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Immuno-Informatic Prediction of Immuno - Genetic DNA Vaccine From Pfm18aapof "Plasmodium Falciparum"

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Abstract: *"Malaria" a protozoan parasite disease confined mostly to the tropical areas caused by Plasmodium parasites & transmitted by Anopheles mosquito. This parasite mainly infect in blood of human being. Antigenic protein of Plasmodium falciparum i.e.**pfM18AAP** is a metalloaminopeptidase that function in affecting complete degradation of host haemoglobin. Hence vaccination with **pfM18AAP** protein would be ideal for disease prevention. The second major feature of malarial **pfM18AAP**protein is its capacity to induce neutralizing antibodies and protective immunity, and it is thereby considered as a potential target for vaccine development. In this present study, an effort was taken to design a candidate vaccine applying the techniques of vaccinology. The method includes identification of potential candidate vaccine, epitope prediction, peptide designing, and energy evaluation of the candidate vaccine followed by validation study. The high scoring vaccine candidate was selected .The result revealed that the designed candidate vaccine has a high binding affinity with T-cell receptor.*

Keywords*: -Malaria, Vaccine, Epitope, Vaccinology, Metalloamino peptidase,*

I. Introduction:-

Malaria, a vector-borne infectious disease, is currently a grave and universal concern with a significant social, economic, and human cost, mainly in developing countries **(*13*).**Malaria, a disease which can be transmitted to people of all ages, is caused by parasites of the species *Plasmodium* that are spread from person to person through the bites of infected mosquitoes**.(*14*)** It was indicated that there are four types of human malaria: *Plasmodium falciparum*, *P.vivax*, *P.malariae*, and *P.ovale*, among which *P. falciparum* and *P.vivax*are the most common and particularly *P. falciparum* is by far the most deadly type of malaria infection. The Plasmodium parasite is spread to humans by the bite of an infected female Anopheles mosquito. This disease mainly infect RBC by rupturing it & by decreasing it numbers in blood circulation . It reduces absorptive capacity of oxygen and causes anemia &finally leads to death. Among all, the**pfM18AAP** antigenic protein plays an essential role in effecting complete degradation or turnover of host hemoglobin which provide a free amino acid pool for the growing parasite. Its molecular weight is 67k Da, and contain 570 amino acid with 12 active sites that control substrate entry into the catalytic site.**PfM18AAP** thus resemble a proteosomal like machine with multiple active sites able to degrade peptide substrate that enter in to catalytic sites **(*19*).** Because of the rapid emergence of drug resistance and unclear mechanisms, much money has been wasted in many malaria endemic sites. Therefore a vaccine seems to be an alternative and pragmatic approach to eradicate the disease. Even a modestly efficacious malaria vaccine may protect hundreds of thousands of people from disease and death each year **(*14*).** A vaccine is a molecular or supra molecular agent that elicits specific, protective immunity, and enhanced adaptive immune response to re-infection against pathogenic microbes and the diseases they cause, by the potential on of immune memory and ultimately mitigating the effect of subsequent infection. But till now no effective vaccine have been developed for malaria. Four types of malaria vaccines are there, among them SPf66 and MSP/RESA vaccines, which are essentially designed against the asexual stages of the Plasmodium parasite, and CS-NANP and RTS, S vaccines designed against the sporozoite stages, have been tested in randomized controlled trials in endemic areas. However, the results are not that encouraging. Important issue in the formulation of blood-stage vaccines against malaria is the fact that immunity in this stage highly depends on the presence of antibodies and CD4 T cells. So, any vaccines used or formulated for this stage should have the capability of inducing immune system to generate both humoral and cell mediated immunity. We are using **pfM18AAP** protein as vaccine designing candidate in order to stop its activity & avoid the rupturing of RBC. Immuno-informatics had provided the freedom to immune diagnose the disease. It is important to understand the pathogenesis of the disease, including the life cycle of the parasite and the interaction between the host immune response and the parasite, for better preventive and therapeutic modalities against malaria. Initial stage of infection is inoculation of sporozoit in to human blood by infected anopheles mosquito.

II. Material and Methodology:

Antigenic sequence

The FASTA format number of the pfM18AAP having gi|124507185|. The sequence is 570 amino acids long.

Prediction of amino acids residue : The use of ProtParam

The pfM18AAP proyein sequence (570 amino acids) is analysed either by giving TrEMBL accession number or by giving its FASTA sequence obtained from NCBI.The protein sequence is of molecular weight 65635.1 & Theoretical Pi value 6.60 . Total number of negatively charged residues (Asp+Glu) is 66 & total number of positively charged residues (Arg +Lys) is 61. Protein sequence pfM18AAP consists of mostly asparagine (67 residues),followed by isoleucine (49 residues) & leucine (48 residues) respectively. Minnimum amino acids were alanine, arginine , cysteine followed by proline & methionine.

