DOI: 10.55522/jmpas.V13I4.6589 **ISSN NO. 2320 – 7418**

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Epitope prediction of malarial MSP-1 protein of plasmodium sp. using *in silico* method

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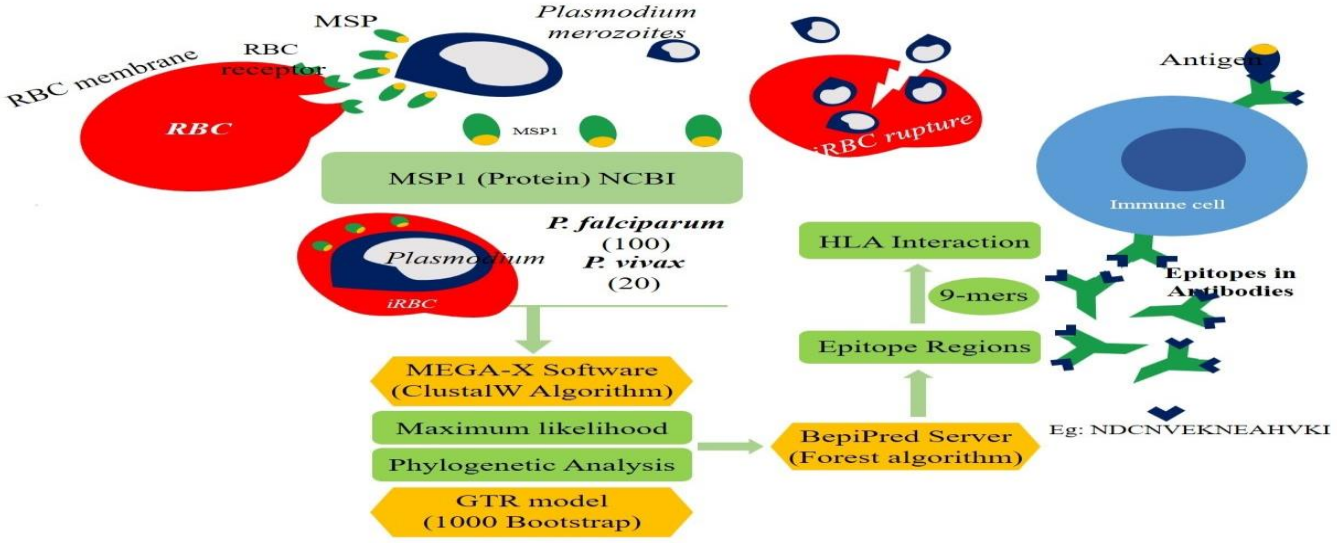
Received -23 -06-2024**, Revised** - 17-07-2024, **Accepted** - 16-08-2024 **(DD-MM-YYYY)**

Refer This Article

Anoopkrishna Rai, Fathimath Zohara, Rama Adiga, 2024. Epitope prediction of malarial MSP-1 protein of plasmodium sp. using in silico method . Journal of medical pharmaceutical and allied sciences, V 13 - I 4, Pages - 6696 – 6703. Doi: https://doi.org/10.55522/jmpas.V13I4.6589.

ABSTRACT

Due to its pivotal function in parasite adherence to the RBC membrane during erythrocyte invasion, Msp1 represents a potential vaccine candidate. Three or four fragments of MSP1 are produced during the proteolytic maturation process, and these fragments stay together as a complex on the cell surface. Msp1 fragments have been investigated as potential vaccine candidate, and malaria antibody immunoassays have made use of the fragmented polypeptide, which exhibits a considerable degree of intra-species conservation. Because of its polymorphism, Msp1 is also useful in strain differentiation and carries a crucial genetic marker. To comprehend genetic variation among species and find epitopes for the development of a disease vaccine, Msp1 needs to be investigated. The preliminary investigation on the evolutionary history using the Maximum Likelihood method and the General Time Reversible model (GTR) models were used. The phylogenetic relationship of the MSP1 gene was inferred and it represents the level of similarity between the MSP1 gene of Plasmodium species from various locations in South-East Asia. The top 30 MHC class II epitopic regions were predicted using Bepipred server with epitope mean score above 0.05.



