INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide. HPV is the causative agent of cervical cancer and the highest infection rate was reported among young women aged between 15-19 y [1, 2, 47]. The greatest burden of HPV infection occurs in developing countries due to the lack of organized screening programs [3, 4, 48]. In India, it has been reported that 130,000 cases and 70-75,000 death occurred annually, suggesting that the cervical cancer is one of the major cancers in India [5]. Based on the carcinogenicity, HPV can be divided into two groups: high-risk types such as HPV 16 and 18 and low-risk types such as HPV type 6 and 11 [6]. More than 70% of cervical cancer is caused by both HPV type 16 and 18. Currently, two vaccines (Cervarix and Gardasil) are commercially administrated to prevent HPV infection by both HPV type 16 and 18. Currently, two vaccines (Cervarix and Gardasil) are commercially administrated to prevent HPV infection.

The efficacy of these peptides in inducing a stronger immune response needs to be tested using in vitro and in vivo assays. The identified epitopes could be used in designing a novel epitope vaccine for HPV.

Keywords: Epitope prediction, CTL epitopes, Human papilloma virus, BIMAS, SYFPEITHI, RANKPEP.

MATERIALS AND METHODS

Source data

In the present study, the sensitivity and the specificity of the algorithms were evaluated by known binders and non-binding peptides. A set of 311 known binders were obtained from the HIV epitope database of Los Alamos National Laboratory and immune epitope database (IEDB). Totally 222 non-binding peptides were derived from MHC class I and II. The complete set of HPV type 16 and 18 proteins (Early proteins E1, E2, E5, E6 and E7; Late proteins L1 and L2) were retrieved from the human papillomavirus type 16 and 18 proteomes database.

Tools used for prediction of HPV 16 and 18 CTL epitopes

The complete set of HPV type 16 and 18 proteomes were analyzed for the MHC class I HLA A_0201 binding peptides using three matrix based prediction algorithms namely BIMAS (http://www.bimas.cit.nih.gov/molbio/hla_bind/), SYFPEITHI (http://www.syfpeithi.de/) and RANKPEP (http://imed.med.ucm.es/Tools/rankpep.html). All individual protein sequences of HPV serotypes 16 and 18 were parsed into the algorithms, and the binding efficiencies of the nine amino acid peptides were calculated.

Calculation of sensitivity and specificity of the algorithms

For the calculation of sensitivity and specificity, each binding and non-binding peptide was analyzed using the three matrix based prediction algorithms (SYFPEITHI, BIMAS, and RANKPEP) and the results were compared. The cut-off score for binding of these peptides to the HLA A_0201 was fixed as 20, ≥ 50 and ≥ 60 for
SYFPEITHI, BIMAS and RANKPEP respectively. A peptide scoring less than this was considered as a non-binder.

The sensitivity of the computational algorithms [13, 34] was calculated using the formula:

\[
\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}
\]

The specificity of the computational algorithms [32, 44] was calculated using the formula:

\[
\text{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}}
\]

Overlapping epitope prediction

Instead of using a single prediction tools for MHC-peptide binding prediction, using a combination of prediction tools could improve the efficiency of epitope prediction. A peptide predicted as an epitope in more than one tool was considered to be an overlapping epitope. The binders predicted in all three prediction tools were further manually compared with one another for the prediction of overlapping epitopes.

Identification of consensus epitopes

A peptide which is present in more than one genotype was considered to be a consensus epitope. Based on the occurrence, all predicted binders of HPV 16 and 18 were compared with one another for prediction of consensus epitopes. The level of conservation (single amino acid variation) in predicted epitopes was also assessed among the HPV 16 and 18 genotypes.

Molecular docking

Molecular docking studies were carried out using AutoDock4.2. The crystal structure of human HLA-A2 (PDB ID: 4NO3) was downloaded from the Protein Data Bank. A known CTL epitope from influenza virus, GILGFVFTL, was taken as a reference peptide. Two predicted binders from this study, QLFVTVVDT (QLF) and KLPQLCTEL (KLP) along with the reference peptide were docked against HLA-A2.

RESULTS

Sensitivity and specificity of the algorithm

When the known binders for HLA A_0201 were analyzed, BIMAS could predict only 176 out of 311 with a sensitivity of 57.56%. The sensitivity of SYFPEITHI and RANKPEP was calculated as 77.49% and 67.52% respectively. The combination of more than one algorithm improved the sensitivity; SYFPEITHI and BIMAS when combined together could predict 252 of the 311 peptides with a sensitivity of 81.02%; However, combining all the three programs increased the sensitivity from 57.56% to 81.99% (255 out of 311) (fig. 1).

