INNOVARE JOURNAL OF LIFE SCIENCE

Vol 8, Issue 3, 2020

ISSN - 2321-550X

Research Article

IN SILICO IDENTIFICATION AND CHARACTERIZATION OF MUTUAL EPITOPE-BASED SUBUNIT VACCINE AGAINST EBOLA VIRUS AND MARBURG VIRUS

SAMEER SHARMA*, SUPRIYA JADHAV, POOJA YADAV

Department of Biotechnology, Dronacharya Government College, Gurugram, Haryana, India. Email: Sameer21.97@gmail.com

Received: 21 February 2020, Revised and Accepted: 25 March 2020

ABSTRACT

Objective: The objective of the study was to analyze the mutual epitope-based vaccine that can evoke immune response against hemorrhagic fever caused by Ebola virus (EBOV) and Marburg virus (MARV).

Methodology: VP40, VP24, VP35, VP30, and NP proteins of Ebola and MARV were recovered from the protein database and subjected to many bioinformatics tools to predict the best B- and T-cell epitopes. And finally, the selected proteins were subjected to molecular docking human leukocyte antigen (HLA)-DR (major histocompatibility complex [MHC] Class I and II) to confirm their antigenicity *in silico.*

Results: The epitopes from EBOV were stable while were unstable from MARV. Further, molecular docking simulation using most significant MHC Class II and Class I molecules demonstrated that their epitopes may bind within HLA-binding affinity to evoke an immune response.

Conclusions: In this study, the data revealed the epitopes from VP40 protein could be the specific target for peptide-based vaccine design against Ebola and MARV.

Keywords: Epitopes, Ebola virus, Marburg virus, Molecular Docking.

INTRODUCTION

The first human outbreak of Ebola virus (EBOV) was in 1976, however, the natural reservoir of this virus remains unknown. Ebola hemorrhagic fever is generated by a negative strand of RNA virus. The viral genome encodes 7 structural proteins and 1 non-structural protein (dissolved glycoprotein) [1]. Morphologically, the virus is made up of a limited genome completely surrounded in an envelope, which is coated by the membrane glycoprotein coordinated in homotrimers [2]. Genetic and antigenic characterization of EBOV isolates during human outbreaks has caused the identification of four subtypes *Ebola Sudan, E. Zaire, E. Ivory Coast,* and *E. Reston*. In assessment to Ebola Reston, which originates in Asia and has no other way been stated to originate the human sickness, the alternative three subtypes distributed on the African subcontinent and are pathogenic for people, originating a particular fevered drained disease [3-5]. After an incubation period of approximately a week, subjects unexpectedly develop high fever, diarrhea, vomiting, respiration issues, and hemorrhaging. Death arises within a few days. The reported death case rates are approximately 80% with *E. Zaire* and 50% with *E. Sudan* [6,7], resulting approximately 1850 people and brought about almost 1300 deaths [8-10]. EBOV hemorrhagic fever is a zoonotic disorder that spreads by direct contact with infected alive or dead animals. The natural reservoir of both Ebola and Marburg virus (MARV) is unknown. A study done in 1968 though confirmed that MARV could persist for more than 3 weeks in *Aedes* mosquitoes after spatial inoculation [11-15]. Since the first recorded human outbreak in 1976, several laboratory and field studies have been conducted to identify the animal(s) – vertebrate or invertebrate – that can harbor the virus asymptomatically. EBOV and MARV belong to the family *Filoviridae* and cause a severe fever with high case fatality rates [16-18]. The nucleocapsid of EBOV is made up of the nucleoprotein NP, which directly encapsulates the viral genome [19]. EBOV nucleocapsid attaches with the viral protein VP24 and the polymerase cofactor VP35, to form a long helical nucleocapsid ~1000 nm in length and 50 nm in diameter [20-22]. In addition, the nucleocapsid can also associate with viral polymerase L and the transcription factor VP30 [23]. Nucleocapsid formation takes place in inclusion bodies within the perinuclear domain of EBOV- and MARV-infected cells, which operated as virus factories [24,25]. The enrollment of nucleocapsid in cell periphery and for developing progeny virions is the filoviral matrix protein VP40 [20,26-28] which is arranged in lower place of plasma membrane in an ordered lattice. This study aims to check the common epitope of EBOV and MARV for the development of subunit vaccines to treat the virus induced zoonosis in affected humans.

