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Immunoinformatics approach for the study of CD_4+ epitopes of HIV-1 Gag protein restricted to HLA-DRB1*07 Allele

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ABSTRACT

*Clade specific vaccine construction strategy would be ideal solution for currently available HIV/AIDS therapy related problems. Immunoinformatics approach based on Stabilized Matrix Method and Neural Network algorithms were adopted to identify Gag epitope based vaccine candidates namely P1-P6(GLNKIVRMYSPTSIL, LGLNKIVRMYSPTSI, LNKIVRMYSPTSILD, NKIVRMYSPTSILDI, IVRMYSPTSILDIKQ,KIVRMYSPTSILDI) restricted to HLA-DRB1*07, a commonly distributed allele among the south Indian population allele would aid significant CD4+ T cell immune response against HIV infection. Three dimensional structure of epitopes P1-P6 modeled using de nova based I Tasser server, and Epitopes binding affinity on HLA-DRB1*07 allele's binding groove were analyzed using ClusPro based docking strategy. Finally HLA and Epitope interactions, binding energy potential calculated for resulting docked complexes and molecular interactions like conventional hydrogen bonding, non classical carbon hydrogen bonding ,salt bridges were analyzed using DS visualize 4.0. This systematic Insilico study would be helpful in designing Gag epitope based vaccine candidate for HIV infection, further in vitro and in vivo experiments needed for validating these vaccine candidates.*

Keywords: HIV Vaccine, HLA-DRB1*07, Gag Gene, CD4+ T cell response, Human Leukocyte Antigen, Epitope.

INTRODUCTION

Human immunodeficiency virus (HIV), a retrovirus that belongs to the Lentiviridae family is causative agent for Acquired immunodeficiency syndrome (AIDS),a major health concern throughout the world. Nearly 75 million people were infected Human immunodeficiency virus-1 (HIV-1) 36 million deaths happens worldwide due to HIV infection [2]. India has the third highest number of estimated people living with HIV in the world and it is estimated that 20.89 lakh people living with HIV/AIDS in India according to recent report (2013-14) of National AIDS control organization[3].Currently Highly active anti retro viral therapy (HAART) based on combination antiretroviral drugs used to treat individuals with HIV infection but each with its own side effects and resistance development in patients [4].Viral load is reduced due to HAART therapy and leads to a declination in morbidity and mortality of HIV-infected individuals, but cannot eradicate the virus[4].HIV vaccine has long been a key area for research and numerous resources have been directed for HIV vaccines development[5-10]. Architecture of the HIV-1 genome is complex and comprised of three functional groups of genes like structural genes (Gag, Env ,Pol) ,regulatory genes (Tat,Rev) and accessory genes (Vpu,Vpr,Vif,Nef)[11]. Major obstacles in the HIV research reveals a fact that designing of vaccine for globally HIV infected people is not possible, due to genetic diversity of HIV and clade /subtype differences seen among the affected population [12, 13]. There were three distinct groups: M (Major), O (Outliers), and N (non-M and non-O) of HIV-1 circulating in a global level among M is the most predominant group

of HIV-1 around the world , Within the M group there are nine subtypes: A–D, F–H, J, and K [14].Global HIV burden is due to commonly known clade HIV-1C[15] ,Thus this systematic immunoinformatics study restricted to analysis to HIV-1 subtype C-Gag specific $CD4^+$epitopes.Due to hypermutation capacity of HIV and HLA polymorphism vaccine design for HIV need to consider these two keyfactors.So conserved fragments in the Gag sequences among Indian population and their restriction to HLA alleles need to be analyzed[16,17]. HLA alleles like HLA-A02, HLA-A11, HLAB27, HLA-B*2705, HLA-B51, HLA-B*5701 of HLA Class I have been reported for their association with resistance or slow progression to HIV/AIDS [18-27].Recent studies on DRB1 allele expression among HIV controllers broader the significant role this heterodimeric HLA class II DRB1 allele restricted $CD4^+$ T cell responses in HIV disease outcome[29-30].Current study is based on DRB1*07 a commonly distributed alleles among south Indian population and their affinity for Gag based CD4+ epitopes[31]. Rational design of HIV vaccine needs the insights of HLA role in the outcome of HIV disease.Immunoinformatics based epitope prediction based on various algorithms would aid playing promising role in screening of and selection of CD4+ T cell Gag epitopes restricted to DRB1*07.

