ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology

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Indo-American Journal of Life Sciences and Biotechnology

Variation in the Genomic Composition of Plasmodium falciparum Merozoite Surface Proteins 1 and 2 in Nigerian Children Less Than 5 Years Old in the State of Delta

Vaishnavi, Vidyasagar

Departments of [1]Medical Biochemistry and Faculty of Basic Medical Sciences, Delta State University, Abraka,
[2]Department of Biochemistry, School of Biomedical Sciences, Novena University, Ogume, Delta State, Nigeria

Received on: 03 Apr 2025 Accepted Date: 10 May 2025 Published on:31 May 2025

Abstract:

In context, Nigeria has the greatest malaria burden among African countries, and the continent itself is still the malaria hub. Pharmacogenomics, genetic reference panels, and malaria biomarkers all need an upgrade to meet the current worldwide issues of malaria resistance. Children under the age of five in certain regions of Delta State, southern Nigeria, were studied to determine the genetic diversity and structure of Plasmodium falciparum merozoite surface proteins (msps 1 and 2). Methods: Due to low parasite density, P. falciparum deoxyribonucleic acid was recovered from 86 samples that tested positive for malaria out of 690 people. The msp1 and msp2 allele genotyping was examined by electrophoresis, polymerase chain reaction, and an ultraviolet-trans illumination gel method. Findings: MAD20+RO33+K1 (67.4% of cases) and 3D7+FC27 (90% of cases) were the most common forms of polyclonal infection for msp1 and msp2, respectively. Ro33 (36.3% of the total) and FC27 (53.4% of the total) were the most common msp1 and msp2 alleles, respectively. Furthermore, the allelic makeup of the research population did not vary significantly between men and females. But msp1 had an infection multiplicity of 1.10 and msp2 had an infection multiplicity of 1.08, whereas the corresponding heterozygosity values were 0.67 and 0.50, respectively. Conclusion: In Delta State, Nigeria, a high transmission rate of P. falciparum infection was observed, suggesting a large degree of genetic middling heterozygous alleles. These results emphasize the need for targeted and locally sourced anti-malaria treatments to be developed.

Plasmodium falciparum, genetic diversity, heterozygosity, merozoite surface proteins, malaria, infection multiplicity, pharmacogenomics

INTRODUCTION

Some kinds of Anopheles mosquitoes transmit the protozoan parasite Plasmodium falciparum, which is indigenous to that region. It is the most common and dangerous species of Plasmodium, and it causes malaria in the majority of Nigerian cases. (2, 3). Children under the age of five are disproportionately vulnerable to the devastating effects of malaria, despite the fact that there has been a marked increase in focus, initiatives, and funding directed at reducing the disease's impact on African areas. [4] Malaria has devastating effects on children's health and well-being, including retardation in physical and mental development, impaired learning capacity, and even death. [5] Recently, the World Health Organization released a study about malaria.

The majority of the anticipated 597,000 fatalities and 263,000,000 cases of malaria in 2023 occurred in the African area. Among these countries, Nigeria had the largest burden of the illness, accounting for 95% of the deaths and 94% of the cases. [6]

Indo-American Journal of Life Sciences and Biotechnology

One of the 36 states in Nigeria, Delta state is located in the southern part of the nation between 5°00 and 6°45 longitude and 5°00 and 6°30 latitude [Figure 1a]. [7] There are 25 LGAs in the state [Figure 1b], and out of a total geographical area of 17,440 km2, one-third is marshy and waterlogged, making it an ideal habitat for malaria vectors. Those two In Nigeria, 17% of all cases in children fewer than 5 years old occur in Delta State, as reported in the 2024 Malaria Report. [6] There has been a dramatic uptick in malaria cases in Delta state as of late, and one major contributing factor is the ineffectiveness of antimalarial treatments. This is due, in part, to the parasites' high degree of genetic diversity, which allows them to evade the immune system, and, more specifically, to the emergence of variant species that are resistant to chemotherapeutic drugs used to treat malaria. Research on this genetic variant takes into account a lot of factors, such as the host's immunity and the level of transmission. I have read [9,10] There are two allelic families for merozoite surface protein-2 (3D7 and FC27) and three for merozoite surface protein-1 (msp1) (K1, MAD20, and RO33) based on differences in the repetitive sequences of msp1 and polymorphisms in the central block of msp2. the eleventh Significant malaria indicators, including Plasmodium transmission, Plasmodium genetic diversity, medication resistance in parasites, and pesticide resistance in mosquito vectors, must be continuously monitored if malaria eradication is to be achieved. References [12,13]; If molecular research are not carried out to understand the genetic structure, variety, and complexity of P. falciparum within regional populations, this aim will remain far-fetched. Research on this topic is still limited. Therefore, the purpose of this research was to examine the genetic diversity and structure of Plasmodium falciparum msp1 and msp2 in children in Nigeria's Delta State who were less than five years old.

