Original Article

Genetic Diversity of *Plasmodium falciparum* Field Isolates in Central Sudan Inferred by PCR Genotyping of Merozoite Surface Protein 1 and 2

Muzamil M Abdel Hamid[1], Sara B Mohammed[2], Ibrahim M El Hassan[1,3]

[1]Department of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan, [2]Faculty of Veterinary Medicine, Sudan University of Science and Technology, Sudan, [3]Faculty of Medicine, Jazan University, Saudi Arabia

Abstract

Background: Characterization of *Plasmodium falciparum* diversity is commonly achieved by amplification of the polymorphic regions of the *merozoite surface proteins 1 (MSP1)* and *2 (MSP2)* genes. **Aims**: The present study aimed to determine the allelic variants distribution of *MSP1* and *MSP2* and multiplicity of infection in *P. falciparum* field isolates from Kosti, central Sudan, an area characterized by seasonal malaria transmission. **Materials and Methods**: Total 121 samples ($N = 121$) were collected during a cross-sectional survey between March and April 2003. DNA was extracted and *MSP1* and *MSP2* polymorphic loci were genotyped. **Results**: The total number of alleles identified in *MSP1* block 2 was 11, while 16 alleles were observed in *MSP2* block 3. In *MSP1*, RO33 was found to be the predominant allelic type, carried alone or in combination with MAD20 and K1 types, whereas FC27 family was the most prevalent in *MSP2*. Sixty two percent of isolates had multiple genotypes and the overall mean multiplicity of infection was 1.93 (CI 95% 1.66-2.20). Age correlated with parasite density ($P = 0.017$). In addition, a positive correlation was observed between parasite densities and the number of alleles ($P = 0.022$). **Conclusion**: Genetic diversity in *P. falciparum* field isolates in central Sudan was high and consisted of multiple clones.

Keywords: Genetic diversity, Merozoite surface protein, Multiplicity of infection

Address for correspondence: Dr. Muzamil Mahdi Abdel Hamid, Institute of Endemic Diseases, University of Khartoum, P.O. Box 102, Khartoum, Sudan. E-mail: mahdi@iend.org

Introduction

Malaria is a major health-threatening disease that is responsible for 0.8 million deaths; the vast majority of these deaths occur in Africa in children <5 years old.[1] *Plasmodium falciparum* is the most virulent species of the human malaria and is responsible for most malaria-related deaths. To control and eventually eradicate malaria, an effective vaccine is considered to be needed in addition to the existing strategies such as insecticide-treated bed nets (ITNs) and chemotherapy.[2] Extensive genetic diversity in the parasite field isolates have been reported in several studies.[3-5] This diversity hampers development of effective vaccine as it limits the efficacy of protective immunity (i.e., antibody-mediated parasite inhibition).[6] Therefore, it is important to determine the genetic diversity of antigens that are vaccine targets in different transmission settings to assist the development of effective malaria vaccines.

Access this article online

Quick Response Code:

Website: www.najms.org

DOI: 10.4103/1947-2714.107524

Merozoite surface protein 1 (MSP1) and *2 (MSP2)* of *P. falciparum* are principal blood-stage malaria vaccine targets.[7] *MSP1* is major surface protein of approximately 190 kDa size. It plays a pivotal role in erythrocyte invasion by merozoite.[8] The protein is a major target of immune responses.[9] *MSP1* contains 17 blocks of sequence flanked by conserved regions.[10] Block 2, which is the most polymorphic part of *MSP1,* is grouped into

three allelic families namely K1, MDA20, and RO33 type.[11] *MSP2* is a glycoprotein consisting of 5 blocks, where the central block is the most polymorphic. The *MSP2* alleles are grouped into two allelic families, FC27 and 3D7/IC1.[12,13] Due to the polymorphic features, *MSP1* and *MSP2* have been widely exploited as markers of great use to determine the diversity of *P. falciparum* in field studies[14-16] and also to determine association with disease severity.[17,18]

Malaria incidence in Sudan is estimated to be about 9 million episodes and the number of deaths is about 44000 every year.[19] The morbidity and mortality rates due to malaria have been declining steadily in Sudan during the last recent years, yet malaria remains one of the major health problems in the country.

