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Prevalence of Asymptomatic Falciparum Malaria, Risk Factors, and Absence of PfHRP2 Gene Deletion in Makurdi, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria remains a persistent public health challenge, particularly in sub-Saharan Africa, where asymptomatic falciparum malaria poses a significant threat. Asymptomatic cases serve as a crucial parasite reservoir, contributing to ongoing transmission.
Aim: The study investigates the prevalence of asymptomatic falciparum malaria and assesses the occurrence of gene deletion in the *Plasmodium falciparum* Histidine Rich Protein 2 (Pf HRP2) gene in Makurdi, Benue State, Nigeria.
Methodology: A cross-sectional study was conducted between September and October 2019, which involved 374 apparently healthy individuals from five communities. Malaria diagnosis utilized Rapid Diagnostic Test (RDT) kits, microscopy, and polymerase chain reaction (PCR) for Pf HRP2 gene assessment.
Results: The study found a prevalence of 25.4% by RDT and 28.1% by microscopy. Asymptomatic falciparum malaria was significantly influenced by location, proximity to water bodies, bed net usage, and history of malaria treatment, while, age, and insecticide usage showed no significant

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impact. PCR results revealed amplified fragments with band sizes ranging from 600 to 900 base pairs in 40 positive isolates, eliminating Pf HRP2 gene deletion as a cause for false negatives observed between RDT and microscopy results.
Discussion/Conclusion: The study highlights a high malaria transmission rate in Nigeria, emphasizing the role of location-specific factors and bed net usage in the proliferation of asymptomatic falciparum malaria. Importantly, no gene deletion was identified in the Pf HRP2 gene among the studied *Plasmodium falciparum* isolates.

Keywords: Malaria; Plasmodium falciparum; gene deletion; histidine rich protein 2 gene; Rapid Diagnostic Test kits (RDT).

1. INTRODUCTION

Malaria poses a significant public health challenge, causing widespread suffering, illness, and premature death in subtropical regions worldwide [1]. This parasitic disease is caused by single-celled protozoan parasites of the genus Plasmodium. Transmission occurs through the bites of female Anopheles mosquitoes, predominantly found in tropical and subtropical areas [2,3].

Asymptomatic malaria, characterized by the presence of Plasmodium parasites in peripheral blood without apparent symptoms, poses a diagnostic challenge due to the absence of clinical manifestations and often low parasite levels [4,5]. Its prevalence in malaria-endemic areas, especially sub-patent malaria, remains a significant concern for efforts aimed at parasite eradication [4]. Earlier studies highlight a notably high prevalence of asymptomatic malaria among school students aged 10-19 [6,7]. In Aba town, Abia state, Eke et al. [7] reported a prevalence of 39.1% and 43.5% among 10-14 and 15-19 age groups, respectively. This underscores the epidemiological concern of asymptomatic parasitemia, disrupting ongoing control programs.

Current malaria control strategies emphasize early detection and treatment of parasites in suspected individuals. However, individuals with asymptomatic parasitemia evade detection, serving as ongoing sources of infection for mosquitoes and complicating control efforts [8]. The use of malaria Rapid Diagnostic Tests (RDTs) for early detection requires high sensitivity and specificity. Unfortunately, genetic diversity within the PfHRP2 antigens used in RDTs may affect their sensitivity [9]. Understanding the population structure of this gene is crucial for designing more effective tests and minimizing false negatives [10].

This study aims to investigate the prevalence of asymptomatic *Plasmodium falciparum* malaria and its major risk factors among rural inhabitants in Makurdi, Benue state. Additionally, it seeks to assess the status of gene deletion in the histidine-rich protein 2 gene of parasite isolates.

2. MATERIALS AND METHODS

2.1 Study Design and Area

The study was conducted in the Makurdi Local Government Area of Benue State, North Central Nigeria, with coordinates 7°43′50″N 8°32′10″E. The area experiences an average annual rainfall of about 1077 mm, coinciding with a high prevalence of malaria, particularly during the rainy season from April to September yearly [11].

