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Research Article

ASSOCIATION BETWEEN PLASMODIUM FALCIPARUM MEROZOITE SURFACE PROTEIN-1 (PFMSP-1) AND SEVERITY OF FALCIPARUM MALARIA AMONG SUDANESE CHILDREN

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Abstract

Background: Malaria is a global health problem, with 241 million cases and 627,000 deaths in 2020 according to the WHO. In Sudan, The estimated malaria prevalence is 5.9% in which *P. falciparum* Incriminates 87.6% of cases and 12.4 % with *P. vivax*. Clinical manifestation of reported malaria cases in Sudan varied widely from asymptomatic, mild uncomplicated to severe and fatal complications. Merozoite surface protein polymorphism (MSP) is an important parasite factor that has been correlated with the severity of falciparum malaria. **Objectives:** The present study aimed to investigate the allelic diversity of merozoite surface protein-1 gene (MSP-1) among the *Plasmodium falciparum* isolates in Sudanese childrenand to determine their potential correlation with the severity of falciparum malaria. **Materials and Methods**: This study was conducted in Wad Medani Pediatric Teaching Hospital during September to December 2022. 89 children admitted with severe falciparum malaria were selected for this study. DNA was extracted from dry blood spots collected from them and polymerase chain reaction (PCR) amplified for the three MSP-1 allelic subfamilies. SPSS computer program (v 20.0) was used for data analysis. **Results:** The results found thatK1 allele was the common MSP-1 allele followed by RO33 and MAD20 with frequencies of 65 (73%), 55 (61.8%), and 17 (19.1%) respectively. There was strong association between RO33 allele and severity of infection with odd ratio (2.571) and *P value* (0.046). Also, in MAD20 allele there was high risk to develop severe malaria (Odd ratio = 1.2) but without significant association (*P value* = 0.70). Furthermore, K1 allele was less risky factor for severity of infection *(P. value* = 0.559, Odd ratio = 0.955). Regarding the common complications and their association with MSP-1 alleles; the hypoglycemia had high risk with higher odd ratio 4.2 and 2.9 for K1 and MAD20 alleles respectively. Moreover, severe anemia was minor *P. falciparum* risk factor for all MSP-1 alleles (K1, MAD20 and RO33), other WHO criteria of sever malaria were less common. **Conclusion:** K1 allele was the most predominant circulating allelic family. There was strong association between RO33 and MAD20 alleles with the severity of infection. K1 allele was less risk factor for severity of infection. The hypoglycemia has high risk with K1 and MAD20 alleles.

Keywords: Falciparum malaria, MSP-1, Children, Sudan.

INTRODUCTION

Plasmodium falciparum is a single-celled eukaryotic parasite causing malaria disease in humans. Falciparum malaria exhibits a wide range of clinical manifestations ranging from asymptomatic parasitemia, uncomplicated (mild) include fever, headache, fatigue, abdominal pain, vomiting and diarrhea and complicated (severe) disease which may be life threatening if untreated, Malaria burden remains high worldwide, with 241 million cases and 627,000 deaths in 2020 according to the WHO(1). Death occurs by falciparum malaria account for 90% of in sub-Saharan Africa in which the children considered the most vulnerable group(2) In Sudan, overall malaria prevalence is 5.9% in which *P. falciparum* constitute 87.6% of cases and 5% mixed infection with *P. vivax(3)*.

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The severity of malaria infection rely on the host, parasite and environmental factors. Parasite factors such as merozoite surface protein polymorphism (MSP), *Plasmodium falciparum* surface protein (Pfs47), and apical membrane antigen (AMA1) may influence treatment outcomes. Merozoite surface protein diversity Controls transmission dynamics, *P. falciparum* immunity Circumvention and virulence(4). There are evidences of an association of severe malaria with specific genetic characteristics of parasites that open the way for intervention strategies targeting key virulence factors of parasites (5). During the blood stage, the parasite expresses arrays of proteins and among others are merozoite surface protein 1 (MSP-1) and MSP-2. These proteins are involved in erythrocyte invasion and are targeted by the immune responses, and therefore have been used as target for vaccine development. The MSP-1 and MSP-2 genes also exhibit high polymorphisms hence play important role in identification of genetically distinct *P. falciparum* parasite sub-populations. The

MSP-1 gene is located on chromosome 9 and contains 17 blocks of sequences; block 2 is most polymorphic and is categorized into three allelic families MAD20, K1 and RO33(6). The microscopic examination of thick and thin blood films is a "gold standard" test that is used to detect parasitemia in the blood and guiding appropriate treatment (7). However, to our knowledge, very few studies have investigated the genetic diversity of *msp* in malaria parasites circulating in many endemic countries including Sudan. the current study reports on the genetic diversity and allelic frequency of *MSP-1* gene alleles and their association with severity of infection in *Plasmodium falciparum* parasites isolated from positive patients in Sudan.