METHODOLOGY

Prediction of antigenicity

All the entire amino acid sequences of every vp40, vp24, vp30, vp35, and NP proteins of EBOV and MARV have been retrieved from protein databases (http://www.us.expasy.org/sprot; http://www.ncbi.nlm.nih.gov/protein/) and non-identical sequences had been analyzed with VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) antigen prediction server [29]. For optimum efficiency, a threshold value of 0.5 was accustomed to test the antigenicity of every full length protein. Amino acid sequences for each proteins that have antigenic score >0.5 had been chosen and less than 0.5 score proteins also selected for further studies [30]. All expected B-cell epitopes (16-mer) having a BCPreds cutoff score >0.8 were selected and afterward checked for membrane topology by comparing with Transmembrane Helices Hidden Markov Models (TMHMM) results for exo-membrane amino acid sequences. Surface defined as a B-cell epitope sequence having the cutoff value >0.8 for BCPreds was then analyzed using VaxiJen at threshold 0.5 to test the antigenicity. Finally, 2–3 epitopes with the highest VaxiJen scores were selected to use in prediction of T-cell epitopes.

Prediction of T-cell epitopes from selected B-cell epitopes

T-cell epitopes were predicted from the chosen B-cell epitopes and two screening steps were followed. Within the screening, the selection criteria were: (i) The T-cell epitope sequence should bind to both the major histocompatibility complex (MHC) I and MHC II molecules and therefore the least number of total interacting MHC molecules should be >15, (ii) the T-cell epitope sequence must interact with human leukocyte antigen (HLA)-DRB1×0101 of MHC-II, and (iii) T-cell epitope sequence should be antigenic supported on VaxiJen

score. Propred-1 (http://www.imtech.res.in/raghava/propred1/) [31] and Propred (http://www.imtech.res.in/raghava/propred/) [32] servers that utilize amino acid position coefficients inferred from literature employing linear prediction model [33] were want to identify common epitopes that bind to both MHC I and MHC II molecules. Total numbers of interacting MHC alleles were counted. For quantitative structure–activity relationship (QSAR) simulation approach, the half maximal (50%) inhibitory concentration (IC_{50}) and antigenicity of common epitopes (predicted by Propred-1 and Propred) were calculated using MHCPred v.2 (http://www.ddg-pharmfac.net/mhcpred/MHCPred/) server [34]. and VaxiJen v2.0, respectively. Epitopes with high antigenicity were selected and bound with predicted 15 molecules of both MHC I and II allels. The second screening was supported on structure and QSAR simulation methods using T-Epitope Designer (http://www.bioinformation.net/ted/) [35] and MHCPred, respectively. T-epitope designer can screen peptides for >1000 HLA alleles. Within the second screening, the criteria were as follows: The peptide should bind >75% of total HLA molecules; the peptide must bind with high scores to (i) HLA-DRB1×0101. T-epitope Designer was used for the first two criteria and MHCPred was used for the ultimate criteria. The ultimate list of T-cell epitopes was made with peptide sequences that pass these above-mentioned criteria and VaxiJen and IC_{50} scores. Physicochemical properties of identified B- and T-cell epitopes (15-mer) were analyzed with the ProtParam computer program (http://web.expasy.org/protparam/).

Molecular docking of predicted epitopes with HLA-DR

The predicted epitopes of B and T cells subjected for molecular docking with MHC I molecules (HLA-B7 and HLA-B44) and MHC Class-II molecules (HLA-DRB1×0101 and HLA-DRA) [34]. Multiple sequence alignment was used for HLA super types B7, B44. (https://www.ebi.ac.uk/Tools/msa/clustalw2/). SWISS-MODEL was used for 3D structure of these seven proteins (http://www.swissmodel.expasy.org) [35,36]. Moreover, similarity among these molecules, HLA molecules were identified by multiple sequence alignment and the best molecular docking orientation was chosen based on binding free energy and hydrogen bond.

