



Molecular assessment of Merozoite Surface Protein-2a (MSP2a) Amongst Malaria Infected Children in Yenagoa District of Bayelsa State, Nigeria

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Abstract

Malaria continues to be a significant public health challenge, particularly in tropical and subtropical regions. The clinical manifestations and severity of malaria is connected to Merozoite Surface Protein-2A (MSP2A), expressed on the surface of merozoites, the invasive form of Plasmodium parasites. This study investigates Merozoite Surface Protein-2A (MSP2A), among malaria-infected children in Yenagoa, Bayelsa State, Nigeria. Utilizing polymerase chain reaction (PCR) techniques, blood samples collected from a cross section of randomly selected malaria-infected children were analyzed to determine presence of MSP2A. The results indicated a 20% prevalence of MSP2A in the tested children population, spread across ages 1, 2, 4 and 5, with highest prevalence (40%) for age five and generally skewed more towards male sex. Although further investigations with larger sample size to enhance reliability of the results is recommended.

Keywords: Malaria, MSPs, MSP2A, Plasmodium falciparum, Erythrocyte, Age.

Introduction

Malaria remains a significant public health challenge globally, particularly affecting populations in tropical and subtropical regions. According to the World Health Organization (WHO), an estimated 229 million cases of malaria occurred worldwide in 2019, with approximately 409,000 deaths, the majority of which were in children under the age of five (WHO, 2020). Malaria is caused by Plasmodium parasites, transmitted through the bites of infected Anopheles mosquitoes. Among the various stages of the Plasmodium life cycle, the merozoite stage is critical for the pathogenesis of the disease, as it attacks erythrocytes leading to the clinical manifestations of malaria. Merozoite Surface Protein-2A (MSP2A) is a protein expressed on the surface of merozoites, the invasive form of Plasmodium parasites responsible for the clinical manifestations of malaria.

The MSP2A plays a crucial role in the invasion of RBCs by the parasite and has been identified as a potential vaccine candidate due to its immunogenic properties. By targeting MSP2A, researchers aim to develop a vaccine that can prevent merozoite invasion and thereby provide protection against malaria infection.

Understanding the genetic diversity of MSPs and their implications for vaccine development is essential. Merozoite Surface Protein 2A (MSP2A), a variant of MSP2 found in certain strains of Plasmodium falciparum, has drawn particular interest due to its unique characteristics. As with other MSP variants, MSP2A's role in immune evasion and malaria pathogenesis makes it a valuable target for research.

Previous research has highlighted the importance of MSPs in malaria pathogenesis, but limited data exist on the prevalence of specific MSP variants, such as MSP2A, for endemic regions in developing nations (Taylor et al., 2012). As such, MSP2A is a key target for vaccine development efforts aimed at preventing merozoite invasion of red blood cells

(RBCs). (Smith et al., 2015). Taylor et al. (2012) highlighted the ability of MSP2A to cause immuno-cellular reactions, suggesting its potential for inducing protective immunity against malaria. Similarly, findings from Smith et al. (2015) supported the immunogenicity of MSP2A and its capacity to stimulate both antibody production and T cell responses in vaccinated individuals.

In recent years, efforts have intensified to further evaluate MSP2A as a vaccine candidate and to advance its development towards clinical trials. Studies have focused on optimizing vaccine formulations, assessing vaccine efficacy in preclinical models, and investigating the genetic diversity of MSP2A in malaria-endemic regions. Despite challenges associated with genetic diversity and antigenic variation, MSP2A remains a promising target for malaria vaccine development, with the potential to contribute to the global efforts to control and eliminate malaria (Holder et al., 1987; Holder et al., 1993).

Merozoite Surface Protein-2A (MSP2A) is a key antigen expressed on the surface of merozoites, the invasive form of Plasmodium parasites responsible for malaria infection. The prevalence of MSP2A varies across different malaria-endemic regions, influenced by factors such as parasite strain diversity and host immune responses. Epidemiological studies have been conducted to assess the prevalence and distribution of MSP2A alleles among malaria parasite populations (Taylor et al., 2015).

