Original Research Article

Predominance of K1 Allele in Erythrocytic form of *Plasmodium falciparum* MSP-1 Gene Signifies Severe Malaria in Jos, Nigeria

ABSTRACT

Aims: The aim of the study was to explore the diversity in the *Plasmodium falciparum* Merozoite Surface Protein 1 (*msp*-1) gene and delineate the K1 allele occurrence and profile The Merozoite Surface Protein 1 (*msp*-1) gene of *Plasmodium falciparum* encodes a major antigen being proposed as a major vaccine candidate against the parasite infections.

Study design: The design of the study was experimental.

Place and Duration of Study: Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Jos, Nigeria, between October 2018 and June 2019.

Methodology: Parasite DNA was extracted from 117 blood samples collected from patients confirmed by microscopy to be *Plasmodium falciparum*-positive in three different hospitals in Jos. The MSP-1 (block 2) allelic family's genotyping was carried out using PCR and nested PCR techniques. Sequencing and Bioinformatics of the K1 alleles were done to further identify the K1 alleles.

Results: Out of the 117 DNA extracted, 13 samples were positive for *Plasmodium falciparum* and were successfully genotyped for MSP 1. K1was the most predominant allele (6/13) followed by MAD20 (4/13) while R033 had the least frequency (2/13). The allelic frequency of K1 was calculated to be 46.15%. The 225 bp *Plasmodium falciparum* K1 allele of *msp*-1 gene obtained exhibited polymorphism and was highly identical to those of already characterized *Plasmodium falciparum*.

Conclusion: Predominance of K1 allele is an indication that Jos is endemic to severe *Plasmodium falciparum*malaria.

Keywords: Plasmodium falciparum, Merozoite Surface Protein-1 gene, K1 allele

1. INTRODUCTION

Malaria continues to be a global health problem. Nearly half of the world's population was at risk; about 247 million cases in 2021 worldwide, resulting in 619,000 fatalities (mostly in children and pregnant women) that could have been avoided [1]. In some countries where the disease is prevalent or endemic, infections have been reported, but its burden, clinical significance and public health impact remain elusive mostly due to the low sensitivity of malaria detection methods [2,3,4]. In hyper endemic areas, each individual host may harbor multiple malaria parasite strains with different genotypes, including those that confer drug resistance [4,5]. Sexual reproduction involving malaria parasites of different genotypes, which can occur when a mosquito feeds on an individual infected with multiple parasite strains or when the same mosquito feeds on more than one human bearing distinct parasite genotypes, favors genetic recombination and generation of higher diversity [5]. Thus, it is expected that parasite genetic diversity tends to correlate with the transmission intensity. Several *Plasmodium falciparum* genes show extensive genetic polymorphism. The most widely

used techniques for genotyping malaria infections are based on amplification by PCR of the polymorphic genes encoding the merozoite surface proteins 1 (MSP-1) and 2 (MSP-2). MSP1 gene is located on chromosome 9 and contains17 blocks of sequences, of which 7 are variable, flanked by conserved regions [6]; block 2 is the most polymorphic and is grouped into three allelic families MAD20, K1 and RO33. The polymorphic regions of merozoite surface proteins-1 (*msp-1*) and glutamate-rich protein (*glurp*) have been the targeted markers for parasite genotyping in antimalarial drug trials to distinguish between recrudescence and reinfection [7]. MSP-1 of *P. falciparum* is a major surface protein, with an approximate molecular size of 190 kDa that plays an important role in erythrocyte invasion by the merozoite [8]. MSP1 block 2 allelic variants fall under three major types - MAD20, K1 and RO33 [9]. This protein is a major target of immune responses and is considered a noteworthy candidate for the development of erythrocytic phase malaria vaccines [10]. In previous studies, the dominance of K1 allelic family has been associated with severe malaria [11], and also with asymptomatic malaria [12]. Plasmodium merozoite surface protein 1 (msp1) is a highly abundant and the most polymorphic antigen, which has been extensively studied in the parasite population. *Plasmodium falciparum* has seven variable blocks that are separated either by conserved or semi-conserved regions. The variable block 2 of pfmsp1 is the most polymorphic region of the antigen [13]. In this study, the genetic diversity of the *Plasmodium falciparum* will be determined using nested PCR method while focusing onK1 of MSP 1 antigenic marker.

2. MATERIAL AND METHODS

2.1 Ethical approval

Ethical clearance with reference no PSSH/ADM/ETH.CO/2018/005 was obtained from the Health Research Ethics Committee of the Plateau state ministry of health, Jos.

2.2 Experimental Subject Sample Collections

Blood samples were collected from human subjects from the selected healthcare centres in Jos metropolitan city. Patients with suspected cases of malaria were identified and their blood collected. Clinical identification was done using microscopy and Rapid Diagnostic Test methods.