RESULTS AND DISCUSSION

Antigenicity and topology of selected proteins

Ebola and MARV have 65–70% amino acid homology in the untranslated regions [20]. The homology of vp40, vp24, vp30, vp35, and NP is approximately above 80% for all proteins between Ebola and MARV [37]. Because of high percentage of homology present between these two virus, the present study focuses on identification of common epitope for Ebola and MARV. Moreover, the antigenicity was observed based on autocross covariance transformation of protein sequence using VaxiJen. Moreover, identification of exo-membrane sequence selected for this present study exhibits various degrees of antigenicity. According to VaxiJen score, the highest VaxiJen score showing the highest antigenicity of an amino acid sequence of vp40, vp24, vp30, vp35, and NP of Ebola was 0.4831, 0.4779, 0.5129, 0.5079, and 0.4379 and for MARV was 0.4454, 0.5348,0.5408, 0.4380, and 0.4639. Although Ebola virus and Marburg virus scored less than 0.5 which leads to non-antigenic in nature using VaxiJen. Analysis of transmembrane topology analysis of these proteins was done employing TMHMM and the results revealed that the lengths of exo-membrane sequences for selected proteins of EBOV were 1–326, 1–251, 1–288, 1–340, and 1–739 and for MARV 1–303, 1–253, 1–277, 1–329, and 1–629.

Antigenic B-cell epitope

To make a proper vaccine, a peptide must be producing B-cell and T-cell-mediated immunity as well as peptide must be hydrophilic in nature [38]. BCPreds were used to analyze for B-cell epitope prediction using BCPreds and all predicted B-cell epitopes were selected on the based as already mentioned in methodology. In general, BCPreds containing 16-mer epitopes [30] and VaxiJen minimal values more than 0.8 and 0.5 accordingly were listed. After the BCPreds and VaxiJen minimal values, single epitope was selected from each protein for further analysis and each listed B-cell epitope from a protein exposed 100% similarity with all amino acid sequences of that protein.

T-cell epitope selected from B-cell epitope

T-cell epitope selection is based on B-cell epitopes. In the initial screening, MHCPred was used for identified 9-mer epitopes which

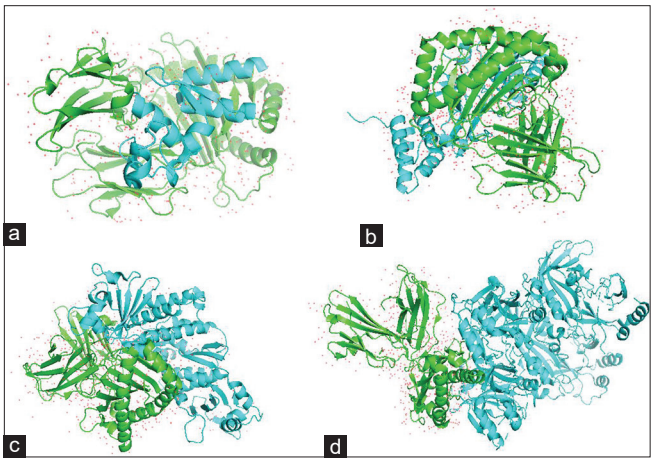


Fig. 1: (a-d) Molecular docking simulation of major histocompatibility complex Class1 molecules with selected proteins

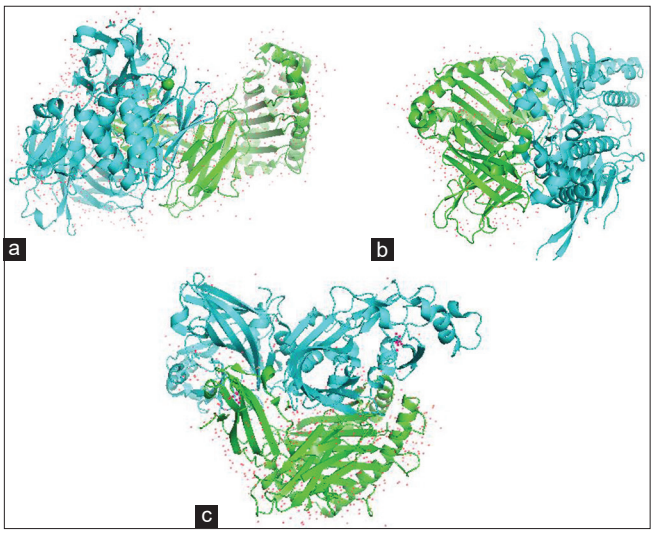


Fig 2: (a-c) Molecular docking simulation with major histocompatibility complex Class II molecules with selected proteins

Table 1: Accession numbers, VaxiJen score and exo-membrane sequences of VP40, VP24, VP30, VP35, NP of Ebola virus and Marburg virus

