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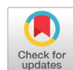
Experimental Research Article

# Designing a novel multi-epitope chimeric vaccine candidate for human papillomavirus by vaccinomic approach



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## ABSTRACT

**Introduction:** Human Papillomavirus (HPV) with small size and double-stranded DNA is the most important cause of sexually transmitted infections and cervical carcinoma. Controlling the spread of papillomavirus infection and protecting people against the pathogenicity of this virus are key steps in reducing the number of cervical cancer patients. One of the effective ways to achieve this goal is to design a suitable vaccine. In the present study, computer-aided methods were used to suggest a potential vaccine candidate against HPV.

**Methods:** Oncoproteins L1 and E5 of the high-risk strain HPV 16 were utilized to predict the linear B-cell epitopes, cytotoxic T lymphocytes (CTL), and helper T lymphocytes (HTL) epitopes. From the obtained epitopes, non-allergenic and non-toxic peptides with acceptable antigenicity were selected and subsequently converted into 3D structures. The epitopes were subjected to molecular docking using the PDB format. In the next step, short amino acid sequences as spacers were used to join peptides together. Finally, computational analysis including allergenicity and antigenicity studies, physicochemical properties, secondary and tertiary structure prediction, molecular interaction pattern, and cloning analyses were conducted for the vaccine construct.

**Results:** Our findings revealed that the designed vaccine with suitable antigenicity and physicochemical properties, shows proper interaction with four types of Toll-like receptors (TLR3, TLR4, TLR5, and TLR8), and Escherichia coli (strain K12) is the suitable host for it.

**Conclusion:** Overall, the vaccine designed in the present study showed a promising immune response. However, further validation through laboratory investigations is required.


**Keywords:**
Multi-epitope
In-silico
HPV
Vaccine
Papillomavirus

## Introduction

Human papillomavirus (HPV), with a diameter of 52–55 nm, is responsible for many infectious agent-related cancers worldwide. Based on the global epidemiology reports of human papillomavirus, about 80% of sexually active men and women will be infected with HPV at least once in their lifetime and this virus is responsible for 97% of cervical cancer. HPV 16 and 18

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are high-risk strains in 100 HPV types and cause 90% of HPV-related cancers. the Caribbean (35.4%) and Eastern Africa (33.6%) have reported the highest percentage of the prevalence of HPV-related diseases (Milano et al., 2023; Soheili et al., 2021). HPV is one of the most common causes of reproductive tract infections. Genital wart (condyloma acuminatum) is a sexually transmitted infection related to this virus. The persistence of HPV infection causes progression to precancerous lesions and eventually causes cervical carcinomas in women. Therefore, prevention, early diagnosis, and treatment of papillomavirus infection are very important (Araldi et al., 2018; Giroglou et al., 2001; Ratanasiripong 2012).

This group of viruses belongs to the *Papillomaviridae* family, which has double-stranded circular DNA. The most common way of transmitting the virus is through sexual activity with a person who has an active HPV infection (Panatto et al., 2012). The size of DNA in HPV is estimated to be eight kilobase pairs (Zaravinos et al., 2009). The proteins coded by the virus genome are classified into early and late proteins. Early promoters like P97 transcribe E proteins while L proteins are mainly transcribed from late promoters like P742. Nine important proteins of the virus include E1, E2, E4, E5, E6, E7, L1, and L2 (Choi et al., 2023; Graham 2017; Morshed et al., 2014). Early proteins are responsible for regulating viral DNA replication, while late proteins are involved in making the icosahedral capsids of the virus. The virus uses E1 and E2 proteins to detect the origin of replication, and the E2 protein is also a master regulator of expression. Research has shown that both E4 and E5 are involved in the second phase of the virus life cycle. Negative regulators of the viral cell cycle are targeted by E6 and E7 proteins (Choi et al., 2023; Morshed et al., 2014). In research by Leechanachai and co-workers, E5 increased the activity of epidermal growth factor receptor (EGFR) (Leechanachai et al., 1992). After increasing expression of EGFR, the E5 protein can complex with it. The role of this protein in relation to the mitogen-activated protein kinase (MAP) has also been proven. Therefore, it plays important and key roles in the life cycle and pathogenesis of the virus (Choi et al., 2023; Leechanachai et al., 1992; Morshed et al., 2014).

L1 is the main protein in capsid formation, and L2 is the minor capsid protein. The molecular weight of L1 protein is approximately 55 kDa, and it can form virus-like particles (VLPs). Overexpression of L1 in tissue culture cells created VLPs that are empty capsids. The study of L1 sequences in different types of papillomaviruses has shown that they have protected regions, making them suitable subjects for vaccine design (Hagensee et al., 1993).