MATERIALS AND METHODS

Study setting and population

A cross-sectional hospital-based study done in Wad Medani Pediatric Teaching Hospital, Wad Medani, Gezira state, Sudan. Wad Medani City is located in Central Sudan in the Western Bank of the Blue Nile river, in 187 Km south of Khartoum. Wad Medani is the main City of the irrigated area of Gezira Agricultural Scheme where malaria is stratified as mesoendemic to hyper endemic with unstable transmission pattern. In this area, malaria occurs year round with one peak transmission from August to November with *P. falciparum* as the predominant species and the second species are *P. vivax.* The study population is children attending Wad Medani Pediatric Teaching Hospital according to inclusion and exclusion criteria. All children whom were diagnosed with *P. falciparum* by microscopic examination from both gender and age up to 18 years were included in the study. The children were selected to participateafter agreement of their guardians.

Data collection and processing

A designed questionnaire used to collect all data of demographic, clinical and laboratory findings.

Sample collection

3 ml venous blood was collected in EDTA containers from each enrolled case. Blood samples were equally spotted on labeled filter paper (Whatmann® No.3, Sigma-Aldrich, Germany), air dried, and stored in opaque envelopes alongside desiccant (8).

DNA extraction

Dry blood spots (DBSs) on the filter paper were cut into small pieces with scissors and transferred into 1.5-mL microtubes. About 500 µL dH2O added and the tubes kept at room temperature for 5 minutes. The dH2O discarded and the procedure was repeated twice. The filter papers soaked in 500 µL extraction buffer [phosphate-buffered saline (PBS) or 10 mMTris-EDTA (TE). For lysis, red blood cell lysis buffer (100 µL) was added to the filter papers and then incubated with 10 µL (1 mg/mL) proteinase K at 37°C overnight. The filter papers were pressed against the bottom of the tube several times with a clean pipette tip. For increased extraction efficiency, cell lysates were incubated at 95°C for 15 minutes. Filter papers were removed after brief centrifugation (2–3 seconds), and the DNA-containing supernatant was stored at −80°C until analysis (9).

Polymerase chain reaction

In the reaction, primer pairs corresponding to the conserved sequences spanning the polymorphic regions (Table 1).

Table 1. Sequences of the primers

Amplification/Gene	Primer	Primer sequence
Primary PCR		
MSP-1	M1-OF	F:5- -CTAGAAGCTTTAGAAGATGCAGTATTG-3-
	M1-OR	R:5- -CTTAAATAGTATTCTAATTCAAGTGGATCA-3-
Secondary PCR		
	M1-KF	5- -AAATGAAGAAGAAATTACTACAAAAGGTGC-3-
	M1-KR	5- -GCTTGCATCAGCTGGAGGGCTTGCACCAGA-3-
	M1-MF	5- -AAATGAAGGAACAAGTGGAACAGCTGTTAC-3-
	M1-MR	5- -ATCTGAAGGATTTGTACGTCTTGAATTACC-3-
	M1-RF	5- -TAAAGGATGGAGCAAATACTCAAGTTGTTG-3-
	M1-RR	5- -CATCTGAAGGATTTGCAGCACCTGGAGATC-3-

The amplification conditions were as 5 min at 94°C, followed by 30 cycles with 30 sec of denaturation at 94°C, annealing at 45°C for 30 sec, and elongation at 72°C for 1 min. After 30 cycles, a final elongation step at 72°C for 7.0 min was carried out. The amplified product of around 1200-1400 bp (the total length of the gene with variation due to the number of repeats) will purified and sequenced (Table 2, 3) (10).