Extensive work on the genetic diversity of *P. falciparum* field isolates in Gadaref state, eastern Sudan, an area characterized by seasonal and low malaria transmission has been thoroughly investigated.[3,20] Parasites were genetically complex, consisting of multiple clones with large repertoire of *MSP1* and *MSP2* alleles. In these studies, fluctuations in allele prevalence from 1 year were also reported.

This study presents the genetic diversity and multiplicity of infection of *P. falciparum* field isolates collected from Kosti site, in the White Nile state, central Sudan, in 2003. Malaria in the White Nile region and, in general, in central Sudan is characterized by low to moderate malaria transmission with highly seasonal outbreaks from July to November.[21] Genotyping of the polymorphic loci of the *MSP1* and *MSP2* was performed and data was used to examine the relationship between genetic diversity and multiplicity of infection according to parasite density and age.

Materials and Methods

Study site

This study was conducted in Kosti, White Nile state in central Sudan.The study region is situated on the west bank of the White Nile, approximately 340 km south of Khartoum. Kosti region experiences one long rainy season, which usually extends from June to September with slight year-to-year variation.

Samples collection and processing

Blood samples were collected from a total of 121 individuals during a cross-sectional survey following transmission season in March and April 2003. All patients with malaria complaints reported to Kosti Teaching Hospital and El-Tigani Health Center in Kosti city were interviewed. Clinical data was recorded including patient age. Microscopy thick and thin blood film slides were prepared using 10% Giemsa solution for 30 min. The stained slides were examined under a light microscope using 100× oil immersion by an experienced laboratory technician. Parasitaemia was calculated per 200 white blood cells (WBC) assuming 8000 WBC/µl of blood stage.[22] Based on the microscopy results, the study subjects were classified into two groups: Group 1 that had hyperparasitaemia (total number of parasite >5000 parasites/µl of blood), group 2 that had hypoparasitaemia (total number of parasite <5000 parasites/µl of blood).[23] Malaria case was diagnosed if a patient had fever (auxiliary temperature >37.5°C) and microscopically detectable asexual parasites. The study was approved by the Institute of Endemic Diseases Ethical Committee. Informed consent was obtained from each participant or guardians for the children prior to recruitment.

DNA preparation

Genomic DNA was extracted from frozen blood samples (approximately 200 µl) using 0.5% saponin (Gimsa-Aldrich, Germany) to free parasites from red blood cells, followed by phenol extraction and ethanol precipitation as previously described,[24] DNA was suspended in 100 µl 1X TE and kept frozen at −20°C until needed.

Identification of plasmodium species

All 121 DNA samples were genotyped using *P. falciparum* genus and species-specific primers in a nested PCR approach (rPLUS1 and rPLUS5; rFAL1 and rFAL2). Details of the methodology have been described elsewhere.[24]

Allelic typing of *P. falciparum MSP1* and *MSP2*

The primers specific for the polymorphic regions (block 2 of *MSP1* and block 3 of *MSP2*) were described previously.[25] The two genes were amplified using nested-PCR. An initial amplification of the outer regions of the two genes was followed by a nested-PCR with allelic family specific primer pairs. All PCR reactions were carried out in a total volume of 50 µl containing 0.2 mM dNTPs, 2 mM $MgCl_2$, 1 µM each primer, and 1 unit of AmpliTaq Polymerase (Perkin Elmer, England). In the first round reaction, 2 µl of genomic DNA was added as a template. In the nested reaction, 1 µl of the outer PCR product was added.

The cycling parameters for both the outer and nested PCR were 39 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min, followed by an additional extension cycle of 72°C for 10 min. Positive controls and negative controls were incorporated in each set of reaction.

PCR analysis

PCR products were analyzed on 3% agarose gels. The DNA was stained with ethidium bromide and visualized by UV trans-illumination (BioDoc-It UVP, Cambridge, UK). The number and size of resulting DNA bands were analyzed using Gene Ruler 50 bp DNA ladder marker (Thermo scientific, Germany).

