test tools ($p > 0.05$). **Conclusion:** CareStart™ Malaria HRP2/pLDH(*Pf*) can reliably be used for malaria diagnosis in areas where microscopy is not easily accessible. Malaria prevention activities should be put in place by the University management.

Key words: Asymptomatic malaria parasite, *Plasmodium falciparum*, microscopic examination, rapid diagnostic test, prevention activities, infection, blood

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria remains one of the oldest and deadliest diseases in the world. Healthy subjects recorded wide prevalence of asymptomatic malaria parasites wherever malaria disease is endemic. Each year, more than 400,000 people die of malaria—a treatable disease. Early diagnosis and treatment of malaria is essential for both rapid and effective case management. High-quality malaria diagnosis, through microscopy or rapid diagnostic testing, is important in all malaria-endemic settings. WHO recommends prompt malaria diagnosis either through microscopy or rapid diagnostic tests (RDTs) for all patients with suspected malaria, before treatment is administered. Diagnostic testing enables health providers to swiftly distinguish between malarial and non-malarial fevers, facilitating appropriate treatment¹.

In 2016, there were 216 million cases of malaria in 91 countries, 5 million more than the 211 million cases reported in 2015, this marks a return to 2012 levels². Malaria continues to claim a significant number of lives: in 2016, 445,000 people died from malaria globally, compared to 446,000 estimated deaths in 2015. The burden is more in WHO African Regions³. Records reveal that African Regions continues to bear 89.81% (approximately 90%) (194 million) of malaria cases and 91% (407,000) of malaria deaths worldwide². Nigeria, the continent's most populous country, accounted for 27% of malaria cases and 24% of malaria deaths globally in 2016. Malaria is transmitted through the bites of infected female *Anopheles* mosquitoes and caused by *Plasmodium* parasites. Two of the parasite species that cause malaria in humans pose the greatest threat. The species are *Plasmodium falciparum* and *Plasmodium vivax*². Malaria has caused fever, low weight of new born babies, frequent hospital attendance, school absenteeism and increase in health seeking movements^{4,5}.

Asymptomatic malaria is as a result of prevalence of gametocytes at various stages of a malarial infection, the limit of infectiousness of the human host, the period of survival of the malaria parasite in human host and the development of immunity through repeated infection. Infected individuals who do not present symptoms remain capable of transmitting malaria parasites and in fact may account for 20-50% of onward transmission. In recent years, reports were published of numerous malaria surveys conducted in various parts of the world. In most of these, blood examinations of clearly healthy subjects recorded wide prevalence of asymptomatic parasitaemia wherever the disease is endemic⁶⁻¹¹. The relation of parasitaemia to severity of illness is different in different populations and age groups. In children, in areas of unstable

endemicity, a peripheral parasitaemia of 4% or more ($\geq 4\%$ of circulating red cells contain parasites) carried an increased risk of death⁸. A 4% parasitaemia in non-immune children or adults should be considered an indicator of high risk requiring supervised management. The present study therefore aimed to determine the asymptomatic malaria parasites among University students on Campus using light microscope and CareStart™ malaria HRP2/pLDH(Pf) as diagnostic tools.

MATERIALS AND METHODS

Study area: The study was conducted between April to September, 2018 at Evangel University, Akaeze. Evangel University is one of the private Universities granted approval by the Federal Government of Nigeria on February 28, 2012 and it started academic activities on November 17 of same year. Presently the University operates at its take off campus located in Okpoto community. Okpoto Community is a small community farthest away from the center of the town and cited near Village Rivers and surrounded by bushes and marshy lands. It is located in Ishielu Local Government Area of Ebonyi State, Southeast Nigeria (Fig. 1). Ishielu Local Government Area is made up of thirteen Communities or Districts which are Ezillo community, Azunyaba, Nkalaha, Nkalagu, Iyonu, Ntezi community, Okpoto community, Agba, Ezzagu, Emuhu-Ali, Amazu, Egge-Egde and Ebeagu Autonomous community. Ishielu lies between longitudes 7°45' and 7°50'E and 6°3' and 6°35'N with a population of 151,048, from 2006 census¹². The people of the Local Government area get their livelihood from farming and petty trading. Most of the communities in this Local Government including Okpoto are sparsely populated. Their level of hygiene is very low. They have land mass covered by bushes, streams, rivers, stagnant water. They have inadequate means of sewage disposal and sewage drainage system. Hence there was stagnant water within and around the homesteads. On campus, students' hostels are surrounded by overgrown grasses and students store water in buckets and bowels due to lack of steady water supply. These are features that promote breeding of vectors of parasites as well as infestation and infiltration of infective stages of some parasitic organisms.

