Padavu et al. | Article 8109
J Pure Appl Microbiol. 2023;17(1):554-566. doi: 10.22207/JPAM.17.1.53
Received: 17 September 2022 | Accepted: 17 January 2023
Published Online: 03 March 2023

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

In-silico Analysis of Human Papillomavirus – 45 E6, E7 & L1 Proteins as Potential Immunogens

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Abstract

Globally, cervical cancer is the fourth most common cancer among women. After being cloned from a recurring cervical lesion in 1987, Human papillomavirus (HPV) type-45 was identified as a high-risk HPV type. It is the third most common cancer-causing HPV subtype, after HPV-16 and HPV-18. Immunogenic epitopes and structural features provide the most useful information for vaccine development. Computational algorithms provide quick, simple, trustworthy, and cost-efficient methods for predicting immunogenic epitopes. In this study, both B and T cell epitopes have been identified as potential immunogens that can elicit a response from the host system. Three potential B-cell epitopes, i.e., SIAGQYRGQCNTCCDQ, LQEIVLHLEPQNELDP, and DSTVYLPPPSVARVVS, were identified in this study. A potential epitope for E6 (ATLERTEVY) was predicted to 8 MHC-I alleles (HLA-A*30:02, HLA-B*15:01, HLA-A*01:01, HLA-A*26:01, HLA-A*32:01, HLA-B*35:01, HLA-B*58:01, HLA-A*11:01) and for L1 epitope (NVFPIFLQM) was predicted for 4 MHC-I alleles (HLA-A*30:02, HLA-A*32:01, HLA-B*53:01, HLA-B*51:01). To conclude, the epitopes identified here might potentially be useful for developing a cervical cancer vaccine against HPV-45 strains, but *in vitro* and *in vivo* trials are needed to validate their safety and efficacy.

Keywords: Human Papillomavirus, Cervical Cancer, Immunogenic Epitopes, Vaccine, *In silico*

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Citation: Padavu S, Kumar BK, Kumar A, Rai P. *In-silico* Analysis of Human Papillomavirus – 45 E6, E7 & L1 Proteins as Potential Immunogens. *J Pure Appl Microbiol.* 2023;17(1):554-566. doi: 10.22207/JPAM.17.1.53

INTRODUCTION

Globally, cervical cancer ranks as the fourth most frequently occurring malignancy among women population.[1] Over 500,000 women worldwide are diagnosed with cervical cancer each year, with low-income nations bearing the burden of mortality.[2] Nearly all cervical malignancies contain oncogenic human papillomavirus (HPV) DNA. With the highest universally attributable percentage ever reported for a particular etiology of a major human malignancy, researchers concluded that HPV is an essential element in the development of cervical cancer.[3] A working committee of the International Agency for Research on Cancer (IARC) Monographs categorized 14 types of HPV as "carcinogenic to humans" out of 200 different types. While the majority of HPV infections are asymptomatic and are eventually removed by our immune system, the virus can remain in some situations, thus leading to cancer.[4]

A persistent cervical lesion seen in a woman in the United States led to the discovery of the high-risk (HR) HPV type HPV-45 in 1987. HPV-45 is more frequent in adenocarcinoma of the cervix. After HPV-16 and HPV-18, HPV-45 has been ranked as the third most oncogenic type, which accounts for around 10% of cervical cancer cases.[4] The cellular structure of this virus is made up of 8,000 bp of circular double-stranded DNA that contains early regions (E1, E2, E4, E5, E6, E7, and E8) encoding early viral proteins, late regions (L1 and L2) that codes for the capsid proteins, and a non-coding region known as the long control region (LCR), which plays a key role in replication and transcription.[3,5]

The oncoproteins of genes E6 and E7 are identified as the key causes of HPV-associated cervical cancer; elevated expression of E6 and E7 is necessary for the onset and maintenance of the malignant phenotype.[6] p53 and pRb (retinoblastoma) tumour suppressors are inactivated when E6 and E7 genes are expressed, respectively.[7] These oncoproteins are tumour-specific antigens, and hence there is no risk of autoimmunity. They are expressed in all the phases of cervical cancer, making them ideal targets for prophylactic vaccination.[8] The icosahedral capsid structure is formed by the major capsid protein L1. Charged residues (K and R) are concentrated near the C-terminus, and there is often >60% L1 amino acid sequence homology between HPV variants that infect the genital epithelia. It indicates that the majority of the L1 protein is conserved among different types of HPV.[9,10] The protein can self-assemble into an icosahedral capsid by forming 72 pentameric capsomers. Because of its icosahedral form, L1 protein is equally distributed on the surface of the capsid, making it highly immunogenic.[11] This protein is capable of forming virus-like particles (VLPs) by self-assembling spontaneously. VLPs that have been assembled are thought to be potent immunogens that B-cells can recognise quickly.[12]

The comprehensive cervical cancer control strategy involves HPV vaccination as primary prevention, screening and treating precancerous lesions as secondary prevention. The Food and Drug Administration (FDA) has approved the use of three forms of prophylactic vaccines: Cervarix® (bivalent), Gardasil® (quadrivalent), and Gardasil®9 (nonavalent). These vaccines are efficient in protecting against HPV infection and neoplasms. However, they are prophylactic vaccines that offer no therapeutic benefit and have limited benefits in eradicating pre-existing infections. As a result, therapeutic vaccinations are gaining popularity due to their capacity to trigger cell-mediated immune responses and destroy infected cells rather than neutralising antibodies (nAbs).[13] All of the aforementioned studies suggest that E6, E7, and L1 are key proteins that can be used as a potential vaccine candidate against HPV-45. Using several bioinformatics tools and programmes, we attempted to examine the E6, E7, and L1 proteins of HPV-45 as a potential vaccine candidate in this work.