EXPERIMENTAL SECTION

Retrieval of Gag amino acid sequence and conservancy analysis:
HIV Sequence database [32] was used to retrieve the Gag protein sequences, conserved fragment of Gag sequences among Indian sample were retrieved based on the literature survey of our earlier work.[33] Based on the conservancy score for each amino acid position of Gag sequence conserved fragment region considered for Epitope prediction.

CTL epitope prediction, Population Coverage assessment:
Immune Epitope Database (IEDB) predicted CTL epitopes of Gag protein restricted to HLA-DRB1*07 allele were retrieved based on their low percentile rank [34].IEDB prediction server includes various modules like Consensus method, combinatorial library, NN-align [35] (netMHCII-2.2)[36], SMM-align (netMHCII-1.1)[37], Sturniolo [38],and NetMHCIIpan [39]. Resulting output includes units of IC_{50}nM for combinatorial library and SMM_align. Therefore a lower IC_{50}nM values indicates higher affinity. Generally peptides with IC_{50}values <50 nM are considered high affinity, <500 nM intermediate affinity and <5000 nM low affinity. Raw score values of Sturniolo output indicates higher score in turn implies higher affinity.. NetMHCIIpan method is used when Consensus and other methods such as SMM_align, NN_align,COMBLIB and/or Sturniolo are not available for a particular allele. However, if only one or two of these methods are available, NetMHC II pan is used as second or third method.Low percentile ranked epitopes were screened and assessed for population coverage among Indian population.Population coverage of the conserved gag epitopes with the corresponding HLA-DRB1*07 alleles were analyzed based on population coverage analysis tool of IEDB [40] depending on allele frequencies.net database [41] a huge population dataset on the web.

3D Modelling of Gag Epitopes structure:
Three dimensional structure of DRB1*07 restricted Gag Epitopes were modeled using I-Tasser [42]. I Tasser explores template search based on locally implemented meta server LOMETS, and TM-align allows fragment assembly simulation and finally function predictions are concluded from the consensus hits among the top structural matches along with function scores calculated based on the confidence score of I–TASSER structural models [42] .TM-Score and sequence identity in the structurally aligned regions were used to evaluate structural similarity between target and template models.

HLA-DRB1*07 allele and Gag Epitope affinity analysis:
Promiscuous Gag Epitopes were assessed for their interaction ability with the HLADRB1*07 Allele using ClusPro server[43] ,a fully integrated Docking server which recruits PIPER and FFT(Fast Fourier Trans-form) based rigid docking program. Complete protocol includes two stages , generation of low energy docked complexes based on pairwise interaction potential as first stage and clustering of docked complexes and low energy clusters assessment using SDU(Semi-Definite programming based Underestimation) program which predicts clusters stability using medium range optimization algorithm as second stage and finally stable clusters are further refined using Monte-Carlo simulation.ClusPro server results retrieved for four different categories like Balanced, Electrostatic favored, Hydrophobic favored and Vdw+Elec,top ranked models in all categories considered for HLADRB1*07 allele and Gag Epitope interaction.DS Visulalizer 4.0[44] was used to assess interaction and visualization of HLA and epitope interaction.

.RESULTS AND DISCUSSION

Retrieval of Gag amino acid sequence and conservancy analysis:
Gag protein sequences of Indian patients were retrieved from HIV sequence database and consensus fragment were retrieved based on literature our earlier work [33].

Population Coverage assessment of HLA-DRB1*07 specific CD4+ CTL Gag epitopes:
CD_4+ Gag epitopes restricted to HLA-DRB1*07 allele were predicted using IEDB server,among 1862 resulted epitopes based IC_{50} value, percentile rank and scores we selected low percentile ranked epitopes namelyP1(GLNKIVRMYSPTSIL),P2(LGLNKIVRMYSPTSI), P3(LNKIVRMYSPTSILD),P4(NKIVRMYSPTSILDI),P5(IVRMYSPTSILDIKQ),P6(KIVRMYSPTSILDIK) as promiscuous epitopes in Gag protein and were listed in **Table.1**

Table1 Gag CD_4+ epitopes and prediction score:

Allele	Peptide	NetmhcII Ic50	Sturniolo Score	Smm_Align Ic50
HLA-DRB1*07 allele	GLNKIVRMYSPTSIL	9.3	7	23
	NKIVRMYSPTSILDI	9.9	7	23
	IVRMYSPTSILDIKQ	10.3	7	26
	LGLNKIVRMYSPTSI	12.5	7	28
	KIVRMYSPTSILDIK	12.7	7	29
	LNKIVRMYSPTSILD	12.8	7	30

Population conservancy analysis of screened CTL epitopes:
An Promiscuous vaccine candidate from a pool of epitopes is selected based on their binding affinity towards the restricted HLA-DRB1*07 allele since the frequency of human MHC-HLA alleles differ among different ethinicities.The IEDB population conservancy analysis tool analyzed the conservancy of the predicted Gag epitopes, which are listed in **Table 2**.

Table 2 Population Coverage of HLA-DRB1*07 allele

Population / Area	Coverage	Average hit	PC90
India	28.59%	1.63	0.76
Average (Standard deviation)	28.52% (0.00%)	1.54 (0.00)	0.76 (0.00)

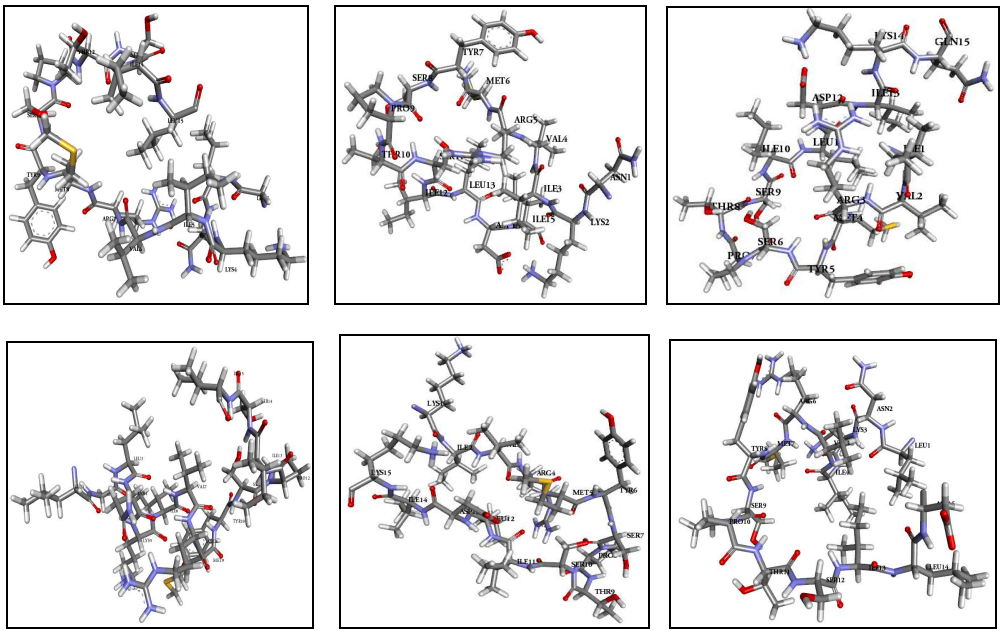
Table 3 Gag Epitope 3D structure prediction scores

Gag Epitopes	C-score	Exp. TM-Score	Exp. RMSD	No. of decoys	Cluster density
GLNKIVRMYSPTSI-P1	-0.80	0.61+-0.14	1.9+-1.6	8725	0.4550
NKIVRMYSPTSILDI-P2	-0.70	0.62+-0.14	1.7+-1.5	9288	0.5388
IVRMYSPTSILDIKQ-P3	-0.85	0.61+-0.14	2.0+-1.6	9660	0.4597
LGLNKIVRMYSPISI-P4	-0.68	0.63+-0.14	1.7+-1.5	9537	0.4947
KIVRMYSPTSILDIK-P5	-0.65	0.63+-0.14	1.7+-1.4	10012	0.5609
LNKIVRMYSPTSILD-P6	-0.85	0.61+-0.14	2.0+-1.6	9205	0.4217