Table.1. Result through ProtParam for the analysis of residue ratio

AMINO ACIDS	PERCENTAGE
Alanine (A)	3.0%
Asparagine (N)	11.8%
Aspartic acid (D)	5.3%
Arginine (R)	2.6%
Cysteine (C)	2.6%
Glutamic acid% (E)	6.3%
Glycine (G)	4.7%
Histidine (H)	4.6%
Glutamin (Q)	3.5%
Leucine (L)	8.4%
Lysine (K)	8.1%
Isoleucine (I)	8.6%
Methionine (M)	1.4%
Phenylalanine (F)	5.3%
Proline (P)	2.1%
Serine (S)	7.0%
Threonine (T)	5.1%
Tryptophan (W)	0.5%
Tyrosine (Y)	4.0%
Valine (V)	5.1%

Table.2.Analyses of atomic composition of input sequence through ProtParam

ATOM	SYMBOL	COMPOSITION
CARBON	C	2913
HYDROGEN	H	4515
NITROGEN	N	803
OXYGEN	O	882
SULPHUR	S	23

The protein sequence has molecular formula$C_{2913}H_{4515}N_{803}O_{882}S_{23}$& total number of atoms 9136. The instability index (II) is computed to be 35.96 & aliphatic index is 84.11. This classifies the protein as stable.

Prediction of B cell epitope: The use of IEDB

The prediction of B-Cell epitope was done by using Immuno epitope database. Hydrophilicity, flexibility accessibility, turns , exposed surface , polarity and antigenic propensity parameters were used to predicted B cell epitope.

Table.3. Prediction of B Cell epitope by Kolaskar and tongaonkar antigenicity methods through IEDB (Immuno Epitope Database Analysis resources)

S.NO.	START POSITION	END POSITION	PEPTIDES	PEPTIDES LENGTH
1.	3	9	KKAREYA	7
2.	29	34	LKERLE	6
3.	48	53	NLNKNE	6
4.	10	16	QDALKFI	7
5.	22	28	NFLACKNL	8
6.	54	59	GYVLCK	6
7.	78	94	GSILISIGHIDSCALKI	17
8.	102	115	KKKIHQINVECYGSG	15
9.	125	135	SLGLSGQVLYK	11

10.	144	162	LIQINKSVLFLPSLAIHLQ	19
11.	168	174	DFSVKIN	7
12.	178	186	HIKPIISTT	9
13.	188	193	FNQLNK	6
14.	223	229	DQMCHSF	7
15.	244	249	IEHLTN	6
16.	266	272	KDIVEHI	7
17.	284	289	LSKELN	6
18.	297	304	DFELCLMD	8
19.	307	316	EPCFTGVYEE	10
20.	326	343	LLGSFCVFEGFIELVNSI	18
21.	369	376	NLYISIGY	8
22.	381	396	IGSLSEVGARSYCTKN	16
23.	399	408	DRIISSVFKK	10
24.	430	442	ILNVDMAHCSHPN	13
25.	449	462	DNHQLFFHEGIAIK	14
26.	525	534	DIGIPQLAMH	10

Prediction of T-Cell: The use of MHCPred

The prediction of promiscuous T-Cell Epitope was done by using MHCPred. The results weretaken in HTML and tabular forms. A0101,A0201,A0202 ,A0203,A0206,A0301,A1101,A3101,A6801, A6802 , B3501 , DRB0101 ,DRB0401 and DRB0701 were the alleles chosen for this computational analyses. Peptides with the lowest prediction IC50, corresponding to the best prediction binding affinities are shown in table 4.

Table.4. Peptide of pfM18AAP protein with best predicted binding affinity for each allele

S.NO.	Alleles	Amino acids groups	Predicted IC50(Nm)	Confidence of prediction (Max=1)
1.	A0101	KSVLFLPSL	13.15	0.89
2.	A0101	TTDTKFSHK	5.62	0.89
3.	A0202	LNKCKRNNV	5.02	0.89
4.	A0203	KISPNNNVI	1.74	1.00
5.	A0206	AIHLQNRTR	13.03	0.78
6.	A0301	HINTDNSYP	7.71	0.89
7.	A1101	AVHDVFFLI	3.30	0.78
8.	A3101	LSMPGIDIG	94.84	0.56
9.	A6801	QLFFHEGIA	21.93	0.67
10.	A6802	NHIKPIIST	8.49	0.67
11.	B3501	FIQRSGSNF	92.68	0.78
12.	DRB0101	HIDSCALKI	0.49	0.89
13.	DRB0401	VSNHNLDKN	31.77	1.00
14.	DRB0701	KKKIHQINV	34.59	1.00

According to this computer based prediction the result from A1101 and DRB0101 reveals lower IC50 than other alleles . for A1101 alleles, the three peptides with the best binding affinities are AVHDVFFLI (IC50=3.30) , SPNNNVIKK (IC50=5.06) and SHKENSQNK (IC50=6.75) Respectively . For DRB0101 allele, the three peptides with the best binding affinities are HIDSCALKI (IC50= 0.49), FIDRIISSV (IC50=0.62) and YVTSPLHAS (IC50=0.85) Respectively.