Keywords: Malaria, Plasmodium, MSP-1, Vaccine, Epitope Cineole.

INTRODUCTION

Merozoite surface protein (MSP) is a protein class that is significant in understanding human malaria pathogenesis. MSP1 is a major target protein of the host immune system due to its abundance and conspicuous presentation on the cell surface. The antibodies recognizing various sections of the protein are found in a substantial percentage of people from endemic locations. MSP1 is required for *Plasmodium* blood stages and is involved in erythrocyte invasion, particularly the first interaction of merozoites with RBCs, as well as RBC rupture accompanied bu parasite escape [1]. MSP1 genes from *Plasmodium* species that infect mammals are 5 kb in size. The copy of *msp1* of *Plasmodium falciparum* and *Plasmodium vivax* is divided into blocks based on interspecies sequence diversity analysis. *Msp1* of *P. falciparum* is located on chromosome 9 and consists of 17 blocks, 5 of which are conserved, 5 of which are semi-conserved, and 7 of which are variable. Block 2 was discovered to be polymorphic, with three prominent allelic families identified, including K1, RO33, and MAD20. MSP1-block 2 and MSP2-block 3 are recommended as the best tools for analyzing *P. falciparum* structure, frequency of distinct allelic families, and heterozygosity in the population [2].

The blood-stage malaria vaccine candidate region of MSP1 which are anchored to the parasite membrane and referred to as P19 peptide epitopes. The p19 peptide's epitopes are mostly conformational, likely dependent on the appropriate folding of two conserved epidermal growth factor (EGF)-like domains held together by many disulfide bonds. *Msp1* is a novel vaccine candidate because it plays a crucial role in parasite adhesion to the RBC membrane during erythrocyte invasion. *Msp1* fragments have been examined as vaccine candidates, and the p19 polypeptide, which has relatively significant intra-species conservation, has been used in malaria antibody immunoassays [3]. *Msp1* is also helpful in strain distinction and contains an essential genetic marker due to its polymorphism [4]. *Msp1* must be studied to understand genetic diversity among species and identify epitopes to develop a vaccine for the disease.

Three main MSP1 blocks 2 allele families, K1, MAD20, and RO33, were identified in MSP1 block2 and the ultrastructure of block 2 alleles revealed that mutation and repeat instability were the primary causes of allelic variability in K1 and MAD20 [5]. This suggests that natural both natural selection and intensity of transmission both play a role in limiting MSP1 allelic diversity in Indian parasites [6]. The structure, processing, and function of the MSP1 protein have been identified in *P. falciparum* and other malaria species. The protein is synthesized from intracellular schizont of asexual blood and liver stages and expressed on the Merozoite surface released by rupture of an infected cell. *P. falciparum* MSP1 is primarily divided into 17 blocks, in which some sequence was highly conserved, and some were different compared to distinct serotypes [7]. Block 2 consists of a short but highly repetitive structure of variable length and composition sequenced genes. MSP1 is processed at the end of schizogony just before the release of merozoites [8]. The MSP1 plays a significant role in erythrocyte invasion and is considered a promising candidate for malaria vaccines [8, 9, and 10]. MSP-1 acts as a platform for different peripheral MSPs, such as MSP3, MSP6, MSP7, MSPDBL-1, and MSPDBL-2.MSP1 induces a humoral immune response in natural infections. MSP1 can elicit CD8+ T-cell-mediated cellular immune responses against liver-stage parasites [7].

MATERIALS AND METHOD

Retrieving Protein Sequences and Multiple Sequence Alignment

The sequences of merozoite surface protein from several isolates of *P. falciparum* and *P. vivax* were obtained in FASTA format from UniProt (http://www.uniprot.org) and the NCBI protein database (http://www.ncbi.nlm.nih.gov/protein). These sequences were deposited in databases collected from several malaria-endemic areas of India. A total of 100 nucleotide sequences of *P. falciparum* and about 20 are in *P. vivax*, retrieved from NCBI of MSP1 proteins of different isolates from different locations like India, China, Thailand, Sudan, Philippines, have been retrieved. Multiple sequence alignment was performed on the extracted sequences using the MEGA X software tool (http://www.megasoftware.net). The alignment was created using the CLUSTALW algorithm, 1000 bootstrap values, and other default settings. The sequences were evaluated to identify immunologically relevant areas, which was accomplished by predicting epitopic peptides. For an amino acid stretch to be classified as an epitope, it must have at least a certain length. The conserved sequence length were nonamers which were chosen as the minimal length for the prediction of epitope-based peptides in this study due to their typical length of peptide that interacts with HLA molecules.