De nova modeling of Gag Epitopes structure:
I-TASSER predicted Gag Epitope models quality were estimated based on C-score which is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of [-5,2], where a C-score of higher value signifies a model with a high confidence and vice-versa [45]. TM-score and RMSD Values reported in Column 3 & 4 in the [**Table 3**] are the estimated values of based on their correlation with C-score [46]. I-TASSER generates full length model of proteins by excising continuous fragments from threading alignments and then reassembling them using replica-exchanged Monte Carlo simulations. Low temperature replicas (decoys) generated during the simulation are clustered by SPICKER and top five cluster centroids are selected for generating full atomic models. The cluster density is defined as the number of structure decoys at a unit of space in the SPICKER cluster. A higher cluster density means the structure occurs more often in the simulation trajectory and therefore signifies a better quality model. The values

in the second last columns of the above mentioned table represents the number of structural decoys that are used in generating each model. The last column represents the density of cluster [46].

Figure 1 3 Dimensional structures of Gag Epitopes P1, P2, P3, P4, P5, P6



HLA-DRB1*07 allele Gag Epitope Interaction analysis:

HLA-DRB1*07 allele structure was retrieved from Protein Data Bank [47].HLA and Gag Epitopes interaction assessed based on their binding energy scores generated from an energy function of PIPER docking program. Scoring of binding energy potential is based on sum of potential terms of shape complementaity,electrostatics,desolvation contributions, and Decoys as reference states(DARS)[48].Thus we selected docked complexes with low binding energy score for our HLA and epitope interaction assessment and considered as a promiscuous epitope candidate for vaccine construction. Largest clusters lowest binding energy values of balanced, electrostatic favored clusters, hydrophobic-favored and VdW+Elec clusters were included for analysis .Based on binding energy scores of promiscuous epitopes P1-P6 binding efficiency were analyzed.HLA and epitope interaction were visualized and analyzed using DS visualize 4.0, it was observed that there were 3 subcategories of hydrogen bonding like Conventional Hydrogen Bond ,Carbon Hydrogen Bond and Salt Bridge interactions were existing and their by implies the stability of interaction and would aid CD_4+ regulated immune response against HIV infection[49].Donor and acceptors atoms of HLA and Gag epitopes and their bonding distance were listed in the Table-,carbon Hydrogen Bond interactions were considered as weaker since the donor is a polarized carbon atom and these interactions were determined using the same geomentric criteria used for classical hydrogen bonds with the exception of the default distance criterion being 3.8 Å.[50].