Table 1: Overview of epitope prediction analysis in HPV 16 and 18 proteomes

| S. No. | Protein | Total number of amino acids | Number of peptides analyzed |
|--------|---------|-----------------------------|----------------------------|
|        |         | HPV 16                      | HPV 18                      |
|        |         | 649                         | 657                         |
|        |         | 365                         | 365                         |
|        |         | 95                          | 88                          |
|        |         | 83                          | 73                          |
|        |         | 158                         | 158                         |
|        |         | 98                          | 105                         |
|        |         | 531                         | 568                         |
|        |         | 473                         | 462                         |

Based on the cut-off criteria, each of the non-binders was tested using all three algorithms and based on the results the specificity was calculated based on the formula described in method’s section. When 222 non-binders were analyzed, the specificities of BIMAS, SYFPEITHI and RANKPEP were 93.69%, 77.03% and 74.78% respectively. The specificity were improved when a combination of two or more algorithms were used (fig. 2).

Fig. 1: Sensitivity of the selected algorithms in the prediction of CTL epitopes. The sensitivity of individual algorithms (SYFPEITHI, BIMAS and RANKPEP) and the combination were analyzed. Sensitivity increased (81.99%) when all the three algorithms were combined with a minimal error rate.
Fig. 2: Specificity of the selected algorithms in the prediction of CTL epitopes. Specificity of individual algorithms (SYFPEITHI, BIMAS and RANKPEP) and the combination was calculated using known non-binders. Improved specificity was observed when two or more of the algorithms were used in combination.

HPV 16 and 18 epitope mapping

The proteomes of HPV type 16 and 18 serotypes were analyzed for the prediction of CTL epitopes using all the three algorithms. In HPV 16, a total of 2388 peptides were analyzed, and 249 of them were predicted as binders by all three algorithms together (fig. 3D). SYFPEITHI alone could predict 115 peptides as binders (fig. 3A), whereas 45 and 89 binders were predicted by BIMAS and RANKPEP respectively (fig. 3B and 3C).

When 2412 peptides were analyzed in HPV 18 proteome, all the three algorithms together predicted 215 peptides as binders (fig. 4D). In which, 102, 44 and 69 binders were predicted by SYFPEITHI (fig. 4A), BIMAS (fig. 4B) and RANKPEP (fig. 4C) respectively.

Fig. 3: Prediction of CTL epitopes for HPV 16 serotype. H. PV 16 serotype proteins were analyzed by SYFPEITHI, BIMAS and RANKPEP. A. Analysis of the proteome of HPV 16 by SYFPEITHI. B. Analysis of the proteome of HPV 16 by BIMAS analysis C. Analysis of the proteome of HPV 16 by RANKPEP D. Prediction of peptides as binders in HPV 16 proteome using SYFPEITHI, BIMAS and RANKPEP algorithms based on the fixed criteria.
Fig. 4: Prediction of CTL epitopes for HPV 18 serotype. HPV 18 serotype proteins were analysed by SYFPEITHI, BIMAS and RANKPEP. A. Analysis of the proteome of HPV 18 by SYFPEITHI. B. Analysis of the proteome of HPV 18 by BIMAS analysis. C. Analysis of the proteome of HPV 18 by RANKPEP. D. Prediction of peptides as binders in HPV 18 proteome using SYFPEITHI, BIMAS and RANKPEP algorithms based on the fixed criteria.

Overlapping epitope prediction

Though 249 peptides were found to be predicted as binders by the three matrix-based algorithms viz. BIMAS, SYFPEITHI and RANKPEP, only 25 of them were considered as overlapping peptides in HPV 16 proteome as predicted by all three prediction tools. The highest number of overlapping peptides were predicted in E1 and L1 proteins (table 2). Likewise, 20 overlapping binders were predicted in HPV 18 analysis and L1 protein showed the highest number of overlapping peptides (table 3).