MATERIALS AND METHODS

Amino acid sequence

E6, E7 and L1 amino acid sequences of HPV-45 having GenBank accession numbers CAA52573.1, CAA52574.1 and CAA52578.1 (Genome ID: X74479), respectively, were retrieved from the NCBI databank.

Sequence analysis

Protparam (http://web.expasy.org/

protparam/) is an online web tool used for the analysis of the different physical and chemical characteristics of E6, E7 and L1 protein sequences of HPV-45, including molecular mass, amino acid composition, and atomic composition.[14]

Prediction of secondary structure

PSIPRED is an internet server (http://bioinf.cs.ucl.ac.uk/psipred) that incorporates two feed-forward neural networks that analyse PSI-BLAST (Position-Specific Iterated-BLAST) output to predict concise and precise secondary structure of E6, E7 and L1 proteins of HPV-45.[15]

T-cell epitope prediction

Immune Epitope Database (IEDB) is a resource (http://www.iedb.org/) funded by the National Institute of Allergy and Infectious Diseases (NIAID), a division of the National Institutes of Health. This tool was used for the prediction of T-cell epitopes for E6, E7 and L1 protein sequences. The IEDB recommended 2020.09 (NetMHCpan El 4.1) prediction method was used to predict the epitopes for MHC-I alleles, while the IEDB recommended 2.22 prediction method was used for MHC-II alleles. The reference set of HLA alleles were selected for predicting both MHC-I and MHC-II binding in various human populations.[16] The antigenicity of the predicted epitopes for MHC-I alleles were calculated using Vaxigen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html).[17] IFNepitope web server (http://crdd.osdd.net/raghava/ifnepitope/) was used to investigate the ability of epitopes for MHC-II alleles to stimulate interferon-gamma (IFN-γ) production. The parameters for this study were set as IFN-γ versus non-IFN-γ model and Motif and SVM hybrid algorithms.[18]

B-cell epitope prediction

The antigenic epitope within the oncogenic proteins (E6 and E7) and major capsid (L1) protein molecule of HPV-45 is predicted using ABCpred server (https://webs.iiitd.edu.in/raghava/abcpred/ABC_submission.html), a standard bioinformatics technique. All the parameters were in their default settings, but the epitopes selected had a score of more than 0.7. The ABCpred web server uses an artificial neural network to predict B-cell epitopes. This