Table 4 .HLA-DRB1*07 allele and Gag Epitope Interaction

Epitope	Donor	Acceptor	Bond Type	Bond distance
	DRB1*07:GLU55:H	Gag Epitope:ASN3:OD1		1.95566
	DRB1*07:TRP61:HE1	Gag Epitope:PRO11:O		2.06179
	DRB1*07:ARG71:HH11	Gag Epitope:MET8:O		2.29957
	DRB1*07:ARG71:HH11	Gag Epitope:SER10:O		2.49825
	DRB1*07:ARG71:HH21	Gag Epitope:SER10:O		1.86339
	Gag Epitope:ASN3:HD22	DRB1*07:GLU55:O		1.96371
GLNKIVRMYSPTSIL-P1	Gag Epitope:ARG7:HE	DRB1*07:ASN62:OD1	Conventional Hydrogen Bond	3.00453
	Gag Epitope:ARG7:HH11	DRB1*07:ALA61:O		1.72084
	Gag Epitope:ARG7:HH12	DRB1*07:CYS65:SG		2.08967
	Gag Epitope:TYR9:HH	DRB1*07:GLU28:OE1		1.97184
	Gag Epitope:SER10:HG	DRB1*07:ASN62:OD1		1.84364
	Gag Epitope:THR12:HG1	DRB1*07:ASN69:OD1		1.83697
	Gag Epitope:SER13:HG	DRB1*07:CYS65:SG		2.31726
NKIVRMYSPTSILDI –P2	DRB1*07:ARG71:HH11	Gag Epitope:ASP14:OD2		1.89414
	DRB1*07:ARG71:HH21	Gag Epitope:ASP14:OD2	Salt Bridge	1.81589
	Gag Epitope:ARG5:HH22	DRB1*07:GLU28:OE1		1.8359
	DRB1*07:GLU55:H	Gag Epitope:LYS2:O		2.38636
	DRB1*07:ASN62:HD22	Gag Epitope:ARG5:O		1.95572
	DRB1*07:ASN69:HD22	Gag Epitope:TYR7:O		2.05903
	DRB1*07:TYR60:HH	Gag Epitope:THR10:O		1.8401
	DRB1*07:TRP61:HE1	Gag Epitope:SER8:O		2.05782
	DRB1*07:GLN70:HE21	Gag Epitope:ASP14:O	Conventional Hydrogen Bond	2.13189
	Gag Epitope:ASN1:HD21	DRB1*07:THR77:O		2.07031
	Gag Epitope:LYS2:HZ3	DRB1*07:SER53:OG		1.68019
	Gag Epitope:ARG5:H	DRB1*07:ASN62:OD1		2.61463
	Gag Epitope:TYR7:HH	DRB1*07:SER30:OG		1.8239
	Gag Epitope:SER8:HG	DRB1*07:ASN69:OD1		1.84511
	Gag Epitope:PRO9:CD	DRB1*07:ASN69:OD1	Carbon Hydrogen Bond	2.98184
IVRMYSPTSILDIKQ-P3	Gag Epitope:ARG3:HN	DRB1*07:ASN62:OD1	Conventional Hydrogen Bond	1.932
	Gag Epitope:SER9:HN	DRB1*07:ASN82:OD1		1.98022
	DRB1*07:GLY58:CA	Gag Epitope:ARG3:O		3.13173
	Gag Epitope:VAL2:CA	DRB1*07:ASN62:OD1	Carbon Hydrogen Bond	3.0325
	Gag Epitope:GLN15:C	DRB1*07:ASP66:OD2		2.96787
	DRB1*07:HIS81:CD2	Gag Epitope:PRO7:O		3.16261
LGLNKIVRMYSPTSI- P4	DRB1*07:TYR60:HH	Gag Epitope:THR13:OG1		1.87516
	DRB1*07:TRP61:HE1	Gag Epitope:TYR10:O		1.90371
	DRB1*07:GLN64:HE22	Gag Epitope:THR13:O		2.02845
	DRB1*07:ARG71:HH11	Gag Epitope:ILE6:O		2.04085
	DRB1*07:ARG71:HH11	Gag Epitope:MET9:SD		2.56631
	DRB1*07:ARG71:HH21	Gag Epitope:ILE6:O	Conventional Hydrogen Bond	1.97121
	Gag Epitope:ARG8:HE	DRB1*07:ALA61:O		2.00496
	Gag Epitope:ARG8:HH11	DRB1*07:ALA61:O		2.51362
	Gag Epitope:TYR10:HH	DRB1*07:SER30:OG		1.84613
	Gag Epitope:SER11:HG	DRB1*07:ASN69:OD1		1.83983
	Gag Epitope:ILE15:H	DRB1*07:ASP66:OD2		2.17311
KIVRMYSPTSILDIK-P5	Gag Epitope:ARG4:HH12	DRB1*07:GLU28:OE1	Salt Bridge	1.92621
	Gag Epitope:ARG4:HH22	DRB1*07:GLU28:OE1		1.77624
	DRB1*07:ASN69:HD22	Gag Epitope:TYR6:O	Conventional Hydrogen Bond	1.96538
	DRB1*07:TYR60:HH	Gag Epitope:THR9:O		1.81908
	DRB1*07:TRP61:HE1	Gag Epitope:SER7:O		1.88451
	DRB1*07:ARG71:HH11	Gag Epitope: LEU12:O		1.81793
	DRB1*07:ARG71:HH21	Gag Epitope:LEU12:O		2.04939
	Gag Epitope:TYR6:HH	DRB1*07:GLU28:OE2		1.9218
	DRB1*07:ARG71:HH22	Gag Epitope:TYR6:OH		1.7473
	Gag Epitope:SER7:CA	DRB1*07:ASN69:OD1	Carbon Hydrogen Bond	3.2257
LNKIVRMYSPTSID-P6	Gag Epitope:LYS3:HZ3	DRB1*07:GLU55:OE1	Salt Bridge	1.68459
	DRB1*07:GLU55:HN	Gag Epitope:LYS3:O	Conventional Hydrogen Bond	2.01705
	DRB1*07:ARG71:HH11	Gag Epitope:THR11:OG1		1.75362
	DRB1*07:ASN82:HD21	Gag Epitope:MET7:O		2.16189
	Gag Epitope:ARG6:HE	DRB1*07:SER53:O	Conventional Hydrogen Bond	2.77849
	Gag Epitope:ARG6:HH11	DRB1*07:ASN82:OD1		2.50059
	Gag Epitope:ARG6:HH12	DRB1*07:ASN82:OD1		2.31906
	Gag Epitope:TYR8:HH	DRB1*07:GLU11:OE1		1.87576

