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Research Article

ALLELIC DIVERSITY OF PLASMODIUM *FALCIPARUM MEROZOITE* SURFACE PROTEIN-2 (PFMSP-2) AND MULTIPLICITY OF INFECTION IN SUDANESE CHILDREN WITH *FALCIPARUM* MALARIA

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Abstract

Background: Malaria is a major public health problem in the Sudan including Gezira State. The genetic diversity of malaria parasite may affect its transmission model and control strategies. Merozoite Surface Protein-2 (MSP-2) is a glycoprotein expressed on the surface of merozoites that has been considered as one of the candidates for blood stage malaria vaccines. The MSP-2 gene is located on chromosome 2 and is composed of five blocks the most polymorphic of which is the central block 3. The gene is encoded by highly divergent alleles, grouped into two dimorphic families FC27 and IC/3D7. **Methods:** Filter paper blood spot samples (89) of *P. falciparum* isolates were collected from the children attending Wad Medani Pediatric Teaching Hospital, Gezira State, Sudan from September 2022 to February 2023. Parasite density was estimated per microliter using thick blood film. The genomic DNA was extracted, and the MSP-2 gene was genotyped by nested PCR using allele-specific primers for *P. falciparum*. **Results:** For the MSP-2 gene, the most frequent allele was the FC27 (68.5%) allelic family in the study area compared to IC1/3D7 (59.6%) allelic family, and samples positive for the both alleles IC1/3D7 and FC27 were (32 and 35.9% respectively). The total multiplicity of infection (MOI) of MSP-22 was (1.48). 14.61% of studied samples were found to have multi-clonal isolates and the mixed infections were 40.45%. In regarding to parasitemia, the level 1 – 5000 parasite/µl were (58.9%) for IC1/3D7 and (70.0%) for FC27. The mixed infections represent (75%) and the MOI was 1.58. On the other hand, in the parasitemia level >10000 parasite/µl there were (62.1%) for IC1/3D7 and (65.6%) for FC27. The mixed infections represent (65.5%) and the MOI was (1.27). **Conclusion:** The study found highly genetic polymorphisms with diverse allele types of MSP-2 as well as the high MOI of *P. falciparum* isolates in Gezira State, Sudan.

Keywords: *Plasmodium falciparum,* Merozoite Surface Protein-2 (PfMSP-2) – Sudan – MOI.

INTRODUCTION

Malaria is an infectious disease caused by the Plasmodium parasite and is transmitted among humans through bites of female Anopheles mosquitoes. The infection has been one of the most global health challenges throughout the world. About 40% of the world's population specially, Africans and some other developing countries live in malaria endemic area. Africa shares 80% of the cases and 90% of deaths (1). Most malaria infections in Africa are caused by *P. falciparum* which is also more virulent and causes the majority of malaria-related mortality worldwide (Al-Awadhi *et al.,* 2021). In 2020, there were 241 million in malaria cases in the 85 malaria-endemic countries, leading to 627, 000 deaths, of these cases, 96% occurred in Africa. Estimated malaria-related morbidity and mortality have increased by 6% and 12% respectively (2). In Sudan, the prevalence of malaria is about 5.9%, on the other hand *P. falciparum* is most common causes (87.6%) of cases (3).

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Plasmodium falciparum is the most virulent malaria parasite species. It exhibits a complex genetic polymorphism which may explain its ability to present with different clinical manifestations of the disease spectrum. Moreover, some have stated that certain *P. falciparum* genotypes can be linked with more virulent infections, and the presence of multiple infections may affect the release of different pro-inflammatory cytokines, that makes it more difficult for the immune system to deal with, resulting in severe malaria (4). The MSP-2 gene is located on chromosome 2 and is encoding the merozoite surface protein 2 which is a glycoprotein with an approximately 30 kDa. It is composed of five blocks whereby the central block (block 3) is the most polymorphic. The msp2 alleles are grouped into two allelic families, namely, FC27 and 3D7/IC1. Fragment size polymorphisms in FC27 and 3D7 (for MSP-2) are used as molecular markers in studying *P. falciparum* malaria transmission dynamics and virulence. Genotyping of MSP-2 is also employed in differentiating recrudescence from reinfection in antimalarial efficacy studies (5). The multiplicity of infection (MOI) is an important parameter in malaria epidemiology. Thus, a person could be infected with many strains of *P. falciparum* at the same time.