Prediction of Post translational modification:-

Prediction of disulphides sites: The use of Disulfind tools:-

Our input epitope doesn't contain any Disulphide sites because for prediction of Disulphide sites the input must contain at least two cysteine molecule.

Prediction of Glycosylation sites: The use ofNetoGly 1.0 server

Glycosylation is a co- and post-translational modification involving the covalent addition of carbohydrates to proteins. Carbohydrates (also referred to as glycan's, sugars, or saccharides) are adopting linear and branched structures and are composed of monosaccharide's, which are covalently linked by glycosidic bonds. Therefore number of asparagine residues in the input sequence provide glycosylation sites .As no asparaginesresidues present in the input sequence and N-glycosylation is only predicted on ASN residues thus the prediction was not made.

Prediction of protein kinase C phosphorylation & Casein Kinase II phosphorylation: The use of Kinasephos 2.0

Protein phosphorylation, which is an important reversible mechanism in post-translational modifications, is involved in many essential cellular processes including cellular regulation, cellular signal

pathways, metabolism, growth, differentiation and membrane transport (1). Phosphorylation of substrate sites at serine, threonine and tyrosine residues of eukaryotic proteins is performed by members of the protein kinase family. Additionally, phosphorylation on plays an important role in signal transduction in organism. Our input sequence contains three residues of Serines.

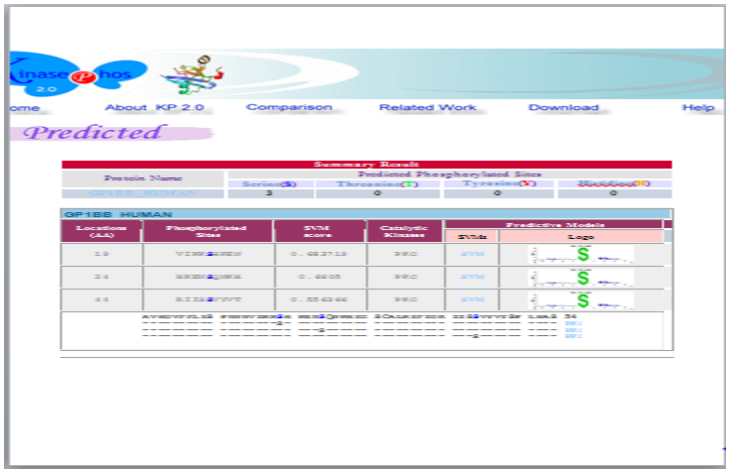


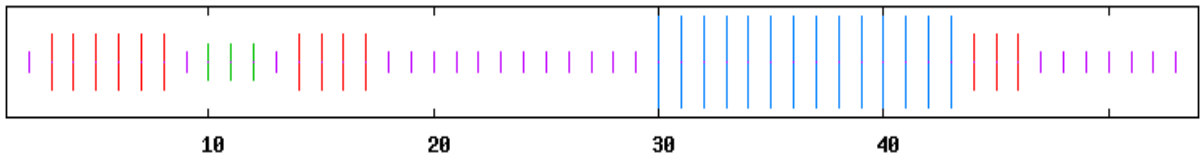
Fig 1:- Serine residues predicted by kinase phos 2.0 server

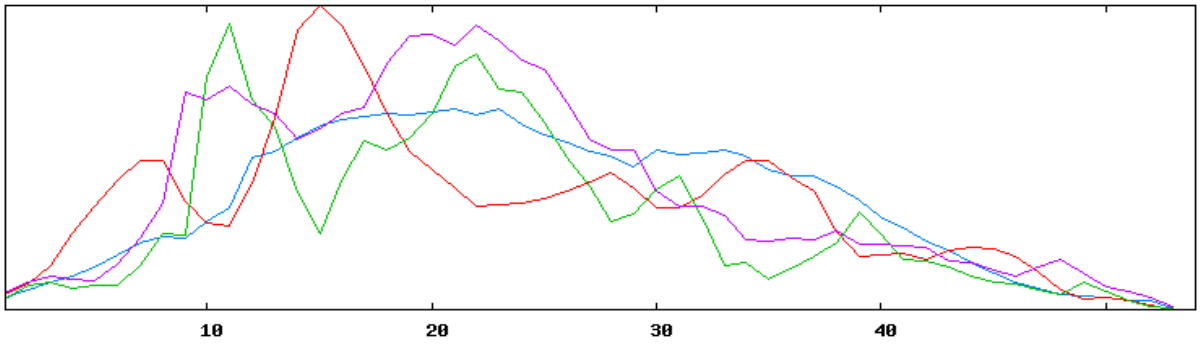
Insilico prediction of phosphorylation sites with high predictive performance could be a promising strategy to conduct preliminary analyses and could heavily reduce the number of potential targets that need further *in vivo* or *in vitro* confirmation.