	Gag Epitope:SER9:HN	DRB1*07:GLN9:OE1		2.11862
	Gag Epitope:SER9:HG	DRB1*07:GLN9:OE1		1.88333
	Gag Epitope:SER12:HG	DRB1*07:ASN62:OD1		1.84036
	DRB1*07:ALA59:CA	Gag Epitope:TYR8:OH	Carbon Hydrogen Bond	3.03691
	DRB1*07:HIS81:CD2	Gag Epitope:VAL5:		3.10517

Based on the binding affinity pattern among the 6 predicted epitopes, it was concluded that P1, P2 and P4 ,P6 could be considered as the potential epitopes, since their binding orientations within the binding groove of DRB1*07 allele ,stable molecular interaction.

Figure 2 HLA-DRB1*07 and epitope P1 interaction analysis and the epitope GLNKIVRMYSPTSIL-P1 binds in the groove of the HLA-DRB1*07 allele.

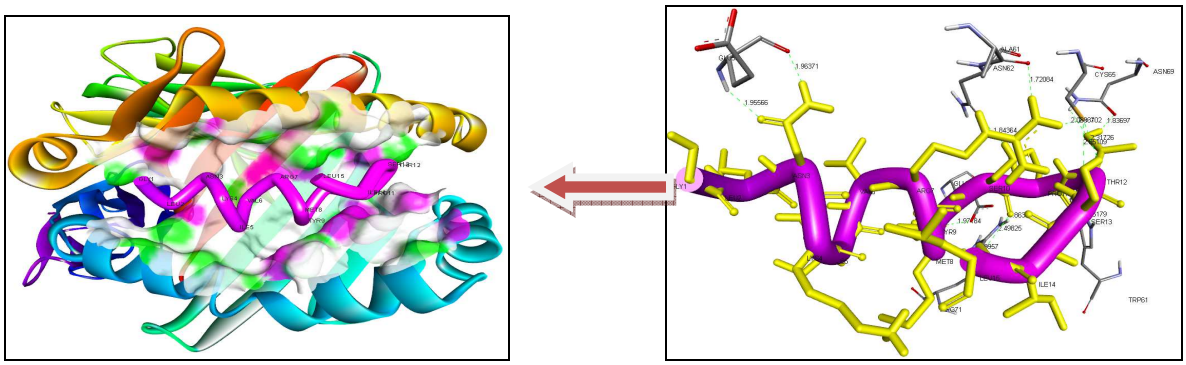


Figure 3. HLA-DRB1*07 and epitope P2 interaction analysis and the epitope NKIVRMYSPTSILDI-P2 binds in the groove of the HLA-DRB1*07 allele.