METHODS

Ethical consideration

This study was carried out in line with the Helsinki rules and was authenticated by Research, Ethics and Grant Committee, Faculty of Basic Medical Sciences, Delta State University(Ref. No.: RBC/FBMC/DELSU/24/501) in synergy with the Ethical Committee, Asaba Specialist Hospital, Delta State (Ref. No.: A24OVO/11/31) on 2nd and 5th of August, 2024, respectively.

Patient consent statement

Filled consent forms were obtained from each participant's guardian. Only children with approved consent were enrolled into the study.

Type of sampling and reasons for selection

A cross-sectional sampling design was adopted to assess the genetic structure and prevalence of *msp1* and *msp2* alleles in a defined population of uncomplicated symptomatic individuals with malarial infection aged 5 years and younger.

Inclusion criteria

Participants who presented with symptoms such as cephalalgia, axillary temperature ≥37.5°C and/or fever history within 72 h of presentation, anemia, and malaise in the absence of other diseases, aged under 5 years were included. Sociodemographic information was also obtained from the participant's guardian.

Exclusion criteria

Participants below 5 years of age who presented with the symptoms of complicated malaria and children above 5 years of age were excluded from the study.

Sample collection and microscopic parasite density determination

A total of 690 participants[14] were recruited for this research in a randomized cross-sectional method. Arterial blood samples (5 mL) were collected from these participants in ethylenediaminetetraacetic acid bottles. Microscopic examination of 10% Giemsa stained blood films were performed to confirm the positive samples. Blood spots were made from the samples with parasite densities >999 μL[15] and then air dried.

Analysis of samples

DNA extraction and 18s ribosomal RNA detection

The parasite DNA was extracted from the DBS samples using a QIAamp DNA Blood kit (QIAGEN, Germany) in accordance with the manufacturer's methods.[12] A confirmation detection test was carried out. *P. falciparum* small subunit

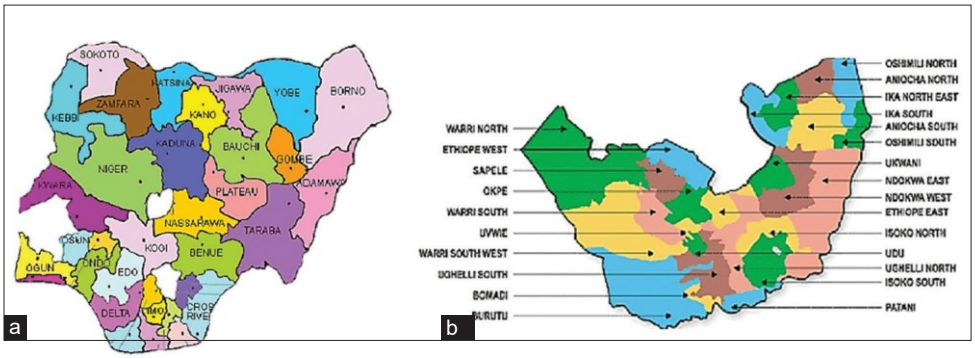

Figure 1: Map of Nigeria, showing the location of Delta State (a) and the local government areas in Delta state (b)[7,8]

Indo-American Journal of Life Sciences and Biotechnology

ribosomal RNA, template free (negative control), and 3D7 strains (positive control) were amplified via primary and nested primers. Only positive isolates with *P. falciparum* were processed further.

Genotyping of Plasmodium falciparum

The first step was amplification of the positive isolates containing genes coding for *msp1* and *msp2* in line with the polymerase chain reaction (PCR) method reported by Ajibaye *et al*.[16] Genotyping *msp1* and *msp2* with the aid of nested-PCR, block 2 of *msp1 (*3 families) and block 3 of *msp2* which consists of two families.[16]

Fragment analysis of merozoite surface proteins

The PCR amplified products were analyzed via electrophoresis on 2% agarose gels and viewed under ultraviolet (UV) transillumination. The various DNA fragment sizes of each allele were observed alongside a DNA ladder marker on an UV-trans illumination gel documentation system (Invitogen, Kalsruhe, Germany). The assessment of parasite strains by the PCR fragment length polymorphism was based on the differences in the sizes of the alleles. Alleles with similar band length fluctuations of <18 base pairs were judged to be identical. The samples with more than one allele were recorded as having a polyclonal infection.