Prediction of secondary structure: The use of SOPMA

The prediction of secondary structure was done by using SOPMA Server. The result of this analyses for pfM18AAP protein predicted that 14 region are Alfa helix and three regions are beta turn. Obtained analyses results are shown on next page:-

```
10        20        30        40        50
        |         |         |         |         |
AVHDVFFLISPNNNVIKKSHKENSQNKHIDSCALKIFIDRIISSVYVTSPLHAS
ccceeeeeectttceeeecccccccccccchhhhhhhhhhhhhheeecccccc
Sequence length:    54
SOPMA:
   Alpha helix     (Hh) :    14 is  25.93%
3(10) helix        (Gg) :     0 is   0.00%
   Pi helix        (Ii) :     0 is   0.00%
   Beta bridge     (Bb) :     0 is   0.00%
   Extended strand (Ee) :    13 is  24.07%
   Beta turn       (Tt) :     3 is   5.56%
   Bend region     (Ss) :     0 is   0.00%
   Random coil     (Cc) :    24 is  44.44%
Ambigous states (?)  :     0 is   0.00%
   Other states         :     0 is   0.00%
```





Parameters:
Window width : 17
Similarity threshold: 8
Number of states : 4

Fig.2:-Secondary structure prediction of pfM18AAP protein from SOPMA server

In proteins, turns are found on the surface ; these parts are accessible and hydrophilic. In contrast the core is mostly devoid of water molecules. In case of pfM18AAP, it has been shown that the secondary structure is important to antibody binding and even a minor modification of the secondary structure can affect the immune identification of antigens. Like any other protein, prediction of secondary structure of pfM18AAP can provide us important information about the interactions and functions of this protein. Since the residue composition of any protein is important, in the present investigation the residue composition for pfM18AAP has been calculated.

Similarity search prediction: The use of EMBOSS

Translation of vector DNA sequence was done by using EMBOSS Back transeq. We are using Plasmid vectors for vaccine designing. Result of its reverse transcriptase is shown below.

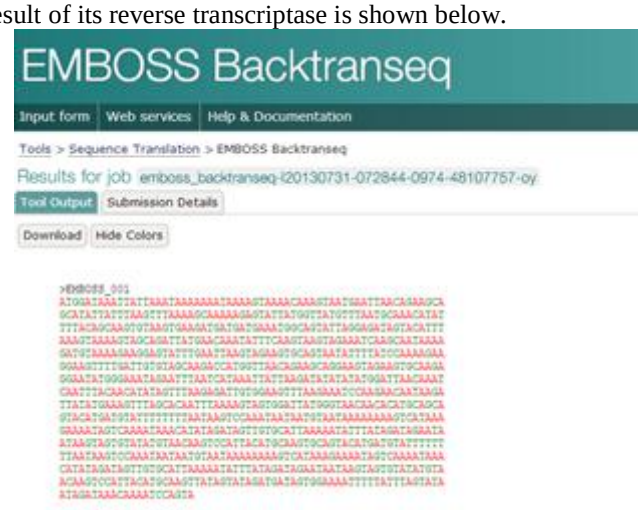


Fig.3:- Results of EMBOSS Back transeq showing protein sequence of Plasmid Vector

After obtaining protein sequence of Plasmid vectors ,similarity search was done between protein sequence of epitope and protein sequence of vectors by using EMBOSS pairwise sequence alignment server. The results of EMBOSS pairwise sequence alignment server for Plasmid vectors are shown below

EMBOSS_001 1 ---------- 0

EMBOSS_001 1 MDKLLNKKIKVKQSNELTEAAYYLSLKAKRVLWLCLMQTYFTASVSEDDD 50

EMBOSS_001 1 ---------- 0

EMBOSS_001 51 EMAVLGDSTFKVKVADYEQIFQVSRNQAIKDVKEGVFELSRSAVIFYPKE 100

```
EMBOSS_001       1 --------------------------------------------------      0

EMBOSS_001     101 GSFDCVARPWLTEAGSRSARGIWEIEFNHKLLRYIYGLTNQFTTYSLRDC
150

EMBOSS_001       1 ------------------------------AVHDVFFLISPNNNVIKKSHK     21
                                                 .::|.|.|
EMBOSS_001     151 GSLRNPRTIRLYESLAQFKSSGLWVTTHAWLNDRFLL----------     187

EMBOSS_001      22 ENSQNKHIDSCALKIFIDRIISSVYVTSPLHAS----------------     54
                   ..||.|::..... :.|:|..:..:...:||.|.
EMBOSS_001     188 PESQQKNLAELK-RSFLDPALKQINEKTPLLAKYSIDDSGKFLFSIIDKQ    236

EMBOSS_001      55 ---     54

EMBOSS_001     237 NPV    239
```