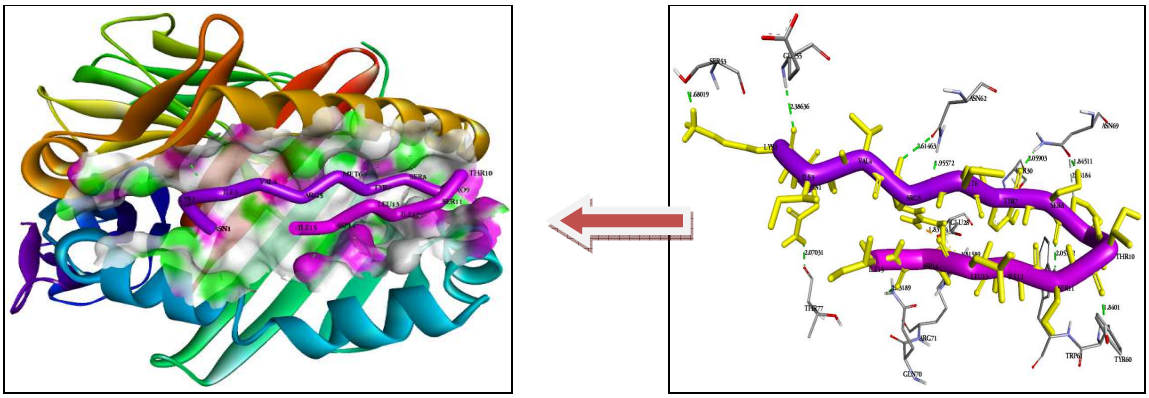


Figure 4
HLA-DRB1* 07 and epitope P3 interaction analysis and the epitope IVRMYSPTSILDIKQ binds in the groove of the HLA-DRB1*07 allele

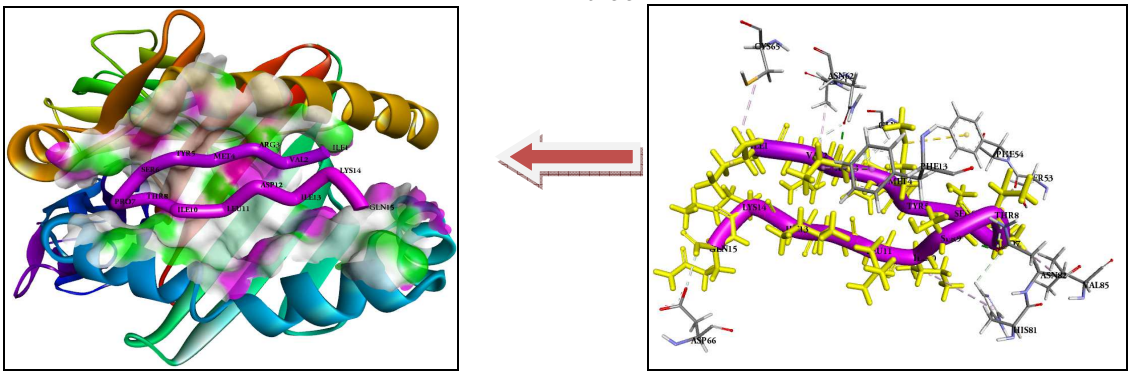


Figure 5. HLA-DRB1*07 and epitope P4 interaction analysis and the epitope LGLNKIVRMYSPTSI binds in the groove of the HLA-DRB1*07 allele

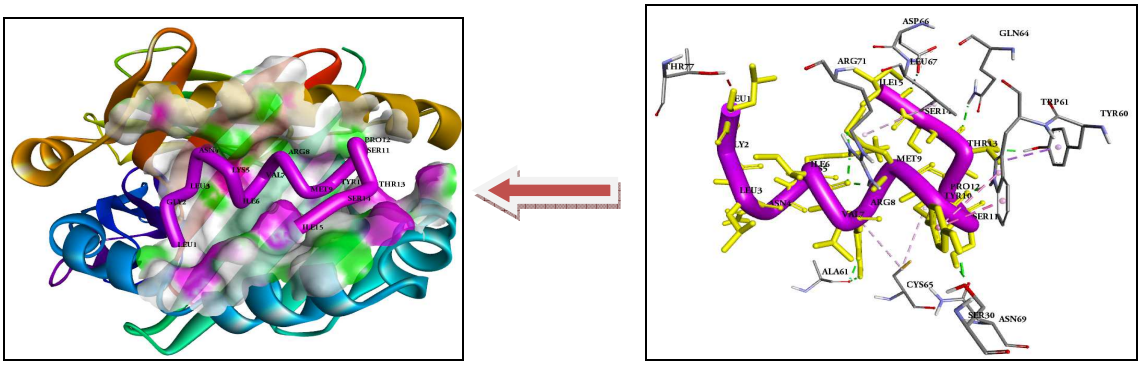


Figure 6. HLA-DRB1*07 and epitope P5 interaction analysis and the epitope KIVRMYSPTSILDI binds in the groove of the HLA-DRB1*07 allele.