In-silico epitope prediction.

BepiPred, a web server, predicts B-cell epitopes from antigen sequences. It is based on the random forest algorithm, which was trained on antigen-antibody protein structure epitopes. This program may predict epitopes indirectly based on sequences extracted from the solved 3D structure and a vast library of linear epitopes retrieved from the IEDB database. This program employs epitope because antibodies against this epitope exhibit substantial anti-invasion activity, implying that this epitope might form the basis of a successful malaria vaccine. Protective antibody responses are directed toward epitopes. Using quantitative matrices, which predict MHC Class-II binding sites in an antigen sequence. The server aids in the identification of promiscuous binding areas that may be used to select vaccine candidates. MHC Class II genes encode cell surface glycoproteins that resemble MHC Class I molecules in structure. These chemicals are exclusively found in Antigen Presenting Cells (APC). Class II proteins create epitopes that T-helper cells (CD4+) detect

when combined with antigenic fragments. As a result, MHC Class II proteins play a vital role in almost all antigen responses. T cell epitope differentiation was predicted for four MHC class II HLA alleles: HLA-DRB1*0101, DRB1*0401, DRB1*0701, and DRB1*1101.

Figure1: Phylogenetic analysis of MSP1 gene 0f *P. falciparum* using Mega software

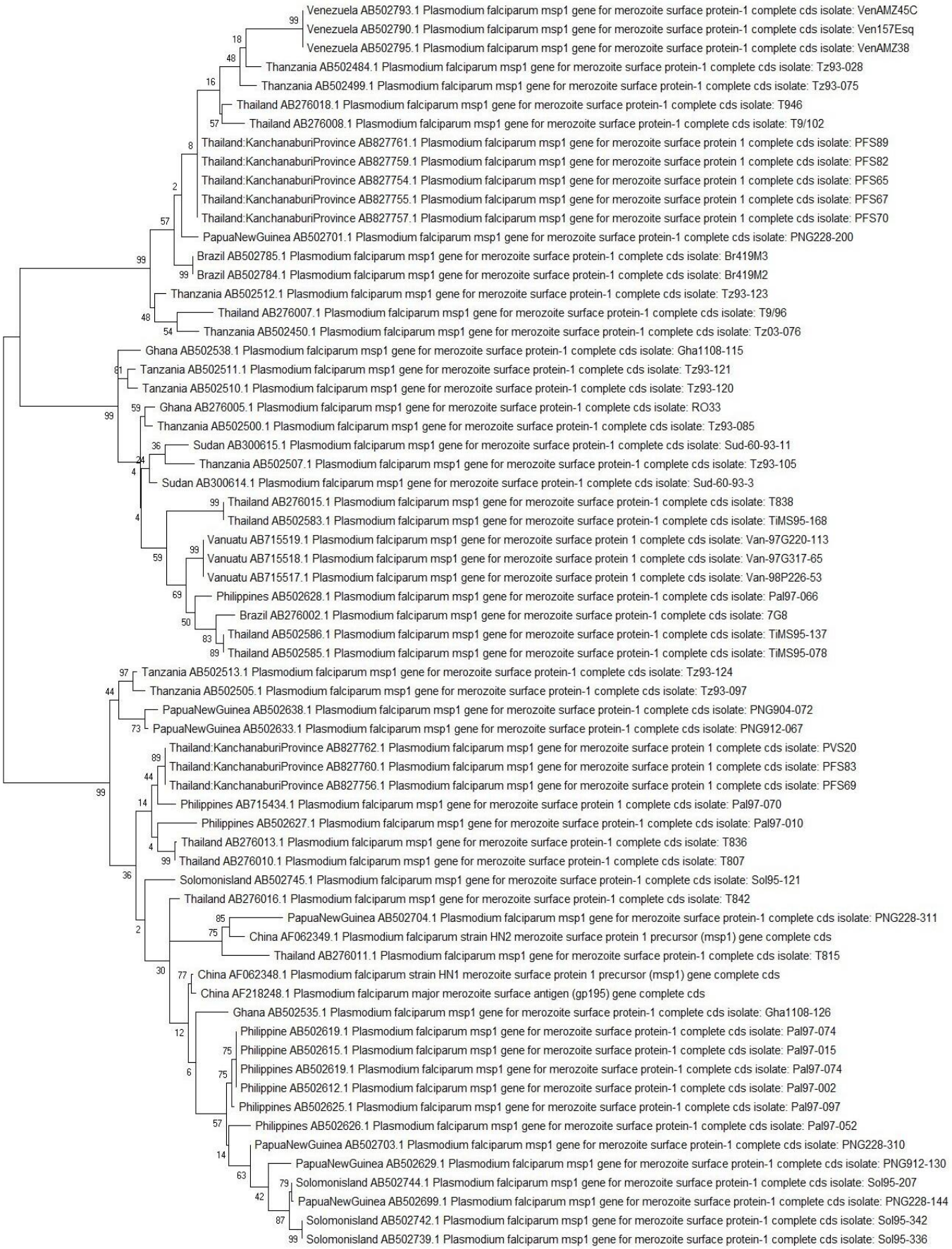


Figure 2: Phylogenetic analysis of MSP1 gene 0f *P.vivax* in Mega software

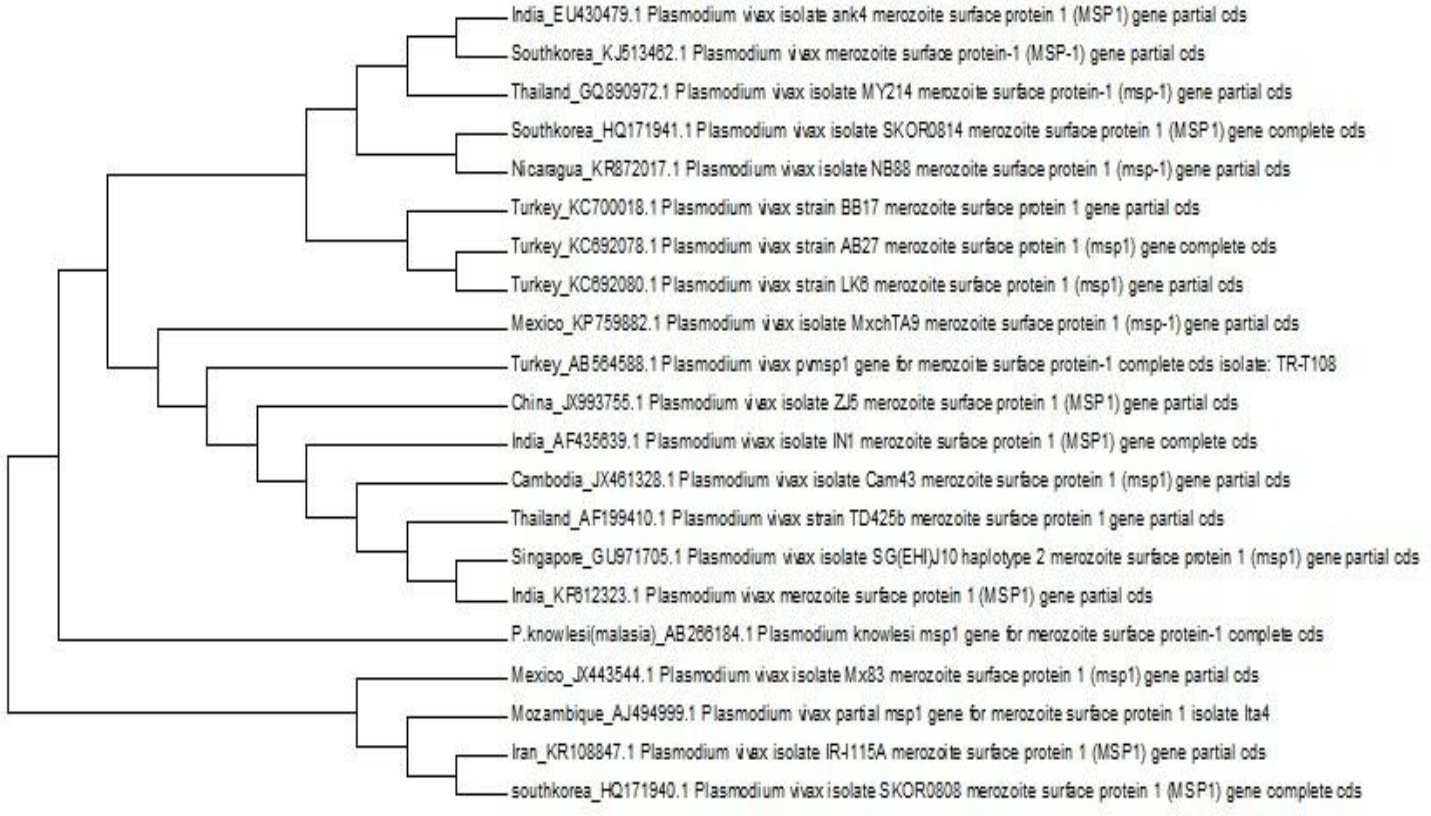


Table 1: Epitope prediction table for MSP1 gene of *P. falciparum*

Epitope prediction	Length	Accession no	Epitope mean score
TYYNKMGELYKTH	13		0.06473
RYLDGTE	7		0.03485
TFKEEGGKCVPA	12	**AHB11181.1**	0.05975
NVTCKDNNGGC	11		0.05477
EGSEPLFE	8		0.03983
KKKLIGSYK	9		0.12321
NKMEEL	6	**AWI97898.1**	0.0.0821
QKEYES	6		0.0.0821
AYYNKMGELYKTHL	14		0.06724
TQLLTMSSEHTCIDTN	16	**AGT38437.1**	0.07684
RYLDGTE	7		0.03362
GSEPLFEG	8		0.03842
TYYNKMGELYKTH	13		0.06360
RYLDGTE	7	**ADX86894.1**	0.03424
KEEGGKCVPA	10		0.04892
NVTCKDNNGGCA	12		0.05871
GASIDKDLATA	11		0.050576
TYYNKMGELYKTH	13		0.059771
MSSEHTCIDTNVP	13	**AGN05322.1**	0.059771
CYRYLDGTE	9		0.041380
EGSEPL	6		0.027587
TQLLTMSSEHTCIDTN	16		0.07684