Fig 4:-Result of EMBOSS pairwise sequence alignment for plasmid vectors

From the above result we can design the sequence of designed plasmid vector vaccine (MDKLLNKKIKVKQSNELTEAAYYLSLKAKRVLWLCLMQTYFTASVSEDDDEMAVLGDSTFKVKVA DYEQIFQVSRNQAIKDVKEGVFELSRSAVIFYPKEGSFDCVARPWLTEAGSRSARGIWEIEFNHKLLRYI YGLTNQFTTYSLRDCGSLRNPRTIRLYESLAQFKSSGLWVTTHAAVHDVFFLISPNNNVIKKSHKENSQ NKHIDSCALKIFIDRIISSVYVTSPLHASAVHDVFFLISPNNNVIKKSHKENSQNKHIDSCALKIFIDRIISSV YVTSPLHASYSIDDSGKFLFSIIDKQNPV)

Ig Blast search: The use of Ig Blast tools

IgBLAST output presents a clear and informative view of the search result. In addition to showing the actual detailed alignment between the query and various hits, the report includes a tabulated summary of results based on the alignments between the query and the top matched germ line V, D and J gene. The summary information includes the identifiers of the best matched V, D and J gene, the relationship between the coding frames of the V and J genes, the details of the V-(D)-J junctions and the match statistics for various FR/CDR.

Query Length=921

Table 5:Sequences producing significant alignments:-

Germline gene	Score (Bits)	E – Value
lcl|IGHV4-34*11	26.8	0.96
lcl|IGHV4-59*04	26.8	0.96
lcl|IGHV4-61*03	23.7	8.3
lcl|IGHD1-7*01	14.4	22
lcl|IGHD1-20*01	14.4	22
lcl|IGHD2-2*02	12.4	87
lcl|IGHJ4*01	14.4	48
lcl|IGHJ4*02	14.4	48

Table 6:- V-(D)-J rearrangement summary for query sequence (multiple equivalent top matches having the same score and percent identity, if present, are separated by a comma):-

Top V gene match	Top D gene match	Top J gene match	Chain type	stop codon	V-J frame	Productive	Strand
IGHV4-34*11,IGHV4-59*04	IGHD1-7*01,IGHD1-20*01	IGHJ4*01,IGHJ4*02	VH	No	In-of-frame	No	-

The alignment section uses a familiar multiple alignment view with the hits aligned to the query ,shown in table 7. The alignments show the three top hits from the V, D and J gene matches by default, but this is user adjustable. The far left column indicates the gene category (i.e. V, D or J) for the germ line gene hits. Our queries represent minus strand of V gene and has been converted to the plus strand. The sequence positions shown in table 6 is referred to their converted sequence. Therefore it is concluded that this sequence can promote antibody activity.

Table 7:-V-(D)-J junction details based on top germline gene matches:-

V region end	V-D junction*	D region	D-J junction*	J region start
TTATA	CTAAATAAAAATTTTCCACTATCATCTATAC	TATAACT	TGCATGTAATGGACTTGTTACATATAC	ACTAC

Table 8 shows the percent identity between the query and each hit.*: Overlapping nucleotides may exist at V-D-J junction (i.e., nucleotides that could be assigned to either rearranging gene). Such nucleotides are indicated inside a parenthesis (i.e., (TACAT)) but are not included under the V, D or J gene itself.

Table 8:-Alignment summary between query and top germ line V gene hit (shows the number of matches and the alignment length):-

	from	to	length	matches	mismatches	gaps	identity(%)
FR2-IMGT	4	15	12	9	3	0	75
CDR2-IMGT	16	25	10	10	0	0	100
Total			22	19	3	0	86.4

Alignments

<-FR2-IMGT-><-----CDR2-IMGT----->

W I L F I Y Y T K * K F S T I I Y T I T C M * W T C Y I Y T

lcl|Query_1_reversed 4
TGGATTTTGTTTATCTATTATACTAAATAAAAATTTTCCACTATCATCTATACTATAACTTGCATGT
AATGGACTTGTTACATATACACT 93

V 86.4% (19/22) IGHV4-34*11 139 .....GG..A.....---------- -------- 160

W I G Y I Y Y

V 86.4% (19/22) IGHV4-59*04 139 .....GG..A.....---------- -------- 160

V 85.0% (17/20) IGHV4-61*03 145 .....GG..A.....---------- -------- 164

D 100.0% (7/7) IGHD1-7*01 3 ----------.....---------- ---- 9

D 100.0% (7/7) IGHD1-20*01 3 ----------.....---------- ----- 9

D 100.0% (6/6) IGHD2-2*02 25 ----------.....---------- ----- 30

J 100.0% (7/7) IGHJ4*01 1 ---------- ... 3

J 100.0% (7/7) IGHJ4*02 1 ---------- ... 3

T

lcl|Query_1_reversed 94 ACTT 97

J 100.0% (7/7) IGHJ4*01 4 7

J 100.0% (7/7) IGHJ4*02 4 7

A quick examination of Ig Blast result for plasmid vector sequence of titles suggests that two hits come from different sources for J region i.e IGHJ4*01 and IGHJ4*02 for 'ACTT' sequence and three hits come from different sources for D region i.e. IGHD1-7*01, IGHD1-20*02 & IGHD2-2*02 showing 100% hit.

