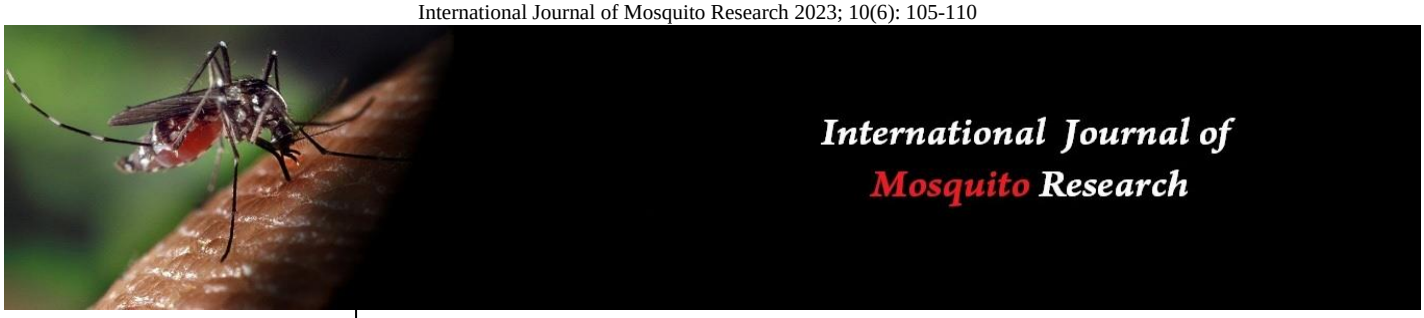

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Cross-sectional survey of *plasmodium falciparum* and *p. vivax* malaria among Nnamdi Azikiwe University undergraduates

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Abstract
Plasmodim falciparum and *P. vivax* pose the greatest malaria threat especially in developing countries. The study was aimed at investigating *P. falciparum* and *P. vivax* malaria infection among Nnamdi Azikiwe University students. Blood samples from 192 students were collected and examined using microscopy and Rapid Diagnostic Test cassettes. Questionnaire was distributed among participants to acertain their perception, treatment and preventive practices. Of the 192 samples collected, 108 (56.25%) were males and 84 (43.75%) were females. Thin blood film examinations revealed about 48 (25.00%) of the participants were positive and 144 (75.00%) negative for *P. falciparum* while the results from Rapid Diagnostic Test (RDT) gave 34 (17.71%) positive cases and 158 (82.29%) negative cases of *P. falciparum*. There was no case of *P. vivax* recorded both for microscopy and RDT. Results indicated that the students were knowledgeable about malaria, its signs and symptoms, mode of transmission and treatment practices.

Keywords: Malaria, *Plasmodium falciparum*, *Plasmodium vivax*, students

1. Introduction
Malaria, a preventable disease caused by the blood dwelling protozoan parasite *Plasmodium*, is a serious health issue in many nations throughout the world (Dayananda *et al*., 2018) [6]. It is transmitted to man when bitten by infected female *Anopheles* mosquito (Umeanaeto *et al*., 2022) [18]. The female *Anopheles* mosquitoes are able to spread the diseases due to their ability to feed on blood unlike the male mosquitoes which feed on plant nectar (Onyido *et al*., 2011) [16]. The extended life span and strong human biting habit of *Anopheles* mosquitoes have been related to the worrisome rate of malaria incidence in Africa (Onah *et al*., 2017) [15], as all it takes is one bite to develop the disease. Malaria could also be transmitted congenitally from a mother to her unborn infant before or during delivery or through contaminated needle and infected blood (Oluput-Oluput *et al*., 2018; WHO, 2020) [14, 20]. In 2021, there were 247 million cases of malaria and 619,000 estimated malaria death (WHO, 2022) [21]. Children under 5 years of age were the most endangered group. In 2020 they accounted for 80% of all malaria deaths. *Plasmodium falciparum* and *Plasmodium vivax* have a high transmission rate in Nigeria and primarily cause severe and deadly malaria in sub-Saharan Africa.
Over 200 species of *Plasmodium* have been identified. Of these number, only five species infect man namely; *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* (Sato, 2021) [17]. Two of these species: *P. falciparum* and *P. vivax* pose the greatest threat especially in Africa (WHO, 2023) [22].
Currently, other human malaria receives little research attention while *P. falciparum* and *P. vivax* receive majority of the attention (Lalremruata *et al*., 2017) [9]. This is because Infections with other *Plasmodium* species present low parasitaemia however, they are able to persist for a very long time and in most cases remain asymptomatic. Although *falciparum* malaria should be the focus, *vivax* malaria should also receive attention because it threatens roughly 40% of the world's population (Naing *et al*., 2014) [12].