Table 2: Predicted CTL epitopes in HPV 16 proteome

| Protein | Accession No. | Peptide Sequence | SYFPEITHI score | BIMAS score | RANKPEP score |
|---------|---------------|------------------|----------------|-------------|---------------|
| E1      | P03114        | KLLSKLLEV        | 29             | 207.606     | 93            |
|         |               | YLVSPLSDI        | 25             | 110.379     | 89            |
|         |               | LLIQVCLYL        | 24             | 199.738     | 81            |
|         |               | CLLYHQLISL       | 27             | 157.227     | 72            |
|         |               | AMKAFKREL        | 24             | 108.462     | 69            |
|         |               | FLALKRPL         | 21             | 108.094     | 65            |
| E2      | P03120        | TLQDVSSLVEV      | 24             | 285.163     | 97            |
|         |               | TLYTAVSST        | 21             | 54.847      | 80            |
| E5      | P06927        | VLLCVCLLI        | 22             | 65.622      | 78            |
|         |               | IILVLLLWI        | 26             | 114.142     | 75            |
|         |               | FLLEFCVIL        | 26             | 130.1635    | 68            |
| E6      | P03126        | KLIQQLCTEL       | 24             | 74.768      | 68            |
| E7      | P03129        | LLMGLTGIV        | 29             | 53.631      | 92            |
|         |               | TLHEYMLDL        | 24             | 201.447     | 86            |
|         |               | RLQVSTHV         | 20             | 69.552      | 75            |
| L1      | P03101        | TLQANKSEV        | 22             | 69.552      | 81            |
|         |               | LLVPKVSGL        | 30             | 83.527      | 75            |
|         |               | YLLRREQMFV       | 22             | 133.735     | 73            |
|         |               | GLOQYRQFR        | 22             | 139.174     | 70            |
|         |               | QLPTTVTVD        | 21             | 63.417      | 62            |
| L2      | P03107        | SLVEETSFI        | 24             | 235.26      | 96            |
|         |               | YLHPSYVML        | 25             | 147.401     | 76            |
|         |               | AILDINNTV        | 26             | 145.077     | 77            |
|         |               | ILQYGSGLV        | 24             | 118.238     | 64            |
Table 3: Predicted CTL epitopes in HPV 18 proteome

| Protein | Accession no. | Peptide sequence | SYFPEITHI score | BIMAS score | RANKPEP score |
|---------|--------------|-----------------|-----------------|-------------|---------------|
| E1      | P06789       | ILYAHIQCL       | 27              | 267.286     | 75            |
|         |              | FLGALKSFL       | 22              | 540.469     | 69            |
| E2      | P06790       | TLSERLSCV       | 26              | 655.875     | 88            |
| E4      | P06791       | RLLHDLDTV       | 28              | 290.025     | 70            |
| E5      | P06792       | VLVFFYIVV       | 20              | 72.717      | 82            |
|         |              | MLLHHA II       | 26              | 150.931     | 80            |
|         |              | LLLHHAII        | 26              | 55.091      | 67            |
|         |              | WVLVPNYYV       | 22              | 37.117      | 64            |
| E6      | P06463       | KLPDLCTEL       | 25              | 306.55      | 70            |
| E7      | P06788       | TLQDVIVHL       | 26              | 201.447     | 94            |
| L1      | P06794       | SLVDTYRFV       | 23              | 470.519     | 90            |
|         |              | CILTFRVLIL      | 26              | 64.463      | 86            |
|         |              | TLQDTKCEV       | 23              | 285.163     | 80            |
|         |              | ILFEVNNV        | 25              | 437.462     | 71            |
|         |              | YVILFRLNV       | 27              | 76.897      | 68            |
|         |              | VILHLYHLL       | 24              | 54.747      | 64            |
|         |              | QLFVTVVDT       | 21              | 63.417      | 62            |
|         |              | RLYWACAGV       | 23              | 69.552      | 62            |
| L2      | P06793       | TLIEDSSSVV      | 24              | 11.6917     | 88            |
|         |              | YLWPLYFYI       | 25              | 328.176     | 69            |

Table 4: Consensus epitopes predicted in HPV 16 and 18

| HPV protein | Amino acid position/Peptide Sequence |
|-------------|-------------------------------------|
| HPV 16, L1  | RLYWACGV                           |
| HPV 18, L1  | RLYWACGV                           |
| HPV 16, E6  | KLPQLTCTEL                         |
| HPV 18, E6  | KLPQLTCTEL                         |

Letters in BOLD indicates single amino acid variation in HPV 16 and 18.

Identification of consensus peptide

A total of 45 overlapping peptides were predicted in this study, among five peptides were considered as consensus peptides (table 4); 100% sequence similarity was found in L1 peptide-QLFVTVVDT354-662 and four other peptides exhibited a single amino acid variation (HPV 16 E6 peptide- KLPQLTCTEL12-21 and L1 peptide-RLYWACGV123-131; HPV 18 E6 peptide-KLPQLTCTEL12-21 and L1 peptide-RLYWACGV158-66).