Immunoinformatics-based computational vaccinology, a fast and cheap method, allows researchers to design vaccines with high effect potential. In this way, immunodominant B and T epitopes are selected from the main and key proteins of the virus, and appropriate sequences are integrated with linkers to create a multi-epitope vaccine construct. Then, the ability and effectiveness of this designed structure are measured (Oli et al., 2020).

In the present study, Due to the importance of preventing high-risk papillomavirus infection in reducing the number of cervical cancer patients, we introduce a novel multi-epitope chimeric vaccine candidate against human papillomavirus using a vaccinomic approach. Considering the key structural and functional roles of L1 and E5 proteins (Haghshenas et al., 2017; Kim et al., 2010) in the pathogenicity of high-risk human papillomavirus type 16, these two proteins were used as target sequences for epitope selection and vaccine design.

## Material and Methods

*Amino acid sequence of target proteins*

The UniProtKB database was utilized to retrieve the complete sequence of target proteins, including E1 and L1, from human papillomavirus in FASTA format. The Multalin Server was then employed to align and identify conserved positions in the protein sequences (Corpet 1988).

*Detecting candidate B-cell epitopes*

Generating both humoral and cellular immune responses is crucial in the design of a recombinant multi-epitope subunit vaccine. Humoral immunity in mammals is orchestrated by B cells or B lymphocytes, pivotal components of the adaptive immune response. In this study, candidate B-cell epitopes were identified using ABCpred, a machine-based technique. This database predicts epitopes ranging from 10 to 20 amino acids in length, and sequences with scores exceeding 0.7 are considered suitable options for selection (Saha and Raghava 2006).

*Prediction of CTL Epitopes*

The role of cytotoxic T lymphocytes (CTLs) is crucial in combating viral infections and pathogens as they directly target and eliminate infected cells. To identify these epitopes, the CTLPred was employed in this study. The server utilized a threshold of 0.75 for epitope identification, producing epitopes with 9 residues that can be recognized by 12 human leukocyte antigen allele class I supertypes. Subsequently, epitopes with a score of less than 2 were filtered using the Immune Epitope Database (IEDB) (Bhasin and Raghava 2004).

*HTL epitope prediction*

T lymphocyte (HTL) epitopes play a crucial role in immune responses as they are involved in activating both B cells and cytotoxic T cells. To predict HTL epitopes, the IEDB was employed in this study. Searches were narrowed down by selecting 10 alleles of the human leukocyte antigen (HLA), and the protein sequence was submitted for analysis.

*Prediction of the antigenicity, toxicity, and allergenicity of the epitopes*

The antigenicity of the predicted epitopes was evaluated using the VaxiJen server, with a threshold of 0.4. Additionally, to ensure safety, it was important to verify that the epitopes did not possess allergenic or toxic properties. For this purpose, the AllerTOP v.2.0 server, known for its 94% sensitivity, was employed to predict allergenicity. Moreover, the ToxinPred online server was utilized to assess the toxicity of the epitopes.

*Molecular docking study of epitopes*

The first structure of the selected epitopes was converted into three-dimensional structures using PEP-FOLD 2.0. Subsequently, the structures of the studied receptors were obtained from the protein data bank database, and protein preparation was conducted using Discovery StudioVisualizer 3.5. Following this, the PDB formats of the epitopes and receptors were imported as input files to the HDOCK server for docking operations. Finally, the output file was analyzed using Accelrys software.

*Vaccine construction*

The final selected epitopes were linked together using appropriate linkers. Additionally, a suitable adjuvant was incorporated at the beginning of the multi-epitope chimeric vaccine to enhance its immunogenic activity.

*Antigenic, allergenic, and physicochemical properties of the vaccine*

The vaccine's allergenicity and antigenic property were assessed using Vaxijen V2.0 and AllerTOP V2.0 servers, respectively. Additionally, the ProtParam server was used to evaluate the vaccine's physicochemical characteristics.

*Two and three-dimensional structures Prediction and 3D structure refinement*

In this section, we determined the structural characteristics of the vaccine, focusing on the ratio of regular structures such as α-helix and β-sheet to irregular structures such as random coils, as well as the three-dimensional structure of the vaccine after final folding. We achieved these goals by using the online server SOPMA, which has an accuracy above 80%, and the I-TASSER web server. Finally, we refined the best output 3D model from the I-TASSER web server using the online GalaxyRefine tool.

