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Original Article

Prevalence of Human Papillomavirus (HPV) in patients with Cervical Cancer and Chronic Cervicitis in Isfahan, Iran

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Abstract

Background and Objectives: HPV is classified into cutaneous and mucosal groups based on the infection site and into high-risk and low-risk groups based on its association with cervical cancer. It is estimated that over 80% of sexually active individuals acquire HPV at least once in their lifetime, and numerous new cases of cervical cancer attributed to HPV high-risk types occur each year globally. This study aimed to determine the prevalence of HPV in cervical tumor specimens and chronic inflammation samples in Isfahan, Iran.

Materials & Methods: A total of 122 formalin-fixed, paraffin-embedded (FFPE) tissue samples were collected, including 43 malignant cervical tumors (twenty-one adenocarcinoma, two cervical dysplasia, and two cutaneous squamous cell carcinomas (SCC)) and 79 chronic cervical inflammation samples. After cutting 4-µm sections, the samples were deparaffinized with xylene, viral DNA was extracted using the phenol-chloroform-isoamyl alcohol method. Nested-PCR was performed on the extracted DNAs using universal primers MY09/MY11 and GP5/GP6 which amplify a conserved region of the L1 gene in HPV genome. Second-stage PCR products were visualized by electrophoresis on a 2% agarose gel. Statistical analyses were performed using SPSS and GraphPad Prism software. Four PCR products were randomly selected and sequenced. Sequence alignments were performed in EMBL using WU-BLAST2.

Results: Neither of the two SCC samples showed HPV infection. Among the 21 adenocarcinoma samples, 23.8%, and among 20 cervical dysplasia samples, 40% were HPV-positive. Notably, 98.7% of the chronic cervical inflammation samples were HPV-positive. The sequences of four randomly selected strains were deposited in the GenBank with accession numbers of GQ179958, GQ179959, GQ452049, and GQ452050.

Conclusion: This study highlights the importance of HPV screening in cervical inflammation and precancerous lesions and can help formulate health policies and cervical cancer prevention programs for the Iranian population.

Keywords: HPV, cervical cancer, cervical inflammation.

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Introduction

Papillomaviruses are small, double-stranded DNA viruses that specifically cause infection in squamous epithelium or cells with capability of squamous maturation. Risk factors for HPV infection include gender, age, and the number of sexual partners. In general, women are more susceptible to HPV infection than men, with the highest incidence observed in females, married and unmarried under 20 years of age (1). HPV infection, particularly low-risk types, also affects older women over 55 years, which may be related to the persistence or reactivation of previously acquired infections rather than new exposures. An increased number of full-term pregnancies is associated with a higher risk of invasive cervical carcinoma and chronic HPV infection. This association may be explained by pregnancy-induced immunosuppression, which can favor HPV infection or enhance its carcinogenic potential (2). In addition to lifestyle and nutritional factors, infectious agents play a role in cancer development. Notably, HPV causes some cancers in both men and women (3). HPV infection is a global health challenge, with an estimated prevalence of 11.7% among women without cytological abnormalities. Rates vary by socioeconomic level and, more recently, by the impact of vaccination introduced in 2007. While in countries such as Australia and Britain declines reported in vaccine-type HPV prevalence, high rates have been reported in regions including Sub-Saharan Africa (24%), Eastern Europe (21.4%), Latin America (16.1%), and South-East Asia (14%). The distribution differs in different age groups (1,4).

Cells infected with high-risk HPV exhibit increased replication rates and may progress to precancerous lesions or invasive tumors. Factors such as long-term oral contraceptive use, multiple pregnancies, smoking, immunodeficiency, co-infection with other sexually transmitted diseases, and high-risk HPV types increase the risk of progression from precancerous cervical lesions to cancer. Almost all cervical cancer cases are associated with HPV infection. Beyond cervical cancer, HPV is linked to anogenital cancers (vulva, vagina, penis, anus), head and neck cancers, and genital warts. Globally, high-risk HPV types account for approximately 5% of all cancer cases, with about 570,000 new infections in women and 60,000 in men, annually (3).

The absence of routine screening programs and inadequate vaccination coverage necessitate policy decisions based on local epidemiological data in Iran. Therefore, this study was conducted to determine the prevalence of HPV in patients with cervical cancer and cervicitis in Isfahan, Iran.

Materials and Methods

A) Sample collecting: After obtaining ethical approval (code: IR.IAU.FALA.REC.1404.031) from the Research Ethics Committee of Falavarjan Branch, Islamic Azad University, Isfahan, Iran, a total of 122 formalin-fixed, paraffin-embedded (FFPE) tissue samples were collected from patients diagnosed with uterine cancer or cervicitis (5). Tumor and inflammation samples were obtained from the archives of pathology departments in Isfahan hospitals between 2024 and 2025.