Table 2. The amplification conditions used to amplify the MSP-1 gene of *P. falciparum* isolates

MSP-1 outer			
	X1	Temperature profile for outer MSP-1	35 cycles
H20	9.0	95°C/5 min	
Mix	4.0	94°C /30 sec	
MSP1-O1	1.0	55°C/30 sec	
MSP1-O2	1.0	72°C/1.0 min	
DNA	5.0	72°C/5 min	

Table 3. The amplification conditions used to amplify the MSP-1 gene of *P. falciparum* isolates

MSP-1 nested			
	X1	Temperature profile for nested MSP-1 (K1, RO33, and Mad 20)	35 cycles
H20	12.0	95°C/5 min	
Mix	4.0	94°C /30 sec	
MSP1-O1	1.0	56°C/30 sec	
MSP1-O2	1.0	72°C/1.5 min	
DNA	2.0	72°C/5 min	

Agarose gel electrophoresis

About 5 µl of each PCR will be mixed on parafilm paper with the loading buffer (0.25% bromophenol blue, 25% ficoll, 10mM Tres and 1mM EDTA) and then placed into the gel lanes. The products will be electrophoretically separated on 2% agarose gel in Tris-Borate-EDTA (TBE) (0.09M boric acid, 0.09 M Tris, 0.002M EDTA) buffer containing ethidium bromide (5mg/ml) to visualize the DNAs. DNA molecular weight markers (Boehringer Mannheim, U.K), or a 100 bp ladder, will be run in parallel wells. The gel will be run for about 10-120 minutes in 1x TBE at 120 volts or until the color front reached two-thirds of the distance. The gels will be examined using photo documentation system. R fragment sizes will be then estimated from their distance and migration relative to size (will be added and then 5-7 µl of each PCR product. The run will be performed for 20 minutes under 120V (Amersham Pharmacia Biotech).(11).

Data analysis

SPSS version 20.0 was used for analysis. The 95% confidence level and confidence intervals were used.

Ethical statement

Ethical clearance was obtained from the Scientific and Research Ethics Committee, Ministry of Health, Gezira State, Sudan. Informed consent was obtained from children parent. Anonymity and confidentiality of patient information were maintained throughout.

RESULTS

A total of 89 children with falciparum malaria participate in this study. (60.7%) were boys. The most age group was 1–5 years 48 (53.9%) (Table 4).

Table 4. Characteristics of study subjects

		Frequency	Percent (%)
Age Groups	1 - 5 Years	48	53.9
	6 - 11 Years	34	38.2
	>12 years	7	7.9
	Total	89	100
Gender	Male	54	60.7
	Female	35	39.3
	Total	89	100

K1 was common MSP-1 alleles followed by RO33 and MAD20 with frequencies 65 (73%), 55 (61.8%), and 17 (19.1%) respectively (Table 5; Figures 1,2,3).

Table 5. Allelic frequency of the MSP-1 genes in study subjects:

Alleles	Status	Frequency	Percent (%)
K1	Present	65	73
	Absent	24	27
MAD20	Present	17	19.1
	Absent	72	80.9
RO33	Present	55	61.8
	Absent	34	38.2

For MSP-1 Alleles and their association with Severity of falciparum malaria there was strong association between RO33 allele and severity of infection with Odd ratio (2.571) and P value (0.046), and also in MAD20 allele there was high risk (Odd ratio = 1.2) but without significant association (P value = 0.700). Furthermore, K1 allele was less risk factor for severity of infection (Table 6). Regarding to common complications and their association with MSP-1 alleles; hypoglycemia has high risk with higher Odd ratio 4.2 and 2.9 a for K1 and MAD20 alleles respectively. Moreover, severe anemia was minor *P. falciparum* risk factor for all MSP-1 alleles (Table 7).

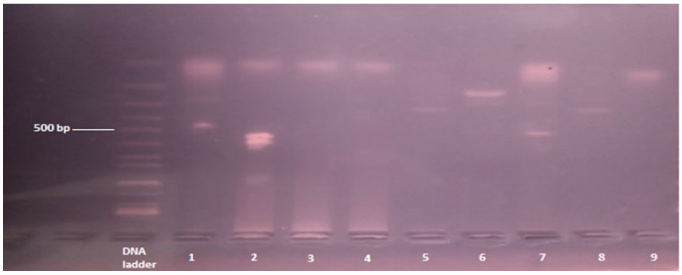

Figure 1. PCR genotyping of MSP -1 (RO33 allele). nPCR typing of RO33 allele band size range (100–500 bp) MW: 100 bp ladder (iNTRON, Biotechology). Lanes 1, 2 and 7 are positive for RO33 allelic family and showing multiple clones (100/250, 100/500 and 100/500 bp respectively), lanes 3, 4, 5, 6, 8 and 9 are positive for RO33 allelic family.