*Interaction analysis of vaccine and receptors*

The vaccine's final refined structure in PDB format was used as the input ligand molecule in the HDOCK server. To check their interactions, the TLR3, TLR4, TLR5, and TLR8 receptors were obtained from the PDB site with identification codes 5gs0, 4g8a, 3j0a, and 3w3g, respectively, and were used as the input receptor molecule. After docking, among the ten conformations of the vaccine-receptor complex, the one with the most negative energy level was filtered, and the monitoring of H-bonds and pi interactions was performed using Discovery Studio software 3.5.

*Computer-aided cloning*

In order to make the vaccine computationally suitable, the Sequence Manipulation Suite (SMS) server and the Java Codon Matching Tool (JCat) were used for reverse translation and codon optimization. The host organism chosen for this process was Escherichia coli (strain K12).

## Results

*Amino acid sequence of target proteins*

The amino acid sequences of the major capsid protein L1 (UniProtID: P03101) and the smallest oncoprotein E5 (UniProtID: P06927) of HPV were retrieved from

**TABLE 1:** The number and sequence of amino acids of L1 and E5 proteins.

| Protein Name | Source | Amino Acid number | Consensus sequence |
|---|---|---|---|
| L1 | Human papillomavirus type 16 | **505** | MSLWLPSEATVYLPPVPVSKVVSTDEYVARTNIYYHAGTSRLLAVGH-PYFPIKKPNNNKILVPKVSGLQYRVFRIHLPDPNKFGFPDTSFYNP-DTQRLVWACVGVEVGRGQPLGVGISGHPLLNKLDDTENASAYAANAG-VDNRECISMDYKQTQLCLIGCKPPIGEHWGKGSPCTNVAVNPGDCP-PLELINTVIQDGDMVDTGFGAMDFTTLQANKSEVPLDICTSICKYPDYIK-MVSEPYGDSLFFYLRREQMFVRHLFNRAGAVGENVPDDLYIKGSGSTAN-LASSNYFPTPSGSMVTSDAQIFNKPYWLQRAQGHNNGICWGNQLFVTV-VDTTRSTNMSLCAAISTSETTYKNTNFKEYLRHGEEYDLQFIFQLCKITL-TADVMTYIHSMNSTILEDWNFGLQPPPGGTLEDTYRFVTSQAIACQKHTP-PAPKEDPLKKYTFWEVNLKEKFSADLDQFPLGRKFLLQAGLKAKPKFTL-GKRKATPTTSSTSTTAKRKKRKL |
| E5 | Human papillomavirus type 16 | 83 | MTNLDTASTTLLACFLLCFCVLLCVCLLIRPLLLSVSTYTSLIILVLLLWI-TAASAFRCFIVYIIFVYIPLFLIHTHARFLIT |

**TABLE 2:** Linear B-cell epitope prediction of L1 and E5 proteins.

| Protein name | Sequence | Start position | Score | Antigenicity | ALLergenicity | Toxicity |
|---|---|---|---|---|---|---|
| protein L1 | RIHLPDPNKFGFPDTS | 74 | 0.92 | 0.5073 | NON-ALLERGEN | NON-Toxin |
| | EATVYLPPVPVSKVVS | 8 | 0.9 | 0.5634 | NON-ALLERGEN | NON-Toxin |
| | KGSPCTNVAVNPGDCP | 171 | 0.9 | 0.5214 | NON-ALLERGEN | NON-Toxin |
| | GQPLGVGISGHPLLNK | 110 | 0.85 | 0.5668 | NON-ALLERGEN | NON-Toxin |
| | GFGAMDFTTLQANKSE | 204 | 0.83 | 1.1201 | NON-ALLERGEN | NON-Toxin |
| | LELINTVIQDGDMVDT | 188 | 0.81 | 0.6016 | NON-ALLERGEN | NON-Toxin |
| | TTSSTSTTAKRKKRKL | 490 | 0.8 | 0.9333 | NON-ALLERGEN | NON-Toxin |
| | FWEVNLKEKFSADLDQ | 446 | 0.76 | 0.5490 | NON-ALLERGEN | NON-Toxin |
| | NSTILEDWNFGLQPPP | 395 | 0.75 | 1.1302 | NON-ALLERGEN | NON-Toxin |
| | AKPKFTLGKRKATPTT | 476 | 0.74 | 0.7234 | NON-ALLERGEN | NON-Toxin |
| | AGAVGENVPDDLYIKG | 264 | 0.71 | 0.4394 | NON-ALLERGEN | NON-Toxin |
| | AVNPGDCPPLELINTV | 179 | 0.66 | 0.7965 | NON-ALLERGEN | NON-Toxin |
| | GVEVGRGQPLGVGISG | 104 | 0.63 | 1.5726 | NON-ALLERGEN | NON-Toxin |
| | - | 336 | 0.6 | 1.0718 | NON-ALLERGEN | NON-Toxin |
| | SETTYKNTNFKEYLRH | 351 | 0.53 | 0.4963 | NON-ALLERGEN | NON-Toxin |
| protein E5 | LDTASTTLLACFLLCF | 4 | 0.74 | 0.4188 | NON-ALLERGEN | NON-Toxin |
| | CLLIRPLLLSVSTYTS | 26 | 0.7 | 0.4961 | NON-ALLERGEN | NON-Toxin |
| | CVLLCVCLLIRPLLLS | 20 | 0.56 | 0.6020 | NON-ALLERGEN | NON-Toxin |
| | LLLSVSTYTSLIILVL | 32 | 0.51 | 0.4100 | NON-ALLERGEN | NON-Toxin |