2.2 Selected Communities

Five communities were selected for the survey: Ijaha, Agbough, Kua, Akpehe, and Terwase-Agbadu.

2.3 Sample Population

The sample population comprised 374 individuals from diverse demographics, including various age groups, occupations, marital statuses, educational backgrounds, social classes, and religious and cultural affiliations. The survey was conducted from September to October 2019.

2.4 Sample Collection

Blood samples were collected under sterile conditions by swabbing the area with 70% alcohol. Capillary blood was obtained from willing participants for microscopic diagnosis, rapid diagnostic test (RDT), and polymerase chain reaction (PCR).

2.5 Diagnostic Methods

2.5.1 Rapid Diagnostic Test (RDT)

PFHRP2 Carestart™ by Access BIO, Inc., was used to detect *PLASMODIUM FALCIPARUM*. The test, based on lateral flow immunochromatography, was conducted in the field and read within 20 minutes.

2.5.2 Microscopy

Thick and thin blood films were stained with 10% Geimsa stain for parasite identification. Experienced microscopists examined slides under a light microscope (100X – oil immersion objective) for parasite quantification.

2.5.3 DNA extraction and molecular detection

Genomic DNA was isolated using Zymo DNA Mini Kit USA. PCR diagnosis was performed using species-specific primer pairs targeting small subunit ribosomal ribonucleic acid (ssRNA) genes of *Plasmodium falciparum*.

The presence or absence of *Plasmodium falciparum* was confirmed by representative amplicon size, and variations in the histidine-rich protein II gene (HRP2) were analyzed using gel analyzer software.

2.6 Statistical Analysis

Data were entered into Microsoft Excel and analyzed using the chi-square test in Statistical Package for the Social Sciences (SPSS) Version 20. The analysis focused on exploring relationships between age, sex, bed-net usage, nearness to water bodies/standing water, and the prevalence of *Plasmodium falciparum* malaria. A p-value of 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Prevalence of Asymptomatic falciparum Malaria by Location

Table 1 shows a breakdown of the frequency of data obtained according to the various parameters measured.

The study assessed the prevalence of asymptomatic falciparum malaria across five communities: Ijaha, Agbough, Kua, Akpehe, and Terwase-Agbadu. The overall prevalence was 25.4% for RDT diagnosis and 28.1% for microscopy (Table 1, Table 2). A Chi-square test revealed a significant association between the prevalence of asymptomatic malaria and the location in Makurdi (P = .02 for RDT and P = .001 for microscopy). The significant P values indicate that location is a critical factor in the prevalence of asymptomatic malaria. This could be due to varying environmental conditions, healthcare access, or community practices across these locations.

3.2 Prevalence by Age Group

3.2.1 Age-specific prevalence

Children aged 0-4 years showed the highest prevalence rate of 50%, while no statistical significance was found between age and prevalence (P = .41) (Table 3). Though not statistically significant, the higher prevalence in the 0-4 age group highlights the need for targeted interventions for this vulnerable population.

3.3 Prevalence by Exposure to Water Bodies

3.3.1 Water exposure rates

Statistical analysis revealed a significant relationship between exposure to water bodies and the prevalence of asymptomatic malaria (P = .017, Table 4). The significant P value suggests that exposure to water bodies is a risk factor, and environmental control could be an effective preventive measure.

3.4 Prevalence by Bed-Net Utilization

3.4.1 Bed-net usage rates

Statistical analysis showed a significant relationship between bed-net usage and the prevalence of asymptomatic malaria (P < .001, Table 5). The usage of bed nets appears to be a highly effective preventive measure, as supported by the significant P value.

3.5 Prevalence by Insecticide Usage

3.5.1 Insecticide Usage Rates

No significant relationship was found between insecticide usage and the prevalence of

asymptomatic malaria (P = .29, Table 6). Insecticides alone may not be sufficient as a preventive measure, as indicated by the non-significant P value.

3.6 Prevalence by History of Malaria Treatment

3.6.1 Treatment history rates

A significant relationship was observed between the history of malaria treatment and the prevalence of asymptomatic malaria (P = .025, Table 7). This finding suggests that previous treatment history could influence current malaria status, raising concerns about treatment effectiveness or adherence.

