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MEROZOITE SURFACE PROTEIN - 1 POLYMORPHISM IN MALARIA INFECTED PATIENTS ATTENDING FEDERAL MEDICAL CENTRE, YENAGOA BAYELSA STATE

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ABSTRACT

Malaria is a major public health issue in Nigeria and the genetic structure of malaria parasite may affect its transmission model and control strategies. Merozoite surface protein (MSP – 1) is a vitral protein found on the surface of the merozoites and plays a crucial role in the invasion of new erythrocytes. This study was aimed at determining the polymorphism of Plasmodium falciparum MSP-1 gene in malaria infected patients who visited a federal medical centre. A total of 50 blood samples were collected and analyzed using standard parasitological and genotypic techniques. Out of the samples obtained for this study, 3 (6%), and 13 (26%) were positive in RDT and PCR in that order. All the 13 PCR positive samples were also positive for MSP-1-K gene whereas none was positive for MSP-1-M, and MSP-1-R genes. It may be inferred from this that MSP 1-K appears the most prevalent of the allele of plasmodium falciparum MSP-1 in the study population.

KEYWORD: MSP-1 genes, polymorphism, PCR, plasmodium falciparum, sex, age.

INTRODUCTION

Malaria is a disease of global concern transmitted in approximately 87 countries and territories particularly the tropical and sub Saharan regions of the world; where it contributes hugely to motility and morbidity (WHO, 2021). And currently, there is recorded decline in its cases credited to efficient preventive and treatment measures such as insecticide-treated bed nets and the use of artemisinin-based combination therapies as the first-line therapy (WHO, 2022).

Although some of these advances are glaring, a huge burden of malaria still plagues Africa as a continent with about 10 million cases and 200,000 fatalities annually and about 31.9% of the global malaria index; with attendant challenges including risk for expectant mothers, young children, complicated by emergence of medication and insecticide resistant strains of the parasite/vector (WHO, 2015; Ashley et al., 2014).

In the face of current realities and developmental strides by the global scientific community, a successful development of a malaria vaccine would no doubt be a perfect complement to the current strategies implored for malaria control and eradication. However, the extensive genetic variety of parasite population is a rate limiting step/factor in the effectiveness of acquired protective immunity to malaria. More so, multiple parasite clones that are related to the intensity of transmission, affecting the host immunological status are frequently simultaneously infecting a single individual (Yavo et al., 2016). Thus, it appears the clinical aspects of this disease and the effectiveness of prospective malaria vaccination may be affected by this scenario.

In order to modify malaria control and extermination techniques, it is crucial to understand the parasite population, and in depth knowledge of the parasite's genomic variation is necessary for the possible elimination of malaria (Yavo et al., 2016). Clinical manifestations of malaria is directly associated with a repeated cycle of invasion of the red blood cell (RBC) by merozoites, followed by its development into schizont which end up in the rupture of the (RBC)and release of daughter merozoites (Cowman et al., 2017; Yavo et al., 2016). During the blood stage the parasites express arrays of protein which include merozoites surface protein (MSP) 1 and 2 that are being considered top candidates for the development of a malaria vaccine and useful markers for identification of genetically distinct plasmodium falciparum parasite populations (Cowman et al., 2017; Yavo et al., 2016).

Meanwhile one of the several identified obstacles or impediment to successful emergence of a malaria vaccine is the extremely polymorphic genetic variation of plasmodium falciparum MSP 1 that helps it avoid or evade immune detection, thus adding to its capacity to infect the same individual repeatedly (Lin et al., 2016; Cowman et al., 2016). Hence researches to further the

understanding of diversity of this parasite in molecular/genetic dimensions are of significance not just for vaccine development but also more precise / accurate therapies.

In lieu of the foregoing, this study was aimed at assessing malaria prevalence with specific objective to determine allele distribution of MSP-1 genes for a study population in Yenagoa, a south southern Nigerian state capital.

METHODS

The study was carried out at the Federal Medical Centre, Yenagoa, the capital city of Bayelsa State in Nigeria, adopting a cross sectional design using random sampling technique; following approval by the Ethical committee of the Federal Medical Centre and signing of written informed consent form by participants.

Sample size was determined in accordance with Taro Yamane formula, and a total of fifty blood samples were collected by standard operational protocol from patients with symptoms of malaria referred by physician for screening of malaria infection within last 48 hours. Those patients who did not meet this inclusion criterion were not enrolled in the study.

The parasites were identified using rapid diagnostic test (RDT) and molecular detection (PCR) methods; nested PCR was done on the samples to detect MSP-1 K, MSP-1 M, and MSP-1 R genes following established procedure of DNA extraction, DNA quantification, and MSP-1 amplifications as well as for the allele variants.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) version 24 was implored in the statistical analysis involving chi-square to determine statistical significance ($P<0.05$) in observed differences of the infection while portion was used to present the prevalence of the infection and distribution of different allelic families.

RESULT

Table 1: Malaria parasite infection by age.