Multiplicity of infection

The mean multiplicity of infection was estimated by dividing the total number of distinct *MSP1* or *MSP2* genotypes detected by the number of positive samples for the same marker.[26,27]

Statistical analysis

The R software version 2.13.1 (R foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses. For descriptive analysis, proportion was used to present the distribution of different allelic families. Comparison of means was performed using non parametric Mann–Whitney U test. Continuous variables were compared using Spearman's rank correlation coefficient. Furthermore, linear regression (glm) was performed to test for independent association between age, parasite density, and multiplicity of infection. *P* values ≤ 0.05 were considered to indicate statistical significance.

Results

Detection of malaria infections and parasite densities

Of the 121 blood samples collected, 39 (32%) samples were confirmed as *P. falciparum* positive by nested-PCR. In contrast to the number of 30 microscopically positive samples, an additional 9 positive samples were detected by PCR resulting in frequency of 23% as submicroscopic parasites. One individual was positive for *P. vivax*, another was positive for *P. malariae*. Eighty samples (66%) were considered negative for all *Plasmodium* species by both PCR and microscopy. For the samples that were microscopically positive, the mean parasite density was 7302 parasites/μl of blood with the range of 165–19800. Children <10 years of age had the highest mean parasite density (10285 parasites/μl ± 8061). The mean parasite density in adults >20 years was 2663 parasites/μl (±4151) [Table 1].

Allelic diversity of *P. falciparum MSP1* and *MSP2*

All 39 isolates were all successfully examined for the presence of *MSP1* allelic families (MAD20, K1, and RO33) and *MSP2* allelic families (3D7/IC1 and FC27). Alleles of *MSP1* and *MSP2* were classified according to the size of their PCR amplified fragments [Figure 1]. Both genetic markers and corresponding allelic family were very diverse [Table 2]. In *MSP1*, 11 different *MSP1* alleles were identified by size differences on agarose gels representing MAD20 (3 alleles), K1 (4 alleles), and RO33 family (4 alleles). Allelic family RO33 was positive in 16 of the isolates (41%) and yielded four different fragments (150-250 bp). Amplification of the allelic family MAD20 was positive in 15 isolates (39%) and yielded three different bands (200–300 bp).

