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# Characterization and Structural Analysis of the Human Papilloma Virus L1 Protein in Iran

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**Abstract**

**Background:** Human papillomavirus (HPV) is a small, non-enveloped DNA virus related to human cervical cancer. The genome is maintained within the basal epithelium where the primary infection is latent. During the late phase of infection, the capsid proteins (L1 and L2) are expressed to encapsidate the viral genome, generating the infectious virion particles required for HPV propagation. HPV genome encodes six proteins, namely E6 oncoprotein, E7 oncoprotein, E1 replication protein, E2 regulatory protein, L1 major capsid protein, and L2 minor capsid protein. L1 is the principal part of the current vaccines, and any changes in this region can decrease vaccine efficiency. The aim of this research was to conduct a comparative analysis among Iranian L1 protein sequences with reference sequences to determine the possible substation in this region and to find the physicochemical and structural properties of L1 by using bioinformatics tools to provide comprehensive comprehension of the HPV L1 protein.

**Methods:** Thirteen Iranian PV sequences of the L1 protein and reference sequences were selected and obtained from the NCBI data bank. CLC Sequence Viewer software was used to translate the alignment. PrediSi and Phobius were employed to predict the signal peptide. The secondary and tertiary structures and structure validations of all sequences were analyzed by Qmean, (PS)2-v2, Phyre2server, Discovery Studio, and I-TASSER.

**Results:** The findings showed that L1 is highly conserved, and only two mutations were found in this region. No signal peptide was described, and this region's main part included a random coil. The tertiary structure was mapped using different software, and five distinct loops were found.

**Conclusion:** This study is the first report that investigated the changes in the L1 protein of Iranian patients and provided helpful comprehension of the L1 properties vital for cloning and producing the new generation of virus-like particle (VLP) vaccines. Furthermore, the structural analysis showed several loops that had an indispensable role in antibody binding and the prevention of HPV infections.

**Keywords:** HPV, In silico, L1, Structural analysis, Physicochemical properties, Bioinformatics

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## Introduction

Currently, sexually transmitted infections (STIs) are becoming more common in societies, and human papillomavirus (HPV) infection is among the most prevalent STIs (1). While most HPV infections remain transient and do not cause disease, few of them can develop high-grade precancerous or invasive cervical lesions (2-5). Much research has established the vital role of HPV in the development of cervical cancer and nearly all precursor lesions related to cervical cancers are infected by high-risk HPV types (6,7). Among all cancers, in women, cervical cancer is ranked fourth and affects many individuals globally (8). In 2012, 528 000 new cases and 266 000 deaths were reported (9). Studies have indicated that around 2.5 per 100 000 Iranian women have cervical cancer, which is a low rate; its mortality rate has also been reported at 1.04 per 100 000 women. The high-risk age for Iranian women is between 55 and 65 years (10).

HPV is a small non-enveloped DNA-tumor virus with a virion size of ~55 nm in diameter; its genome codes 6 nonstructural viral regulatory proteins (E1, E2, E4, E5, E6, and E7) and two structural viral capsid proteins (L1 and L2) (11). The virus capsid, which is responsible for the transmission, spread, and survival of the virus in the environment, differs by at least 10% in different types and 2–10% in different subtypes based on the L1 genome (12).

The potential to cause cancer is an index to classify HPV types as high-risk HPV (HR-HPV) and low-risk HPV (LR-HPV) groups; types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 are commonly considered as HR-HPV, and HPV-6 and HPV-11, which typically cause warts, are the most common LR-HPV types (13,14).

Based on previous studies among all HPV types, HPV 16 and 18 are responsible for around 70% of universal cases, and 68 and 73 are considered "possibly" cancer-causing (15). Globally, HPV-16 is the most frequent genotype;

however, the distribution of HPV genotypes, which contributes to HPV infection-monitoring programs, may be related to several factors, including the geographic region and the severity of the cervical lesion (16).

