IJEMD-BMCR, (3) 2 (2025) https://doi.org/ 10.54938/ijemdbmcr.2025.03.1.425

International Journal of Emerging Multidisciplinaries:
Biomedical and Clinical Research

Research Paper
Journal Homepage: www.ojs.ijemd.com
ISSN (print): 2957-8620 ISSN (online): 2960-0731

PRELIMINARY STUDY OF VARIANT K ALLELE - MEROZOITE SURFACE PROTEIN 1 OF PLASMODIUM FALCIPARUM AMONG PATIENTS IN YENAGOA, BAYELSA STATE

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ABSTRACT

Genetic polymorphism imposes a huge challenge in quest for effective vaccines, and enhanced malaria control. This research aimed at detecting frequency of the K allele - msp-1 gene in malaria infected patients. A total of 25 samples from children (52% males and 48% females) from ages 5 and below was used. Malaria parasites identification was carried out using rapid diagnostic test (RDT) technique. Genotyping of Plasmodium specie was done by Polymerase Chain reaction (PCR). Results from RDT showed 48% of the sampleswere infected with plasmodium falciparum,24% in both males and females respectively.PCR showed that the Msp variant k is more prevalent in males than females with the percentage of 20% and 16% respectively. Molecular diagnostic test is more precise and accurate. Further research should be made on the prevalence of the Msp variant k, for enhanced malaria control.

Key Words: Plasmodium Falciparum, Genetic polymorphism, Msp variant k, Malaria.

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INTRODUCTION

Malaria, despite huge controlling strides that has been made is still burdensome especially in Africa [37]. Pregnant women and their unborn children are particularly vulnerable to the disease, which causes anemia, low birth weight, premature birth, and infant deaths.

Attention is now on vaccine [21]. To develop a vaccine, it is imperative to understand protective immune mechanisms, identify antigenic targets, and establish robust and reliable assays measuring correlates of protection. Persons who live in regions were malaria is endemic naturally acquire immunity against the disease with increasing numbers of survived infections. This sought of protection is majorly mediated byserum antibodies which control levels of blood-stage parasites [12].
Studies have shown various adversities towards attaining absolute control against the menace. [68], [60], [31]. Molecular characterization of P. falciparum enables the investigation ofthe genetic diversity of its infection within alignment with various factors, such as disease phenotype, age and host immunity [32]. In tandem with current focus, molecular investigations garners support globally [3], [25], [30]. However, dearth of information exists in Bayelsa state, Nigeria .

MATERIALS AND METHODS

Current research ensued at Nucleometrix Research Laboratory, within ultra-modern Tobis clinic, Yenagoa, Bayelsa state. The State is cosmopolitan in natureand located in the southern part of Nigeria, geopolitically located within Latitude 415 North, 523 South, Latitude 522 West and 645 East. It has an area of 706km.

In cross section investigation, random samples were selected following ethical committee approval, and statistical package, SPSS was used for analysis of the data. The research was done using Rapid Diagnosis test and molecular diagnosis (PCR technique) methods.The Rapid Diagnosis test is a simple, quick, and cost-effective test usedto determine the presence of malaria parasites and utilizes the principle of immuno-chromatography. It has the test strip coated with monoclonal Anti - HRP - II (Test line Pf) which is specific to the histidine-rich protein II of Plasmodium falciparum. The result is read 20 minutes after the sample is applied to the test strip. The appearance of lines or colors gives the test result.

The PCR technique involved extraction of the DNA by a Chemical Method and the extracted DNAquantified using Nanodrop 1000 spectrophotometer.

MSP 1 was carried out using forward and reverse primers; MSP1-OF: 5'-CTAGAAGCTTTAGAAGATGCAGTATTG -3' and MSP1-OR: 5'-CTTAAATAGTATTCTAATTCAAGTGGATCA-3' respectively on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 25 cycles. The PCR then primes below.

MSP1 K genes were amplified using the MSP1 K F: 5-AATGAAGAAGAAATTACTACAAAAGGTGC-3’ and MSP1 K R: 5'-GCTTGCATCAGCTGGAGGGCTTGCACCAGA-3' as forward and reverse primers respectively on the ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles.