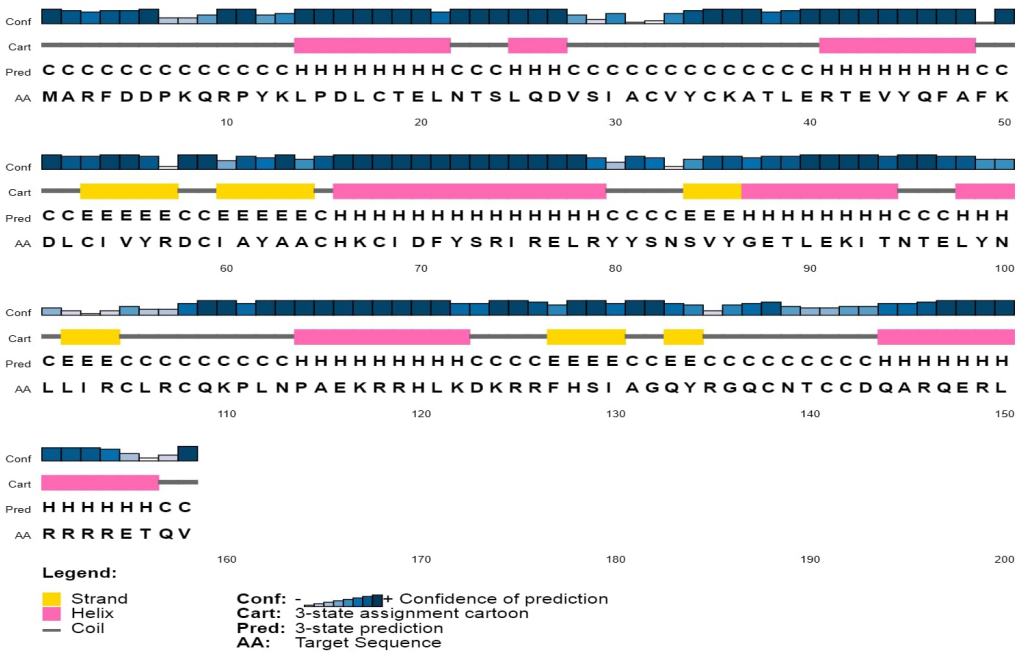

Figure 1. Secondary structure prediction of HPV-45 E6 protein through PSIPRED

Table 1. Predicted MHC-I epitopes for HPV-45 E6, E7 and L1 proteins through IEDB

No.	Start	End	Length	Peptide	Allele	Percentile Rank	Immunogenicity Score	Antigenicity
					E6			
1.	37	45	9	ATLERTEVY	HLA-A*30:02, HLA-B*15:01, HLA-A*01:01, HLA-A*26:01, HLA-A*32:01, HLA-B*35:01, HLA-B*58:01, HLA-A*11:01	0.06-0.46	0.29271	0.6093 (Probable ANTIGEN)
2.	90	98	9	LEKITNTEL	HLA-B*40:01	0.08	0.18689	0.4303 (Probable ANTIGEN)
3.	99	107	9	YNLLIRCLR	HLA-A*33:01	0.34	0.11158	0.5910 (Probable ANTIGEN)
4.	70	78	9	DFYSRIREL	HLA-B*08:01	0.05	0.11019	0.5919 (Probable ANTIGEN)
5.	122	130	9	KDKRRFHSI	HLA-B*08:01	0.43	0.07332	0.9519 (Probable ANTIGEN)
6.	44	52	9	VYQFAFKDL	HLA-A*24:02, HLA-A*23:01	0.2-0.27	0.05946	0.9584 (Probable ANTIGEN)
7.	13	21	9	KLPDLCTEL	HLA-A*02:01, HLA-A*02:03, HLA-A*02:06	0.1-0.14	0.04843	0.9489 (Probable ANTIGEN)
8.	48	56	9	AFKDLCIVY	HLA-A*30:02	0.15	0.02721	1.8184 (Probable ANTIGEN)
9.	113	121	9	NPAEKRRHL	HLA-B*08:01, HLA-B*07:02	0.03-0.04	0.01475	0.9487 (Probable ANTIGEN)
					E7			
10.	19	27	9	NELDPVDLL	HLA-B*40:01, HLA-B*44:03, HLA-B*44:02	0.05-0.26	0.05902	1.7631 (Probable ANTIGEN)
					L1			
11.	428	436	9	ILENWNFGV	HLA-A*30:02	0.25	0.3542	1.1761 (Probable ANTIGEN)
12.	473	481	9	KLKFWTVDL	HLA-A*32:01	0.29	0.34784	0.5705 (Probable ANTIGEN)
13.	17	25	9	NVNVFPIFL	HLA-A*30:02	0.17	0.32372	0.5018 (Probable ANTIGEN)
14.	426	434	9	SSILENWNF	HLA-A*30:02, HLA-A*32:01	0.19-0.4	0.30661	0.7650 (Probable ANTIGEN)
15.	409	417	9	CTITLTAEV	HLA-A*30:02	0.08	0.19952	0.6661 (Probable ANTIGEN)
16.	19	27	9	NVFPIFLQM	HLA-A*30:02, HLA-A*32:01, HLA-B*53:01, HLA-B*51:01	0.04-0.48	0.1896	0.8028 (Probable ANTIGEN)
17.	157	165	9	ESAHAATAV	HLA-A*30:02	0.03	0.1758	0.5028 (Probable ANTIGEN)
18.	359	367	9	FVTVVDTTR	HLA-A*30:02-0.42	0.19-0.42	0.17066	0.9193 (Probable ANTIGEN)
19.	271	279	9	DSMFFCLRR	HLA-A*30:02	0.06-0.35	0.14493	0.8574 (Probable ANTIGEN)
20.	70	78	9	TVGNPYFRV	HLA-A*30:02, HLA-A*02:06	0.13-0.5	0.11925	0.8608 (Probable ANTIGEN)
21.	499	507	9	LVQAGLRRR	HLA-A*30:02	0.29	0.09825	1.1916 (Probable ANTIGEN)
22.	69	77	9	LTVGNPYFR	HLA-A*68:01, HLA-A*30:02, HLA-A*33:01	0.05-0.21	0.09604	1.2652 (Probable ANTIGEN)

Table 1. Cont...

No.	Start	End	Length	Peptide	Allele	Percentile Rank	Immunogenicity Score	Antigenicity
23.	67	75	9	RLLTVGNPY	HLA-A*30:02, HLA-A*32:01	0.11-0.24	0.09562	0.5385 (Probable ANTIGEN)
24.	10	18	9	GIIIFLKNV	HLA-A*02:03, HLA-A*02:06	0.45-0.5	0.0949	0.6342 (Probable ANTIGEN)
25.	274	282	9	FFCLRREQL	HLA-B*08:01	0.22	0.08728	1.8410 (Probable ANTIGEN)
26.	398	406	9	EEYDLQFIF	HLA-B*44:03, HLA-A*30:02	0.01-0.14	0.07784	1.7384 (Probable ANTIGEN)
27.	396	404	9	HVEEYDLQF	HLA-B*35:01, HLA-B*53:01, HLA-A*26:01, HLA-A*01:01	0.24-0.47	0.07349	1.4499 (Probable ANTIGEN)
28.	132	140	9	MEIGRGQPL	HLA-A*30:02, HLA-B*44:03, HLA-B*44:02	0.07-0.36	0.05536	0.9861 (Probable ANTIGEN)
29.	441	449	9	TTSLVDTYR	HLA-A*68:01, HLA-A*33:01, HLA-A*31:01	0.02-0.41	0.02682	0.4094 (Probable ANTIGEN)
30.	452	460	9	QSVAVTCQK	HLA-A*30:02	0.26-0.32	0.01633	1.3682 (Probable ANTIGEN)

server is the first to use fixed-length patterns with a recurrent neural network (machine-based approach). This server can anticipate continuous (linear) B-cell epitopes. A linear B-cell epitope is a short peptide that binds to a conformational epitope and cross-reacts with an antibody. This server has a 65.93% accuracy rate in predicting epitopes.[19,20]