UniProt database. These sequences were then analyzed to identify conserved regions using the Multalin Server. Table 1 shows the number and sequences of protein L1 and E5.

*Detecting candidate B-cell epitopes*

The complete sequences of protein L1 and E5 were scanned for B cell epitopes using the ABCpred server. Then, non-allergenic and non-toxic peptides with suitable antigenic levels were selected for further analysis. The results of this screening process are presented in Table 2.

*Prediction of CTL Epitopes and HTL epitopes*

Using the CTLPred server, CTL epitopes capable of binding to at least two different subtypes were identi-

**TABLE 3:** Prediction of CTL epitopes for L1 and E5 proteins.

| Protein Name | Peptide | Supertype | MHC class 1 allele | Antigenicity | ALLergenicity | Toxicity |
|---|---|---|---|---|---|---|
| protein L1 | YKNTNFKEY | A1,B62 | HLA-A*30:02<br>HLA-B*15:01<br>HLA-B*44:03<br>HLA-B*35:01<br>HLA-B*44:02<br>HLA-A*26:01 | 0.7601 | NON-ALLERGEN | NON-Toxin |
| | ILVPKVSGL | A2,B8 | HLA-B*15:01<br>HLA-A*26:01<br>HLA-B*35:01<br>HLA-A*30:02<br>HLA-A*01:01<br>HLA-A*02:03<br>HLA-A*02:01<br>HLA-A*02:06<br>HLA-B*08:01<br>HLA-B*15:01<br>HLA-A*32:01 | 0.4006 | NON-ALLERGEN | NON-Toxin |
| | FYLRREQMF | B8,B24 | HLA-A*24:02<br>HLA-A*23:01<br>HLA-B*08:01 | 1.1062 | NON-ALLERGEN | NON-Toxin |
| | YDLQFIFQL | B44,B39 | HLA-B*40:01<br>HLA-A*02:06 | 1.6395 | NON-ALLERGEN | NON-Toxin |
| | TANLASSNY | A1,B62,B58 | HLA-B*35:01<br>HLA-A*30:02<br>HLA-A*01:01<br>HLA-A*26:01<br>HLA-B*15:01<br>HLA-B*53:01<br>HLA-B*58:01 | 0.6186 | NON-ALLERGEN | NON-Toxin |
| Protein E5 | YTSLIILVL | B62,B58,B39,B8,A2, A1 | HLA-A*68:02<br>HLA-B*58:01<br>HLA-A*32:01<br>HLA-A*02:06 | 0.6175 | NON-ALLERGEN | NON-Toxin |
| | FIVYIIFVY | A1<br>A26<br>A3<br>B62 | HLA-A*26:01<br><br>HLA-B*35:01 | 0.4718 | NON-ALLERGEN | NON-Toxin |
| | NLDTASTTL | A1,A2,B39 | HLA-A*02:01<br>HLA-A*02:06<br>HLA-B*08:01<br>HLA-A*02:03 | 0.4122 | NON-ALLERGEN | NON-Toxin |
| | YIIFVYIPL | A2,A26,B8,B39,B62 | HLA-A*68:02 | 0.5795 | NON-ALLERGEN | NON-Toxin |
| | STYTSLIIL | A2, B39 | HLA-A*32:01<br>HLA-A*68:02<br>HLA-A*30:01<br>HLA-A*02:06<br>HLA-B*58:01 | 0.4878 | NON-ALLERGEN | NON-Toxin |
| | FLIHTHARF | A26,B8,B39,B58 | HLA-B*15:01<br>HLA-A*23:01<br>HLA-A*24:02<br>HLA-A*26:01<br>HLA-A*32:01<br>HLA-B*35:01 | 0.6381 | NON-ALLERGEN | NON-Toxin |