HPV L1 has an indispensable role in HPV entry, and the initial interaction is remarkably attributable to L1 interactions with proteoglycans (17). L1 protein is highly conserved among different HPV types and can self-assemble in virus-like particles (VLPs); it seems that a surface cell protein is involved in VLP binding. Based on VLPs, VLP-based vaccines have been introduced that offer highly effective protection against HPV infections (17,18).

Humoral immunity always has a pivotal role in preventing virus infections (19,20), and recently, two vaccines have become available to prevent cervical cancer, a quadrivalent vaccine (HPV 6, 11, 16, and 18 types) called Gardasil® and a bivalent vaccine (HPV 16 and 18 types) called Cervarix® (21,22). The vaccines are composed of the HPV major late protein (L1) for each type, and any substitutions in the L1 structure can lead to a decrease in the efficacy and effectiveness of the vaccine. Therefore, monitoring mutations for L1 protein in any society is a requirement. This study aimed to find a substitution in HPV L1 protein sequences obtained from Iranian patients, evaluate their physiochemical properties, and perform structural analysis using several reliable bioinformatics software.

## Materials and Methods

### *L1 protein sequences availability*

Thirteen selected L1 sequences from Iranian patients (KP16098- KP161014, KM058644.1, KM058642.1, KM058663.1, KM058639.1, KP160988.1, KM058636.1, KM058647.1, KM058654.1, KP161010.1, KM058637.1, KM058660.1, KM058648.1, and KM058651.1) and a reference sequence (K02718.1) were obtained from the NCBI databank (http://www.ncbi.nlm.nih.gov).

### *Signal peptide prediction*

To predict the signal peptide of the L1 protein, PrediSi (http://www.predisi.de/ ) and Phobius (http://phobius.sbc.su.se/) were employed.

### *Physicochemical properties*

Prediction of instability index, aliphatic index, theoretical isoelectric point (pI), and Grand average of hydropathy (GRAVY) was done by ExPASy ProtParam (23) (http://expasy.org/tools/protparam.html).

### *Secondary and tertiary structure*

Secondary structure prediction was done by Scratch, Porter, I-TASSER, Phyre2, and (PS)2-v2 software to utilize the tertiary prediction structure of the selected sequences. All predicted 3D structures were evaluated for the stereochemistry, reliability, and quality by Qmean and Rampage tools. Discovery Studio software was used to find the loops on the L1 protein.

## Results

### *Sequences analysis*

Compared with the reference sequence, all selected sequences showed 2 mutations in amino acids 202 (T to N), 228 (H to D), 292 (T to A), and 501 (L to F).

### *Signal peptide*

Two reliable software could not show any cleavage position and signal peptide for the selected sequences (Figure 1).