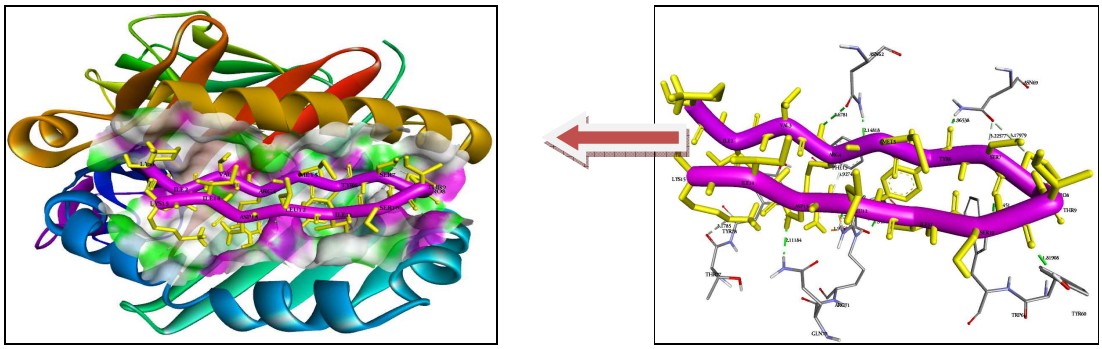
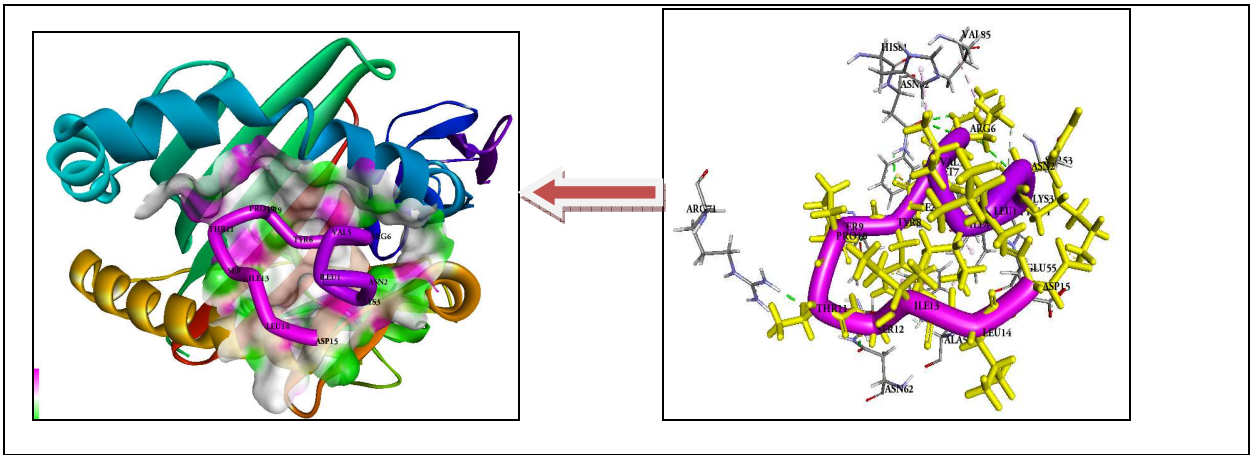


Figure 7. HLA-DRB1*07 and epitope P6 interaction analysis and the epitope LNKIVRMYSPTSILD binds in the groove of the HLA-DRB1*07 allele.



CONCLUSION

Current studies based on Immunoinformatics approach includes both epitope prediction and their affinity analysis towards DRB1*07 using docking studies provides the structural insight of Gag CTL epitopes namely P1(GLNKIVRMYSPTSIL), P2(LGLNKIVRMYSPTSI), P3(LNKIVRMYSPTSILD), P4(NKIVRMYSPTSILDI), P5(IVRMYSPTSILDIKQ), P6(KIVRMYSP TSILDIK).These CD_4+ Gag based epitopes can be considered as vaccine candidates for HIV infection due to their stable molecular interaction namely conventional hydrogen bonding and salt bridges with the HLA ,thus would aid both humoral and cell-mediated immunity.Gag epitope anchor residues showed greater affinity and interaction with the binding pocket residues of HLA-DRB1*07 a more highly distributed allele among southern population in India. Invitro and in vivo studies are needed to evaluate their efficiency as vaccine candidate to construct an ideal HIV vaccine for Indian population.

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