RESULTS

Twenty-five samples (aged range one to five) were obtained from male and female patients in the ultra-modern, Tobis clinic,. Five was from one year olds, seven samples from age 2, three samples from age 3, five from age 4, and five from age 5.

Table1: **Distribution of patients' age and gender.**

Age	Male	Female	Total
1	2	3	5
2	4	3	7
3	1	2	3
4	3	2	5
5	3	2	5
Total	13	12	25
Percentage	52%	48%	100%

The table below shows the results of malaria tests using the Rapid Diagnosis test method indicating the patients' age and gender. Where; NE= persons assessed , NI= persons Infected

Table 2: Result of Rapid Diagnosis test

Age	Male		Female		Total	
	NE	NI	NE	NI	NE	NI
1	2	1	3	2	5	3
2	4	2	3	1	7	3
3	1	1	2	2	3	3
4	3	1	2	1	5	2
5	3	1	2	0	5	1
Total	13	6	12	6	25	12
Percentage %	52%	24%	48%	24%	100%	48%

Table 3: Distribution table showing the results of molecular diagnosis

Age	Male		Female		Total	
	NE	NI	NE	NI	NE	NI
1	2	1	3	1	5	1
2	4	1	3	0	7	3
3	1	1	2	2	3	3
4	3	2	2	1	5	2
5	3	0	2	0	5	0
Total	13	5	12	4	25	9
Percentage %	52%	20%	48%	16%	100%	36%

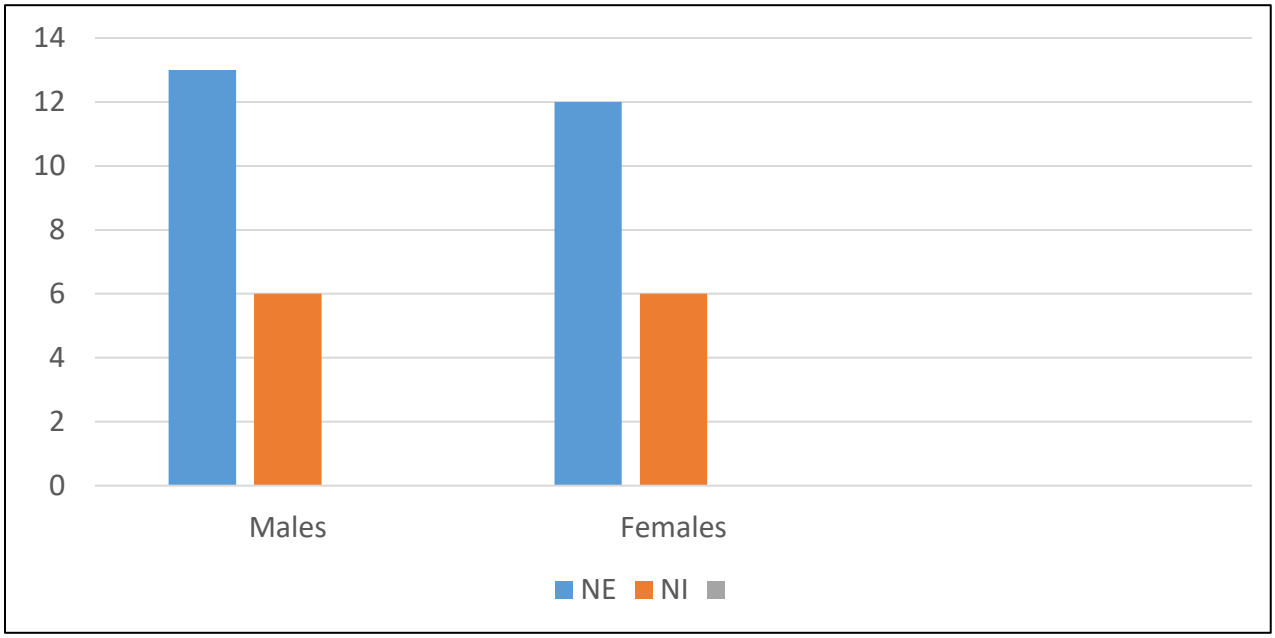

Figure 1: Chart showing results from RDT.