Molecular docking

The reference peptide binds with HLA-A2 with a binding energy of -2.37 kcal/mol and the interaction is mediated through two hydrogen bonds. The peptides, QLF and KLP, bind with HLA-A2 with the binding energies of -3.57 kcal/mol and -3.55 kcal/mol respectively, indicating that these two predicted peptides bind efficiently than the reference peptide.

The interaction of QLF with HLA-A2 is through five hydrogen bonds (fig. 5), whereas the interaction of the reference peptide is only by two hydrogen bonds; this confirms that the binding of QLF is stronger than that of the reference peptide. The interacting residues are presented in table 5. The binding poses of the QLF peptide along with the reference peptide is shown in fig. 6. This indicates that the QLF peptide binds at the same binding site (peptide binding groove) where the reference peptide binds.

Fig. 5: Interactions of QLF with HLA-A2
DISCUSSION

Modern immunoinformatics tools provide the new platform for designing peptide vaccines against pathogenic microorganisms [33]. Though many tools are available for predicting immunogenic CTL epitopes, the accuracy of any of these tools is not appreciative. Hence with a concept that a combination of two or more tools could solve the problem [45]; this study was undertaken with three well-known matrix based algorithms. The specificity and sensitivity of the algorithms were evaluated using a known set of binders and non-binders, and the results indicated that combination of algorithms increased the specificity without affecting the sensitivity of the tested tools.

Based on this approach, a total of 249 (10.42%) binders were predicted out of 2388 peptides in HPV 16. Similarly, 215 (8.91%) binders were predicted out of 2412 peptides analyzed in HPV 18. Among the predicted epitopes, 45 were promiscuous overlapping peptides that were predicted by all three algorithms. Some of the peptides predicted in the study were already reported as CTL epitopes. HPV 16 peptides E1-LLQQYCLYL254-262 [34], E5-FLLCFCVLL15-23, VLLCVCLLI21-29 [35], E6-KLPQLCTEL18-26 [36, 37] and E7-TLHEYMLDL7-15 [38] are known CTL epitopes. E6-KLPQLCTEL13-22 [41] and E7-TLQDIVLHL 7-15 were proved to be CTL epitopes for HPV 18.

One of the predicted peptides, QLFVTVVDVT \[^{154-162}\] from L1 protein is conserved in both HPV 16 and 18 genotypes. Peptide KLPQLCTEL \[^{18-26}\] from E6 has a single amino acid variation in the fourth position; the variation glutamine (HPV 16) instead of aspartic acid (HPV 18) has already been reported [8]. Similarly, alanine (HPV 16) instead of valine (HPV 18) was observed in L1-RLVWACAGV \[^{158-166}\] at the 7th position. The results were further confirmed using docking studies of the peptide with the MHC.

CONCLUSION

The results of the present study revealed the use of computational algorithms in the prediction of CTL epitopes based on the binding to MHC Class I MHC molecules. Combination of more than one tool increases the chance to predict potent CTL epitopes against viral diseases. Using this approach few epitopes were predicted for HPV 16 and 18. Further confirmation of the efficacy of these epitopes in inducing a stronger immune response needs to be done based on in vitro and in vivo assays

ACKNOWLEDGEMENT

The work was supported by a grant from Science and Engineering Research Board, New Delhi (SR/50/HS-0248/2012) to Krishnan Sundar. Manikandan Mohan thank Indian Council of Medical Research, New Delhi for a Senior Research Fellowship (45/18/2011-IMM-BMS). The authors thanks, Mrs. J. Christina Rosy for her help in docking studies.

AUTHORS CONTRIBUTION

All the in silico analyses and written part of the manuscript was carried out by the first author Mr. Manikandan Mohan. The study was conceived, designed, correction and communications of the manuscript were done by the corresponding author Prof. Krishnan Sundar.

CONFLICT OF INTERESTS

The authors declared that they have no conflict of interests

REFERENCES

1. Garland SM, Smith JS. Human papilloma virus vaccines. Drugs 2010;70:1079-98.
2. Gatto M, Agmon-Levin N, Soriano A, Manna R, Maoz-Segal R, Rivty S, et al. Human papilloma virus vaccine and systemic lupus erythematosus. Clin Rheumatol 2013;32:1301-7.
3. Soliman PT, Slomovitz BM, Wolf JK. Mechanism of cervical cancer. Drug Discovery Today: Dis Mech 2004;1:253-8.