RYLDGTE	7		0.03362
GSEPLFEG	8		0.03842
TYYNKMGELYKTH	13	**ADX86894.1**	0.06360
RYLDGTE	7		0.03424
KEEGGKCVPA	10		0.04892
NVTCKDNNGGCA	12		0.05871
GASIDKDLATA	11	**AGN05322.1**	0.050576
TYYNKMGELYKTH	13		0.059771
MSSEHTCIDTNVP	13		0.059771
CYRYLDGTE	9		0.041380
EGSEPL	6		0.027587

Table 2: Epitope prediction table for MSP1 gene of *P.vivax*

Epitope prediction	Length	Accession no	Epitope mean score
IETLYNN	7	**AAX08034.1**	0.06338
NDCNVEKNEAHVKI	14		0.12674
KQEGDKCVE	9		0.08149
TCNENNGG	8		0.07243
EEDSGSNGKKIT	12		0.10865
NILSGFENEY	10	**ABF18744.1**	0.05022
NIETLYNNLVNKIDD	15		0.07534
NDCNVEKDEAHVKIT	15		0.07534
LSDL	4		0.02009
TGLVQNFPNT	10		0.05022
KQEGDKCVEN	10		0.05022
NPTCNENNGG	10		0.05022
EEDSGSSGKKI	11		0.05525
NIETLYNNLVNKIDD	15	**AXG32312.1**	0.07218
NDCNVEKDEAHVKIT	15		0.07218
LSDL	4		0.01924
GLVQNFP	7		0.03368
RHLDEREECK	10		0.04812
EEDSGSSRKK	10		0.04812
SLFQK	5	**ADQ74287.1**	0.07328
ELKYPELF	8		0.11725
KDDLES	6	**AXG32311.1**	0.02889
TLYNNLVN	8		0.03852
VEKDEAHVKIT	11		0.05297
EGKFQDMLNI	10		0.04816
QHQCVKKQCPEN	12		0.05779
RHLDEREECK	10		0.04816
KQEGDKCVEN	10		0.04816
NPTCNENNGG	10		0.04816
EEDSGSNGKKI	11		0.05297
SYPLF	5		0.02408