fied for further analysis. The results from the CTLPred server were then used as the input of the IEDB to check the MHC class 1 allele (Table 3). Helper T lymphocyte epitopes were predicted using the Immune Epitope Database MHC II (Table 4). Finally, the antigenicity, allergenicity, and toxicity of all epitopes were assessed.

**TABLE 4:** Prediction of HTL epitopes for L1 and E5 proteins.

| Protein Name | Peptide | IEDB ALLELE | Position | Antigenicity | ALLergenicity | Toxicity |
|---|---|---|---|---|---|---|
| protein L1 | GRKFLLQA-GLKAKPK | LA-DRB1*01:09<br>HLA-DRB1*01:10<br>HLA-DRB1*01:01 | 465-479 | 0.8115 | NON-ALLERGEN | NON-Toxin |
| | AMDFT-TLQANKSEVP | HLA-DRB1*01:10<br>HLA-DRB1*01:01<br>HLA-DRB1*01:05<br>HLA-DRB1*01:07<br>HLA-DRB1*01:08 | 207-221 | 0.6328 | NON-ALLERGEN | NON-Toxin |
| | LGRKFLLQA-GLKAKP | HLA-DRB1*01:10<br>HLA-DRB1*01:09<br>HLA-DRB1*01:01<br>HLA-DRB1*01:05<br>HLA-DRB1*01:07<br>HLA-DRB1*01:08 | 464-478 | 0.7769 | NON-ALLERGEN | NON-Toxin |
| protein E5 | YTSLIILVLLL-WITA | HLA-DRB4*01:01 | 39-53 | 0.4381 | NON-ALLERGEN | NON-Toxin |
| | TSLIILVLLLWI-TAA | HLA-DRB4*01:01 | 40-54 | 0.4541 | NON-ALLERGEN | NON-Toxin |
| | TYTSLIILVLLL-WIT | HLA-DRB4*01:01 | 38-52 | 0.5137 | NON-ALLERGEN | NON-Toxin |

**TABLE 5:** Docking results of selected epitopes

| Peptide | Docking score | Ligand RMS-D(Å) | number of H-bonds | amino acids involved in hydrogen bonds |
|---|---|---|---|---|
| YTSLIILVL | -176.36 | 51.41 | 5 | THR2,TYR1,ILE5 |
| NLDTASTTL | -160.74 | 49.11 | 8 | ASN1,ASP3,LEU2,THR7 |
| YIIFVYIPL | -221.29 | 56.47 | 2 | TYR1,TYR6 |
| STYTSLIIL | -185.81 | 54.29 | 3 | TYR3,THR2,SER5 |
| ILVPKVSGL | -178.87 | 53.22 | 5 | ILE1, LEU2,LYS5,VAL6 |
| TANLASSNY | -200.49 | 85.30 | 2 | SER6,TYR9 |
| AMDFTTLQANKSEVP | -176.05 | 57.58 | 4 | ASP3,THR5,GLU13,GLN8 |
| LGRKFLLQAGLKAKP | -210.08 | 60.48 | 6 | LYS14,PRO15,ARG3,LYS4 |
| YTSLIILVLLLWITA | -238.83 | 67.70 | 6 | ILE5,THR14,TRP12 |
| TSLIILVLLLWITAA | -227.77 | 58.90 | 3 | ILE4,ILE5,SER2 |
| TYTSLIILVLLLWIT | -241.85 | 58.67 | 4 | ILE14,THR3,LEU11,TRP13 |