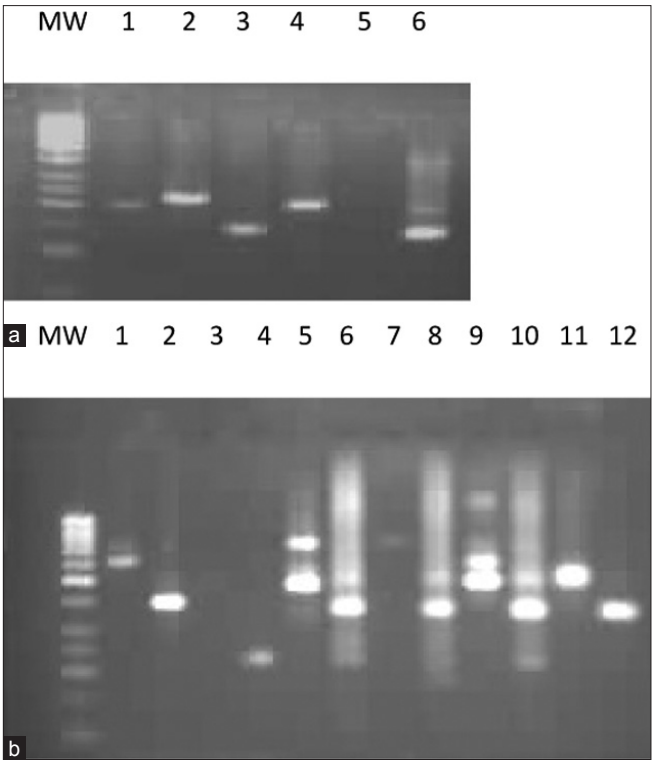

Figure 1: PCR genotyping of polymorphic alleles of *MSP1*: Merozoite surface proteins 1 (a) and *MSP2*: Merozoite surface proteins 2 (b). (a) PCR typing of *MSP1* (MAD20) band size (200 bp), (K1) band size (250 bp), and (RO33) band size (150 bp). Molecular weight marker (MW) (50-bp ladder), Lane: 1 and 4 positive sample (MAD20); 200 bp, Lane: 2 positive sample (K1), 5 negative samples (K1); 250 bp, Lane: 3 and 6 positive sample (RO33); 150 bp, (b) PCR typing of *MSP2* multiple clones (FC27) band size (250-400 bp) and (IC1) band size (500–750 bp). Molecular weight marker (MW) (50-bp ladder). Lane: 1, 5, 9, and 11 positive samples (IC1), 3 and 7 negative sample (IC1). Lane: 2, 4, 6,8,10, and 12 positive samples (FC27). Lane: 5 positive sample (IC1) multiple clones (500 and 750 bp), 9 positive sample (IC1) multiple clones (500 and 600 bp)

Table 1: Mean of *plasmodium falciparum* density (parasite/μl) and multiplicity of infection in *MSP1* and *MSP2* genes stratified by age group (*N*=39)

Age (years)	*N*	Parasite Density	Gene	
			MSP1	*MSP2*
<10	18	10285	1.93	2.1
10–20	9	7636	1.6	2.4
>20	12	2663	1.6	1.0

N: Number of malaria cases; *MSP1*: Merozoite surface proteins 1

Allelic family K1 was positive in 12 isolates (31%) and produced up to four different fragments (150–300 bp). Some 15% ($N = 6$) of the infections carried two allelic types (MAD20/K1, RO33/K1, MAD20/RO33), whereas only three samples (7%) contained all three allelic types.

In *MSP2*, a total of 16 different alleles were identified [Table 2], of which 8 alleles belonged to each allelic family (3D7/IC1 or FC27). Alleles sizes ranged from (400-750 bp) for 3D7/IC1 and (200-700 bp) for FC27 allelic families. The FC27 family was more prevalent with 25 samples positive (64%) of overall detected *MSP2* alleles as compared to 23 samples (59%) positive for 3D7/IC1 family. Eighteen of the isolates (46%) carried both *MSP2* allelic families. A significant decrease in number of alleles with increase in age was observed for 3D7/IC1 ($P = 0.05$) and FC27 ($P = 0.002$). Parasite densities were decreased with age (correlation coefficient = −0.408, $P = 0.025$) [Figure 2].

Association between mean multiplicity of infection, parasite density, and age

Of the 39 positive samples, 24 (62%) harbored more than one parasite genotype identified by the presence of two or more alleles of one or both genes. The overall mean multiplicity of infection was 1.94 (CI 95% 1.1-2.76). When considering *MSP1* and *MSP2* separately, the estimated multiplicity was 1.86 (CI 95% 1.46–2.26) and 2.0 (CI 95% 1.60–2.39) for *MSP1* and *MSP2*, respectively. Thirty patients (30/39) had parasitaemia detectable in blood film. Nineteen isolates (63%) harbored multiclonal infection (mean parasite density of 9463 parasites/µl) as compared to 11 isolates (37%) with monoclonal infection (mean parasite density 4478 parasites/µl).

In *MSP1*, the mean parasite density was significantly higher (9368 parasites/µl) in patients ($N = 15$) carrying multiple *MSP1* genotypes as compared with patients ($N = 15$) with monoclonal infections (mean density of 5238 parasites/µl) ($P = 0.05$). Furthermore, in *MSP2*, much higher parasite density was observed in patients ($N = 17$) harboring multiple clonal infections as compared to patients ($N = 13$) with single *MSP2* genotype infections, mean parasite densities were 9463 parasites/µl versus 4478 parasites/µl, respectively ($P = .025$).