Research design: The study was a descriptive survey design done from April-September, 2018, to assess the occurrence of asymptomatic malaria parasites among the new students of ages 16-27 years' age range.

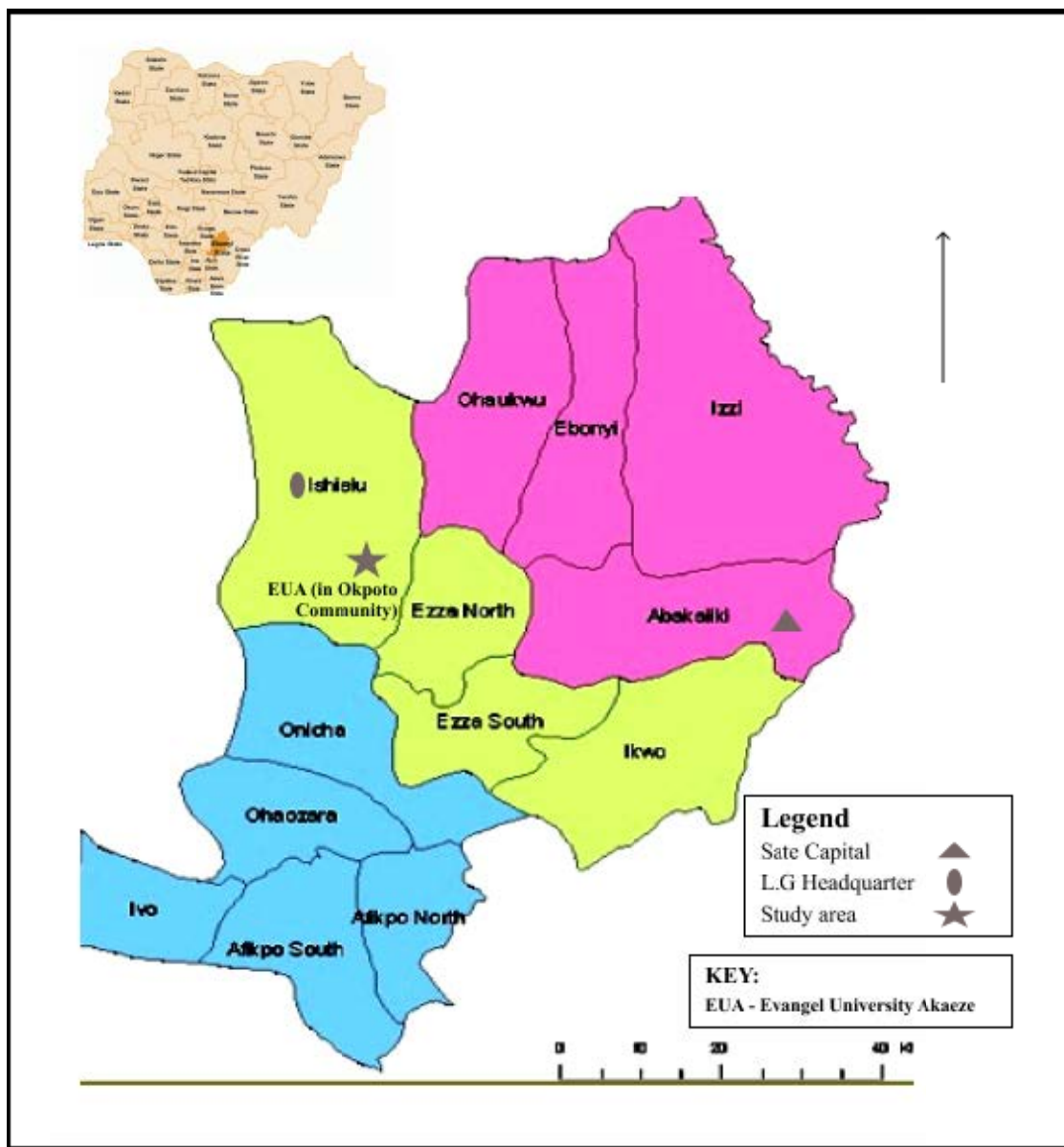


Fig. 1: Map of Ebonyi State of Nigeria showing the study area (Okpoto)

Health service: The health service in the University is the University Health center. There is also one health center in Okpoto community.