Protein	Accession number	VaxiJen score	Exo-membrane sequence
Ebola VP40	AKB09553.1	0.4831	1–326
Ebola VP24	AKB09558.1	0.4779	1–251
Ebola VP30	AKB09553.1	0.5129	1–288
Ebola VP35	AKB09552.1	0.5079	1–340
Ebola NP	AKB09551.1	0.4379	1–739
Marburg VP40	CAA78116.1	0.4454	1–303
Marburg VP24	CAA78119.1	0.5348	1–253
Marburg VP30	CAA78118.1	0.5408	1–277
MarburgVP35	CAA78115.1	0.4380	1–329
Marburg NP	CAA78114.1	0.4639	1–692

Table 2: B-cell epitopes from full length VP40, VP24, VP30, VP35, NP

Protein	BCPred epitope sequence	BCPred score	VaxiJen score	Exo-membrane sequence
Ebola VP40	PNKSGKKGNSADLTSP	1.567	0.4831	1–326
Ebola VP24	MRTQRVKEQLSLKMLS	1.286	0.4779	1–251
Ebola VP30	SSRENYRGEYRQSRSA	1.756	0.5129	1–288
Ebola VP35	SQMQQTKPNPKTRNSQ	1.194	0.5079	1–340
Ebola NP	LDDQEKKILMNFHQKK	1.354	0.4379	1–739
Marburg VP40	GVIRSMSKEKENYAMA	1.287	0.4454	1–303
Marburg VP24	GMTLLHHLKSNFVVPE	1.787	0.5348	1–253
Marburg VP30	ENYAMQQPRGRSRTRN	1.998	0.5408	1–277
MarburgVP35	GANPLEKLYKRRKPKG	1.355	0.4380	1–329
Marburg NP	HSLLELGTKPTAPHVR	1.457	0.4639	1–692

Table 3: T-cell epitopes predicted from selected B-cell epitopes

Protein	Predicted T-cell epitope	VaxiJen score	IC50 value of T-cell epitopes for DRBI×0101 (MHCPred)
Ebola VP40	SGKKGNSAD	1.567	70.15
Ebola VP24	QRVKEQLSL	1.286	90.23
Ebola VP30	ENYRGEYRQ	1.756	65.14
Ebola VP35	QQTKPNPKT	1.194	53.13
Ebola NP	QEKKILMNF	1.354	35.12
Marburg VP40	SMSKEKENY	1.287	51.02
Marburg VP24	LHHLKSNFV	1.787	48.24
Marburg VP30	YAMQQPRGR	1.998	74.25
MarburgVP35	PLEKLYKRR	1.355	65.24
Marburg NP	LELGTKPTA	1.457	50.05

Table 4: Molecular docking score with selected proteins

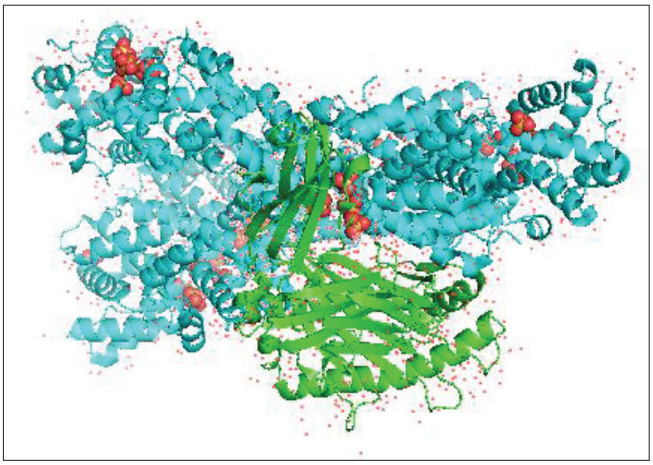
Protein	Docking score
Ebola VP40	20,008
Ebola VP24	17,776
Ebola VP30	16,222
Ebola VP35	15,050
Ebola NP	14,502
Marburg VP40	20,382
Marburg VP24	19,444
Marburg VP30	16,228
MarburgVP35	18,125
Marburg NP	15,234

were all antigenic with best VaxiJen score. In the second screening level, selected peptides from the first screening were used to analyze and predict their binding capabilities to more than 1000 MHC alleles with the help of T-epitope designer and epitopes that bound more than 75% MHC alleles were listed. For mostly used A×0101 alleles, the minimal value was set accordingly that listed peptides must bind to these HLA molecules. The final list of epitopes was made with peptide sequences that confirmed the criteria of the second screening level.