Age (Years)	No. Examined (%)	No. Infected In Microscopy (%)	No. Infected in PCR (%)	No. Infected in RDT (%)	p-value (chi-square)
1-10	6(12)	3(50.0)	0(0)	0(0)	p=0.0551
11-20	9(18)	0(0)	6(66.67)	3(33.3)	
21-30	16(32)	3(18.75)	3(18.75)	0(0)	
31-40	13(26)	9(69.23)	3(23.07)	0(0)	
≥41	6(12)	4(66.67)	1(16)	0(0)	
Total	50	22(44)	13(26)	3(6)	

Majority of the subjects 16 (32%) were between 21-30 years, followed by 31-40 years that was 13 (26%), 11-20 years was 9 (18%) while 1-10 and those ≥ 41 were both 6 (12%) the least in the study population. All infected as captured in RDT 3 (6%) were within age brackets 11-20 years. Out of the 13 infected from PCR test, 11-20 years was more.

Table 2: Malaria parasite infections along sex profile.

Sex	No. Examined	No. Infected in RDT	No. Infected in Microscopy	No. Infected	in PCR P-value
Male	31(62)	3(9.7)	9(29.03)[b]	6(19.35)	p= 0.0646
Female	19(38)	0(0)	13(68.42)[a]	6(31.58)	p= 0.3259
Total	50	3(6)	22(44)	12(24)	

Male subjects 31 (62%) were more in the study population than female 19 (38%); and RDT captured no female infected whereas 3 (9.7%) male were infected. But female were more infected 13 (68.42%) than male 6 (19.35%) as captured by PCR. The PCR captured difference between sexes was significant (p> 0.05).

Table 3: Alleles of MSP-1 genes and base pair range in the study area.

MSP-1(n=13)	Allele Size (bp)	No. of different genotype/allele (%)	Total No. per allele	MOI (%)
K1	180-240	13 (100)	13 (100)	1.0
MAD20		0 (0)	0 (0)	
RO33		0 (0)	0 (0)	
K1/MAD20		0 (0)	0 (0)	
K1/RO33		0 (0)	0 (0)	
RO33/MAD20		0 (0)	0 (0)	
K1/RO33/MAD20		0 (0)	0 (0)	
Total		13	13	
Out of the 13 positive subjects in PCR, all (100%) were observed with MSP-1 K. All the infections were monoclonal with no MSP-1 M or MSP-1 R found in the study population.				

DISCUSSION AND CONCLUSION

An inaccurate malaria diagnosis represents one worrisome public concern, as this could lead to ineffective treatment outcomes and consequently drug resistance and recurrence, particularly in malaria endemic regions such as Nigeria. Investigations focusing on discoveries for improvement of malaria treatment, control and especially prevention through development of malaria vaccines have been among frontline research.

In lieu of this, the present study considered blood samples of fifty individuals showing malaria symptoms for which they were referred to the laboratory to undergo malaria screening test. Preliminarily the RDT carried out for the samples captured 3 (6%) positive cases of plasmodium falciparum infection (see table 1) which was a contrast of previous report by Dahal et al (2021); as they observed and reported 13.6% cases of malaria infection from RDT screening of subjects in Nepal.

Regarding malaria infection and age profile, the observation from table 1 showed 11 to 20 years 3 (33.3%) to be prone to the infection as captured in RDT which may be comparable to the results of independent investigations by Umunakwe et al (2019) in Lagos, Nigeria and Khagayi et al (2019) in Uganda that most malaria infections were between the ages of 6-14 years and 5-14 years respectively.

Similarly, the PCR screening captured ages between 11 and 20 as being more prone to malaria infection, which may also be comparable with independent research of Umunakwe et al (2019) and Khagayi et al (2019). Although, little difference exists when comparing 11-20 with 6-14 and 5-14, possible causes for such discrepancies are explored for reporting in further investigations outside this current scope.

On the evaluations regarding polymorphism of merozoite surface protein which is a salient objective in the study, the malaria parasite when in the blood is known with identity of releasing varieties of proteins which include MSP 1 and 2 that currently are top targeted candidates for malaria vaccine emergence (Cowman et al., 2017). Besides, they are also relevant as markers for identifying of genetic varieties in plasmodium falciparum parasite populations (Yavo et al., 2016). More succinctly, is the submission that polymorphic genetic variation of plasmodium falciparum MSP 1 enables this parasite evade any possible detection/identification by immune system, thereby enhancing its capacity to repeatedly invade and infect the same person (Lin et al., 2016).

In this investigation, carried out within Yenagoa, a malaria endemic region, it was observed that twenty-six percent of individuals were positive for the parasite from the PCR screening, among whom those aged 11-20 were most prevalent (see table 1). Also, from table 2, female were more infected 13 (68.42%) than male 6 (19.35%). The PCR captured difference between sexes was significant ($p > 0.05$). Meanwhile, out of this whole proportion captured in PCR, one hundred percent (100%) was observed with MSP-1 K (see table 3). This further presents all the infections as monoclonal with no MSP-1 M or MSP-1 R found in the study population in this malaria endemic region.

Conclusively, MSP-1 K is most prevalent in this study population of malaria endemic area, and this awareness with further investigations could contribute viable knowledge to better malaria control measures.

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