Statistical analysis

The MOI was obtained from the total number of alleles for *msp1* and *msp2* divided by the number of samples that tested positive for the marker.[11] The formula $[n/(n-1)]$ $[(1-\Sigma Pi^2)]$ was used to calculate the expected heterozygosity (Hz), where Pi is the allele frequency and n is the sample size.[17] The comparisons of the allele proportions between sexes were performed through the Chi-squared test. A value of *P* (0.05) was considered statistically significant.

Data availability statement

Data is with correspond author, upon request it will share.

RESULTS

A total of 690 participants were recruited for this research, of which only 86 (12.5%) were enrolled for PCR analysis and gel electrophoresis analysis on the basis of low parasite density. Among the participants, 38 (44.2%) were males, while 48 (55.8%) were females [Table 1]. A total of 86 isolates were positive for *msp1,* whereas 80 were positive for *msp2*. Overall analysis of 86 *P. falciparum* isolates within the *msp1* allelic families, yielded 240 unique fragments, of which 32.74%, 30.97%, and 36.28% of the total fragments were K1, MAD20, and RO33 alleles, respectively. The overall 80 *P. falciparum* isolates within the *msp2* allelic families, yielded 164 unique fragments, 52.4% and 47.6% of which were FC27 and 3D7 alleles, respectively [Figure 2].

Table 1: Allelic composition of *Plasmodium falciparum* merozoite surface protein-1 gene among male and female malaria infected under five children in Delta State

Gene	Alleles	Total, *n* (%)	Males, *n* (%)	Females, *n* (%)	*P*
msp1					
Monoclonal	K1	4 (4.6)	2 (2.3)	2 (2.3)	0.642
	MAD20	-	-	-	-
	RO33	-	-	-	-
	MAD20 + K1	-	-	-	-
Polyclonal	RO33 + K1	12 (14.0)	8 (9.3)	4 (4.7)	0.564
	MAD20 + RO33	12 (14.0)	6 (7.0)	6 (7.0)	0.522
	MAD20 + RO33 + K1	58 (67.4)	22 (25.5)	36 (41.9)	0.728

*P<0.05 was significant. *msp1*: Merozoite surface protein-1

Allelic variability in *Plasmodium falciparum*'s merozoite surface protein 1 in Delta state

Among the 240 fragments detected for the *msp1* gene, 4.6% were K1 monoclonal alleles with band sizes within 200–220 bp and 14.0% were polyclonal k1+RO33 alleles having band sizes ranging from 180 to 220 bp; 14.0% of MAD20+RO33 alleles with band sizes ranging from 180 to 200 bp; and 67.4% were MAD20+RO33+K1 alleles with band range, ranging from 180 to 220 bp. In addition, the different alleles MAD20, K1 and RO33 in the female population were not significantly ($P < 0.05$) different from the alleles in the male population [Table 1].

Plasmodium falciparum merozoite surface protein 2 allelic variability in the study population

A total of 80 positive samples were obtained from the PCR fragments and gel electrophoresis analysis of msp2 [Table 2] in the study population, of which 10.0% were FC27 monoclonal alleles with a band size range of 405 bp and 90.0% multiple allelic infection of 3D7+FC27 within the range of 270–405 bp. The *msp2* polyclonal allele FC27+3D7 (90.0%) was higher in the study population than the monoclonal allele (10.0%) [Figure 3]. In addition, the monoclonal 3D7 allele was not detected in the study population [Table 2]. Furthermore, the 3D7 and FC27 alleles in the female population were not significantly ($P < 0.05$) different from the alleles in the male population [Table 2].

Multiplicity of infection and expected heterozygosity The MOIs for *msp1* and *msp2* were 1.10 and 1.08, respectively. The Hz values for *msp1* and *msp2* were 0.67 and 0.50, respectively [Table 3].