3D structure prediction: The use of PHYRE 2 tools

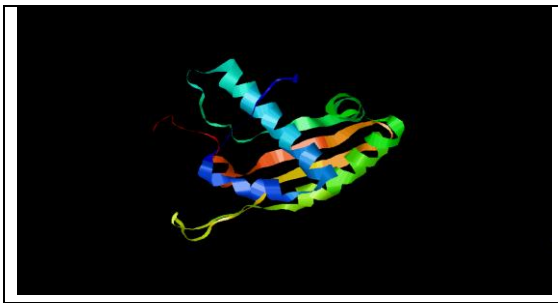
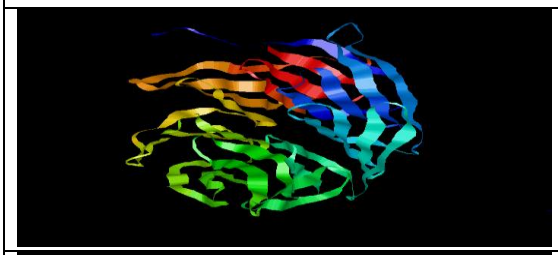
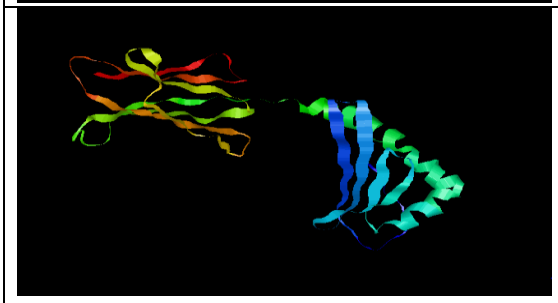
The prediction of 3Dstructure of designed vaccine sequence is done by PHYRE 2 tools .

Validation of 3Dstructure:The use of SAVES Server:

Two models of Plasmid vector vaccine had passed Validation steps i.e d1hkqa_.4. and d2nrac1.3.pdb. Among this two models of Plasmid d2nrac.1.3.pdb had shown high percentage validation result i.e 85.5%. Validation of MHC I and MHC II molecule is also preformed which is needed for docking purpose.

Visualization of validated models: The use of RASMOL

Visualization of validated model was done by RASMOL. The visualized structure of validated model has shown below.

	Visualized model of Plasmid Vector designed vaccine
	Visualized model of Validated MHC I molecule
	Visualized model of Validated MHC II molecule

Docking of Visualised model :The use of Patch dock

The result of epitope molecule, docked with MHC I molecule successfully is shown below. The epitope molecule docked with MHC-I molecule successfully this shows that MHC I molecule represent the epitope to B cells.

Table 9:- Patch Dock result showing binding affinity between epitope and MHC I molecules

SOLUTION NO.	SCORE	AREA	ACE
1.	18460	2946.70	433.87
2.	16880	3013.60	267.93
3.	16116	2584.10	294.46
4.	16054	2386.20	478.44
5.	15684	2354.80	255.85
6.	15534	2668.80	255.99
7.	15410	2360.10	245.24
8.	14876	1996.60	441.92
9.	14790	2274.10	414.03
10.	14706	1993.70	152.12
11.	14642	2029.50	462.37
12.	14588	2284.40	496.41
13.	14540	2200.70	233.62
14.	14538	2229.70	425.15
15.	14496	2154.80	402.87
16.	14432	2154.80	304.62
17.	14410	1935.80	399.82
18.	14366	2228.10	314.21
19.	14300	1887.20	133.79
20.	14266	1856.60	304.65

The Patch Dock results showing binding between epitope and MHC II molecule has shown below

Table 10:- Patch DOCK result showing binding affinity between epitope and MHC II molecule

SOLTION NO.	SCORE	AREA	ACE
1.	15162	2030.90	2.37
2.	14860	2162.20	142.19
3.	14628	2108.30	463.29
4.	14492	2589.60	22.95
5.	14490	2272.80	263.31
6.	14100	2275.30	375.42
7.	14076	1970.90	12.74
8.	13972	3191.80	-444.45
9.	13942	2333.60	3.72
10.	13856	1844.70	399.68
11.	13854	2107.50	174.46
12.	13734	2250.90	266.30
13.	13710	2031.90	465.95
14.	13592	1760.40	127.55
15.	13586	1975.10	40.25
16.	13514	1622.80	436.04
17.	13414	1709.50	142.58
18.	13354	1836.70	-31.24
19.	13348	2030.60	79.51
20.	13232	1586.20	200.97

Patch dock score ≥ 8741 having good binding solutions. Best docking model from obtained Patch Dock model was obtained from Fire Dock. The idea is that additional correct results are hidden somewhere among the 100-1000 best PATCHDOCK results was obtained from Fire Dock. The FIREDOCK output page is similar to the PATCHDOCK, but the results are sorted by Global Energy obtained, the table shows also the Solution Number column, which displays the numbers corresponding to the original sorted solutions from PATCHDOCK. Best model obtained from fire dock for epitope and MHC I molecule has shown below.