4. Schiffman M, Castle PE. Human papilloma virus: epidemiology and public health. Arch Pathol Lab Med 2003;127:930-4.

5. Agarwal SM, Raghav D, Singh H, Raghava GPS. CDDB: a curated database of sequences involved in cervix cancer. Nucleic Acids Res 2011;39:975-9.

6. Glahder JA, Hansen CN, Vinther J, Madsen BS, Norrild B. A promoter within the E6 ORF of human papilloma virus type 16 contributes to the expression of the E6 oncoprotein from a monocistronic mRNA. J Gen Virol 2003;84:3429-41.

7. Vu LT, Bui DT. Prevalence of cervical infection with HPV type 16 and 18 in Vietnam: implications for vaccine campaign. BMC Cancer 2013;13:1-7.

8. Nirmala S, Sudandiradoss C. Prediction of promiscuous epitopes in the E6 protein of three high risk human papilloma viruses: a computational approach. Asian Pac J Cancer Prev 2013;14:4167-75.

9. Yao Y, Huang W, Yang X, Sun W, Liu X, Cun W, et al. HPV-16 E6 and E7 protein T cell epitopes prediction analysis based on distributions of HLA-A loci across populations: an in silico approach. Vaccine 2013;31:2289.

10. Suzich JA, Ghiu SJ, Palmer-Hill PJ, White WI, Tamura JK, Bell JA, et al. Systemic immunization with papilloma virus L1 protein completely prevents the development of viral mucosal papillomas. Proc Natl Acad Sci USA 1995;92:11553-7.

11. Van der Burg SH, de Jong A, Welthers MJ, Offringa R, Melief CJ. The status of HPV16-specific T-cell reactivity in health and disease as a guide to HPV vaccine development. Virus Res 2002;89:275-84.

12. Sette A, Newman M, Livingston B, McKinney D, Sidky J, Ishioka O, et al. Optimizing vaccine design for cellular processing, MHC binding and TCR recognition. HLA 2002;59:443-51.

13. Irini AD, Guan P, Flower DR. Epjlen: a server for multistep T cell epitope prediction. BMC Bioinformatics 2006;7:131.

14. Hudson AW, Ploegh HL. The cell biology of antigenic peptides. Annu Rev Immunol 2005;23:229-55.

15. Srinivasan KN, Zhang G, Khan AM, August JT, Brusic V. Prediction of class I T-cell epitopes: evidence of presence of immunogenic hot spots inside antigens. Bioinformatics 2004;20:297-302.

16. Van Kaer L. Major histocompatibility complex class restricted antigen processing and presentation. Tissue Antigens 2002;60:1-9.

17. Larsen MV, Lundegaard C, Lambricht K, Buus S, Brunak S, Lund O, et al. An immunoproteome approach to CTL epitope prediction: a combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions. Eur J Immunol 2005;35:2295-303.

18. Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu Rev Immunol 1999;17:51-88.

19. Riedl P, Reimann J, Schirmeck R. Complexes of DNA viruses with cationic, antigenic peptides are potent, polymvalent CD8 (+) T-cell-stimulating immunogens. Meth Mol Biol 2006;127:159-69.

20. Brusic V, Bajic VB, Petrovsky N. Computational methods for prediction of T-cell epitopes-a framework for modelling, testing, and applications. Methods 2004;34:436-43.

21. De Groot AS, Moise L. Prediction of immunogenecity for therapeutic proteins: state of the art. Curr Opin Drug Discovery Dev 2007;10:332-40.

22. Rammecke H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S. SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 1999;50:213-9.

23. Yu K, Petrovsly N, Schonbach C, Koh JL, Brusic V. Methods for prediction of peptide binding to MHC molecules: a comparative study. Mol Med 2002;8:137-48.

24. Brusic V, Bajic VB, Honyman MC, Hammer J, Harrison LC. Prediction of MHC class-II binding peptides using an evolutionary algorithm and artificial neural network. Bioinformatics 1998;14:121-30.

25. Mimatisuka H. Predicting peptides that bind to MHC molecules using supervised learning of hidden Markov models. Proteins Struct Funct Genet 1998;33:460-74.

26. Lim JS, Kim S, Lee HG. Selection of peptides that bind to the HLA A2.1 molecule by molecular modeling. Mol Immunol 1996;33:221-30.