DISCUSSION

P. falciparum genetic multiplicity and diversity are currently significant endemicity indices in epidemiology studies and are recommended as the standard profiles for understanding parasite population structure to design new preventive and treatment strategies.[17,18] Reports have shown that geological locations and cities with high genetic diversity exhibit high transmission intensity and weak population structure, while regions with reduced genetic diversity exhibit low transmission intensity and a defined population structure.[19,20] Although notable strategies have been implemented to prevent, control, and eliminate malaria from Nigeria, the disease continues to be

Indo-American Journal of Life Sciences and Biotechnology

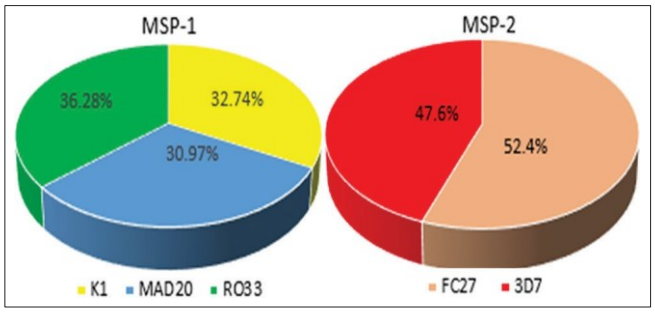

Figure 2: Allelic frequency of *Plasmodium falciparum* merozoite surface protein-1 gene and merozoite surface protein-2 gene in Delta State. *msp1*: Merozoite surface protein-1, *msp2*: Merozoite surface protein-2

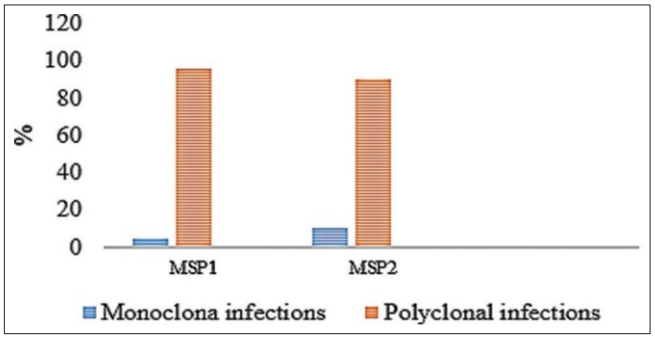

Figure 3: Proportions of monoclonal and polyclonal infections. *msp1*: Merozoite surface protein-1, *msp2*: Merozoite surface protein-2

Table 2: Allelic composition of *Plasmodium falciparum* merozoite surface protein-2 gene in Delta State

Gene	Alleles	Total, *n* (%)	Male, *n* (%)	Female, *n* (%)	*P*
msp2					
Monoclonal	3D7	-	-	-	-
	FC27	8 (10.0)	6 (7.5)	2 (2.5)	0.484
Polyclonal	FC27 + 3D7	72 (90.0)	32 (40.02)	40 (50.0)	0.682

*P<0.05 was significant. *msp2*: Merozoite surface protein-2

Table 3: Expected heterozygosity and multiplicity of infection of *Plasmodium falciparum* merozoite surface protein-1 and merozoite surface protein-2 alleles in Delta State, Nigeria

	Total	*P*
MOI		
msp1	1.10	0.001*
msp2	1.08	0.102
Overall	1.07	0.000*
Mean expected (Hz)		
msp1	0.67	0.000*
msp2	0.50	0.000*

*P<0.05 was significant. MOI: Multiplicity of infection, Hz: Heterozygosity, *msp1*: Merozoite surface protein-1, *msp2*: Merozoite surface protein-2

transmitted and spread across the country. Thus, this research evaluated the genetic structure and diversity of *P. falciparum* *msp1* and *msp2* among children under 5 years of age in the selected areas of Delta State, Nigeria.