Recent studies show the contribution of *P. vivax* to severe malaria in some endemic countries such as Thailand, Brazil, Indonesia, Papua New Guinea and India.

P. vivax is the primary cause of malaria in Southeast Asia and the South America where it accounts for over 80% of the global burden (Dayananda *et al*., 2018; Chu and White, 2021; Menkin-Smith and Winders, 2022) [5, 6, 10] while *P. falciparum* is predominant in Sub Sahara Africa where it accounts for 99.7% cases of malaria. It is the most distributed worldwide (Ndiaye *et al*., 2020; Baird, 2021) [13, 3] and has fairly strong ability to adapt to more diverse habitats and environmental conditions. It has been assumed that the vast majority of people in sub-Saharan Africa are immune to the endemic *P. vivax* malaria due to their duffy blood group negativity (Baird, 2022) [4]. But, recent evidence indicates widespread transmission of *P. vivax* in duffy-negative Africa (Baird, 2022) [4]. It is against this background that the present study was conducted to determine the species composition of malaria parasites infecting Nnamdi Azikiwe University students with particular reference to *P. falciparum* and *P. vivax*, their prevalence, student's perception, prevention and treatment practices.

2. Materials and Methods

2.1 Study Area

The study was carried out among Nnamdi Azikiwe University undergraduates residing within and outside the university premises. Nnamdi Azikiwe University is a Federal institution domiciled in Awka metropolis, the capital of Anambra State, Nigeria. Awka is in the tropical rainforest region and is located between latitude 6°12'N and longitude 7°06'E. The climate is humid with average daily relative humidity of 79.4%, annual rainfall of 2000-3000 mm and average daily maximum and minimum air temperatures of 32.2 °C and 23.3 °C respectively. There is an abundance of natural vegetation in and around the school environment. Most of the students who live off campus reside in Ifite area which is densely populated and might be referred to as a peri-urban area, located just outside the school campus. In Ifite, Potholes and dirty gutters are common sightings and are good breeding grounds for mosquitoes due to accumulation of stagnant water.

2.2 Study Design and Sampling Procedures

A cross-sectional survey of malaria infections among Nnamdi Azikiwe University students was conducted using Blood microscopy and Micropoint® Rapid diagnostic test kit. Non-random convenient sampling was used to enroll the students who were willing to participate in the study as the exact population could not be determined (infinite population). Blood samples were collected voluntarily from one hundred and ninety-two undergraduate students. Structured questionnaire was also distributed among the study population to study their perception, treatment and prevention practices towards malaria.

2.3 Ethical Consideration

A letter of introduction from the Head of Department of Parasitology and Entomology of Nnamdi Azikiwe University, Awka to the Director of Students' Affairs was issued to obtain permission for the study. Ethical approval was obtained from University Research Ethical Committee (U- REC). In all cases, consent was obtained from volunteers before enrollment into the study. The individuals were enlightened on the aim, objectives, importance and benefits of the study and the need to volunteer.

2.4 Blood Collection

Blood specimens were collected with care and adequate precautions. About 2ml of venous blood samples were collected from the students using syringes from which thick and thin blood films were prepared.

2.5 Preparation and Examination of Blood Films

Thick and thin blood films stained with Giemsa stain were prepared for the determination of the presence of the *P. falciparum* and *P. vivax* parasite as described by Abeje *et al*., (2021) [1].

The thick and thin blood films were examined under a microscope using x10, x40 and x100 oil immersion objective lens (WHO, 2015) [19] to visualize different aspect of the blood cell and detect malaria parasites.