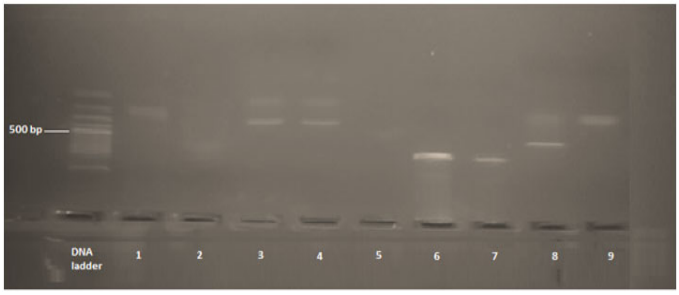

Figure 2. PCR genotyping of MSP -1 (K1 allele). nPCR typing of K1 allele band size range (100–650 bp) MW: 100 bp ladder (iNTRON, Biotechology). Lanes 3, 4 and 8 are positive for K1allelic family and showing multiple clones (300/500, 300/500 and 600/500 bp respectively), lanes 1, 2, 5, 6, 7 and 9 are positive for K1allelic family.

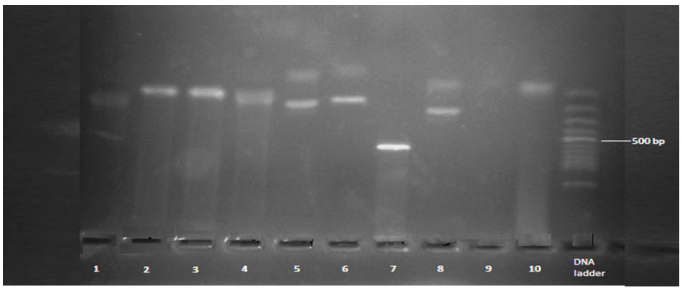

Figure 3. PCR genotyping of MSP -1 (MAD20 allele). nPCR typing of MAD20 allele band size range (90–600 bp) MW: 100 bp ladder (iNTRON, Biotechology). lanes 1, 2,3, 4, 7, and 10 are positive for RO33 allelic family. Lanes 5, 6 and 8 are positive for MAD20allelic family and showing multiple clones (90/100, 90/100 and 100/300 bp respectively). Lane 9 is negative for MAD20allelic family.

Table 6. Association between MSP-1 Allelesand malaria severity

MSP-1 Alleles		Severity of falciparum malaria				Total	P. value	Odd ratio
		Severe		Mild				
		Frequency	%	Frequency	%			
RO33	Present	22	76	33	55	55	0.046	2.571
	Absent	7	24	27	45	34		
	Total	29	100	60	100	89		
K1	Present	21	72	44	73	65	0.559	0.955
	Absent	8	28	16	27	24		
	Total	29	100	60	100	89		
MAD20	Present	24	83	48	80	72	0.700	1.200
	Absent	5	17	12	20	17		
	Total	29	100	60	100	89		

Table 7. Association between MSP-1 alleles and common complications of severe falciparum malaria

Complications		Present		Absent		P. Value	Odd ratio
		Count	%	Count	%		
			K1 allele				
Severe Anemia	Yes	17	26.2	5	20.8	0.414	1.4
	No	48	73.8	19	79.2		
	Total	65	100.0	24	100.0		
Hypoglycemia	Yes	10	15.4	1	4.2	0.142	4.2
	No	55	84.6	23	95.8		
	Total	65	100.0	24	100.0		
MAD20 allele							
Severe Anemia	Yes	4	24	18	25	0.586	0.923
	No	13	76	54	75		
	Total	17	100	72	100		
Hypoglycemia	Yes	4	24	7	10	0.128	2.9
	No	13	76	65	90		
	Total	17	100	72	100		
			RO33 allele				
Severe Anemia	Yes	12	22	10	29	0.228	0.670
	No	43	78	24	71		
	Total	55	100	34	100		
Hypoglycemia	Yes	5	9	6	18	0.194	0.467
	No	50	91	28	82		
	Total	55	100	34	100		