RESULTS

Protein structure analysis

The E6, E7 and L1 proteins of HPV-45 contain 158, 106 and 539 amino acid residues and have a molecular weight of 18.89 kDa, 12.05 kDa and 60.31 kDa, respectively. ProtParam server was used to estimate the amino acid composition of all three proteins (Supplementary Table 1). The most common amino acids in E6 was arginine (R) (20 residues), followed by leucine (L) (15 residues). The secondary structure prediction revealed that 44.3% of the protein is coil (C), 41.8% is helix (H), and 13.9% is strand (E) (Figure 1). The most common amino acids in E7 protein was found to be leucine (L) (15 residues), followed by glutamic acid (E) (14 residues). The secondary structure prediction revealed that 65.1% of the protein is coil (C), 21.7% is helix (H), and 13.2% is strand (E) (Figure 2). The most common amino acids in L1 protein was proline (P), serine (S) and threonine (T) (42 residues), followed by valine (V) and leucine (L) (40 residues). The secondary structure prediction revealed that 63.5% of the protein is coil (C), 17.8% is helix (H), and 18.7% is strand (E). This was found to be the same in both the variant and reference sequences (Figure 3).

Prediction of epitopes for MHC-I alleles

IEDB server was used to predict epitopes for MHC-I alleles. It is crucial to understand the MHC-I and -II alleles that are highly expressed for the development of an efficient immunological response. The HLA allele reference set from the database and most frequently occurring MHC-I alleles were chosen for MHC-I binding. The immunogenicity score (<0.4) and percentile value (<0.5) were used to evaluate the possible epitopes (Table 1) for binding MHC-I alleles. HLA-A*01:01, HLA-A*02:01, HLA-A*02:03, HLA-A*02:06, HLA-B*07:02, HLA-B*15:01,

Table 2. Predicted MHC-II epitopes for HPV-45 E6, E7 and L1 proteins through IEDB

No.	Peptide	Length	Start	End	Allele	Percentile Rank	IFN-γ Score
					E6		
1.	SRIRELRYYSNSVYG	15	73	87	HLA-DRB1*15:01	0.49	1.000
2.	YSRIRELRYYSNSVY	15	72	86	HLA-DRB1*15:01	0.49	1.000
3.	IRELRYYSNSVYGET	15	75	89	HLA-DRB1*15:01	0.54	1.000
4.	RIRELRYYSNSVYGE	15	74	88	HLA-DRB1*15:01	0.54	1.000
5.	RELRYYSNSVYGETL	15	76	90	HLA-DRB1*15:01	1.3	1.000
6.	FHSIAGQYRGQCNTC	15	127	141	HLA-DRB5*01:01	1.6	0.151
7.	SNSVYGETLEKITNT	15	82	96	HLA-DPA1*03:01/DPB1*04:02	1.7	0.474
8.	YSNSVYGETLEKITN	15	81	95	HLA-DPA1*03:01/DPB1*04:02	1.7	0.146
9.	YYSNSVYGETLEKIT	15	80	94	HLA-DPA1*03:01/DPB1*04:02	1.7	0.489
		15			HLA-DPA1*02:01/DPB1*01:01	2.2	0.489
10.	RTEVYQFAFKDLCIV	15	41	55	HLA-DQA1*01:01/DQB1*05:01	1.8	0.541
		15			HLA-DPA1*01:03/DPB1*04:01	2.1	0.541
11.	TEVYQFAFKDLCIVY	15	42	56	HLA-DQA1*01:01/DQB1*05:01	1.8	0.660
		15			HLA-DPA1*01:03/DPB1*04:01	2.5	0.660
12.	ELRYYSNSVYGETLE	15	77	91	HLA-DRB1*15:01	2.0	1.000
13.	RYYSNSVYGETLEKI	15	79	93	HLA-DPA1*02:01/DPB1*01:01	2.1	0.476
14.	ERTEVYQFAFKDLCI	15	40	54	HLA-DQA1*01:01/DQB1*05:01	2.5	0.205
					E7		
15.	RTLQQLFLSTLSFVC	15	85	99	HLA-DPA1*01:03/DPB1*04:01	0.53	0.204
		15			HLA-DPA1*02:01/DPB1*01:01	1.5	0.204
		15			HLA-DPA1*01:03/DPB1*02:01	2.0	0.204
		15			HLA-DPA1*03:01/DPB1*04:02	2.3	0.204
16.	LRTLQQLFLSTLSFV	15	84	98	HLA-DPA1*01:03/DPB1*04:01	0.61	0.264
		15			HLA-DPA1*02:01/DPB1*01:01	1.2	0.264
		15			HLA-DPA1*01:03/DPB1*02:01	1.9	0.264
		15			HLA-DPA1*03:01/DPB1*04:02	2.2	0.264
17.	DLRTLQQLFLSTLSF	15	83	97	HLA-DPA1*01:03/DPB1*04:01	0.99	0.037
18.	QQLFLSTLSFVCPWC	15	88	102	HLA-DPA1*01:03/DPB1*04:01	1.2	0.045
		15			HLA-DPA1*01:03/DPB1*02:01	2.2	0.045
19.	EDLRTLQQLFLSTLS	15	82	96	HLA-DPA1*01:03/DPB1*04:01	1.7	0.179
		15			HLA-DPA1*02:01/DPB1*01:01	2.0	0.179
		15			HLA-DPA1*03:01/DPB1*04:02	2.3	0.179
20.	AEDLRTLQQLFLSTL	15	81	95	HLA-DPA1*01:03/DPB1*04:01	1.8	0.214
		15			HLA-DPA1*02:01/DPB1*01:01	2.0	0.214
		15			HLA-DPA1*03:01/DPB1*04:02	2.3	0.214
21.	QLFLSTLSFVCPWCA	15	89	103	HLA-DPA1*01:03/DPB1*04:01	2.2	0.088
					L1		
21.	AHNIIYGHGIIIFLK	15	2	16	HLA-DRB1*13:02	2	0.679
22.	AYQYRVFRVALPDPN	15	94	108	HLA-DPA1*02:01/DPB1*14:01	1.1	0.748
23.	DDTESAHAATAVITQ	15	154	168	HLA-DQA1*05:01/DQB1*03:01	1.5	0.66
24.	DTESAHAATAVITQD	15	155	169	HLA-DQA1*05:01/DQB1*03:01	1.5	0.843
		15			HLA-DQA1*01:02/DQB1*06:02	2.4	0.843
25.	ESAHAATAVITQDVR	15	157	171	HLA-DQA1*01:02/DQB1*06:02	2.3	1.052
26.	FLKNVNVFPIFLQMA	15	14	28	HLA-DPA1*01:03/DPB1*04:01	2.5	0.178
27.	FLVQAGLRRRPTIGP	15	498	512	HLA-DRB5*01:01	1.1	0.151
28.	GRKFLVQAGLRRRPT	15	495	509	HLA-DRB5*01:01	0.13	0.615
		15			HLA-DPA1*02:01/DPB1*14:01	0.64	0.615
29.	IFLKNVNVFPIFLQM	15	13	27	HLA-DRB3*02:02	1.3	0.137