For the parasite densities between the different allelic families type significantly higher parasite densities were observed for *MSP2* 3D7/IC1 allelic families ($P = 0.015$). The relationship between mean parasite density and number of alleles was analyzed [Figure 3]. The parasite density was correlated with number of *MSP2* alleles (Spearman rank coefficient = 0.470; $P = 0.009$). The

Table 2: Number of *plasmodium falciparum* alleles and base pair ranges observed per allelic family in *MSP1* and *MSP2*

Gene/ Allele	Number of positive samples	Frequency (%)	PCR product size	Number of observed alleles
*MSP1**				
MAD20	15	39	200–300 bp	3
K1	12	31	150–300 bp	4
RO33	16	41	150–250 bp	4
Total				11
*MSP2***				
3D7/IC1	23	59	400–750 bp	8
FC27	25	64	200–700 bp	8
Total				16

*For *MSP1*, 10 (26%) samples showed more than one type of allelic family; **For *MSP2*, 18 (46%) samples showed more than one type of allelic family; ***estimated sizes of alleles in base pairs (bp)

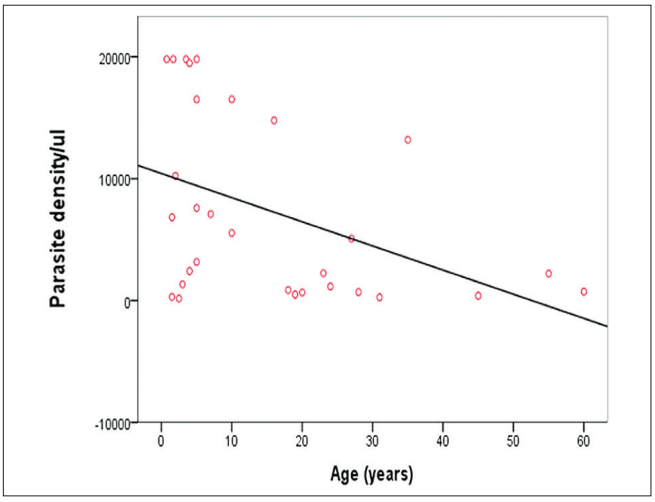

Figure 2: Parasite density of *P. falciparum* infection per microliter blood according to age

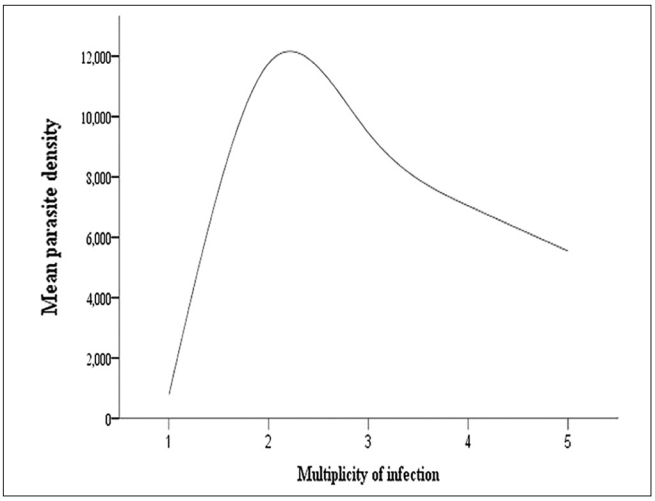

Figure 3: Relationship between mean parasite density and multiplicity of *P. falciparum* infection.

correlation was less pronounced when the two genes are taken together (Spearman rank coefficient = 0.420; $P = 0.022$).

Parasite density and mean multiplicity of infection in *MSP1* and *MSP2* categorized by age groups is presented in Table 1.

Discussion

The present study provides the first report on genetic diversity and multiplicity of *P. falciparum* isolates in an area characterized by low and highly seasonal malaria transmission in central Sudan. Such knowledge will build up on the existing knowledge on genetic characteristics and multiplicity of *P. falciparum* parasite population in Sudan. Genetic diversity has been reported to play key role in the acquisition of anti-malaria parasite immunity.[6,28,29] Therefore, identifying the genotypes circulating in different geographical locations would facilitate the development of effective control strategies (i.e., vaccine or drug).