### *ProtParam results*

The L1 protein had 505 amino acids, a molecular weight

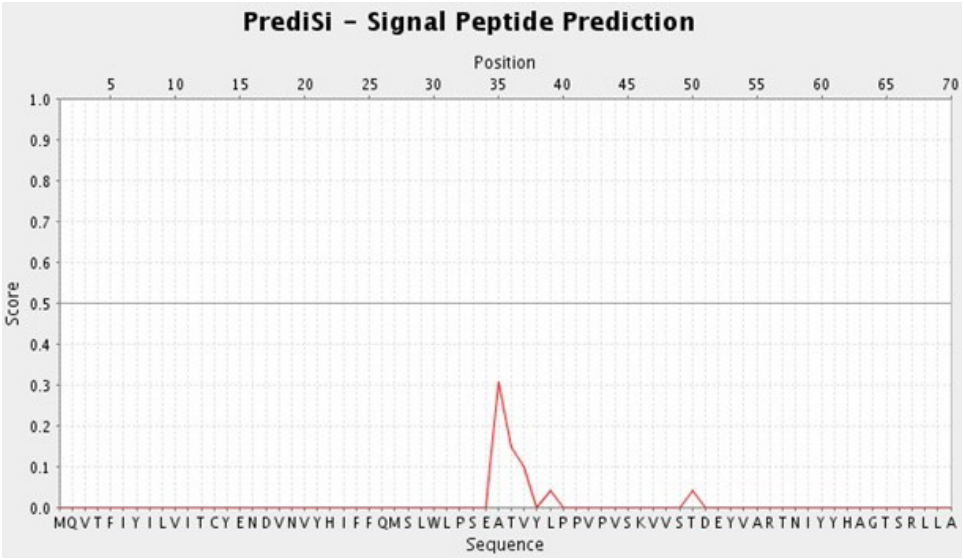


**Figure 1.** Signal peptide perdition by PrediSi which was unable to determine any reliable signal peptide for L1 protein

of 59554.02 Da, and a pI of 8.27, indicating that it was a basic protein. Its in vivo half-life was estimated as 30 hours in mammalian cells and more than 10 hours in *E. coli*. Its instability index and aliphatic index were found to be stable.

### *Secondary structure*

Figure 2 illustrates the secondary structure prediction, showing that most structures included random coil, extended strand, and alpha helix.

### *Tertiary structure prediction*

Figure 3 shows the tertiary structures of the reference and selected sequences predicted by 3 reliable software. The Qmean and Ramachandran plot results are summarized in Table 1.

The percentage of possible regions (favored, allowed, and outlier) showed that around 90% of residues were in the favored region for (PS)2-v2. Using Discovery Studio, five loops were found on the L1 structure; the results are summarized and illustrated in Figure 4.

## Discussion

HPV infection is a major concern for healthcare systems in many countries (24). Several studies have determined the prevalence of HPV genotypes in Iranian patients with different grades of cervical lesions; Mortazavi et al conducted a study in 2002 on 100 patients with uterine cervical carcinomas; they found the majority (73.9%) of HPV-positive tumours contained HPV-16. The rest