3.7 HRP2 Gene Deletion

3.7.1 Gene deletion status

No deletions were observed in the HRP2 gene among the isolates (Table 8). The absence of HRP2 gene deletions suggests that RDT tests targeting this gene are likely to be reliable in this population.

Table 1. Prevalence of Asymptomatic *P. falciparum* infection in different communities based on RDT

Community	Positive (%)	Negetive (%)	Total (%)
Ijaha	16(19.5)	66(80.5)	82(100)
Agbough	12(17.1)	58(82.9)	70(100)
Kua	19(24.7)	58(75.3)	77(100)
Akpehe	28(37.3)	47(62.7)	75(100)
T. Agbadu	23(32.9)	47(67.1)	70(100)
Total	98(26.2)	276(73.8)	374(100)

χ^2=11.371 df^2=4 P^2=0.02

Key: χ^2= Chi-square value; df^2= degree of freedom; P^2= P- VALUE

Table 2. Prevalence of Asymptomatic *P. falciparum* infection in different communities based on Microscopy

Community	Positive (%)	Negetive (%)	Total (%)
Ijaha	15(18.3)	67(81.7)	82(100)
Agbough	12(17.1)	58(82.9)	70(100)
Kua	20(26.0)	57(74.0)	77(100)
Akpehe	32(42.7)	43(57.3)	75(100)
T. Agbadu	26(37.1)	44(62.9)	70(100)
Total	105(28.1)	269(71.9)	374(100)

χ^2=18.956 df^2=4 P^2=0.001

Key: χ^2= Chi-square value; df^2= degree of freedom; P^2= P- Value

Table 3. Prevalence of asymptomatic *P. falciparum* according to age class

Parameter	Microscopy		Total (%)
	Positive (%)	Negetive (%)	
0-4 Years	*4(50)*	4(50)	8(100)
5-9 Years	7(21.9)	25(78.1)	32(100)
10-14 Years	15(31.9)	32(68.1)	47(100)
15 Years Above	79(27.5)	208(72.5)	287(100)
Total	105(28.1)	269(71.9)	374(100)

χ^2= 2.900 df^2=3 p^2= 0.407

Key: χ^2= Chi-square value; df^2= degree of freedom; P^2= P- Value

Table 4. Prevalence of asymptomatic *P. falciparum* in relation to exposure to water body/standing water

Parameter	Measurment	Microscopy		Total (%)
		Positive (%)	Negetive (%)	
Close to	No	*47 (35.6)*	85 (64.4)	132 (100)
Water Body	Yes	58 (24.0)	184 (76.0)	242 (100)
	Total	105(28.1)	269(71.9)	374(100)

χ^2= 5.730 df^2=1 p^2= 0.017 Odd ratio= 0.570 Likelihood ratio= 1.486
Key: χ^2= Chi-square value; df^2= degree of freedom; P^2= P- Value

Table 5. Prevalence of asymptomatic *P. falciparum* in relation to bed-net use

Parameter	Measurment	Microscopy		Total (%)
		Positive (%)	Negetive (%)	
Bednet Use	No	*73 (42.7)*	98 (57.3)	171 (100)
	Yes	32 (15.8)	171 (84.2)	203 (100)
	Total	105(28.1)	269(71.9)	374(100)

χ^2= 33.33 df^2=1 p^2< 0.05 Odd ratio= 0.251 Likelihood ratio= 2.708
Key: χ^2= Chi-square value; df^2= degree of freedom; P^2= P-Value

Table 6. Prevalence of asymptomatic *P. falciparum* in relation to insecticide use

Parameter	Measurment	Microscopy		Total (%)
		Positive (%)	Negetive (%)	
Insecticide	No	38 (31.7)	82 (68.3)	120 (100)
Use	Yes	67 (26.4)	187 (73.6)	254 (100)
	Total	105(28.1)	269(71.9)	374(100)