DISCUSSION

Plasmodium falciparum merozoite surface protein-1 (MSP-1) Imply in the invasion process of these parasites into the human erythrocytes. investigating the polymorphism and the fluctuation in the diversity of this important gene in malaria-endemic areas may help understanding the pathogenicity, epidemiology and diagnosis of malaria (12). *P. falciparum* MSP-1 is major surface protein encoded by *MSP-1* gene on chromosome 9, which contains 17 blocks of sequences bounded by conserved regions. It plays a major role in erythrocyte invasion and is targeted by immune responses (13).

A total of 89 *P. falciparum* rDNA was detected in children with falciparum malaria in Wad Medani Pediatric Teaching Hospital, Gezira State, Wad Medani, Sudan using nested PCR. In our study the males were 54 (60.7%), while the females were 35 (39.3%). The age groups as 1– 5 years 48 (53.9%),6 – 11 years 34 (38.2%) and >12 years 7 (7.9%). Among 89 children with falciparum malaria,K1 family has shown the trend of predominance 65 (73%) frequencies of different MSP-1 alleles, followed by RO33 with 55 (61.8%)and then MAD20 17 (19.1%). This finding was in accordance with previous studies done in Africa including Republic of Congo (14)Tanzania(4), Burkina Faso(15),Gabon (16), and the Asian country, Thailand(17), but in contrast to other study done in Central Africa in Sudan (18). This difference may be due to different study areas that mean RO33 is the predominant allele in Kosti area and fewer samples as they are carried on 39 positive nested PCR *P. falciparum* samples only. There was strong association between RO33 allele and severity of infection (P. value = 0.046) and the presence of RO33 increase the severity more than two times (Odd ratio = 2.571). Association between MSP-1 alleles and severity of infection showed that RO33 allelic family represent (40.0%) with severe malaria and (60.0%) with mild malaria from samples positive for RO33 allelic family. K1 allele represent (32.3%) with severe malaria and 44 (67.7%) with mild malaria from samples positive for K1 allelic family. MAD20 allele represent 24 (33.3%) with severe malaria and 48 (66.7%) with mild malaria from samples positive for MAD20 allelic family (Table 6)

Associations between the allelic families and disease severity were examined. In MSP-1, the K1 allelic family was identified in similar Percentage in uncomplicated and complicated malaria. This differs to previous studies in Senegal where the K1 allelic family had been associated with severe malaria (19). This difference may be Attributed to differences in malaria endemicity or the small size of the current study, particularly in the severe malaria cases (13).This finding is in accordance with the findings in previous study in Bobo-Dioulasso (21)The predominant MSP-1 allelic families were RO33 for the severe malaria and K1 for the mild malaria (22). There was no significant difference between MSP-1 alleles and severe Anemia and hypoglycemia with K1 allele (P. value = 0.414), MAD20 allele (P. value = 0.586), and RO33 allele (P. value = 0.228), which agree with (12). There was strong correlation between K1 allele and MAD20 allele with hypoglycemia and severe anemia with Odd ratio (4.2), (2.9), (0.923) and (1.4) respectively. There was no correlation between RO33 allele with hypoglycemia and severe anemia with Odd ratio (0.670).and this agree with study done in Indonesia(6). Based on the complications as classified by the WHO, subjects carried the allelic families of K1 of MSP-1 had higher chance to have hypoglycemia (Odd ratio = 4.2), and severe anemia (Odd ratio = 1.4). Other alleles revealed either no correlation or insignificant P-value, and this agree with study done in Indonesia(6).

Conclusion

The study concluded that the genetic diversity of *P. falciparum* is high, reflected the high intensity of malaria transmission in Sudan; K1 allele was the most predominant circulating allelic families. There was strong correlation between RO33 and MAD20 alleles with the severity of infection. K1 allele was less risk factor for severity of infection. The hypoglycemia has high risk with K1 and MAD20 alleles. The high level of genetic diversity of *pfmsp-1* in *P. falciparum* population underlined the requirement for continuous monitoring that could have significant implications for the use of this gene in the understanding and development of antimalarial drugs and vaccines.

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Conflict of interest: All authors have declared that they have no conflict of interest.

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