Interventions, including bedding-net treatment, and room sprays against insects, with drug treatment, can show significant effects on the epidemiology of MSP2A in malaria rollback. Studies have assessed the impact of these interventions on the prevalence and genetic diversity of MSP2A alleles, providing insights into the dynamics of parasite populations and the effectiveness of control strategies (Garcia et al., 2019). Monitoring changes in MSP2A prevalence and distribution can help evaluate the success of malaria control programs and guide future intervention efforts.

Investigations into the prevalence of MSP2A in malaria-endemic regions have provided important epidemiological data. Jones et al. (2018) assessed the prevalence of MSP2A alleles in a cohort of malaria-infected individuals in sub-Saharan Africa, revealing significant genetic diversity in MSP2A among parasite populations. These findings underscore the importance of considering genetic variation in MSP2A when designing vaccines targeted against this protein.

Nigeria as a nation is also grappling with malaria scourge, accounting for twenty-seven percent of it globally, and with the most death (24%) in 2019 (World Malaria Report, 2020). Meanwhile, seventy-six percent of the populace are susceptible as they live in high areas malaria is highly transmitted. Incidence and prevalence of malaria among children in Yenagoa, Bayelsa, and the implications for public health interventions is a major concern. Okonofua et al. (2019) investigated the burden of malaria in children under five years of age in Bayelsa State, highlighting the high prevalence of malaria and the need for effective control measures in the region. In view of these, phased studies have been designed to explore MSPs, the allelic variants and interactions in association with some clinical manifestations of the scourge of malaria; to provide scientific information for research data and succor in this locality.

In this study, researchers focused on the prevalence of MSP2A in malaria-infected children in Yenagoa. This region presents unique epidemiological challenges, including high malaria transmission rates and limited access to healthcare services.

Methods

The study was carried out in Nucleometrix Research Laboratory, Yenegoa, Bayelsa state. Bayelsa State is a cosmopolitan state in the southern part of Nigeria, which is geopolitically located within Latitude 415 North, 523 South, Latitude 522 West and 645 East. It has an area of 706km. It shares boundaries with Delta State on the North, Rivers State on the East with the Atlantic Ocean on the West and South. The official language is English language but the major language spoken is the Izon language.

Random selection of 25 samples from cross section; following ethical clearance obtained from the ethical committee department of Nucleometrix Research Laboratory was done. A total number of twenty five (25) blood samples were collected from patients of Nucleometrix Research Laboratory, Yenegoa, Bayelsa state. Taro Yamane formula with 95% confidence level was used to determine the sample size determined by method of Yamane, 1973. The research was carried out using rapid diagnosis test and molecular diagnosis (PCR technique) method.

DNA extraction (Chemical Method)

The samples were lysed by adding four volumes of genomic lysis buffer to the samples (4:1). And vortexed in 6s, kept standing five mins at room temperature. Mixture of individual samples was placed in correspondingly labelled Zymo-spin II CR column of tubes, and centrifuging at 10,000 rpm for 1 minute, with discarding of flow through. The Zymo-spin II CR column was transferred to a new collection tube and DNA pre-wash buffer 200µl added. and centrifuged for one minute; g-DNA wash buffer 500µl was added to the Zymo-spin II CR column and centrifuged for one minute. The spin column was then transferred to a clean microcentrifuge tube, and 90µl DNA elution buffer was added, incubated at

room temperature for two minutes and then centrifuged (10000 rpm for 30 seconds) with a view to eluting the DNA. Then DNA quantification followed accordingly, with subsequent amplifications (Muth et al, 2007; Ezumma et al, 2011).

Results

RESULTS OF MALARIA BLOOD TESTS INDICATING THE PREVALENCE OF M SP2A

SN	Sex	Age	M SP2A
1	F	4	+
2	M	5	+
3	M	5	-
4	M	2	-
5	F	4	-
6	M	1	+
7	F	2	-
8	M	3	-
9	M	4	-
10	F	2	-
11	M	5	+
12	F	1	-
13	M	4	-
14	M	4	-
15	M	2	-
16	F	5	-
17	M	2	-
18	F	1	-
19	F	2	+
20	M	2	-
21	F	1	-
22	M	1	-
23	M	5	-
24	F	3	-
25	F	3	-

Table 1 Where + indicates the presence of MSP2A and - indicates the absence of MSP2A.