Table 2. Cont...

No.	Peptide	Length	Start	End	Allele	Percentile Rank	IFN-γ Score
		15			HLA-DPA1*01:03/DPB1*04:01	2.3	0.137
		15			HLA-DRB1*13:02	2.3	0.137
30.	IFYHAGSSRLLTVGN	15	59	73	HLA-DRB1*09:01	0.09	0.181
		15			HLA-DRB1*07:01	0.75	0.181
		15			HLA-DRB1*01:01	1.2	0.181
		15			HLA-DRB3*02:02	2.2	0.181
31.	IIFLKNVNVFPIFLQ	15	12	26	HLA-DRB1*13:02	0.98	0.243
		15			HLA-DRB3*02:02	0.99	0.243
		15			HLA-DRB1*15:01	2.5	0.243
32.	KFLVQAGLRRRPTIG	15	497	511	HLA-DRB5*01:01	0.88	0.387
33.	KVSAYQYRVFRVALP	15	91	105	HLA-DPA1*01:03/DPB1*04:01	1.9	0.429
34.	LDDTESAHAATAVIT	15	153	167	HLA-DQA1*05:01/DQB1*03:01	2.5	0.58
35.	LGRKFLVQAGLRRRP	15	494	508	HLA-DRB5*01:01	0.13	0.386
		15			HLA-DPA1*02:01/DPB1*14:01	0.56	0.386
36.	MAHNIIYGHGIIIFL	15	1	15	HLA-DRB1*13:02	1.8	0.55
37.	MFFCLRREQLFARHF	15	273	287	HLA-DPA1*02:01/DPB1*05:01	2.4	1
38.	PKVSAYQYRVFRVAL	15	90	104	HLA-DPA1*01:03/DPB1*04:01	2.2	0.775
39.	PLGRKFLVQAGLRRR	15	493	507	HLA-DRB5*01:01	0.13	0.371
		15			HLA-DPA1*02:01/DPB1*14:01	0.66	0.371
40.	QYRVFRVALPDPNKF	15	96	110	HLA-DPA1*02:01/DPB1*14:01	0.6	0.797
41.	RHVEEYDLQFIFQLC	15	395	409	HLA-DQA1*01:01/DQB1*05:01	2	0.115
42.	RKFLVQAGLRRRPTI	15	496	510	HLA-DRB5*01:01	0.13	0.547
		15			HLA-DPA1*02:01/DPB1*14:01	1.2	0.547
43.	RTSIFYHAGSSRLLT	15	56	70	HLA-DRB1*09:01	0.07	0.062
		15			HLA-DRB1*07:01	0.28	0.062
		15			HLA-DRB1*01:01	0.67	0.062
		15			HLA-DRB3*02:02	1.1	0.062
		15			HLA-DRB5*01:01	2.2	0.062
44.	SAYQYRVFRVALPDP	15	93	107	HLA-DPA1*02:01/DPB1*14:01	1.7	0.441
45.	SMFFCLRREQLFARH	15	272	286	HLA-DPA1*02:01/DPB1*05:01	2.5	1
46.	TESAHAATAVITQDV	15	156	170	HLA-DQA1*05:01/DQB1*03:01	1.8	0.957
		15			HLA-DQA1*01:02/DQB1*06:02	2.1	0.957
47.	TSIFYHAGSSRLLTV	15	57	71	HLA-DRB1*09:01	0.07	0.063
		15			HLA-DRB1*07:01	0.28	0.063
		15			HLA-DRB1*01:01	0.51	0.063
		15			HLA-DRB3*02:02	1.1	0.063
		15			HLA-DRB5*01:01	2.1	0.063
48.	YPLGRKFLVQAGLRR	15	492	506	HLA-DRB5*01:01	0.13	0.368
		15			HLA-DPA1*02:01/DPB1*14:01	1.5	0.368
49.	YQYRVFRVALPDPNK	15	95	109	HLA-DPA1*02:01/DPB1*14:01	0.66	1.049
50.	YRVFRVALPDPNKFG	15	97	111	HLA-DPA1*02:01/DPB1*14:01	1.4	1.122