Table 3: *P. falciparum* MHC Class-II Binding Peptide Prediction:with the threshold score provided in two separate boxes

Rank	Sequence	At Position	Score	% of Highest Score
1	YIIEDSFKL	1421	2.4000	40.00
2	YIIEDSFKL	3150	2.4000	40.00
3	YIIEDSFKL	4896	2.4000	40.00
4	YIIEDSFKL	8322	2.4000	40.00
5	YIIEDSFKL	13440	2.4000	40.00
6	MLILYSFIV	13746	2.4000	40.00
7	YIIEDSFKL	15170	2.4000	40.00
8	YIIEDSFKL	16934	2.4000	40.00
9	FNIQNNIPV	6069	2.2000	36.67
10	FNIQNNIPV	9499	2.2000	36.67
11	FNIQNNIPV	11169	2.2000	36.67
12	FFIINTQCV	52	2.1000	35.00
13	FFIINTQCV	1787	2.1000	35.00
14	FFIINTQCV	3521	2.1000	35.00
15	FFIINTQCV	5266	2.1000	35.00
16	FFIINTQCV	6938	2.1000	35.00
17	FFIINTQCV	8690	2.1000	35.00
18	FFIINTQCV	10366	2.1000	35.00
19	FFIINTQCV	12054	2.1000	35.00
20	FFIINTQCV	13807	2.1000	35.00
21	FFIINTQCV	15545	2.1000	35.00
22	FNIQNNIPA	867	1.9000	31.67
23	FNIQNNIPA	2596	1.9000	31.67
24	MLILYSFIT	3457	1.9000	31.67
25	FNIQNNIPA	4342	1.9000	31.67
26	MLILYSFIT	5202	1.9000	31.67
27	MLILYSFIT	6874	1.9000	31.67
28	FNIQNNIPA	7768	1.9000	31.67
29	MLILYSFIT	11974	1.9000	31.67
30	FNIQNNIPA	12886	1.9000	31.67
31	FNIQNNIPA	14616	1.9000	31.67

ALLELE: DRB1_0101	Threshold for 10 % with score: -1.6	Highest Score achievable by any peptide: 6

Rank	Sequence	At Position	Score	% of Highest Score
1	LVSNSSMDQ	833	4.5000	52.33
2	LVSNSSMDQ	2562	4.5000	52.33
3	LVSNSSMDQ	4308	4.5000	52.33

4	LVSNSSMDQ	7734	4.5000	52.33
5	LVSNSSMDQ	12852	4.5000	52.33
6	LVSNSSMDQ	14582	4.5000	52.33
7	LVSNSSMDQ	16346	4.5000	52.33
8	FNIQNNIPA	867	4.4800	52.09
9	FNIQNNIPA	2596	4.4800	52.09
10	FNIQNNIPA	4342	4.4800	52.09
11	FNIQNNIPA	7768	4.4800	52.09
12	FNIQNNIPA	12886	4.4800	52.09
13	FNIQNNIPA	14616	4.4800	52.09
14	FNIQNNIPA	16380	4.4800	52.09
15	FLPEGTDVA	6430	4.4000	51.16
16	FLPEGTDVA	9860	4.4000	51.16
17	FLPEGTDVA	11530	4.4000	51.16
18	FFIINTQCV	52	4.3000	50.00
19	FFIINTQCV	1787	4.3000	50.00
20	FFIINTQCV	3521	4.3000	50.00
21	FFIINTQCV	5266	4.3000	50.00
22	FFIINTQCV	6938	4.3000	50.00
23	FFIINTQCV	8690	4.3000	50.00
24	FFIINTQCV	10366	4.3000	50.00
25	FFIINTQCV	12054	4.3000	50.00
26	FFIINTQCV	13807	4.3000	50.00
27	FFIINTQCV	15545	4.3000	50.00
28	VRVSGSSGS	1277	4.0000	46.51
29	VRVSGSSGS	3006	4.0000	46.51
30	VRVSGSSGS	4752	4.0000	46.51
31	VRVSGSSGS	8178	4.0000	46.51