Study population: Newly admitted students on a University Campus who consented were used for the study. Their ages ranged between 16 years and 27 years.

Sampling method and sample size: Ten out of thirteen Departments in the University were selected by random sampling techniques^{13,14}. The names of the Departments were compiled and the ones to be used selected by balloting. From

the selected Departments, 210 students were engaged for the study, thus adopting the WHO guideline on survey of prevalence or intensity of infection. The guideline recommends that when a survey is organized to assess the need for control measures (e.g., prevalence or intensity), a sample size of 200- 250 people is adequate^{15,16}.

Data collection: Every consented student from each of the Departments was registered. During the registration, oral interview was used to collect information on the students age, weight, sex, health record (number of cases of health seeking visit) and their methods of response to episodes of malaria.

Sample collection: Two milliliter of blood samples, were collected by venipuncture from each sampled student into Ethylene diamine tetra acetic acid (EDTA) tube, by the help of a resident nurse at the University health center, following the WHO recommended standard procedure¹⁷. Blood smears (thin and thick smear) for malaria parasites detection were prepared on clean, grease-free slides. The slides were appropriately labeled for easy identification. They were aired to dry under room temperature and at the end of 30 min, they were packed in slide racks and taken to the University research laboratory for analysis.

Laboratory studies: Microscopic examination of blood samples: The slides were stained with slightly alkaline (pH 7.2) 5% Giemsa stain, subjected to microscopic examination with 1000X oil immersion lenses for detection, identification and quantification of malaria parasite. When the parasite was identified to be present, it was then stained further with Leishman's stain for specie identification. Parasite species and stages were assessed. Parasite density was determined by counting only the asexual stages against 300 white blood cells, multiplied by 25, assume the mean total white blood cell count of individuals is 7500 cells μL^{-1} of blood. The Parasites density was recorded as low (<1000 parasites μL^{-1} of blood), moderate (1000-9999 parasites μL^{-1} of blood) and severe ($\geq 10,000$ parasites μL^{-1} of blood).

Dipstick antigen-capture test: Test was also done using CareStart™ malaria HRP2/pLDH(*Pf*) RDT kit kept at between 1 and 40°C, for detection of malaria parasite antigen (*P. falciparum* histidine-rich protein 2). The test was done perfectly following the manufacturer's instructions, by 2 separate experts, independent of each other's results. The dipsticks were examined separately and each result recorded as positive or negative depending on the precipitated band observed. A senior laboratory technologist at the Federal Medical center Abakaliki was used for quality control assessment of both slide reading and RDT interpretations.

Statistical analysis: Data collected were analyzed using Statistical Package for Social Sciences (SPSS) software, version 19.0 (Chicago, USA). Chi-square (χ^2) was used to determine if the relationships between the results obtain

using microscopy and that of RDT were actually significant. Accuracy of CareStart™ Malaria HRP2/pLDH(*Pf*) (sensitivity, specificity, positive and negative predictive value) were also determined.

RESULTS

Table 1 shows that the 210 newly admitted students comprises of 120 males and 90 females. It equally shows that the ages of these total population ranges from 16-27 years. More than half the total population (54.3%) fell within ages 16-19 years' age range. Those within ages 20-23 and 24-27 years' age range accounted for 24.8 and 20.9%, respectively. The body weight of the total population varied between 50 and 75 kg. The University does not operate off-campus system, all students lived on campus.

Table 2 shows that in the event of malaria episodes, majority of the students seek for remedy at the formal health system (hospital and health center). One noticed from the table that there was low frequency of health seeking movement, which by implication means low rate of malaria crisis. A total of 97 attendances to different health systems were recorded for a period of 92 days (June-August), for 210 sampled students. Attendance to University health center was the highest (46 times) and the least was attendance to chemist shop (4 times).

Whereas the female gender of all the ages seek for health more at Federal Medical Center, the males go to University health center more than any other place (Table 3 and 4). The table shows that the females recorded more attendance (65 times) to health systems than the males (32 times).