Table 3. Predicted potential B-cell epitopes in HPV-45 E6, E7 and L1 proteins by ABCPred

Rank	Sequence	Start position	Score
	E6		
1.	SIAGQYRGQCNTCCDQ	129	0.87
2.	YGETLEKITNTELYNL	86	0.85
2.	SRIRELRYYSNSVYGE	73	0.85
3.	HKCIDFYSRIRELRYY	66	0.83
4.	MARFDDPKQRPYKLPD	1	0.82
5.	CVYCKATLERTEVYQF	32	0.81
5.	PDLCTELNTSLQDVSI	15	0.81
6.	RRHLKDKRRFHSIAGQ	118	0.80
7.	YNLLIRCLRCQKPLNP	99	0.78
8.	QRPYKLPDLCTELNTS	9	0.77
9.	YQFAFKDLCIVYRDCI	45	0.74
10.	DCIAYAACHKCIDFYS	58	0.72
	E7		
1.	LQEIVLHLEPQNELDP	8	0.92
2.	ADGVSHAQLPARRAEP	42	0.89
3.	DGRIELTVESSAEDLR	70	0.88
4.	AEPQRHKILCVCCKCD	55	0.81
5.	ILCVCCKCDGRIELTV	62	0.78
5.	SESEEENDEADGVSHA	33	0.78
	L1		
1.	DSTVYLPPPSVARVVS	34	0.96
2.	VSAYQYRVFRVALPDP	92	0.92
2.	AVTCQKDTTPPEKQDP	455	0.92
2.	CQSICKYPDYLQMSAD	252	0.92
3.	KFLVQAGLRRRPTIGP	497	0.91
4.	RPTIGPRKRPAASTST	507	0.89
4.	PPEKQDPYDKLKFWTV	464	0.89
4.	DSTIYNPETQRLVWAC	114	0.89
5.	LGCVPAIGEHWAKGTL	186	0.88
5.	RVALPDPNKFGLPDST	101	0.88
6.	RHVEEYDLQFIFQLCT	395	0.87
6.	NGICWHNQLFVTVVDT	350	0.87
7.	FWTVDLKEKFSSDLDQ	476	0.86
7.	AHAATAVITQDVRDNV	159	0.86
7.	LGIGLSGHPFYNKLDD	140	0.86
7.	QRLVWACVGMEIGRGQ	123	0.86
8.	SIITSDSQLFNKPYWL	327	0.85
8.	GSCVYSPSPSGSIITS	316	0.85
8.	ANMRETPGSCVYSPSP	309	0.85
8	RHFWNRAGVMGDTVPT	285	0.85
8.	QMALWRPSDSTVYLPP	26	0.85
8.	EHWAKGTLCKPAQLQP	194	0.85
9.	TAEVMSYIHSMNSSIL	414	0.84
9.	PVPSTYDPTKFKQYSR	380	0.84
10.	GVMGDTVPTDLYIKGT	292	0.83
11.	LQPGDCPPLELKNTII	207	0.82
11.	QDVRDNVSVDYKQTQ	167	0.82
12.	YFRVVPNGAGNKQAVP	75	0.81
12.	YGHGIIIFLKNVNVFP	7	0.81
12.	TSTASTASRPAKRVRI	520	0.81
12.	TDLYIKGTSANMRETP	300	0.81
12.	KNTIIEDGDMVDTGYG	218	0.81
13.	TSLVDTYRFVQSVAVT	442	0.80
14.	HSMNSSILENWNFGVP	422	0.79
14.	DYLQMSADPYGDSMFF	260	0.79
14.	GDMVDTGYGAMDFSTL	225	0.79
14.	GMEIGRGQPLGIGLSG	131	0.79
15.	LCTITLTAEVMSYIHS	408	0.78
15.	TLCASTQNPVPSTYDP	372	0.78
15.	SVDYKQTQLCILGCVP	175	0.78
15.	PFYNKLDDTESAHAAT	148	0.78
16.	SRTSIFYHAGSSRLLT	55	0.77
17.	AGNKQAVPKVSAYQYR	83	0.76
17.	SSDLDQYPLGRKFLVQ	486	0.76
18.	TVVDTTRSTNLTLCAS	361	0.75
18.	LHKAQGHNNGICWHNQ	342	0.75
18.	STLQDTKCEVPLDICQ	238	0.75
19.	VARVVSTDDYVSRTSI	44	0.73
19.	YGDSMFFCLRREQLFA	269	0.73
20.	RFVQSVAVTCQKDTTP	449	0.72
21.	RREQLFARHFWNRAGV	278	0.71
21.	YGAMDFSTLQDTKCEV	232	0.71
22.	DPTKFKQYSRHVEEYD	386	0.74