The allele specific *MSP1* and *MSP2* genotyping data reveal considerable allelic diversity in *P. falciparum* isolates in the study area. Nonetheless, the numbers of alleles (bands) detected may have been underestimated due to sensitivity of PCR technique as minor fragments (<50 bp) would not be detected on the agarose gel. Therefore, more robust techniques such as DNA sequencing are necessary to study in depth the molecular structure of the Plasmodium parasite. The number of alleles detected for *MSP1* and *MSP2* were 11 and 16, respectively. This data is consistent with that of previous studies in low and unstable malaria transmission regions in eastern Sudan[3,30] and other areas of seasonal unstable malaria in Sudan (Alyamani L.Y., M.Sc thesis, University of Khartoum, 2002) and in central Africa.[31,32]

In this study, we found that the predominant allele type for *MSP1* was RO33. This finding differs from those of previous studies in Asar area, eastern Sudan,[4,20] where MAD20 was the most predominant allelic family. This discrepancy might be due to difference in the degree of transmission intensity. Regarding *MSP2*, the predominant allele type was FC27; this is consistent with reports from other studies in eastern Sudan.[4,30]

In this study, we have reported that almost two-third of the samples (62%) harbored multiple genotypes with an overall mean multiplicity of 1.94 clones (with 1.7 and 3.3 in *MSP1* and *MSP2,* respectively. These findings are compatible with some previous studies that have shown increased multiplicity in areas of low and highly seasonal transmission in Senegal[27] and Iran.[5]

It has been shown in areas of low malaria transmission that patent parsitaemias decrease significantly during the long dry season (November to June).[33] An interesting question arising out of this is whether infected people maintain the same multiplicity during the wet season; this will help to draw a complete picture of genetic profiles in this area including seasonal variations. In the current study, parasite density was found to be high in children aged <2 years and decreased with age >20 years. Similar result was recorded elsewhere.[34]

The association between age and multiplicity of infection is not well understood. The present study found a significant association between age and the number of parasite genotypes ($P = 0.003$). Similar results were reported elsewhere,[35] suggesting a key role of adaptive immunity (antibody and cell-mediated immunity).

This study found significant positive correlation between parasite density and the number of parasite genotypes (multiplicity of *MSP1* and *MSP2*) ($P = 022$). This finding is well in agreement with that reported by Mayengue *et al.*,[36] suggesting that individuals are exposed to different clones.

With regards to malaria parasites detection, nested-PCR detected 23% *P. falciparum,* which were diagnosed as negative by microscopy, confirming high sensitivity of molecular tools in detecting sub-microscopic infection.

The reported study represents the first attempt to establish molecular technique for malaria research at the Institute of Endemic Diseases, University of Khartoum. Future studies should be designed to take larger sample size, use other *MSP 1, 2* genotype method, and to include different transmission seasons. Further studies should analyze genetic markers related to anti-malarial drug resistance will be taken to gain insight on *P. falciparum* molecular epidemiology in Sudan. In conclusion, this study suggested that *P. falciparum* in the White Nile region, central Sudan is highly diverse and mainly consisting of multiple clones.

Conclusion

Genetic diversity in *P. falciparum* field isolates in central was high and consisted mainly of multiple clones. Significant positive correlation between parasite density and age and parasite density and number of parasite genotypes were observed.

Acknowledgments

We are grateful to all patients who participated in this study as well as to the guardian of participating children. We thank Dr. Ayman Hussain, Mr. Omer Mahjoob, and Mr.

Waleed Omer for providing excellent assistance in the lab and statistics work.