Table 5 reveals that microscopic examination of blood samples of the 210 subjects identified 46 (21.9%) as being positive for malaria parasites. Among the subjects within 16-19 years' age group, 21 (18.4%) students were identified as being positive for malaria parasites, 13 (25%) among ages 20-23 years' age range and 21 (27.3%) among 24-27 years' age range.

Examination of the samples with CareStart™ malaria HRP2/pLDH(*Pf*) RDT showed a total of 40 (19.0%) students as being infected with malaria parasites. It equally showed that 20 (17.5%), 8 (15.4%) and 12 (27.3%) students within ages 16-19, 20-23 and 24-27 years respectively, were infected with malaria parasite (Table 6). The accuracy, sensitivity, specificity, positive and negative predictive

Table 1: Demographic characteristics of respondents

Parameters	Variables	Number	Percentage
Sex	Male	120	57.1
	Female	90	42.9
Total		210	100.0
Age	16-19	114	54.3
	20-23	52	24.8
	24-27	44	20.9
Total		210	100.0
Weight of ages 16- 19 vary from	50-70 kg	114	54.3
Weight of ages 20-23 vary from	50-70 kg	52	24.8
Weight of ages 24-27 vary from	52-75 kg	44	20.9
Total		210	100.0
Place of residence	University Hostel	210	100.0
	Off campus	0	0.0
Total		210	100.0
Levels of student	100	210	100.0
	200	0	0.0
	300	0	0.0
	400	0	0.0
Total		210	100.0

Table 2: Methods of malaria management practices reported by sampled students of different age groups, during the study period (June-August)

		Methods of malaria management practices					
		Number of cases of health seeking visits to:					
Sex and months	Age group	Number of respondents	Chemist shops	Hospital (FMC Abakaliki)	University health center	Use of herbs	Total number
June							
Male	16-19	74	0	3 (4.1%)	4 (5.4%)	0	07
	20-23	32	0	0	6 (18.8%)	0	06
	24-27	14	3 (21.4%)	0	2 (14.3%)	0	05
Female	16-19	40	0	6 (15%)	3 (7.5%)	0	09
	20-23	20	0	2 (10%)	1 (5%)	0	03
	24-27	30	0	1 (3.3%)	1 (3.3%)	2 (6.7%)	04
July							
Male	16-19	74	0	3 (4.1%)	5 (6.8%)	0	08
	20-23	32	0	0	2 (6.3%)	0	02
	24-27	14	0	0	0	0	00
Female	16-19	40	0	8 (20%)	6 (15%)	0	14
	20-23	20	0	5 (25%)	1 (5%)	0	06
	24-27	30	0	1 (3.37%)	3 (10%)	3 (10%)	07
August							
Male	16-19	74	0	0	1 (1.4%)	0	01
	20-23	32	0	0	2 (6.3%)	0	02
	24-27	14	1 (7.1%)	0	0	0	01
Female	16-19	40	0	6 (15%)	5 (12.5%)	0	11
	20-23	20	0	4 (20%)	2 (10%)	0	06
	24-27	30	0	0	2 (6.6%)	3 (10%)	05
Total			4	39	46	8	97

FMC: Federal medical center

values (PPV, NPV) of CareStart™ malaria HRP2/pLDH(*Pf*) were 97.1, 87, 100, 100 and 96.5%, respectively. The Likelihood ratio for positive test results (LR+) and Likelihood ratio for negative test results (LR-) were -87.9 and -86%, respectively.

The female gender recorded higher percentage parasite positive with the two test tools ($p > 0.05$). When

CareStart™ Malaria HRP2/pLDH(*Pf*) RDT was used, 20% of the females were identified as being infected with malaria parasite against the males where 18.3% was recorded. Microscopy revealed that 23.3% female had parasitaemia against 20.8% for male gender (Table 7).