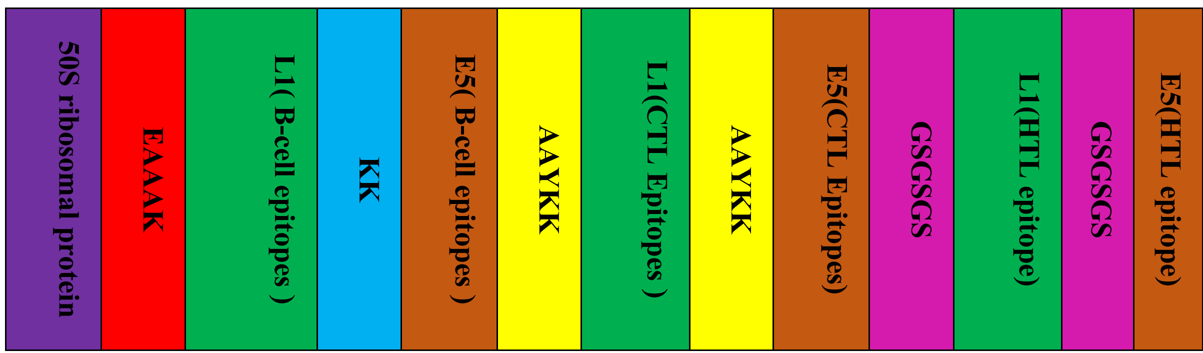
Docking Score: This value is a scoring factor that is used to predict the degree of affinity between ligand and receptor, and more negative means greater affinity.
RMSD: Root Mean Square Deviation

*Molecular docking study of epitopes*

To refine the selection of epitopes based on their interaction and energy levels, docking was performed using the HDOCK server. The docking score, Root Mean Square Deviation of the ligand, and the amino acids of the epitopes involved in hydrogen bonds with receptors are shown in Table 5.

*Multi-epitope vaccine Construction*

Based on the binding mode and energy levels of the peptide sequences, epitope selection was performed, followed by connection using AAYKK, GSGSGS, and KK spacers. The AAYKK linker is a pentapeptide containing alanine, tyrosine, and lysine residues. GSGSGS is the flexible linker with glycine and serine compo-




MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEF
DVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATV
TVKEAAAKGFGAMDFTTLQANKSEKKGVEVGRGQPLGVGISGKKNSTILEDWNFGLQPPPKKC
VLLCVCLLIRPLLLSAAYKKYTSLIILVL AAYKKNLDTASTTL AAYKKYIIFVYIPL AAYKK
STYTSLIILAAYKKILVPKVSGLAAYKKTANLASSNYGSGSGSAMDFTTLQANKSEVPGSGSGSL
GRKFLLQAGLKAKPGSGSGSYTSLIILVLLLWITAGSGSGSTSLIILVLLLWITAAGSGSGSTYTSLI
ILVLLLWIT

**FIGURE 1.** Schematic picture and sequences of multi-epitope vaccine construction

nents. The KK linker consists solely of lysine residues. All linkers increase the immunogenic activity of the vaccine. To construct the final vaccine structure, the 50S ribosomal protein (accession number: P9WHE3) was used as an adjunct. The placement of the adjuvant is to increase the immunogenicity of the vaccine. The adjuvant sequence was added to the N-terminal part with the EAAAK linker. A schematic illustration and the sequences of the multi-epitope vaccine construct are shown in figure 1.

*Antigenic, allergenic, and physicochemical properties of the vaccine*

Based on the results obtained from the Vaxijen V2.0 and AllerTOP V2.0 servers, the designed vaccine is confirmed to be non-allergenic. The antigenic property of the vaccine was estimated to be 0.5640. Based on the results of ProtParam server, the vaccine has a molecular weight of 41.151 kDa and consists of 394 amino acids. The theoretical pI, instability index, grand average of hydropathicity, and aliphatic index of the designed vaccine are 9.19, 23.75, 0.347, and 112.03, respectively. Moreover, the calculated half-life of the vaccine exceeds 20 hours in yeast, more than 30 hours in reticulocytes of mammals, and 10 hours in Escherichia coli.

*Two and three-dimensional structures prediction and 3D structure refinement*

Based on the analysis performed using the SOPMA server, the secondary structure of the vaccine was predicted. The results indicate that the vaccine contains 189 residues in the alpha helix (47.97%), 64 amino acids in the extended strand (16.24%), and 141 amino acids in the random coil (35.79%). The predicted secondary structure of the vaccine is illustrated in Figure 2. Additionally, utilizing the I-TASSER web server, the top five models were generated based on the C score. The C score ranges from -5 to 2 on this server, with the closest and most accurate protein model corresponding to the highest C score. The final structure of model 1, with a C score of -1.12, an estimated TM-score of 0.57±0.14, and an estimated RMSD of 9.3±4.6Å, was saved in PDB format.

*Interaction analysis of vaccine and receptors*

The interaction analysis between the novel chimeric vaccine and Toll-like receptors (TLRs) was conducted using the HDOCK server. Following the docking process, models were selected based on their ability to correctly occupy the binding site of TLRs and their low Docking Scores.