χ^2= 1.13 df^2=1 p^2= 0.29 Odd ratio= 0.77 Likelihood ratio= 1.20
Key: χ^2= Chi-square value; df^2= degree of freedom; P^2= P- Value

Table 7. Prevalence of asymptomatic *P. falciparum* in relation to time of last malaria treatment

Parameter	Measure	Microscopy		Total (%)
		Positive (%)	Negetive (%)	
Time	3 Weeks	*3 (15.0)*	17 (85.0)	20
Of Last	1 Month	2 (8.0)	23 (92.0)	25
Malaria	2 Months	4 (15.4)	22 (84.6)	26
Treatment	3 Months	10 (37.0)	17 (63.0)	27
	4M and Above	86 (31.2)	190 (68.8)	276
	Total	105(28.1)	269(71.9)	374(100)

χ^2= 11.13 df^2=4 p^2= 0.025
Key: χ^2= Chi-square value; df^2= degree of freedom; P^2= P- Value

Table 8. Asymptomatic *P. falciparum* HRP2 deletion by communities

Community	N	HRP2 Deletion	Total	% Of Undeleted HRP2
Ijaha	8	ABSENT	8	100
Agbough	8	ABSENT	8	100
Kua	8	ABSENT	8	100
Akpehe	8	ABSENT	8	100
T. Agbadu	8	ABSENT	8	100
Total	40	0	40	100

4. DISCUSSION

This comprehensive study aimed to evaluate the prevalence of asymptomatic carriers of the malaria parasite *P. falciparum* in Makurdi, Benue State, considering various demographic and environmental variables [12].

The study revealed that the prevalence rates identified in this study were higher than some previous studies but lower than others, indicating geographical variations in malaria prevalence. This underscores the dynamic nature of malaria epidemiology across different regions which aligns with Klinkenberg [13] conclusion, that malaria in urban areas differed from the rural environment and in region that are associated with flooding.

While no significant correlation was observed between age and malaria prevalence, the youngest age group (0-4 years) exhibited the highest prevalence. Location and proximity to water bodies emerged as significant factors, suggesting the influence of environmental conditions conducive to mosquito breeding. This finding agrees with Salako et al. [14] and agrees with the declaration of World Health Organization [15] that children are among some of the vulnerable population groups at higher risk of malaria.

Bed-net usage was found to significantly reduce malaria prevalence, aligning with prior research. Although insecticide usage showed a reduced impact, it was not statistically significant. Notably, a history of treatment emerged as a significant factor, emphasizing the importance of regular, planned malaria treatment to control the disease as reported by Gamble et al. [16] who noted that the use of insecticide treated bed nets (ITNs) is one of the most cost-effective interventions against malaria in endemic areas and is associated with significant reductions in malaria morbidity and mortality.

The study found no evidence of HRP2 gene deletion, indicating the effectiveness of RDTs based on this gene in the community. However, the presence of false negatives in RDT results underscores the necessity for confirmatory tests in certain cases. *P. falciparum* isolates lacking pfhrp2 and pfhrp3 genes may be circulating and contributing to RDT false negativity in Nigeria [17].

The study underscores the critical role of preventive measures, such as bed-net usage and regular treatment, particularly in areas with a high prevalence of malaria.

In conclusion, this study contributes valuable insights into the nuanced dynamics of malaria prevalence in Makurdi, emphasizing the importance of tailored strategies for diagnosis and prevention based on demographic and environmental considerations.

5. CONCLUSION

The study confirms that asymptomatic malaria remains prevalent in Makurdi, Benue State, especially among the 0-4 age group, signaling a lapse in preventive measures for this vulnerable population. It highlights the effectiveness of bed-net usage in reducing malaria prevalence and rules out gene deletion as a cause of false negatives in Rapid Diagnostic Tests (RDTs). The study calls for further exploration into other factors that could influence the prevalence of asymptomatic malaria.

CONSENT AND ETHICAL APPROVAL

The study proposal received approval from the ethical committee of the Benue State Ministry of Health, Makurdi. Written consent was obtained from community members and families after advocacy meetings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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