Original Research Article

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

ABSTRACT

Aim: The study explored the diversity of *Plasmodium falciparum* Merozoite Surface Protein 1 (*msp*-1) gene sequence and delineated the K1 allele occurrence and profile. The *Plasmodium falciparum* msp-1 gene encodes an antigen that is 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: The study was undertaken, between October 2018 and June 2019, in the Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Jos, Nigeria.
Methodology: The DNA of *Plasmodium falciparum* was extracted from 117 blood samples of malaria patients confirmed by microscopy 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 each was genotyped for msp-1. K1 was the most predominant allele (6/13) compared to MAD20 (4/13) and R033 (2/13). The allelic frequency of K1 was calculated to be 46.15%. The 225 bp *Plasmodium falciparum* K1 allele of the *msp*-1 gene studied displayed polymorphism. However, the sequence was very similar to those of *Plasmodium falciparum* already characterized.
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 health problem globally. About half of the population of world 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, there are reports on infections, however, the disease burden and public health impact are not clearly presented. This is mostly due to the low sensitivity of malaria detection methods [2,3,4]. In hyper endemic areas, individual human host may be carrying more than one malaria parasite strains possessing different genotypes, which may include those conferring drug resistance [4,5].

Sexual reproduction may occur among malaria parasites of distinctively different genotypes when a mosquito sucks up an individual's infected blood carrying multiple parasite strains. It may also occur when the same mosquito feeds on more than one human carrying parasites with distinctively different genotypes. These tend to favor generation of higher diversity [5]. This signifies that parasite genetic diversity correlates with the intensity of malaria transmission. Several Plasmodium

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falciparum genes show extensive genetic polymorphism. The most commonly used methods or techniques for genotyping malaria parasites are substantially based on PCR amplification. The target genes are polymorphic encoding the merozoite surface proteins 1 (MSP-1) and 2 (MSP-2).

The msp-1 gene of *Plasmodium falciparum*, situated on chromosome 9, is the most quite abundant; the antigen with highest level of polymorphism; and has been widely studied in *Plasmodium falciparum* population. The msp-1 contains sequences that are organized in 17 blocks out of which Seven (7) are the variable and flanked by semi-conserved or fully conserved regions [6]. The block 2 of the msp-1 displays the highest level polymorphism [7] and it is classified into three allelic families which are K1, MAD20 and RO33 [8]. These merozoite surface proteins-1 (msp-1) regions and the polymorphic glutamate-rich protein (glurp) are good molecular markers targeted for genotyping the parasites during trials of antimalarial drugs. These help in distinguishing between malaria reinfection and recrudescence [9]. The msp-1 is a major surface protein in *P. falciparum* having molecular size of about 190 kDa and playing important role in the parasite's erythrocyte invasion [10].

It is an important protein during immune responses and so being considered as a vaccine candidate against erythrocytic phase malaria [11]. In situations where the K1 allelic family was reported to be dominant, the interpretation was that the malaria was a severe type [12] and asymptomatic [13]. In this study, we undertook a genetic diversity study of the *Plasmodium falciparum* using nested PCR method while focusing on the K1 allele of msp-1 antigenic marker.

2. MATERIAL AND METHODS

2.1 Ethical approval

The Ethical clearance for the study was obtained from the Health Research Ethics Committee of the Plateau State Ministry of health, Jos. The reference no was PSSH/ADM/ETH.CO/2018/005.

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 samples collected. The clinical diagnoses were done with the aid of Rapid Diagnostic Test and Microscopic 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) based on 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". [19]

"Next, the Zymo-spin™ IIC Column was then transferred into a new collection tube. About 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". [19]

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 gene product of 1200bp. This product served as a template amplifying species- specific Plasmodium falciparum product. The primers specific for the species were (F:TTAAATGGTTTGGGAAAACCAAATATATT and R:ACACAATGAACTCAATCATGACTACCCGTA) synthesized in Inqaba Biotech West Africa Limited.

In the procedure, a 25 µl reaction mixture for nest-1 amplification contained 3 µl of genomic DNA extract, 0.5 µl of each 20?M primer (PspF and PspR), 12.5 µl of One Taq® Quick-Load® 2x Master Mix with Standard Buffer (New England BioLabs) and the volume made up with 8.5 µl Nuclease Free Water. Nest-1 amplification conditions were as follows: initial denaturation at 940C for 1 min; denaturation at 94^{0}C for 1 min; annealing at 58^{0}C 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 25 µl 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').

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2.5 Gel Electrophoresis

The products from polymerase chain reaction 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 5 μl 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 K1 nucleotide sequence was accessed using Finch TV® programmes (GeoPiza). The gene sequence was analysed using NCBI BLASTN programme software.

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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 detected from the *msp*-1 gene. The 46.15% occurrence of K1 allele was the predominant (Table 1). The PCR amplification as analysed on 2% agarose gel displayed *Plasmodium falciparum* MSP-1 gene product bands and its alleles. The K1 allele sequence was 225bp of partial length K1 gene of *P. falciparum* (Figure 1).

Table 1: Samples collected, the PCR positive and occurrences of MSP-1 alleles

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 produced blast Hits generating alignments that were significant. There were 4 Hits with *Plasmodium falciparum* subjects that gave 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 study area (Jos, Nigeria) was alike with the sequences of characterized *Plasmodium falciparum* strains responsibility for malaria in humans.

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GGCTCAAGTG  GTGCAGTGCT  CAAAGTGGTG  CAAGTGCTCA
AAGTGGTGCA  AGTGCTCMAA  GTGGTACAWK  TGGTCCAAGT
GGTCCAAGTG  GTCCAWGTG   GTCCAAGTGG  TYCAAGTGGT
MCAAGTCCW   TCATCTSGTT  CAAAYMCTTT  AYCTCGTTCA
AATACTTCAT  CTGGTGCAAG  YCCTCCAKYT  GATGCAAGMC
CTCCATTTKA  TGCAAKYWTC  TTCTTCATTTA
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Figure 1: Primary Nucleotide sequence of K1 gene from *Plasmodium falciparum* (FinchTV (GeoPiza)

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Table 2: Blast Hits on four K1 gene sequences from *Plasmodium falciparum* 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 polymerase chain reaction product of the K1 allele of the msp1 gene obtained from *Plasmodium falciparum* genome was 225 bp in length. This result is falls within the previously reported size range (150 to 300 bp) for K1 allelic families [15].

In the total samples collected for *P. falciparum*, the investigation revealed a predominance of MSP 1 locus alleles from the K1 family. With an allelic frequency of 46.15%, K1 was detected in all samples taken from the three healthcare centers. This report is comparable to others found in the literature. According to reports, K1 is the dominating family for MSP 1, as previously documented in African research such as Cote d'Ivoire, Gabon [16], and Ethiopia [17].

The increasing frequency of MSP-1 alleles observed in Plasmodium falciparum malaria infections is a severe condition. Previous research has linked the dominance of the K1 allelic family to both severe and asymptomatic malaria [12, 13]. These findings have significance for the development of a blood stage malaria vaccine based on MSP-1, as a successful vaccination should elicit an immune response specific for these dominant MSP-1 allelic variants.

Hits with high similarity to Plasmodium falciparum MSP 1 gene sequences in the gene bank database were found in bioinformatics investigations of the Plasmodium falciparum K1 allele sequence. The nucleotide alignment of the sequences revealed numerous polymorphisms that were previously reported [17,18].

Cote d'Ivoire, Gabon [16], and Ethiopia [17] are among the countries involved.

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

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

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.

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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

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