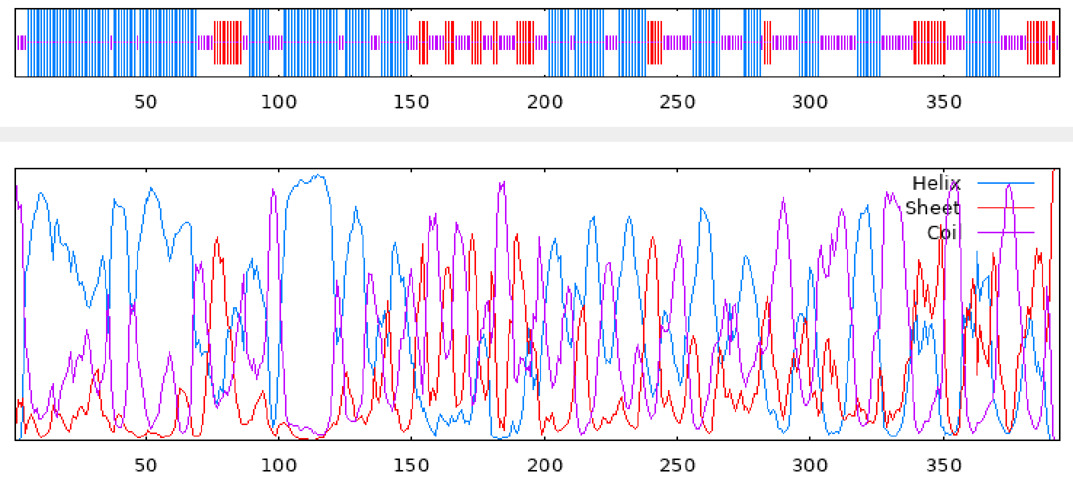



**FIGURE 2.** The predicted secondary structure of the vaccine

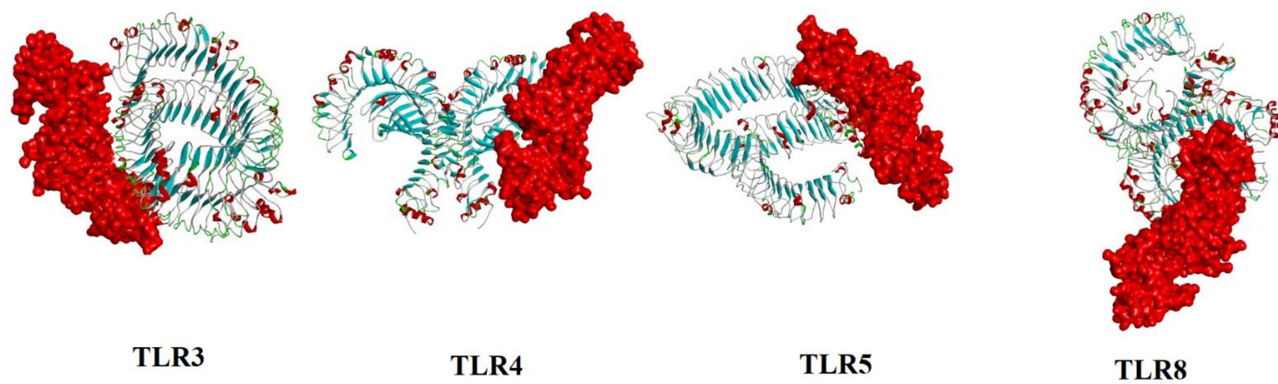



**FIGURE 3.** The interaction pattern between vaccine and TLRs (The vaccine protein is depicted in surface representation and highlighted in red color.)

The Docking Scores calculated for the TLR3-Vaccine, TLR4-Vaccine, TLR5-Vaccine, and TLR8-Vaccine complexes were as follows: -289.47, -292.36, -292.23, and -267.87, respectively. It's important to note that the highest binding affinity in the receptor-vaccine complex is associated with the lowest energy levels.

The best conformation of the complex involving the four types of TLRs with the vaccine is depicted in Figure 3.

*Computer-aided cloning*

The vaccine sequences were translated into DNA using the SMS server and then entered into the JCAT web server for codon optimization. The Codon Adaptation Index (CAI) value and GC content of the optimized sequence were determined to be 1 and 48.86%, respectively, for Escherichia coli (strain K12).