References

1. Breman JG. Eradicating malaria. Sci Prog 2009;92:1-38.
2. Kilama W, Ntoumi F. Malaria: A research agenda for the eradication era. Lancet 2009;374:1480-2.
3. Babiker HA, Abdel-Muhsin AA, Hamad A, Mackinnon MJ, Hill WG, Walliker D. Population dynamics of *Plasmodium falciparum* in an unstable malaria area of eastern Sudan. Parasitology 2000;120:105-11.
4. Babiker HA, Lines J, Hill WG, Walliker D. Population structure of *Plasmodium falciparum* in villages with different malaria endemicity in east Africa. Am J Trop Med Hyg 1997;56:141-7.
5. Zakeri S, Bereczky S, Naimi P, Pedro Gil J, Djadid ND, Farnert A, *et al*. Multiple genotypes of the merozoite surface proteins 1 and 2 in *Plasmodium falciparum* infections in a hypoendemic area in Iran. Trop Med Int Health 2005;10:1060-4.
6. Healer J, Murphy V, Hodder AN, Masciantonio R, Gemmill AW, Anders RF, *et al*. Allelic polymorphisms in apical membrane antigen-1 are responsible for evasion of antibody-mediated inhibition in *Plasmodium falciparum*. Mol Microbiol 2004;52:159-68.
7. Chitarra V, Holm I, Bentley GA, Petres S, Longacre S. The crystal structure of C-terminal merozoite surface protein 1 at 1.8 A resolution, a highly protective malaria vaccine candidate. Mol Cell 1999;3:457-64.
8. Holder AA, Blackman MJ, Burghaus PA, Chappel JA, Ling IT, McCallum-Deighton N, *et al*. A malaria merozoite surface protein (*MSP1*)-structure, processing and function. Mem Inst Oswaldo Cruz 1992;87:37-42.
9. Woehlbier U, Epp C, Kauth CW, Lutz R, Long CA, Coulibaly B, *et al*. Analysis of antibodies directed against merozoite surface protein 1 of the human malaria parasite *Plasmodium falciparum*. Infect Immun 2006;74:1313-22.
10. Tanabe K, Mackay M, Goman M, Scaife JG. Allelic dimorphism in a surface antigen gene of the malaria parasite *Plasmodium falciparum*. J Mol Biol 1987;195:273-87.
11. Kimura E, Mattei D, di Santi SM, Scherf A. Genetic diversity in the major merozoite surface antigen of *Plasmodium falciparum*: High prevalence of a third polymorphic form detected in strains derived from malaria patients. Gene 1990;91:57-62.
12. Smythe JA, Coppel RL, Day KP, Martin RK, Oduola AM, Kemp DJ, *et al*. Structural diversity in the *Plasmodium falciparum* merozoite surface antigen 2. Proc Natl Acad Sci U S A 1991;88:1751-5.
13. Snewin VA, Herrera M, Sanchez G, Scherf A, Langsley G, Herrera S. Polymorphism of the alleles of the merozoite surface antigens MSA1 and MSA2 in *Plasmodium falciparum* wild isolates from Colombia. Mol Biochem Parasitol 1991;49:265-75.
14. Kang J, Robinson HP, Feng J. Diversity of intrinsic frequency encoding patterns in rat cortical neurons-mechanisms and possible functions. PLoS One 2010;5:e9608.
15. Mwingira F, Nkwengulila G, Schoepflin S, Sumari D, Beck HP, Snounou G, *et al*. *Plasmodium falciparum MSP1*, *MSP2* and glurp allele frequency and diversity in sub-Saharan Africa. Malar J 2011;10:79.
16. Soulama I, Nébié I, Ouédraogo A, Gansane A, Diarra A, Tiono AB, *et al*. *Plasmodium falciparum* genotypes diversity in symptomatic malaria of children living in an urban and a rural setting in Burkina Faso. Malar J 2009;8:135.
17. Ariey F, Hommel D, Le Scanf C, Duchemin JB, Peneau C, Hulin A, *et al*. Association of severe malaria with a specific *Plasmodium falciparum* genotype in French Guiana. J Infect Dis 2001;184:237-41.
18. Engelbrecht F, Felger I, Genton B, Alpers M, Beck HP. *Plasmodium falciparum* malaria morbidity is associated with specific merozoite surface antigen 2 genotypes. Exp Parasitol 1995;81:90-6.
19. Abdalla SI, Malik EM, Ali KM. The burden of malaria in Sudan: Incidence, mortality and disability–adjusted life–years. Malar J 2007;6:97.
20. Babiker HA, Creasey AM, Fenton B, Bayoumi RA, Arnot DE, Walliker D. Genetic diversity of *Plasmodium falciparum* in a village in eastern Sudan. 1. Diversity of enzymes, 2D-PAGE proteins and antigens. Trans R Soc Trop Med Hyg 1991;85:572-7.
21. Giha HA, Rosthoj S, Dodoo D, Hviid L, Satti GM, Scheike T, *et al*. The epidemiology of febrile malaria episodes in an area of unstable and seasonal transmission. Trans R Soc Trop Med Hyg 2000;94:645-51.
22. Cheesbrough M. District Laboratory Practice in Tropical Countries; Part.1, UK: Cambridge University Press. 2005. pp. 245-51.
23. Gilles HM. The malaria parasites. In: Gilles HM, Warrell DA, editors. Bruce Chwatt's Essential Malariology; 3rd ed. London: Edward Arnold; 1993. p. s12-34.
24. Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol Biochem Parasitol 1993;58:283-92.
25. Snounou G, Zhu X, Siripoon N, Jarra W, Thaithong S, Brown KN, *et al*. Biased distribution of *MSP1* and *MSP2* allelic variants in *Plasmodium falciparum* populations in Thailand. Trans R Soc Trop Med Hyg 1999;93:369-74.
26. Kobbe R, Neuhoff R, Marks F, Adjei S, Langefeld I, von Reden C, *et al*. Seasonal variation and high multiplicity of first *Plasmodium falciparum* infections in children from a holoendemic area in Ghana, West Africa. Trop Med Int Health 2006;11:613-9.
27. Zwetyenga J, Rogier C, Tall A, Fontenille D, Snounou G, Trape JF, *et al*. No influence of age on infection complexity and allelic distribution in *Plasmodium falciparum* infections in Ndiop, a Senegalese village with seasonal, mesoendemic malaria. Am J Trop Med Hyg 1998;59:726-35.
28. Färnert A, Williams TN, Mwangi TW, Ehlin A, Fegan G, Macharia A, *et al*. Transmission-dependent tolerance to multiclonal *Plasmodium falciparum* infection. J Infect Dis 2009;200:1166-75.
29. Omer S, Khalil E, Ali H, Sharief A. Submicroscopic and multiple *Plasmodium falciparum* infections in pregnant Sudanese women. N Am J Med Sci 2011;3:137-41.
30. A-Elbasit IE, ElGhazali G, A-Elgadir TM, Hamad AA, Babiker HA, Elbashir MI, *et al*. Allelic polymorphism of *MSP2* gene in severe *P. falciparum* malaria in an area of low and seasonal transmission. Parasitol Res 2007;102:29-34.
31. Vafa M, Troye-Blomberg M, Anchang J, Garcia A, Migot-Nabias F. Multiplicity of *Plasmodium falciparum* infection in asymptomatic children in Senegal: Relation to transmission, age and erythrocyte variants. Malar J 2008;7:17.