B) Tissue preparation and DNA extraction: A microtome was used to prepare tissue sections. Before sectioning, paraffin blocks were placed at 4°C for 30 minutes to prevent crumbling. All equipment was disinfected with 70% ethanol prior to use. First, 3-µm sections were prepared and stained with hematoxylin and eosin (H&E). Prepared slides were examined under a light microscope for histopathological changes to

confirm the lesion diagnosis. In addition, after preparing the slides, the boundary between normal and tumor tissues was determined by a pathologist. Subsequently, 20-µm sections were prepared, matched with the 3-µm slides, dissected from each sample, and placed into 1.5 mL tubes (6).

For deparaffinization, each sample was placed in 1 ml of xylene for 15 minutes at 59°C in a Thermomix. Samples were then centrifuged at 11000 rpm for 10 min and the supernatant was discarded. This step was repeated three times. Xylene was removed using 100% ethanol followed by centrifugation at 10000 rpm for 10 minutes. After ethanol evaporation, paraffin was separated from the tissue pellet. DNA extraction was performed using the phenol chloroform method. Briefly, 200 µl of ETDA (0.5 M, pH = 8) was added to each tube. Then, 50 µl of 5 M sodium chloride was added and vortexed for 1 minute. Next, 650 µL of lysis buffer was added and vortexed for 1 minute. The tubes were heated at 59°C and 450 rpm for 15 minutes, then 90 µL of proteinase K (10 mg/mL) was added and vortexed for 2 minutes. The mixture was then heated at 59°C at 500 rpm for 1 hour, vortexed for 2 minutes, and reheated at 59°C at 450 rpm for an additional 1 hour. The tubes were centrifuged at 11,000 rpm for 15 minutes, and the supernatant was transferred to new tubes. An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added, gently shaken for 5 minutes, and centrifuged at 8000 rpm for 10 minutes. This step was repeated once more to ensure that proteins and particles were removed. Sodium acetate (pH 5.2) was added to one-tenth of the sample volume and vortexed for 1 minutes. Then, 2-2.5 volumes of cooled ethanol were added and gently mixed. The tubes were incubated at -20°C for 2 hours, then centrifuged at 14000 rpm for 15 minutes at 4°C, and the supernatant was removed. The pellet was washed with 400 µL of 70% ethanol and centrifuged at 10000 rpm for 6 minutes. Finally, the pellet was air-dried for 30–45 min, and 50 µL of Tris-EDTA buffer was added to each tube and incubated overnight at room temperature to dissolve the DNA. DNA quality was assessed by measuring light absorbance at 260 nm and 280 nm, and the A260/A280 ratio was calculated (7).

C) Nested Polymerase Chain Reaction amplification: Nested PCR was performed using external primers My09 (CGTCCM ARRGGAWACTGATC) and My11 (GCMC AGGGWCATAAYAATGG) (8) and internal primers GpF+ (TTTGTTACTGTGGTAGATA CTAC) and GpR+ (GAAAAATAAACTGTAA ATCATATTC). The PCR mixture contained 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTP mix, 0.4 µM each primer, 1 unit of Taq DNA polymerase, and 1 µg of extracted DNA in a total volume of 50 µl. The first-round amplification protocol consisted of initial denaturation at 95°C for 5 minutes; 44 cycles of denaturation at 95°C for 30 seconds, annealing at 62°C for 45 seconds, and extension at 72°C for 45 seconds; followed by a final extension at 72°C for 10 minutes. The amplification protocol for the second-round amplification protocol included an initial denaturation at 95°C for 5 minutes; a 44-time repeated cycles included a denaturation at 95°C for 30 seconds, a primer annealing at 60°C for 45 seconds, and an extension at 72°C for 45 seconds; and a final step at 72°C for 10 minutes (5). All reactions were performed in an Eppendorf thermal cycler. PCR products were visualized by electrophoresis on 2% agarose gels.

D) Nucleotide sequence analysis: To confirm HPV DNA presence, four randomly selected

positive samples (two cervicitis and two malignant samples) were sequenced (GeneService, UK). The resulting sequences were analyzed using the WU-BLAST2 tool at EMBL to confirm homology.

E) Statistical analysis of data: Fisher's exact test was used to compare frequencies, and one paired t-test was used to compare means. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 3.05 (San Diego, CA, USA) and SPSS version 16.0 (Chicago, IL, USA).

Results

A) Pathological features: Of 122 samples, 43 (34.7%) were diagnosed as malignant tumors, and 79 (65.2%) as chronic cervical inflammation. Among malignant samples, 21 were adenocarcinomas, 20 were cervical dysplasias, and 2 were squamous cell carcinomas (SCC). All inflammation samples were from chronic cervicitis cases. Figure 1 shows the microscopic image of a sample with malignant area in an adenocarcinoma lesion in contrast to a sample of glandular epithelium and a sample of squamous epithelium without signs of malignancy.