Table 3: Methods of malaria management practices reported by male sampled students of different age groups, during the study period (June-August)

		Methods of malaria management practices					
		Number of cases of health seeking visits to:					
Sex and months	Age group	Number of respondents	Chemist shops	Hospital (FMC Abakaliki)	University health center	Use of herbs	Total number
June							
Male	16-19	74	0	3 (4.1%)	4 (5.4%)	0	07
	20-23	32	0	0	6 (18.8%)	0	06
	24-27	14	3 (21.4%)	0	2 (14.3%)	0	05
July							
Male	16-19	74	0	3 (4.1%)	5 (6.8%)	0	08
	20-23	32	0	0	2 (6.3%)	0	02
	24-27	14	0	0	0	0	00
August							
Male	16-19	74	0	0	1 (1.4%)	0	01
	20-23	32	0	0	2 (6.3%)	0	02
	24-27	14	1 (7.1%)	0	0	0	01
Total			4	6	22	0	32

Table 4: Methods of malaria management practices reported by female sampled students of different age groups, during the study period (June-August)

		Methods of malaria management practices					
		Number of cases of health seeking visits to:					
Sex and months	Age group	Number of respondents	Chemist shops	Hospital (FMC Abakaliki)	University health center	Use of herbs	Total (Number)
June							
Female	16-19	40	0	6 (15%)	3 (7.5%)	0	09
	20-23	20	0	2 (10%)	1 (5%)	0	03
	24-27	30	0	1 (3.3%)	1 (3.3%)	2 (6.7%)	04
July							
Female	16-19	40	0	8 (20%)	6 (15%)	0	14
	20-23	20	0	5 (25%)	1 (5%)	0	06
	24-27	30	0	1 (3.37%)	3 (10%)	3 (10%)	07
August							
Female	16-19	40	0	6 (15%)	5 (12.5%)	0	11
	20-23	20	0	4 (20%)	2 (10%)	0	06
	24-27	30	0	0	2 (6.6%)	3 (10%)	05
Total			0	33	24	8	65

Table 5: Prevalence of malaria parasite according to age groups of sampled students as indicated by microscopy

Age group (years)	No examined	No infected (%)
16-19	114	21 (18.4)
20-23	52	13 (25.0)
24-27	44	12 (27.3)
Total	210	46 (21.9)

Table 6: Prevalence of malaria parasite according to age groups of sampled students as indicated by CareStart™ Malaria HRP2/pLDH(Pf)

Age group (years)	No examined	No infected (%)
16-19	114	20 (17.5)
20-23	52	8 (15.4)
24-27	44	12 (27.3)
Total	210	40 (19.0)

From the results summarized in Table 8, we observed a significant ($p < 0.05$) increase in the prevalence of malaria parasites across all the ages. The table shows a mean percentage prevalence of 17.9, 20.2 and 27.3% for sampled students within ages 16-19,

20-23 and 24-27 years' age range respectively. It equally reveals that the highest and lowest percentage prevalence of malaria parasites were recorded among those aged 24-27 (27.3%) and 16-19 (17.9%), respectively.

Table 7: Comparison of the results of microscopic assay and that of CareStart™ malaria HRP2/pLDH(Pf) RDT according to age group and sex of sampled students

Age groups	Total number Examined	Diagnostic methods used							
		Microscopy				Malaria Pfantigen capture			
		Male		Female		Male		Female	
		No examined	No infected	No examined	No infected	No examined	No infected	No examined	No infected
16-19	114	74	13 (18.6%)	40	8 (20.0%)	74	12 (16.2%)	40	8 (20%)
20-23	52	32	6 (18.8%)	20	7 (35.0%)	32	4 (12.5%)	20	4 (20%)
24-27	44	14	6 (42.9%)	30	6 (20.0%)	14	6 (42.9%)	30	6 (20%)
Total	210	120	25 (20.8%)	90	21 (23.3%)	120	22 (18.3%)	90	18 (20%)

Table 8: Mean (%) prevalence of malaria parasite among the sampled students, as indicated by microscopy and Pf. antigen capture rapid diagnostic tests (RDT)

Age groups (years)	Diagnostic methods used		
	Microscopy	Pfantigen capture (RDT)	Mean (%)
16-19	18.4	17.5%	17.9
20-23	25.0	15.4%	20.2
24-27	27.3	27.3%	27.3

DISCUSSION

The present study was conducted during the peak season of malaria transmission in the south-Eastern side of Nigeria, where Evangel University is located in one of the States, Ebonyi. A 21.9% prevalence of malaria parasites was recorded using microscopic examination among the sampled students and 19% was recorded using CareStart™ malaria HRP2/pLDH(Pf) rapid diagnostic test (RDT) at 87% sensitivity and 97.1% accuracy, for the same group of students. Also the mean percentage prevalence of microscopy and RDT performance revealed 17.9, 20.2 and 27.3% among the sampled students of ages 16-19, 20-23 and 24-27 years' age range.