Molecular docking of predicted epitopes

Normally, molecular docking was used to identify the correlation between the epitopes with the MHC Class II molecules. Molecular docking results revealed that the HLA-DRA does not have collection in the peptide-binding sector and serves as the solely alpha chain for HLA-DRB1 to HLA-DRB5. Molecular docking study revealed that epitope side chain attached into the grooves of HLA-DRsB1×0101 and HLA-DRB1×1501 through respective hydrogen bonds.

Docking outcomes also showed predicted epitope might attach with MHC II molecules in antigen presenting cells and also could trigger the B- and T-cell epitopes. Epitope binding within the groove of the MHCII molecules is recommended as director enticement of binding affinity [34,39]. The analysis observed that the selected proteins and listed B- and T-cell epitopes bound with the groove of HLA-B7 and HLA-B44. Moreover, multiple alignment result of MHC II molecules and HLA-DRA exposed that these molecules have difference in particular sequence as well as alignment of super types B7 and B44 showed the dissimilarity of their sequence.



CONCLUSIONS

In the present study, we aimed to design an epitope-based common vaccine for both Ebola and MARV and lead to investigate the genome proteins which are vp40, vp24, vp30, vp35, and NP proteins. In this study, VP40 has the best binding affinity in both Ebola and MARV that lead to design peptide vaccine against hemorrhagic fever. The prediction of non-identical sequence or antigenicity of sequence from selected membrane proteins were retrieved through TMHMM and VaxiJen score methods. For B-cell epitope prediction, both BCPreds and AAP server methods were used, which were lastly used for T-cell epitopes. In this study, we analyzed 9-mer epitope from EBOV and also from MARV. Moreover, we investigated the similarities between both the epitopes. From the selected epitopes, we subjected to molecular docking simulation and the docking result with both the MHC molecules was appropriate with binding affinity.

These selected epitopes were observed to be stable and might accurate for an epitope to be used as a common vaccine and also reflect the common cure and therapeutic treatment for EBOV and MARV.

REFERENCES

1. Sanchez A, Kiley MP, Holloway BP, Auperin DD. Sequence analysis of the Ebola virus genome: Organization, genetic elements, and comparison with the genome of Marburg virus. Virus Res 1993;29:215-40.
2. Volchkov VE. Processing of the Ebola virus glycoprotein. In: Klenk HD, editor. Current Topics in Microbiology and Immunology: Marburg and Ebola Viruses. Vol. 235. Berlin: Springer-Verlag; 1999. p. 35-47.
3. Feldmann H, Klenk HD, Sanchez A. Molecular biology and evolution of filoviruses. Arch Virol Suppl 1993;7:81-100.
4. Rollin PE, Williams RJ, Bressler DS, Pearson S, Cottingham M, Pucak G, *et al*. Ebola (subtype Reston) virus among quarantined