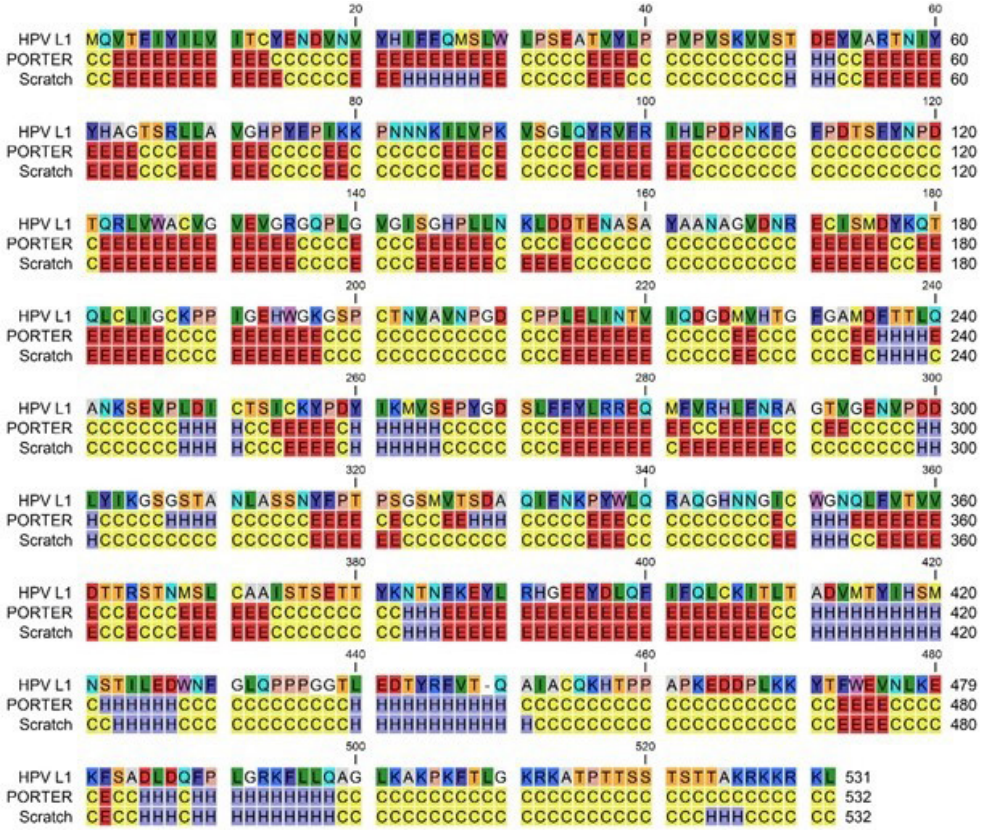


**Figure 2.** Secondary structure of L1 protein predicted by Porter and Scratch. C: coil, E: extended strand, H: helix

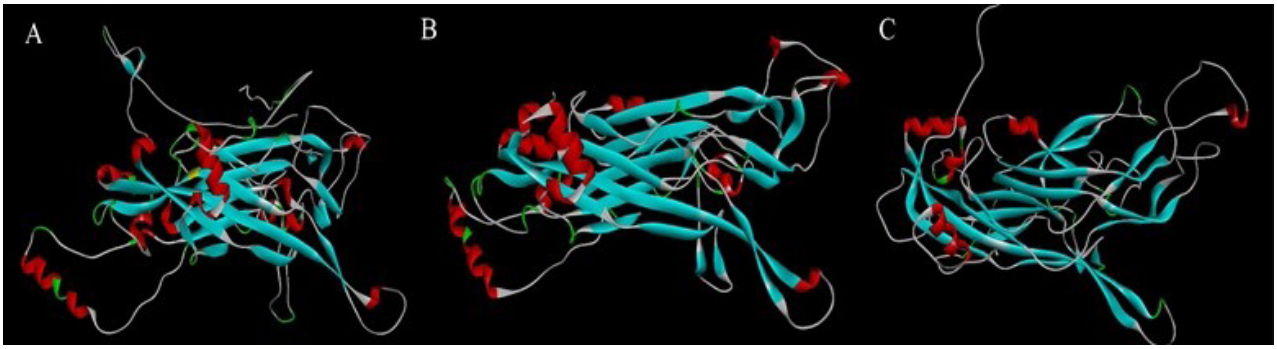


**Figure 3.** Tertiary structures predicted by I-TASSER (A), (PS)2-v2 (B), Phyre2 (C)

**Table 1.** Ramachandran plot and Qmean results for selected and reference sequences

| Tools | Ramachandran plot | | | Qmean |
|---|---|---|---|---|
| | Favored | Allowed | Outlier | Qmean score |
| I-TASSER | 79.2% | 14.40% | 6.40% | -8.79 |
| (PS)2-v2 | 95.4% | 4.2% | 0.4% | -3.73 |
| Phyre2 | 85.6% | 10.4% | 4.0% | -7.7 |

(11.6%) demonstrated type 18 (25). In 2014, Yousefzadeh et al. studied 851 Iranian women aged 18–65 years; they concluded that HPV infection among Iranian females was higher than the previous estimates reported in Iran. The prevalence of HPV-16 and 18 was 7.3% and 2.8%, respectively (26). A total of 436 Iranian women with different cervical lesions or malignancies were investigated by Salehi-Vaziri et al from 2011 to 2013; in 45.4% of cases, HPV infection was detected, and HPV-16 (32.8%) was the most common HR-HPV genotype (16). In the present study, all selected sequences and reference sequences belonged to HPV-16.

Yoshiyuki Ishii, in 2003 described 6 positions (C175, C185, C428, C161, C229, and C379) in the L1 HPV-16 protein with a vital role in the assembly and integrity of L1 capsids through intramolecular bonding (27). Our analysis showed that there were no changes in the mentioned positions and they were completely conserved.