2.5.1 Detection of *P. falciparum* and *P. vivax* using Rapid Diagnostic Test (mRDT)

Using a pasteur pipette, whole blood from EDT bottle was added into sample well "A" on the RDT cassette. About 2-3 drops of assay buffer was added into developer well "B. The sample was read after 10 minutes to obtain results.

2.6 Data Analysis

Statistical Package for Social Sciences (SPSS) as well as Microsoft (MS) EXCEL was used to analyze the data obtained.

3. Results of Analysis

3.1 Result from Blood Examination Using Microscopy and Rapid Diagnostic Test (RDT)

Table 1: Result of Rapid Diagnostic Test Kit (mRDT)

Gender	Number Examined	Number positive for RDT	% Positive	Number Negative for RDT	% Negative
Male	108	20	58.8	88	55.7
Female	84	14	41.2	70	44.3
Total	192	34	17.71	158	82.29

From Table 1, a total of 192 students participated in the study of which 108 (56.25%) were male and 84(43.75%) were female. A total of 34(17.71%) cases of the 192 samples collected were positive with RDT while 20(58.8%) were males and 14(41.2%) were females. Exactly 158(82.29%) of the total number examined tested negative with 88(55.7%) males and 70 (44.3%) females. More males were examined and tested positive for RDT compared to females.

Table 2: Results of Microscopy

Gender	Number Examined	Number positive for Microscopy	% Positive	Number Negative for Microscopy	% Negative
Male	108	26	51.2	82	56.9
Female	84	22	45.8	62	43.1
Total	192	48	25.0	144	75.0

From table 2, we observed that a total of 48 out of 192 samples examined were positive, of which 26(51.2%) were males and 22(45.8%) females. While 144(75.00%) were negative with 82(56.9%) males and 62(43.1%) females recorded negative. The results, however, reveal that more males tested positive and negative for microscopy than females.

Result of Species of *Plasmodium* identified using Microscopy and Rapid Diagnostic Test kit

Table 3: Species of *Plasmodium* identified from the study

Gender	Total number of sample	Microscopy		Rapid Diagnostic Test	
		***P. vivax* (%)**	***P. falciparum* (%)**	***P. vivax* (%)**	***P. falciparum* (%)**
Male	108	0 (0.0%)	26 (51.2%)	0 (0.0%)	20 (58.8%)
Female	84	0 (0.0%)	22(45.8%)	0 (0.0%)	14 (41.2%)
Total	192	0 (0.0%)	48 (100%)	0 (0.0%)	34 (100%)

Table 3 represents species of plasmodium identified using microscopy and rapid diagnostic test kit. We however observed that out of 192 persons examined, 108(56.25%) were males while 84 (43.75%) were females. From this number examined, none of the gender tested positive for *P. vivax* with both microscopy and RDT kit. Out of 48 persons who tested positive for *P. falciparum* with microscopy, 26 (51.2%) were males while 22 (45.8%) were females. When 34 persons were examined and tested positive for *P. falciparum* with RDT kit, 20 (58.8%) were males while 14 (41.2%) were females. This however suggests that more males tested positive for *P. falciparum* than females.

Table 4: Comparison between rapid diagnostic test results and microscopy

RDT n (%)	Microscopy n (%)		
	Positive	**Negative**	**Total**
Positive	34 (17.71%)	0 (0.00%)	34 (17.71%)
Negative	14 (7.30%)	144 (75.00%)	158 (82.29%)
Total	48 (25.01%)	144 (75.00%)	192 (100.00%)

From table 4, we observed Microscopy had 48 (25.01%) positive cases and 144 (75%) negative cases while RDT had 34 (17.71%) positive cases and 158 (82.29%) negative cases. The prevalence of malaria among the students, using microscopy (Gold standard), was 25.01% where 48 out of 192 subjects tested positive. When tested with RDT kit, the prevalence was observed to be 17.1%. This however shows that higher positive results were obtained with microscopy than RDT kit.