2.3 Parasite DNA extraction

Total DNA was extracted from whole blood samples collected using the ZR Quick-gDNA™ Miniprep Extraction Kit (ZYMO RESEARCH USA) according to the manufacturer's instructions. Briefly, 400 µl of Genomic Lysis Buffer was added to 100 µl of whole blood and mixed completely by vortex mixing for 6 seconds and was then allowed to stand for 10 minutes at room temperature. The mixture was transferred into a Zymo-spin™ IIC Column in a collection tube and centrifuged at 10,000 x g for 1 minute. The flow through in the collection tube was discarded with the collection tube. Next, the Zymo-spin™ IIC Column was then transferred into a new collection tube. 200 µl of DNA Pre-wash Buffer was added into the spin column and then centrifuged at 10,000 g for 1 minute. Then, 500 µl of g-DNA Wash Buffer was added in to the spin column and then centrifuged at 10,000 g for 1 minute. The spin column was transferred into a clean 1.5 ml microcentrifuge tube and 50 µl DNA Elution Buffer was added into spin column and was then incubated for 5 minutes at room temperature and then centrifuged at 14, 000 x g for 30 seconds. The eluted DNA was then stored at -70 ^{0}C for further analysis.

2.4 PCR amplification of msp1 gene and K1 allele

The nested PCR assay were carried out according to a modified method [14] using oligonucleotide primers designed based on the Plasmodium small subunit ribosomal RNA (ssrRNA) genes. One set of primers coded PspF (F:TTAAAATTGTTGCGTTAAAAG) and PspR (R:CCTGTTGTTGCCTTAAACTTC) were designed to amplify a primary product of 1200bp which served as a template for species- specific amplification of *Plasmodium falciparum*. The species specific primers were (F:TTAAATGGTTTGGGAAAACCAAATATATT and R:ACACAATGAACTCAATCATGACTACCCGTA) synthesized in Inqaba Biotech West Africa Limited. In the procedure, a 25µl reaction mixture for nest-1 amplification contained 3 µl of genomic DNA extract, 0.5 µl of each 20µM primer (PspF and PspR), 12.5µl of One Taq® Quick-Load® 2x Master Mix with Standard Buffer (New England BioLabs) and the volume made up with 8.5µl Nuclease Free Water. Nest-1 amplification conditions were as follows: initial denaturation at 94^{0}C for 1 min; denaturation at 94^{0}C for 1 min; annealing at 580C for 2 min; extension at 72^{0}C for 2 min for 25 cycles. Final extension was at 72^{0}C for 4 min and rest at 4^{0}C. Two microliters (2 µl) of the nest 1 amplification products served as the DNA template for each of the 25µl nest -2 amplifications. *Plasmodium falciparum* genotypes were further analysed by amplification of the polymorphic regions of *msp-1* (Block 2) using nested-PCR. The cycling conditions for the secondary PCR were as follows: denaturation at 95 °C for 10 min; 35 cycles of denaturation at 94 °C for 30s, annealing at 58 °C for 30s and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. MSP-1 was amplified using primer pairs (F5' – CTAGAA GCTTTAGAAGATGCAGTATTG -3' and R5' –CTTAAATAGTATTCTAATTCAAGTGGAT CA -3') while the

K1-type was done using (F 5'- AAATGAAGAAGAAATTACTACAAAAG GTGC -3' and R 5'- GCTTGCATCAGCTGGAGGGCTTGCACCAGA -3').

2.5 Gel Electrophoresis

The PCR products were analyzed in ethidium bromide stained 2% agarose gel prepared by suspending 2.0 g of agarose powder in 100 ml 1X TBE buffer and boiling to dissolve the agarose. The agarose gel was cast pouring the solution into appropriate tanks, inserting the combs and allowing the solution to gel after adding 5µl ethidium bromide (Fermentas,® USA). A 10 µl PCR product was then mixed uniformly and loaded into the wells. A 10 µl PCR ladder marker (Fermentas®, USA) was loaded into the first well and the PCR products into the subsequent wells. Electrophoresis separation was at 100V and set for ~40 minutes and image capture and gel documentation was done with ChemiGenuis® Gel Documentation System (Synegene Tokyo Japan).

2.6 Bioinformatics Analyses

The Finch TV® programmes (GeoPiza) was used to access the K1 nucleotide sequence while NCBI BLASTN programme software was used to analyse the gene sequence.