HLA-A*30:02, HLA-A*31:01, HLA-A*33:01, HLA-B*35:01, HLA-B*40:01, HLA-B*44:03, HLA-B*57:01, HLA-B*58:01, HLA-A*68:01 are the highly expressed MHC-I alleles.

VaxiJen is the first server that allows antigen classification and predict protective antigens exclusively based on protein physicochemical properties rather than sequence alignment. Based on the high binding affinity score, the epitopes obtained from the IEDB server for MHC-I alleles were submitted to Vaxigen v2.0 for the prediction of probable antigens. The non-antigenic epitopes were removed and probable antigens were retained based on their antigenicity score.

Prediction of epitopes for MHC-II alleles

The epitopes for MHC-II alleles were predicted using IEDB server. The complete HLA reference set was chosen from the database for MHC-II binding. The potential epitopes (Table 2)

for binding MHC-II alleles were assessed based on the percentile rank (<2.5). MHC-II alleles HLA-DRB1*01:01, HLA-DRB5*01:01, HLA-DRB3*02:02, HLA-DRB1*07:01, HLA-DRB1*09:01, HLA-DRB1*13:02, HLA-DRB1*15:01, HLA-DPA1*02:01/DPB1*01:01,HLA-DPA1*01:03/DPB1*02:01,HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*01:01/DQB1*05:01, HLA-DPA1*01:03/DPB1*04:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*02:01/DPB1*14:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DPA1*02:01/DPB1*05:01 are majorly expressed. IFNepitope is an online prediction tool that seeks to predict and build peptides from protein sequences that can cause CD4+ T cells to release IFN-gamma. The MHC-II alleles retrieved from the IEDB server were further tested for IFN-γ production, and those epitopes that were negative for IFN-γ release were eliminated.

Potential B-cell epitope prediction

The B-cell epitopes for HPV-45 E6, E7, and L1 proteins were predicted using the default settings of the ABCpred server (Table 3). B-cell epitopes are essential for cancer immunotherapy. In total, 12 potent B-epitopes were predicted for HPV-45 E6 protein. The most prominent epitope was SIAGQYRGQCNTCCDQ, with a binding score of 0.87. For HPV-45 E7 protein sequences, 6 potent B-epitopes were predicted, with the most prominent epitope LQEIVLHLEPQNELDP, with a binding score of 0.92. Whereas, for HPV-45 L1 protein sequences, 53 potent B-epitopes were predicted. The most prominent epitope was DSTVYLPPPSVARVVS, with a binding score of 0.96.

DISCUSSION

HPV-related cancers account for approximately 4.5% of all cancers, affecting nearly 600,000 people globally every year. Both E6 and E7 proteins of HPV promote excessive cell proliferation. E6 binds to and degrades p53 and other host cell proteins, whereas E7 binds to and degrades Retinoblastoma (Rb) protein. Both p53 and Rb protein are cellular growth repressors. HPV virions use L1 and L2 proteins to attach to the basal cells after infection. Antibodies bind to the virus, preventing infection by stopping it from infecting epithelial cells.[21] An immunoglobulin-coated capsid is formed by high antibody titers, thus preventing the viral particle from attaching to basal cells, which is the initial stage of infection. As a result, neutrophils remove the virus that has been coated with antibodies. The virus particles are partially prevented from adhering to the basal cells in the presence of low antibody titers. The main mechanism of action is triggered by the capsid not binding to the second L1 receptor on the