ALLELE: DRB1_0401	Threshold for 10 % with score: -0.5	Highest Score achievable by any peptide: 8.6

Table 4: *P. vivax* MHC Class-II Binding Peptide Prediction for the allele DRB1_0101

Rank	Sequence	At Position	Score	% of Highest Score
1	FNILFCHAR	38	2.3000	38.33
2	LLFLLCMEL	966	2.2900	38.17
3	VVYLKPLAG	169	2.1000	35.00
4	VVYLKPLAG	598	2.1000	35.00
5	VVYLKPLAG	1067	2.1000	35.00
6	VVYLKPLAG	1515	2.1000	35.00
7	YRYLDGTEE	453	1.7900	29.83
8	YRYLDGTEE	882	1.7900	29.83
9	YRYLDGTEE	1351	1.7900	29.83
10	YRYLDGTEE	1799	1.7900	29.83
11	WKINDCPAR	73	1.7100	28.50
12	FLLLMLLFL	961	1.7000	28.33
13	FLLLMLLFL	1878	1.7000	28.33
14	LLFLPARET	1883	1.5900	26.50
15	YIIKDPYKL	230	1.3000	21.67
16	YIIKDPYKL	659	1.3000	21.67
17	YIIKDPYKL	1128	1.3000	21.67
18	YIIKDPYKL	1576	1.3000	21.67
19	FLSLSFLLL	956	0.8000	13.33
20	FLSLSFLLL	1873	0.8000	13.33
21	FANSIANSI	2	0.6000	10.00
22	LLLMLLFLL	962	0.6000	10.00
23	LLMLLFLLC	963	0.5000	8.33
24	LLMLLFLPA	1880	0.5000	8.33
25	LMLLFLLCM	964	0.3800	6.33
26	IRIGENERA	53	0.3000	5.00
27	YKYIGASID	251	0.3000	5.00
28	WRCLLTFKE	462	0.3000	5.00
29	YKYIGASID	680	0.3000	5.00
30	WRCLLTFKE	891	0.3000	5.00

31	YKYIGASID	1149	0.3000	5.00

ALLELE: DRB1_0101	Threshold for 10 % with score: -1.6	Highest Score achievable by any peptide: 6

Rank	Sequence	At Position	Score	% of Highest Score
1	WIMERITES	1898	4.5000	52.33
2	LQKLGSEVS	733	2.9000	33.72
3	LGSEVSQNS	736	2.9000	33.72
4	LQKLGSEVS	1650	2.9000	33.72
5	LGSEVSQNS	1653	2.9000	33.72
6	LQKLGSEVS	2001	2.9000	33.72
7	LGSEVSQNS	2004	2.9000	33.72
8	IRIGENERA	53	2.6000	30.23
9	LCMELPARS	970	2.6000	30.23
10	VVYLKPLAG	169	2.4000	27.91
11	VVYLKPLAG	598	2.4000	27.91
12	VVYLKPLAG	1067	2.4000	27.91
13	VVYLKPLAG	1515	2.4000	27.91
14	FNTNITDML	193	2.3000	26.74
15	FNTNITDML	622	2.3000	26.74
16	FNTNITDML	1091	2.3000	26.74
17	FNTNITDML	1539	2.3000	26.74
18	LSLMLTFFS	86	2.2000	25.58
19	WRCLLTFKE	462	2.2000	25.58
20	WRCLLTFKE	891	2.2000	25.58
21	WRCLLTFKE	1360	2.2000	25.58
22	WRCLLTFKE	1808	2.2000	25.58
23	FNILFCHAR	38	2.1000	24.42
24	YLPFLNSLQ	330	2.0000	23.26
25	YLPFLNSLQ	759	2.0000	23.26
26	YLPFLNSLQ	1228	2.0000	23.26
27	YLPFLNSLQ	1676	2.0000	23.26
28	YLPFLNSLQ	2027	2.0000	23.26
29	WKINDCPAR	73	1.8000	20.93
30	LLMLLFLLC	963	1.8000	20.93
31	LLMLLFLPA	1880	1.8000	20.93
32	LNSDLNPFK	215	1.7000	19.77
33	YKLLDLEKK	236	1.7000	19.77
34	LNSDLNPFK	644	1.7000	19.77
35	YKLLDLEKK	665	1.7000	19.77
36	FCSSSSFLS	950	1.7000	19.77
37	LNSDLNPFK	1113	1.7000	19.77
38	YKLLDLEKK	1134	1.7000	19.77
39	LNSDLNPFK	1561	1.7000	19.77
40	YKLLDLEKK	1582	1.7000	19.77
41	FCSSSSFLS	1867	1.7000	19.77
42	FANSIANSI	2	1.6000	18.60
43	LKKVINNCQ	355	1.6000	18.60