27. Rogan D, Lauenomler SL, Holm A, Buss S, Tschinke V. Predicting binding affinities of protein ligands from three dimensional models: an application to peptide binding to class I major histocompatibility proteins. J Med Chem 1999;42:4650-8.

28. Parker KC, Rednarek MA, Colgan JE. Scheme for ranking potential HLA A2 binding peptides based on independent binding to individual peptide side chains. J Immunol 1994;152:163-75.

29. Reche PA, Glutting JP, Reinherz EL. Prediction of MHC class I binding peptides using phiie motifs. Hum Immunol 2002;63:701-8.

30. Donnes P, Blobsson A. Prediction of MHC class I binding peptides, using SVMHIC. BMCBioinformatics 2002;3:25-30.

31. Guan P, Doytchino IA, Zygouri C, Flower DR. MHC pred: a server for quantitative prediction of peptide-MHC binding. Nucleic Acids Res 2003;31:3621-4.

32. Antonets DV, Maksyutov AZ. TEpredict: software for T cell epitope prediction. Mol Biol 2010;44:130-43.

33. Cohen T, Moise I, Martin W, Dopson S. Immunoinformatics: the next step in vaccine design. In Infectious Disease Informatics, Springer New York; 2010. p. 223-44.

34. Blundl C, Afgejerstam V, Yuan F, Stubber G, Dihler J. Identification of a cytotoxic T-lymphocyte epitope in the human papilloma virus type 16 E6 protein. J Gen Virol 1997;78:26-15.

35. Liu DW, Yang YC, Lu HF, Liu MF, Cheng YW, Chu CC. Cytotoxic T-lymphocyte responses to human papilloma virus type 16 E5 and E7 proteins and HLA-A*0201-restricted T-cell peptides in cervical cancer patients. J Virol 2007;81:2869-79.

36. Zehbe I, Kaufmann AM, Schmidt M, Holm H, Maeruer M. Human papilloma virus 16 E6-specific CD45RA+CCR7+high avidity CD8+ T cells fail to control tumours expressing HPV-16-transformed interferon-[gamma] production in patients with cervical cancer. J Immunother 2007;30:523-32.

37. Matjevic M, Hedley ML, Urban RG, Chicz RM, Lajoie C, Luby TM. Immunization with a poly (lactide co-glycolide) encapsulated plasmid DNA expressing antigenic regions of HPV 16 and 18 results in an increase in the precursor frequency of T cells that respond to epitopes from HPV 16, 18, 6 and 11. Cell Immunol 2011;270:62-9.

38. Riemer AB, Resikin DB, Zhang G, Handley M, Anderson KS, Brusic V, et al. A conserved E7-derived cytotoxic T lymphocyte epitope expressed on human papilloma virus 16-transformed HLA-A2+epithelial cancers. J Biol Chem 2010;285:29608-22.

39. Korets-Smith E, MacDonald L, Daftarian PM, Mansour M, Pohajdak B, Fuentes-Ortega A, Korets-Smith E, MacDonald L, et al. Selection of peptides that bind to the HLA-A2.1 transgenic rabbit model to study immunity to human papilloma virus type 16 E6 and E7 proteins. J Trans Med 2013;11:320.

40. Rudolf MP, Man S, Melief CJ, Sette A, Kast WM. Human T-cell responses to epitopes from HPV 16, 18, 6 and 11 in healthy donors. Virus Res 1998;54:23-9.

41. Liu Z, Hof J, Han J, Liu R. A computational model for predicting transmembrane regions of retroviruses. J Bioinf Comput Biol 2017;15:17500-10.

42. Boesen A, Sundar K, Coico R. Lassa fever virus peptides predicted by computational analysis indicate epitope-specific cytotoxic T-lymphocyte responses in HLA-A2.1 transgenic mice. Clin Diag Immunol 2005;12:1223-30.

181

Mohan et al. Int J Pharm Pharm Sci, Vol 9, Issue 11, 175-182
46. Kirti, Pranav Kumar P. Human papilloma virus associated cervical cancer: a review. Asian J Pharm Clin Res 2016; 9:14-7.
47. Chozhavel Rajanathan TM, Lakshmikanth G, Agastian P. Evaluating the efficacy of aluminum phosphate formulated l2 based human papilloma virus vaccine. J Pharm Clin Res 2015;8:199-201.
48. Borappa M, Kanakarajan S, Kamalanathan A. In silico docking of quercetin compound against the hela cell line proteins. Int J Curr Pharm Res 2015;7:13-6.