Table.11:- Fire Dock result showing binding affinity of epitope and MHC I molecule

Rank	Solution number	Global energy	Attractive VdW	Repulsive VdW	ACE	HB
1.	5	0.48	-26.34	9.91	12.26	-3.21
2.	6	8.86	-1.03	0.00	1.23	0.00
3.	1	10.72	-4.65	1.88	3.22	-1.33
4.	4	15.27	-8.16	0.22	3.25	0.00
5.	10	15.52	-36.76	52.66	5.59	-1.68
6.	8	372.34	-28.00	449.94	21.71	-3.84
7.	3	729.79	-42.67	927.97	16.12	-6.06
8.	9	2496.25	-64.29	3162.96	19.45	-12.12
9.	7	2563.65	-62.02	3308.69	4.96	-10.24
10.	2	2754.17	-50.27	3498.03	10.62	-10.19

The global energy obtained is in negative form showing that the model obtained by Fire Dock is best showing maximum binding affinity between epitope and MHC I molecule's. Best model obtained from Fire Dock for epitope and MHC II molecule has shown below.

Table.12:- Fire Dock result showing binding affinity of epitope and MHC II molecule

Rank	Solution Number	Global Energy	Attractive VdW	Repulsive VdW	ACE	HB
1	2	-16.91	-15.96	5.70	5.83	-1.27
2	8	7.88	-1.79	0.27	1.32	0.00
3	7	10.96	-27.11	5.32	11.58	-3.29
4	10	16.79	-10.23	19.85	7.93	-2.05
5	6	23.86	-28.79	23.07	12.15	-4.23
6	3	140.00	-22.47	167.02	11.55	-2.60
7	5	140.16	-7.64	155.96	5.57	-1.30
8	9	685.79	-29.29	915.36	2.21	-1.30
9	4	1520.34	-76.40	2028.36	1.22	-15.08
10	1	3637.11	-49.27	4584.44	14.20	-5.31

From above table it is clear that predicted epitope show maximum binding with MHC I &MHC II molecules. Therefore it is cleared that designed epitope based vaccine shows maximum affinity to MHC molecules. Docking result obtained has shown below.

(A) (B)

Fig 5:(A)Fire dock result for Plasmid vector vaccine and MHC I molecule .(B)Fire dock result for Plasmid vector vaccine and MHC II molecule.

III. Result And Discussion:-

Vaccination remains a high priority for animal disease prevention and control especially on account of rising antimicrobial resistant strains of pathogens and frightening increase in new emerging and reemerging pathogens. Vaccine designing involves identification of epitopes of a pathogen which is not an easy task as pathogen encodes for a variety of proteins. Traditionally antigenic peptides have been identified by the overlapping peptide synthesis which is a time consuming and an expensive work. With the advent in the field of computational immunology it is possible now to drastically reduce the time for identification of putative and promiscuous antigenic peptides. The present study has used MHC2Pred web based tool for identification of antigenic epitope.

1). Amino acid residues analysis has shown that our protein sequence contain Asparagine in maximum amount and methionine in minimum amount where Asparagine is a polar amino acids and Methionine is non-polar amino acid. From the study of amino acid residue analyses it is clear that our protein sequence is hydrophilic i.e. water loving molecule.

2).From the analyses of MHC2Pred tools, six MHC I and II binding peptides in pfM18AAP protein of Plasmodium falciparum has predicted. MHC class I and class II predicted T cell epitope are shown below:-

SEQUENCE	AMINO ACIDS RESIDUE	IC50 VALUES
1). MHC Class I binding peptides		
AVHDVFFLI	9	3.30
SPNNNVIKK	9	5.06
SHKENSQNK	9	6.75
2). MHC Class II binding peptides		
HIDSCALKI	9	0.49
FIDRIISSV	9	0.62
YVTSPLHAS	9	0.85

The MHC class I binding consensus peptides AVHDVFFLI has six hydrophobic amino acids residues (75% hydrophobic), and two charged amino acid residues i.e. Histidine and Aspartic acids. Second MHC class I binding consensus peptides SPNNNVIKK has three hydrophobic amino acids residues (25.5% hydrophobic), and three hydrophilic amino acids residue along with a positively charged molecule i.e. Lysine. Third MHC class I binding consensus peptides SHKENSQNK has three hydrophilic amino acids residue along with charged molecule i.e. two positively charged Lysine and Histidine , and one negatively charged Glutamic acids.
The MHC class II binding consensus peptides HIDSCALKI has four hydrophobic amino acids i.e. Isoleucine, Alanine and Leucine (i.e. 45% hydrophobic) and a polar amino acids i.e. cysteine, along with two positively charged amino acids i.e. Lysine and Histidine, and one negatively charged molecule Aspartic acid. Second MHC class II binding consensus peptides FIDRIISSV has five hydrophobic amino acids i.e. Phenylalanine, Isoleucine, and Valine, along with charged molecule i.e. a negatively charged molecule Aspartic acids. Third

MHC class II binding consensus peptides YVTSPLHAS has four hydrophobic amino acids i.e. Proline, Valine, Alanine and Leucine along with charged molecule i.e. a positively charged molecule Histidine.
Together with hydrophobic amino acids, charged amino acid also contributes towards the Interaction with the MHC pockets. The presence of hydrophobic and charged amino acids makes these peptide a good choice to include this peptide into an experimental study. All of the six MHC binding peptides predicted by MHC2Pred tools conform to the criterion to be ideal consensus T cell epitopes.