A lowest and highest mean prevalence of 17.9 and 27.3% respectively, of malaria parasite is an indication that malaria parasites is endemic among the newly admitted students of the University. The high percentage prevalence could be attributed to the rural settings of Okpoto community in which the University is cited, which is similar to earlier finding in Kano, Nigeria, that prevalence of malaria parasites is usually higher in the rural areas where mosquito breeding and transmission is intense¹⁸. Similarly, there was a steep gradient in the prevalence of malaria parasites between the center and edge of town which is adjacent to marshy areas where breeding of *Anopheles* mosquito takes place. In this study, it was noted that the Evangel University is located in a small community farthest away from the center of the town and cited near Village Rivers and surrounded by bushes and marshy lands. This could be attributing to the reason for high

percentage prevalence of malaria parasites recorded among the sampled subjects. However, degree of parasite positivity (+) was not reflected in the sampled students, because at the time of the study, none of the students was having any clinical features (signs or symptoms) of malaria and the total number of cases of visit to health systems was 97 times within the periods of 92 days, by 210 sampled students, when 21.9% and 19.0% prevalence of malaria parasite were blindly recorded by microscopic examination and CareStart™ Malaria HRP2/pLDH(Pf) RDT test respectively. The number of health visit is not commensurate with parasite percentage positive. However, it could be that the students would have developed a high level of immunity as a result of constant attack of malaria infection. In many parts of Africa where malaria infection has been endemic, people are infected so frequently that they develop a certain degree of immunity and in many cases, they carry malaria parasite without showing any symptoms or signs (asymptomatic)^{5,7,19}. It simply means that the individuals can carry the number of *Plasmodium* parasites in their blood without having the disease.

It is quite true that malaria is endemic in many parts of Africa because the level of immunity born by the sampled student which prevented clinical features may have been built up by the individuals from their previous attack of malaria at their different communities/cities before admission to the University. Residents of a malaria region may be exposed to the disease so frequently that they acquire a partial immunity, which can lessen the severity of malaria symptoms. However, this partial immunity can disappear if one moves to a country where he is no longer frequently exposed to the parasite.

It was observed that the sampled students use combined management practices to combat malaria. However, majority of the female students preferred to go to Federal Medical Center in response to malaria attack while majority of the male students preferred to visit the University Health Center for malaria treatments. When the results obtained with microscopy were compared with those of CareStart™ Malaria HRP2/pLDH(Pf) RDT, the results of the two diagnostic tools

were strongly correlated²⁰. This is because all the samples which were positive with RDT kit, were equally positive with microscopy, plus only 6 false-negative results recorded by RDT which microscopy was able to identify. These results correspond with other studies that the Diagnostic performance of RDT in comparison with microscopy was a strong agreement between the results obtained using CareStart and BS microscopy^{20,21,22}. This may be due to the fact that Pf antigen capture kit identifies only the *P. falciparum* parasite antigen especially at the erythrocytic stage when the schizonts rupture out from the liver⁸. However, microscopy was able to determine the quantity (+, ++ etc) of parasites which the RDT did not explain^{8,20,21}.

CONCLUSION

The results of the 2 diagnostic tools were strongly correlated. This shows that asymptomatic malaria parasites were prevalent among the sampled students. The students may not be very aware of the factors that cause it. Environmental factors are mainly responsible for proliferation of mosquitoes, mosquitoes biting rates and consequent high rate of malaria parasitaemia. CareStart™ Malaria HRP2/pLDH(*Pf*) can reliably be used for malaria diagnosis in areas where microscopy is not easily accessible. These findings provide base-line information for planning and implementation of prevention of mosquitoes biting, hence, malaria control activities in the University by the management.

SIGNIFICANCE STATEMENT

This study discovered the occurrence of asymptomatic malaria parasites among a study population on University campus, Southeast Nigeria that can be beneficial for implementation of malaria control methods. This study will help the researchers to uncover the critical areas of malaria epidemiology that many researchers were not able to explore. Thus, a new theory on malaria epidemiology may be arrived at.

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