Subjecting the result to Statistical analysis using Chi-Square Statistical tool in SPSS version 25.0 to determine the associative sensitivity of the two methods employed during the study, it was observed that 192 cases processed are valid and used for the analysis while none is missing. At 5% level of significance, the Chi-square analysis gave the following result; N=192; χ^2= 123.95; DF= 1; p< 0.05, where p = 0.00 as observed from Pearson Chi-Square which shows significant. This however indicates that there is some measure of associative sensitivity between microscopy and RDT kits in producing the positive and negative results among the students seeing that the *p value* is less than the alpha value of 5%. Both however are peculiarly sensitive as well as share some level of relationship as far as testing for malaria is concerned. The later is supported by the *phi value* (which measures the degree of association). The phi-value given as 0.803 opines a strong significant relationship existing between microscopy and RDT test kits.

3.2 Result Obtained Questionnaire Distributed

3.2.1 Students Perception/Knowledge of Malaria

From table 5 below, all the respondents have good knowledge of malaria infection. Of the 192 respondents, 188 (97.9%) knew malaria was transmitted through the bite of infected mosquitoes and all 192 (100%) were aware of the basic signs of malaria. The respondents' answers to whether "anyone can be infected with malaria" and "if recovery from malaria were possible without treatment" depict were well information about the disease.

Table 5: Students Perception / Knowledge of Malaria

Variables	Frequency (%)
Are you aware of malaria?	
Yes	192 (100%)
No	0 (0%)
How is Malaria transmitted?	
Drinking contaminated water	4 (2.1%)
Eating contaminated food	0 (0.0%)
Mosquito bites	188 (97.9%)
Close contact with infected persons	0 (0.0%)
Don't know	0 (0.0%)
Do you know about basic signs of malaria?	
Yes	192 (100%)
No	0 (0.0%)
Can anyone be infected with malaria?	
Strongly agree	108 (56.3%)
Agree	80 (41.7%)
Strongly disagree	4 (2.0%)
Disagree	0 (0%)
Recovery from malaria without getting any treatment is possible	
Strongly agree	8 (4.1%)
Agree	60 (31.3%)
Strongly disagree	60 (31.3%)
Disagree	64 (33.3%)

3.2.2 Students' treatment seeking behaviour

Students' responses on how and when they seek for treatment were commendable as presented in table 6. The students' revealed positive attitude towards malaria treatment. More

than half of the students practice self-medication as 58.3% responded positively when asked while less than half gave a contrast response. However, majority of the students 188 (97.9%) share similar approach/choice of treatment (use of drugs) for similar reason.

Table 6: Treatment seeking habits

Variables	Frequency
Do you get tested before treating malaria	
Yes	92 (47.9%)
No	100 (52.1%)
How long do wait before seeking treatment after malaria onset?	
After 3 days	136 (70.8%)
1week	36 (18.8)
2 weeks	16 (8.3%)
Never	4 (2.1%)
Do you practice self-care	
Yes	112 (58.3)
No	80 (41.7%)
What is your choice of treatment	
Herbs	4 (2.1%)
Drugs	188 (97.9%)
Reason for choosing above treatment behavior?	
It' cheap	8 (4.2%)
It's safe	68 (43.8%)
It's effective	116 (60.4%)
Easily accessible	0 (0.0%)

3.2.3 Students prevention practices

Large number of the students do not use the insecticide treated net as a preventive measure even though they were aware of its ability to prevent mosquitoes from biting them and transmitting the infection to them. This was seen in their responses to the questions "does sleeping under the net at night prevent malaria" and "how often do you sleep under a mosquito net?" as 104 (60.4%) that insecticide nets were capable of preventing malaria transmission and more than half of the respondents 104 (56.3%) never slept using the nets. Lastly, it was observed that the students preferred using insecticides as anti-mosquitoes compared to other available products in the market.

From Cronbach's Alpha value which stood at 0.941 for the standardized item, the questionnaire instrument designed for obtaining the data used for the study has a very high level of consistency or reliability on the scale as seen from the SPSS output. Since $p<0.05$; where p=0.00, we conclude that the overall variance of the items is statistically significant.