The investigation revealed that the RO33 and FC27 alleles of the P. falciparum msp1 gene and msp2 gene, respectively, were the most prevalent in the study region. Consistent with other findings, this finding confirms that the RO33 variant is the most common msp1 gene in Osun state. [21] On the other hand, the most common msp1 and msp2 alleles in Minna, North Central Nigeria, were MAD20 and FC27, while in Nigeria and Southwest Nigeria, K1 and 3D7 were dominant[11, 22]. [23] The In addition, pregnant women in Delta state and the state of Osun have both indicated that the 3D7 allele of the msp2 gene is the most common. [24] This variance in allele distribution might be explained by changes in the primary adaptation of the alleles, which are influenced by factors such as the strength of transmission, population size, and geographical location. 18 and 19 Greater allele variety is seen in areas with high transmission intensity because there are more co-circulating strains and polyclonal alleles, which allow advantageous alleles to thrive. Delta state had a higher prevalence of these polyclonal variants of msp1 (95.4%) and msp2 (90%) P. falciparum infections. The research region had the highest prevalence of msp1 and msp2 polyclonal malaria infections caused by the K1+MAD20+RO33 and 3D7+FC27 co-infections, respectively. Similar to what Usman-Yamman et al. found, the majority of malaria infections in Minna, North Central Nigeria, were caused by polyclonal forms of msp1 (62% of cases) and msp2 (59% of cases). [23] The The majority of P. falciparum infections in Indonesia, Republic of the Congo, and Kinshasa Province were found to be monoclonal, according to the aforementioned investigations. 20 and 25 Variegated results may be attributable to variations in the demographic and epidemiological characteristics, such as asymptomatic migrants and transmission intensity, that determine the structure of the P. falciparum population. The multi-organ infection (MOI) is a consequence of parasite infection with several genotypes, which might be the outcome of super-infection or co-infection. Those two numbers Results showed that the msp1 gene of P. falciparum had a high mean MOI of 1.10 and the msp2 gene of 1.08, respectively. Delta State, in southern Nigeria, is still one of the areas where different P. falciparum alleles are transmitted at significant rates, even though the World Health Organization and the Nigerian government have stepped up their efforts to eliminate malaria in the country. In contrast to other studies, ours found lower mean MOIs for msp1 and msp2 than 1.5 and 1.8 in Osogbo, Southwest Nigeria, 2.1 and 2.2 in Ogun state, 1.32 and 1.24 in Nigeria, 2.0 and 1.6 in Chewaka district, Ethiopia, 1.24 and 1.20 in Oyo state, Western Nigeria, 1.63 and 1.24 in South Africa, and so on. Possible explanations for these discrepancies include variations in malaria endemicity, transmission intensity, parasite density in the research area's population, use of antimalarial drugs, and vectors that transmit malaria to humans. pages 18–28 areas with low levels of infection transmission are indicated by areas with low MOIs, while regions with high MOIs represent regions with significant transmission. [26]

The Hz values that were projected to be achieved were 0.67 (msp1) and 0.50 (msp2), which indicate that Delta State usually has high genetic diversity and moderate genetic diversity, respectively. Although a higher Hz of 0.95 for msp2 was recorded in Chewaka District, Ethiopia, the Hz results in this research were greater than those in a few prior investigations that showed values of 0.54 (msp1) and 0.59 (msp2)

Indo-American Journal of Life Sciences and Biotechnology

in Nigeria[12] and 0.43 (msp1) in Ethiopia. [18] in Hz provides crucial information on the genetic structure of a population and is hence an essential parameter for evaluating the complexity of genetic diversity in natural populations. [8] Study scores ranging from 0 to 1, 2, 3, 4, and 6 are categorized as extremely low, low, moderate, and high, respectively, on a scale from 1 (absence of Hz) to 0 (equal frequency of a large number of alleles). [26] The results show that there is a high transmission rate of certain multiclonal P. falciparum infections in this particular geographic location.

CONCLUSION

This study presents a significant degree of genetic middling heterozygous alleles and a typically high transmission rate of *P. falciparum* infection in Delta state, southern region of Nigeria. These findings highlight the necessity of designing and formulating précised and regionalized antimalarial remedies.

Outcomes of the study

- The mean MOIs of *msp1* and *msp2* gene were 1.10 and 1.08, respectively, which infer high levels of *P. falciparum* transmission in Delta State
- The expected Hz values obtained were 0.67 (*msp1*) and 0.50 (*msp2*), inferring typically high and moderate genetic diversity within Delta State
- Polyclonal infections are the dominant form in Delta State
- The most prevalent monoclonal *msp1* and *msp2* allele were RO33 and FC27.

Rationale of the study

Despite the increasing efforts and interventions to achieve decreased malaria burden and mortality rate in the African regions, this disease remains a major public health burden of which children aged 5 years and younger are mostly at risk of the major complication. The awareness of genetic structure and variations in *P. falciparum* is critical for designing and developing more effective elimination and prevention strategies for malaria and for evaluating the efficiency of antimalarial drugs in Nigeria. There have been reports of

P. falciparum variety in certain sub-Saharan African regions, but parasite genetic structure is yet to be fully established in Delta State, Nigeria. Hence, it is essential to obtain a structural profile, and then build a genetic repertoire of *P. falciparum* for malaria intervention in the study area. Thus, the use of established molecular markers such as *msp1* and *msp2* to assess *P. falciparum* infection's genomic diversity and complexity in clinical isolates in this study reveals the baseline data on *P. falciparum* structural profile and genetic diversity in the Delta State. This finding provides a molecular collection of information for planning successful malaria intervention programs in Nigeria.

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Indo-American Journal of Life Sciences and Biotechnology

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