This can make treating the infection extremely difficult. When only one or two of the numerous strains are treatment-resistant or particularly virulent (6). Studies have shown a positive correlation between MOI and endemicity, and so the MOI has been used to characterize malaria transmission intensity (7). In this regard, determination of MOI in a highly endemic area such as the Gezira State becomes crucial, since it predicts clinical course target population with higher attention (e.g. certain age groups) (4). This is a cross-sectional hospital-based study aimed to investigate the genetic diversity of *P. falciparum* MSP-2 and its relationship with the multiplicity of infection and the parasite density.

METHODOLOGY

Study area

The study was carried out in Wad Medani Pediatric Teaching Hospital in Gezira State, located in the Center of Sudan. Gezira state lies on the west bank of the Blue Nile. The geographical coordinates of the state are 14°30'0"N, 33°30'0"E. *Plasmodium falciparum* is the predominant species (prevalence 90–95 %), and the *Anopheles arabiensis* is the main mosquito vector.

Patients and malaria definition

A total of 89 patients were screened for malaria during a cross-sectional study conducted from September 2022 to February 2023. Then samples were confirmed positive for *P. falciparum* malaria by microscopy and nested PCR. All age groups (from age 1 to 18 years) were targeted. Demographical data were recorded including age and sex.

Microscopic examination and parasite counts

Blood films were stained with 10% Giemsa's at pH 7.2 and then examined microscopically for the presence of malaria parasites; 200 fields under 100× magnification was examined from the thick film before the slide was considered negative. For positive slides, parasitemia (parasite density) was determined by counting only the a sexual stages against 200 white blood cells (WBC) and the number of parasites multiplied by 8000 then divided by 200, assuming the average of total WBC count of individuals equal to 8000 cells. The level of parasitemia was graded as low (< 1000 parasites/µl of blood), moderate (1000–9999 parasites/µl of blood) and severe (≥ 10,000 parasites/µl of blood) (8).

Extraction of parasite DNA

DNA extraction was done using TE Buffer. The circles of DBS were cut off by using sterile surgical plate, then put in a sterile eppendrof tube with 1.5 ml size. Then 100 µl of TE buffer added and the DBS were punched down with tips for many times. The mix was incubated at 97℃ for 15 minutes. Then the tube was centrifuged at high speed for 30 seconds. The supernatant which contained the DNA was used for the rest of the molecular test (9).

Molecular identification, MSP2 genotyping

Plasmodium spp. were identified by 18S rRNA gene based nested PCR using genus and species-specific primers as described by (10). *Plasmodium falciparum* were further analyzed by highly polymorphic region of MSP-2 (block 3) and their allelic types (FC27and IC1/3D7), using nested PCR. In brief PCR reactions were carried out in a final volume of 25 µl containing 2 µl of parasite DNA, 2 mM MgCl2, 0.2 mMdNTPs, 1 µl of each primer and 1 unit of Taq Polymerase (Vivantis, Selangor DarulEhsan, Malaysia). Cycling conditions for the outer PCR were as follows: initial denaturation at 94 °C for 3 min, followed by 37 cycles of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 1 min; and primer extension at 72 °C for 2 min. The final cycle had a prolonged extension at 72 °C for 10 min. PCR reactions were incubated in a thermal cycler (SensoQuest, Göttingen, Germany).

Detection of alleles

The amplified PCR products were analyzed by electrophoresis on 2% molecular grade agarose gel (Caisson, Utah, USA) and visualized by UV trans-illuminator (BioDoc-It UVP, Cambridge, UK), following ethidium bromide staining. The number and size of DNA fragments was estimated using 100 bp DNA ladder (Vivantis, Selangor Darul Ehsan, Malaysia).

Multiplicity of infection

Multiplicity of infection (MOI) was determined by calculating the number of different alleles at each locus; single infections were those with only one allele per locus at all of the genotyped loci. Multi-clonal infections were defined as those having more than one allele in at least one locus out of the loci genotyped (11).

Data analysis

Data were entered and analyzed using the software Statistical Package for Social Sciences version 20 (SPSS, Inc., Chicago, IL, USA). The MSP2 allelic frequency was calculated. The mean multiplicity of infection (MOI) was calculated for MSP-2 alleles.

RESULTS

P. falciparum rDNA was detected in a total of 89 (100%) malaria patients using nested PCR. Of the total 89 samples, 54 (61%) were males, while 35 (39%) were females. The age groups ranged between 1–18 years, with a mean of 5.60 years. The mean parasite density was (21,057 parasites/µl) (Table 1).