Table 7: Study on prevention practices among the students

Variables	Frequency (%)
Sleeping under mosquito nets in the night can prevent malaria	
Strongly agree	72 (37.5%)
Agree	116 (60.4%)
Strongly disagree	0 (0%)
Disagree	4 (2.1%)
How often do you sleep under a mosquito net?	
Always	20 (10.4%)
Sometime	68 (35.4%)
Never	104 (56.3%)
How often do you use anti-mosquito products like repellent coils, spray or cream?	
Always	44 (22.9%)
Sometimes	124 (64.6%)
Never	246 (12.5%)
How often do you use insecticides to kill mosquitoes around your house?	
Always	36 (18.8%)
Sometimes	132 (68.8%)
Never	24 (12.5)

Discussion

The results of this study revealed the presence of *P. falciparum* infection amongst Nnamdi Azikiwe University undergraduates however, no *P. vivax* infection was detected using both microscopy and RDT. This is backed by the findings of Baird (2022) [4] who stated that *P. vivax* has a low incidence in sub-Saharan Africa. The presence of only *P. falciparum* infection was reported amongst the students is in line with the study conducted by Onyido *et al.* (2011) [16] and Ezugbo-Nwobi *et al.* (2011) [7] who reported *P. falciparum* as the only *Plasmodium* species responsible for malaria in Uli and Awka respectively. The prevalence of 25.01% reported from microscopy conducted with the samples was lower than 57.1% and 78.75% reported in a location within the same area

(Onyido *et al.*, 2011; Ezugbo-Nwobi *et al.*, 2011) [16, 7]. However, prevalence of *P. falciparum* 34 (17.71%) recorded through RDT is in line with the studies conducted by Anumudu *et al.* (2006) [2] who reported a low prevalence of 17% for students of University of Ibadan, Oyo State. The high prevalence recorded among the students may be explained by the facts that there are a lot of natural vegetation and stagnant water around them which created favourable breeding site for *Anopheles* species and most of the students belong to the low socio-economic status and avoid sleeping under the insecticidal treated net nor could they install nets on doors and windows.

Comparing the results from RDT and microscopy, it was observed that microscopy was able to detect more blood malaria positive samples than RDT. The difference in positive cases of malaria from microscopy and RDT could be attributed to their individual sensitivity and specificity hence, microscopy is regarded as the gold standard for malaria parasites identification.

In the study, the overall infection was higher in males than in females for both RDT and microscopy. This could be due to the variation in the frequency and intensity of exposure to mosquito vectors responsible for transmitting *Plasmodium* parasites. Male students are fond of removing their clothes during hot weather thus exposing their bodies to bites of mosquito vectors while the females cover their bodies. Also the female students treat themselves at the onset of the infection. They visit medical centres and hospitals more than males who like enduring illness.

All of the students (100%) were knowledgeable about malaria. Majority of them knew it was transmitted through the bites of infected mosquitoes. The observation is comparable with the reports of similar study amongst Nnamdi Azikiwe University students (Ezugbo-Nwobi *et al.*, 2011) [7] where 74.3% of the respondents were aware of mode of transmission of malaria. In another study in Northwestern Ethiopia, 75.4% of the respondents also responded correctly to mode of transmission of malaria (Flatie and Munshea, 2021) [8]. The knowledge level of respondents on mode of transmission of malaria can be attributed to the fact that the study was conducted among students in tertiary level of education. This study demonstrated that respondents had a good knowledge about the signs and symptoms of malaria and is consistent with observations from similar studies conducted by Munzhedzi *et al*., (2021) [11]. This high level of awareness of the clinical symptoms of malaria might be due to increased access to mass media, health education by health workers and self-experience of malaria. It was also deduced from the study that majority of the participants had commendable attitudes towards malaria treatment, with 97.9% of the study population using drugs as a choice of treatment over herbs.

Prevention of malaria through vector control and use of Long-Lasting Insecticide Nets are important preventive measures for malaria. In this study it was observed that the respondents had poor preventive practices towards malaria. This might be due to socio-economic level of the study population and low level of formal education on the importance of adequate preventive practice against malaria.

Conclusion

Findings from this study revealed the presence of *P. falciparum and* absence of *P. vivax* parasites among Nnamdi Azikiwe University undergraduate students. The participants had appropriate knowledge of the infection and a positive treatment seeking attitude. However, substantial portion of them had poor preventive practices against malaria.

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