3). Post translation modification of the predicted peptides was done by using Disulfind tools, NetOGlyc 1.0 tools, Kinase phos 2.0. As we can observe from Kinase phos 2.0 server results page that predicted epitope has three Serine molecules which play an important role in signal transduction in organism. No Asparagine molecule and Histidine molecule is present in our input sequence.

4). Secondary structure prediction was done by using SOPMA server. The results of analyses for pfM18AAP protein predicted that 14 regions are alpha helix and three regions are beta turn. This shows that in protein turns found on the surface are accessible and hydrophilic. In contrast the core is mostly devoid of water molecules. Secondary structure is important to antibody binding and even a minor modification of the secondary structure can affect the immune identification of antigens. Prediction of secondary structure of pfM18AAP protein can provide us important information about the interactions and functions of this protein.
5).Similarity search between vector sequence and epitope sequence was done by using EMBOSS pairwise sequence alignment tools for designing complete vaccine sequence. Designed Plasmid vector based vaccine sequence has shown below:
(MDKLLNKKIKVKQSNELTEAAYYLSLKAKRVLWLCLMQTYFTASVSEDDDEMAVLGDSTFKVKVA
DYEQIFQVSRNQAIKDVKEGVFELSRSAVIFYPKEGSFDCVARPWLTEAGSRSARGIWEIEFNHKLLRYI
YGLTNQFTTYSLRDCGSLRNPRTIRLYESLAQFKSSGLWVTTHAAVHDVFFLISPNNNVIKKSHKENSQ
NKHIDSCALKIFIDRIISSVYVTSPLHASAVHDVFFLISPNNNVIKKSHKENSQNKHIDSCALKIFIDRIISSV
YVTSPLHASYSIDDSGKFLFSIIDKQNPV)
6). Ig Blast sequence search result was done at NCBI IgBlast database. It is worth noting that the IgBLAST report provides information on overlapping nucleotides at a rearrangement junction that might have been contributed by either of the rearranging genes because of homology directed recombination events. A quick examination of Ig Blast result for plasmid vector sequence of titles suggests that two hits come from different sources i.e. IGHJ4*01 and IGHJ4*02 for 'ACTT' sequence and three hits come from different sources for D region i.e. IGHD1-7*01, IGHD1-20*02 & IGHD2-2*02 showing 100% hit. Whereas minus strand is shown in V gene which is converted into plus strand by tools therefore V-D-J gene become productive and able to activate antibody.

7).3D structure of designed sequence was done by using PHYRE tools .Result for our sequence comes in our mail. Obtained models are validate in SAVES server .For Plasmid vector based vaccine fourth model was predicted to be best i.e. d2nrac1.3.pdb.Obtained best models are visualize with the help of Rasmol.

8). Interaction between Obtained models of both vectors and MHC molecules are done by using protein protein docking tools i.e. Patch Dock. 20 results came from Patch dock. To find out the best one among them, the result were filtered by using Fire dock which provide best result of our input. The tool provide information that the selected epitope was docked with the MHC Class1 molecule and the docking energy was found to be 0.48 and between epitope and MHC II molecule was found to be -16.91.The result revealed that the designed candidate vaccine has a high binding affinity with T-cell receptor.

IV. Conclusion:-

The discipline of Immuno-informatics is accelerating the development of vaccines composed of epitope ensembles. Opportunities for epitope discovery and epitope-drived vaccine design are expanding as the number of pathogens. Epitope-driven vaccines that are designed and optimized, based on our current knowledge and understanding of the mechanics of immunogenicity and immune-dominance, are filling the vaccine development pipelines. We have shown through tabulated representation that the Glycoprotein pfM18AAP of Plasmodium falciparum posseses region of solvent accessible and highly conserved which also have epitopic significance. Thus obtained epitopic sequence of pfM18AAPhas high degree of probability as suitable targets for development of peptide vaccines. Antigen determinants was selected based on the least identity and least E value and the epitope was predicted with the help of MHC Pred tools i.e."AVHDVFFLISPNNNVIKKSHKENSQNKHIDSCALKIFIDRIISSVYVTSPLHAS". From which we have designed epitope molecule. This molecule binds with MHC 1 molecule successfully, so the selected epitope was docked with the MHC Class1 molecule and the docking energy was found to be 0.48 and between epitope and

MHC II molecule was found to be -16.91 .From this docking analysis we can conclude that the result revealed that the designed candidate vaccine has a high binding affinity with T-cell receptor.

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