32. Schleiermacher D, Rogier C, Spiegel A, Tall A, Trape JF, Mercereau-Puijalon O. Increased multiplicity of *Plasmodium falciparum* infections and skewed distribution of individual *MSP1* and *MSP2* alleles during pregnancy in Ndiop, a Senegalese village with seasonal, mesoendemic malaria. Am J Trop Med Hyg 2001;64:303-9.
33. Hamad AA, Nugud Ael H, Arnot DE, Giha HA, Abdel-Muhsin AM, Satti GM, *et al*. A marked seasonality of malaria transmission in two rural sites in eastern Sudan. Acta Trop 2002;83:71-82.
34. Peyerl-Hoffmann G, Jelinek T, Kilian A, Kabagambe G, Metzger WG, von Sonnenburg F. Genetic diversity of *Plasmodium falciparum* and its relationship to parasite density in an area with different malaria endemicities in West Uganda. Trop Med Inter Health 2001;6:607-13.
35. Dolmazon V, Matsika-Claquin MD, Manirakiza A, Yapou F, Nambot M, Menard D. Genetic diversity and genotype multiplicity of *Plasmodium falciparum* infections in symptomatic individuals living in Bangui (CAR). Acta Trop 2008;107:37-42.
36. Mayengue PI, Ndounga M, Malonga FV, Bitemo M, Ntoumi F. Genetic polymorphism of merozoite surface protein-1 and merozoite surface protein-2 in *Plasmodium falciparum* isolates from Brazzaville, Republic of Congo. Malar J 2011;10:276.

How to cite this article: Abdel Hamid MM, Mohammed SB, El Hassan IM. Genetic diversity of *Plasmodium falciparum* field isolates in central Sudan inferred by PCR genotyping of Merozoite surface protein 1 and 2. North Am J Med Sci 2013;5:95-101.

Source of Support: This work received financial support from DAAD, Germany. **Conflict of Interest:** None declared.