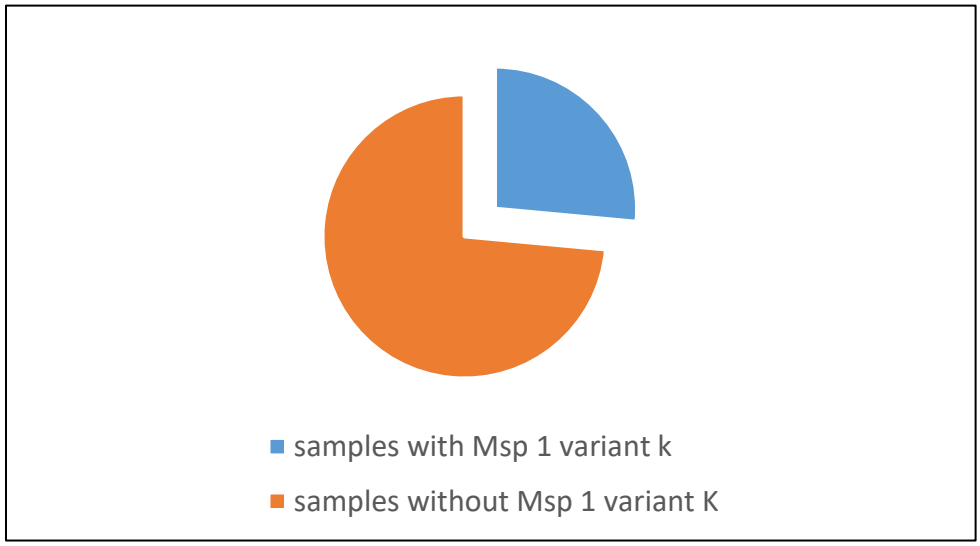

Figure2: Pie chart showing the presence or absence of theMsp variant K.

DISCUSSION

In operation, most RDTs target a P. falciparum-specific protein, such as histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some tests detect both P. falciparum-specific and pan-malarial antigens (e.g., aldolase or pan-malaria pLDH), allowing them to differentiate malaria illnesses [38], [35].

The RDT kit used in this study specifically targets HRP-II. A total of 25 samples were tested, and the results (Table 2) indicate that 12 (48%) samples were infected with P. falciparum. The distribution by age shows that three samples (12%) were from individuals aged 1, 2, and 3 years, two samples (8%) from age 4, and one sample (4%) from age 5, with infections occurring in both genders.

Diversified genetics awareness is relevant in regulating drug- or vaccine-resistant parasites [70],[26],[69]. The availability of polymorphic genetic markers, combined with the ease of characterizing them through sensitive PCR amplification from field-collected samples [43], [58], [64],[20],[65][20],[71], has facilitated such investigations; and nested PCR, has garnered preference for investigations in spite of its sophistication [65],[7].

In this study (Table 3), PCR analysis of 25 samples revealed that 36% carried the Msp variant K, with 20% of cases occurring in males and 16% in females, all within the 1–5 age group. Specifically, one sample (4%) at age 1, three samples (12%) each at ages 2 and 3, and two samples (8%) at age 4 tested positive for Msp variant K, while no cases were detected at age 5. These results align with findings that PCR is more sensitive than QBC and some RDTs [57]. PCR has demonstrated higher sensitivity and

specificity compared to conventional microscopic examination of stained peripheral blood smears and is now considered the most reliable method for malaria diagnosis [39].

CONCLUSION

The Molecular diagnosis test is more precise and accurate thanthe Rapid Diagnostic test. From the result above, 48% of samples are infected with plasmodium falciparum and 36% showed the presence of MSP variant K, there's thus a possibility that the Msp variant K is prevalent in Yenagoa, Bayelsa state.

CONTRIBUTION TO KNOWLEDGE

The findings contribute to the understanding of the distribution and epidemiology of Msp variant K, which can inform future disease control and prevention efforts. The study highlights the importance of utilizing multiple diagnostic approaches to gain a more complete understanding of the distribution and prevalence of specific malaria parasite variants It is thus recommended that further research should be made in the state on the prevalence of theMsp variant k, to ensure rapid control and elimination of the infection. Also, Molecular diagnostic tools should be provided by the government to health sectors to ensure accurate and precise results in molecular testing.

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