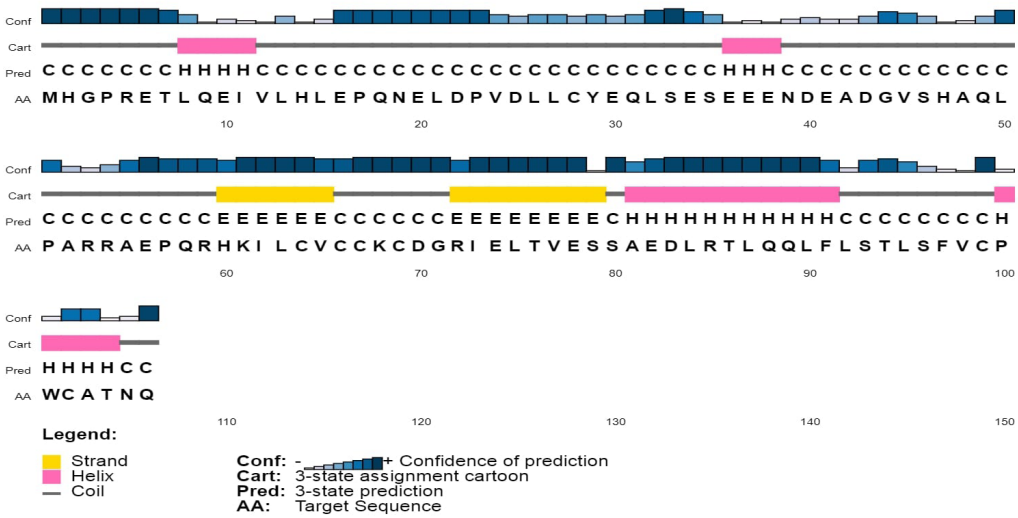

Figure 2. Secondary structure prediction of HPV-45 E7 protein through PSIPRED

surface of the epithelial cell. As a result, the virus is removed from the tissue.[22] E6, E7, and L1 proteins appear to be promising vaccine candidates due to the presence of numerous known neutralising epitopes.[23]

It is crucial to understand the vaccine candidate's structural characteristics, such as its secondary structure. Alpha helix and coil-containing proteins and peptides are significant structural antigens because antibodies can identify

Figure 3. Secondary structure prediction of HPV-45 L1 protein through PSIPRED

them.[24] The amino acid composition has shown that leucine residues are the most frequently occurring amino acids in all three proteins, i.e., E6, E7, and L1 of HPV-45. The most intriguing fact is that leucine residues have been studied earlier due to their significance in histone deacetylases (HDACs) binding. Histones attached to the MHC-I promoter are physically coupled with HDACs, and act as transcriptional co-repressors. It is likely that these HDACs cause MHC-I down-regulation due to the suppression of chromatin activation.[25]

In the present study, three potential B-cell epitopes were identified i.e., SIAGQYRGQCNTCCDQ, LQEIVLHLEPQNELDP, DSTVYLPPPSVARVVS, each in E6, E7 and L1 protein of HPV-45, respectively. The KLPDLCTEL epitope was predicted as a potential epitope for MHC class I alleles (HLA-A*02:01, HLA-A*02:03, HLA-A*02:06), similar to the HPV-18 validated epitopes,[26,27] the predicted epitope can provide the cross-protection against HPV-18. Another potential epitope for E6 (ATLERTEVY) was predicted to 8 different MHC-I alleles (HLA-A*01:01, HLA-A*11:01, HLA-B*15:01, HLA-A*26:01, HLA-A*30:02, HLA-A*32:01, HLA-B*35:01, HLA-B*58:01) and for L1 epitope (NVFPIFLQM) was predicted for 4 MHC-I alleles (HLA-A*30:02, HLA-A*32:01, HLA-B*51:01, HLA-B*53:01).

As experimental procedures are labor-intensive and time-consuming, numerous *in silico* techniques for distinguishing protein epitopes are being developed. Computational techniques, on the other hand, provide quick, simple, cost-effective, and reliable methods for the prediction of immunogenic epitopes. Scientists can use bioinformatics tools to extract epitopes from a protein of interest instead of potential binding sites in epitope-based vaccinations. Moreover, enhanced computational model dependability for the prediction of desired epitopes will undoubtedly aid in the pre-experimental stage of vaccine development. Due to several limitations, such as the occurrence of diverse genotypes and vaccine price and accessibility, HPV prevention has remained a major problem. The most significant drawback appears to be the present vaccine's limited coverage.[28]

A reference set of HLA alleles has been derived for both MHC I and MHC II binding prediction tools, providing more than 95% global population coverage, a significant characteristic for drug development. These techniques are useful for identifying a class of high-affinity binding peptides that could be produced and tested in the lab. The analysis projected the coverage of B and T cell epitope-based vaccinations in the population, allowing vaccines to be designed to maximise coverage.[29]

CONCLUSION

In silico approaches were used in this study to develop a vaccine candidate against oncoproteins and the major capsid protein of HPV-45. These proteins are strong candidates for antigenicity and immunogenicity due to their roles in viral replication, oncogenicity, and virus assembly. In this study, the amino acid sequence of the selected proteins was analysed, and their secondary structure was predicted. MHC-I and MHC-II epitopes for all three proteins were predicted and chosen based on their ability to induce antigenicity and produce IFN-γ, respectively. Further, B-cell epitopes were also predicted for the protein sequences. The epitopes identified by various web servers can be further used to create an effective antigenic vaccine capable of eliciting a significant immunological reaction over HPV-45. The discovery of potential epitopes has aided in developing cancer immunotherapy and detecting a wide range of infectious illnesses. Based on its rational design, we predict that the above-mentioned epitopes might be good candidates for vaccines against HPV-45 strains that are responsible for causing cervical cancer. It is possible to conduct additional molecular docking studies, followed by vaccine construct design using the predicted epitopes. It will require experimental confirmation by *in vivo* and *in vitro* studies, but it can be validated as a universally derived antigen when computationally analysed.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.17.1.53

Additional file: Additional Table S1.

ACKNOWLEDGMENTS

The authors would like to thank Nitte (Deemed to be University) for providing a research facility.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

PR and AK conceptualized the study. SP and AK applied methodology; SP wrote the original draft. PR, AK and BKK wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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