Twenty five (25) blood samples from children aged 1-5 were collected and tested. A total of five (5) samples tested positive for MSP2A. This indicated a 20% prevalence of the MSP2A gene in the test samples.

Distribution of MSP2A gene by age and sex

No. of MSP2A Detected +	Male	Female	Age
1	1	0	1
1	1	0	2
0	0	0	3
1	0	1	4
2	2	0	5

Table 2 Identified MSP2A gene were found in children aged 1 (20%), age 2 (20%), age 4 (20 %) and age 5 (40%). MSP2A gene was not found in children aged 3

Fisher’s Exact test (p = [0.61939] and p = [0.0858]) showed significant association between MSP2A and Age, as well as MSP2A and sex respectively.

Figure 1

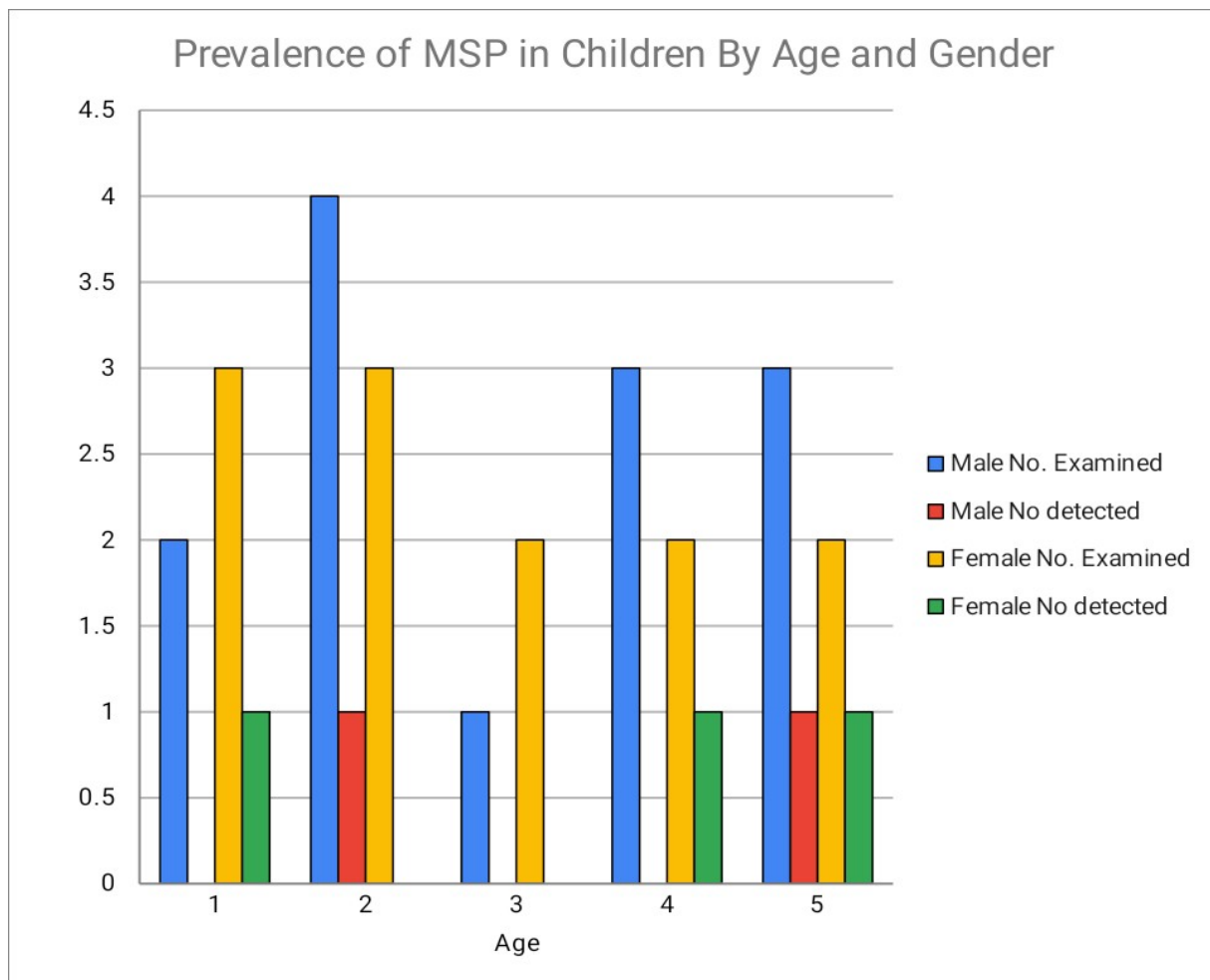


Figure 1: Showing the prevalence of MSP2A by age and sex (gender).