nonhuman primates recently imported from the Philippines to the United States. J Infect Dis 1999;179:S108-14.
5. Hayes CG, Burans JP, Ksiazek TG, Del Rosario RA, Miranda ME, Manaloto CR, *et al*. Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. Am J Trop Med Hyg 1992;46:664-71.
6. Bwaka MA, Bonnet MJ, Calain P, Colebunders R, De Roo A, Guimard Y, *et al*. Ebola hemorrhagic fever in kiwit, Democratic Republic of the Congo: Clinical observations in 103 patients. J Infect Dis 1999;179:S1-7.
7. Nkoghé D, Formenty P, Nnégué S, Mvé MT, Hypolite I, Léonard P, *et al*. Recommandations pratiques pour la prise en charge sur le terrain des patients infectés par le virus Ebola. Med Trop 2004;64:199-204.
8. Smith DI. Ebola haemorrhagic fever in Sudan. 1976. Bull World Health Organ 1978;56:247-70.
9. Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in Southern Sudan: Hospital dissemination and intrafamilial spread. Bull World Health Organ 1983;61:997-1003.
10. Martini GA. Marburg agent disease in man. Trans R Soc Trop Med Hyg 1969;63:295-302.
11. Smith CE, Simpson DI, Bowen ET. Fatal human disease from vervet monkeys. Lancet 1967;2:1119-21.
12. Smith DH, Isaacson M, Johnson KM, Bagshawe A, Johnson BK, Swanapoel R, *et al*. Marburg-virus disease in Kenya. Lancet 1982;1:816-20.
13. Turell MJ, Bressler DS, Rossi CA. Lack of virus replication in arthropods after intrathoracic inoculation of Ebola Reston virus. Am J Trop Med Hyg 1996;55:89-90.
14. Swanepoel R, Leman PA, Burt FJ. Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis 1996;2:321-5.
15. Kunz C, Hofmann H, Aspöck H. Propagation of Marburg-virus (vervet monkey disease agent) in *Aedes* aegypti. Zentralbl Bakteriol Orig 1968;208:347-9.
16. Bowen ET, Platt GS, Lloyd G, Raymond RT, Simpson DI. A comparative study of strains of Ebola virus isolated from Southern Sudan and Northern Zaire in 1976. J Med Virol 1980;6:129-38.
17. Jahrling PB, Geisbert TW, Jaax NK, Hanes MA, Ksiazek TG, Peters CJ. Experimental infection of cynomolgus macaques with Ebola-Reston filoviruses from the 1989-1990 US epizootic. Arch Virol 1996;11:115-34.
18. WHO. Ebola Situation Report; 2016. Available from: http://www.who.int/csr/disease/ebola/en. [Last accessed on 2017 Jul 01].
19. Muhlberger E, Weik M, Volchkov VE, Klenk HD, Becker S. Comparison of the transcription and replication strategies of Marburg virus and Ebola virus by using artificial replication systems. J Virol 1999;73:2333-42.
20. Bharat TA. Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. Proc Natl Acad Sci USA 2012;109:4275-80.
21. Huang Y, Xu L, Sun Y, Nabel GJ. The assembly of Ebola virus nucleocapsid virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein. Mol Cell 2002;10:307-16.
22. Wan W. Structure and assembly of the Ebola virus nucleocapsid. Nature 2017;551:394-7.
23. Hartlieb B, Modrof J, Muhlberger E, Klenk HD, Becker S. Oligomerization of Ebola virus VP30 is essential for viral transcription and can be inhibited by a synthetic peptide. J Biol Chem 2003;278:41830-6.
24. Nanbo A, Watanabe S, Halfmann P, Kawaoka Y. The spatio-temporal distribution dynamics of Ebola virus proteins and RNA in infected cells. Sci Rep 2013;3:1206.
25. Dolnik O, Stevermann L, Kolesnikova L, Becker S. Marburg virus inclusions: A virus-induced microcompartment and interface to multivesicular bodies and the late endosomal compartment. Eur J Cell Biol 2015;94:323-31.
26. Noda T, Watanabe S, Sagara H, Kawaoka Y. Mapping of the VP40-binding regions of the nucleoprotein of Ebola virus. J Virol 2007;81:3554-62.
27. Becker S, Klenk HD, Mühlberger E. Intracellular transport and processing of the Marburg virus surface protein in vertebrate and insect cells. Virology 1996;225:145-55.
28. Booth TF, Rabb MJ, Beniac DR. How do filovirus filaments bend without breaking? Trends Microbiol 2013;21:583-93.
29. Doytchinova IA, Flower DR. VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinform 2007;8:4.
30. Prabhavathy K, Perumal P, Baalaji NS. *In silico* identification of B-and T-cell epitopes on OMPLA and LsrC from *Salmonella* typhi for peptide-based subunit vaccine design. Indian J Biotechnol 2011;10:440-51.
31. Singh H, Raghava G. ProPred1: Prediction of promiscuous MHC Class-I binding sites. Bioinformatics 2003;19:1009-14.
32. Singh H, Raghava G. ProPred: Prediction of HLA-DR bindingsites. Bioinformatics 2001;17:1236-7.
33. Sturniolo T, Bono E, Ding J, Raddrizzani L, Tuereci O, Sahin U, *et al*. Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices. Nat Biotechnol 1999;17:555-61.
34. Guan P, Doytchinova IA, Zygouri C, Flower DR. MHCPred: A server for quantitative prediction of peptide-MHC binding. Nucleic Acids Res 2003;31:3621-4.
35. Kangueane P, Sakharkar MK. T-Epitope designer: A HLA-peptide binding prediction server. Bioinformation 2005;1:21.
36. Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: An automated protein homology-modeling server. Nucleic Acids Res 2003;31:3381-5.
37. Wang LF, Harcourt BH, Yu M, Tamin A, Rota PA, Bellini WJ, *et al*. Molecular biology of hendra and nipah viruses. Microbes Infect 2001;3:279-87.
38. Krogh A, Larsson B, Von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. J Mol Biol 2001;305:567-80.
39. Patronov A, Dimitrov I, Flower DR, Doytchinova I. Peptide binding prediction for the human class II MHC allele HLA-DP2: A molecular docking approach. BMC Struct Biol 2011;11:32.