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3. RESULTS AND DISCUSSION

3.1 Results

Out of the 117 samples that were confirmed by microscopy to be malaria positive, 13 were confirmed to be *Plasmodium falciparum* positive. A total of six K1, four MAD20 and two RO33 alleles were identified from the *msp*-1 gene. The frequency of the positive samples with K1 allele (46.15%) was the predominant as shown in Table 1. The PCR amplification analysed on 2% agarose gel gave product bands of *Plasmodium falciparum* MSP-1 gene and its alleles. The K1 allele sequence obtained is 225bp of partial length K1gene of *falciparum* presented in Figure 1.

Table 1: Distribution of samples collected from the hospitals, the PCR positive samples and frequencies of MSP-1 alleles detected *Plasmodium falciparum.*

Hospital	Detected by microscopy	PCR detected	MAD 20 detected	RO33 detected	K1 allele detected	K1 % Frequency
A	40	6	4	2	3	23.07
B	50	6	0	0	1	7.69
C	27	1	0	0	2	15.38
Total	**117**	**13 (11.11%)**	**4 (30.77%)**	**2 (13.38%)**	**6 (46.15%)**	**46.15**

The NCBI BLAST tool analysis of *Plasmodium falciparum*K1 allele sequence gave blast Hits presenting sequences producing significant alignments. Among the Hits were four *Plasmodium falciparum* subjects having query covers of 93%, 95%, 95%, and 95% and 91% identity to the query sequence as shown in Table 2. This shows that the K1 allele of the Merozoite Surface Protein 1 of *Plasmodium falciparum* gene in Jos, Nigeria is similar to the sequences of the human malaria parasite *Plasmodium falciparum* strains already characterized.

GGCTCAAGTG GTGCAGTGCT CAAAGTGGTG CAAGTGCTCA
AAGTGGTGCA AGTGCTCMAA GTGGTACAWK TGGTCCAAGT
GGTCCAAGTG GTCCAWGTG GTCCAAGTGG TYCAAGTGGT
MCAAGTCCW TCATCTSGTT CAAAYMCTTT AYCTCGTTCA
AATACTTCAT CTGGTGCAAG YCCTCCAKYT GATGCAAGMC
CTCCATTTKA TGCAAKYWTC TTCTTCATTTA

Figure 1: Primary Nucleotide sequence of K1 gene from *Plasmodium falciparum* (FinchTV (GeoPiza)

Table 2: Distribution of the Blast Hits on top 4 K1 gene sequences from *Plasmodium falciparum* producing significant alignments compared with the query sequence

Description	Max Score	Total Score	Query Cover	Identity	Accession Number
Plasmodium falciparum isolate Samp383 (MSP1) gene, partial cds	244	244	93%	91%	MG675555.1
Plasmodium falciparum isolate Samp189 (MSP1) gene, partial cds	244	244	95%	91%	MG675480.1
Plasmodium falciparum isolate Samp163 (MSP1) gene, partial cds	244	244	95%	91%	MG675460.1
Plasmodium falciparum isolate Samp160 (MSP1) gene, partial cds	244	244	95%	91%	MG675458.1

3.2 Discussion

The 225 bp sequence from the PCR product of *Plasmodium falciparum* K1 allele of msp1 gene obtained falls within a size range (150 to 300 bp) for K1 allelic families earlier reported [15]. The investigation revealed a predominant distribution of MSP 1 locus alleles belonging to the K1 family in the total samples collected for *P. falciparum*. K1 was found to be present in all samples collected from the three healthcare centres with an allelic frequency of 46.15%. K1 is the predominant family for MSP 1 as also reported in previous studies in Africa including, Cote d'Ivoire, Gabon [16] and Ethiopia [17]. Of the 50 samples collected from Hospital B, only one tested positive for *P. falciparum* after molecular identification and upon genotyping the sample was found to belong to the K1 allelic family. In previous studies, the dominance of K1 allelic family has been associated with severe malaria and also with asymptomatic malaria [12]. These studies have implications for the design of blood stage malaria vaccine based on MSP-1 because an effective vaccine should induce immune response specific for these dominant related to the MSP-1 allelic sequences. The *Plasmodium falciparum* K1 allele sequence bioinformatics analyses presented Hits having high identity to the sequences in the gene database. The nucleotide alignment of the sequence also demonstrated several polymorphisms similar to findings earlier reported [17,18].

4. CONCLUSION

The predominance of the 225bp *Plasmodium falciparum* K1 allele in the samples studied point to Jos as native to severe and asymptomatic *Plasmodium falciparum*malaria.

CONSENT (WHEREEVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Ethical clearance with reference no PSSH/ADM/ETH.CO/2018/005 was obtained from the Health Research Ethics Committee of the Plateau state ministry of health.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

MSP-1 Merozoite Surface Protein-1; **NCBI** *National Center for Biotechnology Information**;*** **BLASTN** Basic Local Alignment Search Tool Nucleotide; **PCR** Polymerase Chain Reaction; **DNA** Deoxyribonucleic acid; **bp** base pair