ALLELE: DRB1_0401	Threshold for 10 % with score: -0.5	Highest Score achievable by any peptide: 8.6

RESULTS

Phylogenetic analysis of *P. falciparum msp1* (Fig. 1) indicates the phylogenetic relationship of the MSP1 gene of *P. falciparum* and represents the level of similarity between the MSP1 gene of *P. falciparum* based on the location of sequences. It also showed that a few sequences from two different locations in Africa are more related to each other as they have a common ancestor. It also shows branches & sub-branches in the tree. The evolutionary history was inferred using the Maximum Likelihood method and General

Time Reversible model (GTR). The tree (Fig. 2) indicates the phylogenetic relationship of the MSP1 gene of *P. vivax* and it represents the level of similarity between the MSP1 genes of *P. vivax* based on the location of sequences. It also showed that a few sequences from other locations, such as Turkey and South Korea, are more related to each other as they have a common ancestor. It also shows branches & sub-branches in the tree. The evolutionary history was read using the Maximum Likelihood method and General Time Reversible model.

Prediction and Selection of B cell Epitopes

Epitope prediction of both *P. falciparum* (Table 1) and *P. vivax* (Table 2) was performed using BepiPred. Epitopes having amino acid lengths between 4- 16 are only listed in Table The nucleotide sequences of *P. falciparum* having accession numbers AAX08034.1, ABF18744.1, AXG32312.1, ADQ74287.1, and AXG32311.1 were used. Sequences with accession numbers AHB11181.1, AWI97898.1, AGT38437.1, ADX86894.1, and AGN05322.1 were used for *P. vivax* to predict epitope based on the threshold of 0.5. To predict MHC Class-II binding regions corresponding to HLA-DRB1*0101 and DRB1*0401 for *P. falciparum* (Table 3) and *P. vivax* (Table 4), which showed scores of 0.12674, 0.10865, 0.11725 respectively for the epitopes 14 (NDCNVEKNEAHVKI), 12 (EEDSGSNGKKIT), 8 (ELKYPELF) in *P. falciparum* but in the case of *P. vivax* sequence, KKKLIGSYK having epitope length 9 had scored 0.12321.

CONCLUSION

The current study is aimed at identifying polymorphism of MSP1 in *P. falciparum* and *P. vivax* based on performing MSA and phylogenetic analysis to predict epitope and to design a primer. The conserved regions within sequences are predicted showing epitopic regions at threshold 0.5 with varying epitope mean scores. The analysis of the MSP1 gene in Plasmodium species is highly relevant to understand the origin of drug resistance which makes MSP1 a useful vaccine candidate.

ACKNOWLEDGEMENT

We are grateful to Nitte University Centre for Science Education & Research and Nitte (Deemed to be University) and the Director, Dr Anirban Chakraborty for providing a Research environment and facility for carrying out the study.

Ethical Approval and Consent Form:

Not applicable

Funding

This manuscript did not receive any specific grant from funding agencies in the public, commercial, or non-for-profit sectors

Conflict of Interest

The authors declare no conflicts of interest.

Author contributions

RA conceptualized and defined the research idea and created the research design; AR searched the literature; FZ performed the analyses; AR wrote the first draft of the manuscript; AR and RA edited the manuscript.

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