Discussion

This section provides a discussion of the findings from the study investigating the prevalence and distribution of the MSP2A gene in children aged 1-5. A total of 25 blood samples were analyzed, revealing patterns related to age and sex.

Distribution of MSP2A Gene by Age: The distribution of the MSP2A gene across different ages was an important aspect of this study. The gene was detected in 20% of the overall sample population, distributed as follows:

Age 1: Detected in 1 sample, representing 20% of the total detections.

Age 2: Detected in 1 sample, representing another 20%.

Age 3: No detections, indicating 0% presence.

Age 4: Detected in 1 sample, contributing to 20%.

Age 5: Detected in 2 samples, the highest at 40%.

The data indicates that prevalence of the MSP2A gene is not uniform across all age groups. Notably, the gene was absent in 3-year-olds and most prevalent in 5-year-olds. This suggests potential age-related factors influencing gene presence for further investigation.

Prevalence by Age and Sex: The study also examined the prevalence of the MSP2A gene in relation to sex, highlighting significant differences:

Age 1: The gene was detected in males more frequently than females.

Age 2: A similar pattern was observed, with higher male prevalence.

Age 3: No detections in either gender.

Age 4: The prevalence was more among females.

Age 5: Males showed a higher prevalence than females.

The findings suggest sex might be associated with susceptibility to MSP2A gene, showing a higher detection rate among males in most age groups. The absence of the gene in age 3 across both sexes is intriguing and suggests a potential biological or developmental factor that needs further exploration; particularly as Fisher's Exact test seem to affirm statistical significance in both trends – implying association exists between MSP2A gene presence and sex as well as age.

Summary

The research revealed a 20% prevalence of the MSP2A gene among the children tested, with notable variations based on age and sex. Higher detection rates were in ages 1 and 5 specifically, while no detections were found at age 3. Males were generally more affected than females, indicating possible sex-related susceptibility.

The absence of the MSP2A gene in 3-year-olds could indicate a developmental stage where the gene is less likely to be present or detectable. Although, non-captured environmental factors, small sample size, or age range (study only including children aged 1-5), may limit the ability to generalize validity of findings across a broader age range. Precisely, lack of data on environmental, dietary, and other potential contributing factors might affect the MSP2A gene as seen.

The higher prevalence in 5-year-olds may suggest that as children age, the likelihood of detecting the gene increases, particularly in males.

Conclusion

MSP2A is variably prevalent in these children investigated along sex (more in males) and age (highest in 5 year olds) profile in this preliminary research.

Recommendations

*Future studies should include larger sample sizes and age range (older children and adolescents) to validate these findings and provide a more comprehensive understanding of MSP2A gene prevalence.

*Longitudinal Studies, tracking the same cohort over time could provide insights into how the prevalence of the MSP2A gene changes with age.

*Investigating potential environmental exposures or genetic predispositions that might contribute to the presence of the MSP2A gene.

*Developing targeted public health interventions aimed at age groups and genders most at risk.

*Exploring the biological mechanisms underlying age and sex differences in MSP2A gene prevalence

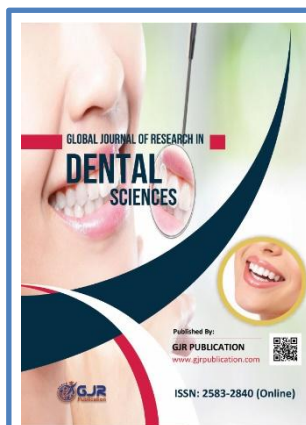
Addressing limitations and these recommendations, future research can provide a deeper understanding of the MSP2A gene and its implications for children's health, leading to more effective prevention and intervention strategies in Bayelsa State health sector.

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