Table 1. Demographic data

		Frequency	Percent (%)
Gender	Male	54	61
	Female	35	39
	Total	89	100.0
Age groups	1 – 4 Years	44	49.4
	5 – 9 Years	31	34.8
	10 – 18 Years	14	15.7
	Total	89	100.0

Means of Age -5.60; Median of Age -5.0;
Means of Parasitemia -21,057 parasite/µl
Median of Parasitemia 924.00 parasite/µl

The frequency of isolates of MSP-2 gene showed that allelic family FC27 was predominate allele (68.5%) in compare with IC1/3D7 allelic family (59.6%), the IC1/3D7/FC27 combinations were found in 35.9% (32/89) of samples. From all studied samples multi-clonal isolates represents 14.61%, mixed infections were 40.45% and MOI was 1.48 and fragments sizes were ranged from 300 – 600 for the two

detected alleles (Table 2). In parasitemia level 1 – 5000 parasite/µl there were 35 IC1/3D7, 42 FC27 and 26 mixed infections and the MOI was 1.58. in the other hand in parasitemia level >10000 parasite/µl there were 18 IC1/3D7, FC27 19 and 10 mixed infections and the MOI was1.27 This may go with unstable seasonality of transmission in most malarious areas of Sudan (Table 3).

Table 2. MSP-2 block 3 allelic type frequencies and multiplicity of infection of *P. falciparum* isolates from Gezira State, Sudan

Allelic Type	Total N=(89)	Fragment size*
IC1/3D7	59.6%(53/89)	300 – 600
FC27	68.5%(61/89)	300 – 600
IC1/3D7/FC27	35.9%(32/89)	
Total IC1/3D7	95.5% (85/89)	
Total FC27	104.5% (93/89)	
Multiclonal isolates	14.61%(13/89)	
Mix infections	40.45%(36/89)	
MOI	1.48	

Table 3. Distribution of MSP-2 block 3 allelic types among parasitemic groups of *P. falciparum* infected patients from Gezira state – Sudan

MSP-2 Alleles	1–5000 parasite/µl N=60	>10000 parasite/µl N= 29	Total
	Frequency and Percent	Frequency and Percent	
IC1/3D7	35 (58.9%)	18 (62.1%)	53
FC27	42(70.0%)	19(65.5%)	61
Total	60	29	89
IC1/3D7/FC27	24(75%)	8(25%)	32
Total IC1/3D7	59	26	
Total FC27	66	27	
Mix	26 (43%)	10 (34%)	
MOI	1.58	1.27	

DISCUSSION

This is study investigated the genetic polymorphism of *P. falciparum* in Gezira state, Sudan; using the most polymorphic region of the *MSP-2* gene. A high genetic diversity of the population of *P. falciparum* isolates from the study area was found. From a total of 89 samples, 54 (61%) were males and 35 (39%) were females. The age groups ranged between 1–18 years, with a mean of 5.60 years. The mean parasite density was (21,057 parasites/µl) as the study conducted in highly endemic area with high transmission of *P. falciparum* species. This is agree with (Mohammed, H., 2017). Different alleles of MSP-2 were identified in Gezira State, Sudan, FC27 alleles (61, 68.5%) and 3D7 alleles (53, 59.6%) and samples positive for the both alleles IC1/3D7 and FC27 were (32, 35.9%). FC27 allelic family was more prevalent in the study area compared to IC1/3D7 allelic family, and this findings are in consistent with studies done in White Nile State in Central Sudan, Gublak, North West Ethiopia and Jazan, Saudi Arabia(14-16) and differ from studies done in North West Ethiopia, Boset and Badewacho Districts, Southern Ethiopia and South western Nigeria(17-19), and this may be due to variation in sample size and study area. In our study, 14.61% of studied samples were found to have Multi-clonal isolates, mixed infections were 40.45% and MOI was 1.483 and this can be due to high malaria transmission levels and the frequency of multiple infections, with individuals living in areas generally harboring multiple parasite strains. This suggests that the diversity of *P. falciparum* infections may differ according to geographical location, transmission intensity and sample population and this agree with study done by(20). In regarding to parasitemia, level 1 – 5000 parasite/µl there were (58.9%) IC1/3D7 and (70.0%) FC27. The mixed infections represent (75%) and the MOI was 1.58. in the other hand in parasitemia level >10000 parasite/µl there were (62.1%) IC1/3D7 and FC27 (65.6%) the mixed infections represent (65.5%) and the MOI was (1.27) This may go with unstable seasonality of transmission in most malarious areas of Sudan.

Conclusion

This study of *P.falciparum* isolates from Gezira state, Sudan found isolates to have ahigh genetic polymorphism and that most infections were from multipleclones. This result underlines the fact that assessment of parasite diversity can play an important role in the evaluation of malaria control interventions. Therefore, further investigation in different transmission settings is required to assess the changes in the genetic parameters and to evaluate malaria control interventions in this endemic area.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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