## Discussion

Many epithelial cancers and lesions on cutaneous and mucosal surfaces have been linked to the HPV (Stanley 2012). Investigations into lung, laryngeal, and oral cancers have shown traces of this virus as a triggering factor (Shukla et al., 2009). With over 100 subtypes, HPV 16 and 18 are considered high-risk variants responsible for causing damage and promoting malignancies. HPV16, a high-risk strain, has been identified in 50 to 60% of cervical carcinomas (Faridi et al., 2011).

One method within the interdisciplinary field for designing enzyme inhibitors, drugs, and vaccines involves in silico studies (Asadzadeh et al., 2023; Asadzadeh et al., 2015; Faridi et al., 2011; Mosalanezhad et al., 2022; Shojaei Barjouei et al., 2022; Sholehvar et al., 2017). The immunoinformatics approach is particularly notable as a rapid and effective means to design more ef-

ficient epitope-based vaccines against infectious pathogens (Shams Moattar et al., 2022). Therefore, a key strategy for preventing HPV-related infectious diseases is the development of chimeric vaccines. Epitope-based vaccines involve identifying suitable peptide sequences within the pathogen's structural or functional proteins and incorporating them into vaccine design.

Viral proteins of the HPV are broadly categorized into two groups: the E proteins, which are part of the early gene, and the L proteins, which correspond to the late gene in the virus genome. The L1 protein of the papillomavirus is used in the design of vaccines against the cancerous HPV, due to its capability for self-assembly and the conserved sequences within its structure. On the other hand, the early phase E5 protein of the virus plays a significant role in cellular pathways and signaling within human cell lines (Choi et al., 2023; Monie et al., 2008; Morshed et al., 2014; Ratanasiripong 2012). Therefore, in this study, a robust recombinant vaccine against the HPV was designed by combining two proteins, L1 and E5. At first, non-allergenic, non-toxic, and antigenic B-cell and T-cell epitopes derived from L1 and E5 proteins were identified. Subsequently, the 3D structure of the selected epitopes was generated using the PEP-FOLD server. Secondary filtering was performed through molecular docking studies based on binding energy levels and interaction modes. In the next step, the final sequence of the vaccine was designed using adjuvant and appropriate linkers for further investigations.

Based on the data obtained from ProtParam server, the vaccine, with a molecular weight of 41.151kDa, is suitable for the purification process. Additionally, according to the values of the Grand average of hydropathicity and aliphatic index, the designed construction is thermostable. Based on the half-life value and instability index, the designed vaccine is classified as a resistant protein.

TLRs are family of Type 1 transmembrane innate immune receptors (Kawai and Akira 2007; Sabroe et al., 2008). These receptors play a crucial role in identifying invading pathogens, as well as recognizing dangerous molecules released by dying cells and damaged tissues. Due to the importance of TLRs in the immune system, the interaction analysis of vaccines with these receptors has been emphasized in the design of multi-epitope vaccines (Kawai and Akira 2007; Sabroe et al., 2008; Van Duin et al., 2006).

The results of molecular docking showed that the designed vaccine has suitable hydrogen bonds with TLR3, TLR4, TLR5, and TLR8. Comparing the docking scores of all four receptors, it is determined that the affinity between the receptor- vaccine complex was in the order of TLR4-vaccine> TLR5-vaccine > TLR3-vaccine> TLR8-vaccine. In the vaccine-TLR3 interaction, seven amino acids of TLR3 are involved in hydrogen bond formation, including ARG64, HIS319, GLN352, TRP353, HIS406, HIS410, and ASN507. On the other hand, amino acids in the vaccine construction bond with GLN115, GLN21, TYR22, ASN47, ASN49, GLN91, ASN160, SER184, GLU266, THR115 in TLR4. Four hydrogen bonds were also observed in the binding site of TLR5, including THR651, ARG664, HIS596, and THR600. In the results of the vaccine-TLR8 docking, ARG338, TYR482, SER368, GLU557, and GLU691 in the receptor participate in the interaction.

Finally, the computer-aided cloning results showed that Escherichia coli (strain K12) is the best organism for the designed vaccine.

## Conclusion

In this study, we employed a vaccinomic approach to design effective vaccines against HPV. The designed vaccine exhibits high antigenic properties and demonstrates a strong binding affinity for TLR3, TLR4, TLR5, and TLR8 receptors. Moreover, it shows good stability and resistance, and is non-allergenic for humans. However, experimental validation is necessary to assess its ability to control HPV infection.

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## Conflict of interest

The authors declared that they have no conflicts of interest.

## Ethics approval

This research was done with bioinformatics approaches and human or animal samples were not used.

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