Teimoori et al in 2008 and Hajmohammadi et al in 2016 expressed papillomavirus 16 L1 protein in *Escherichia coli* and showed the stability of this protein in this host (28,29). Furthermore, in 2011, Coimbra et al. succeeded in producing this protein in a eukaryotic host, *Pichia pastoris*, as an integrative vector (30). Finally, in 2013, Abdoli et al used *Spodoptera frugiperda* (Sf9) cells to express the L1 protein (31). In agreement with the findings from previous studies, our results confirmed the stability of this protein in prokaryotic and eukaryotic cells as well as the thermostability of this protein; also, this indicates that the expression in various hosts can be used to develop self-assembled VLP vaccines as well as diagnostic tests.

Bioinformatics has provided reliable tools to predict virus proteins (32,33). Structural analysis of the L1 protein showed several different loops between strands and several α-helices. Analysis showed mutations could not affect this structure significantly. Many studies have indicated I-TASSER as the most reliable tool to predict 3D structures; however, the present results determined that (PS)2-v2 constructed the most reliable structure for the L1 protein, which was confirmed by Ramachandran plot and Qmean results.

Suhandono et al in 2014 found five loop regions in the

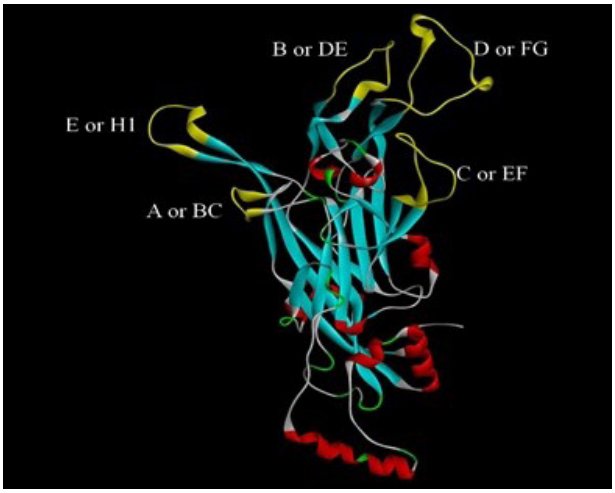


**Figure 4.** HPV L1 3D model structure. Yellow: 5 identified loops, Red: α-helices

model of the HPV L1 (34). Based on Stanley and colleagues' study in 2006, these loops are the exposed antigens, and any shift at the surface of the loop may contribute to changes in the surface antigen determinants and changes in the antibody needed to identify the virus. Each HPV type has specific loop structures and is functionally active regarding antibody binding (35). In 2006, Carter et al showed DE, FG, and HI are the essential regions for binding by neutralizing the antibodies (36). Moreover, Christensen et al in 2001 showed an immunodominant epitope composed of the FG and HI loops (37). Similar to previous studies, the present study showed five distinct loops that are exposed, and in the selected sequences, we found one substitution that can affect neutralizing antibody binding and the effectiveness of antibodies in preventing HPV infections in the FG or D loop.

## Conclusion

To conclude, the results showed L1 was a highly conserved region, and two substitutions, 228 (H to D) and 292 (T to A), did not affect the structure and properties of this protein, which confirmed that the vaccines can still have adequate protection for Iranians. It is suggested that this region should be monitored frequently.

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**Authors' Contribution**

**Conceptualization:** Behzad Dehghani.
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**Supervision:** Ava Hashempour.
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**Writing–original draft:** Ava Hashempour.
**Writing–review & editing:** Elnaz Alamdari.

**Competing Interests**
The authors certify that they have no conflicts of interest.

**Ethical Approval**
This article contains no studies performed on human participants or animals by any of the authors. Shiraz University of Medical Sciences Ethics Committee approved the study (Ethics Code: IR.SUMS.REC.1398.316).

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