B) Nested PCR results: Amplification of the conserved L1 gene region produced a 150-bp product visualized on 2% agarose gels (Figure 2). PCR results showed that 78 out of 79 cervicitis samples (98.7%) and 13 out of 43 malignant samples (30.2%) were HPV-positive. Overall, 91 out of 122 samples (74.5%) harbored HPV DNA. Statistical analysis revealed that HPV infection was significantly more frequent in cervicitis samples than in malignant tumors ($p < 0.0001$). Details of the types of tumors and cases of cervicitis along with the PCR results are presented in Figure 3.

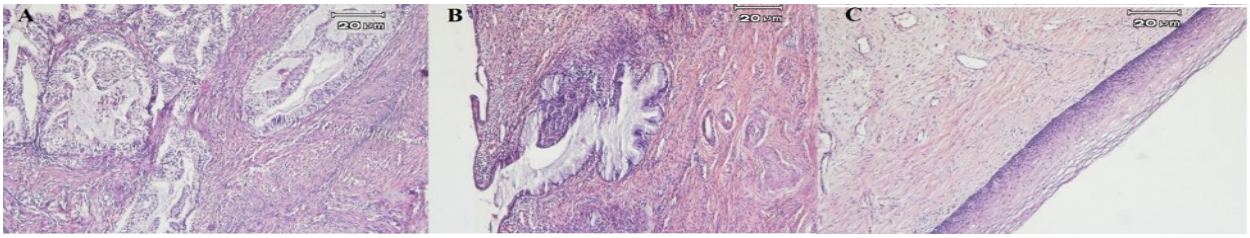

Fig 1. Microscopic images (100 X magnification): a sample with a malignant area with adenocarcinoma lesion (A), glandular epithelium (B), and squamous epithelium without signs of malignancy (C).

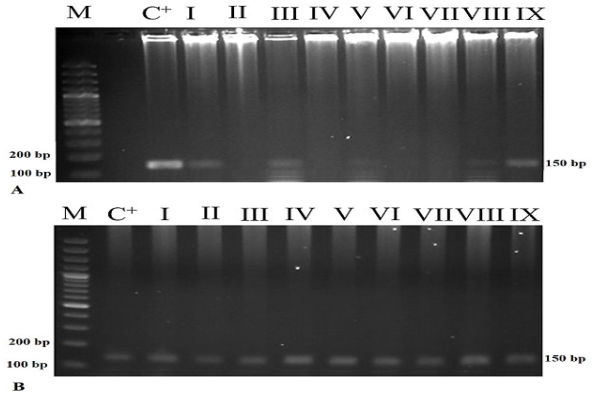

Fig 2. Agarose gel electrophoresis of the amplified fragment of HPV which resulted a 150 bp bound. A: malignant samples. B: chronic cervical inflammation samples. M: 100 bp DNA size marker. C+: positive control. I to IX: positive samples.

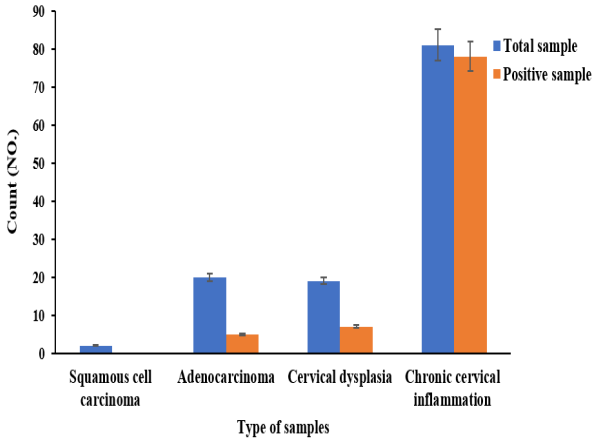

Fig 3. The frequency number of positive nested PCR results in tumor and cervicitis samples.

C) Nucleotide sequence analysis: Four randomly selected HPV sequences (two from cervicitis and two from malignant samples) were deposited in GenBank under accession numbers GQ179958 (strain EL1-R), GQ179959 (strain EL2-R), GQ452049 (strain M3-MF), and GQ452050 (strain M49-MF). Table 1 shows the nucleotide sequence similarity among the four HPV isolates. The sequences showed 95-99% homology, with the highest divergence (5%) observed between M49-MF and both EL1-R and EL2-R.

Tables 2-5 show the nucleotide sequence similarity of different HPV strains (obtained from different studies in different parts of the world) with the sequence of PCR products obtained in the present study. The highest similarity of the isolate EL1-R (GQ179958) was to the strain EU911303 with 97% identity (Table 2), and the highest similarity of the isolate EL2-R (GQ179959) was to the strain EU911303 with 97% identity (Table 3). The highest similarity of the isolate M3-MF (GQ452049) was seen to the strains GQ179958, GQ179958, and DQ31226 with 93% similarity (Table 4), and the highest similarity of the isolate M49-MF (GQ452050) was seen to the strain EU911303 with 97% similarity (Table 5).

Table 1. The similarity report of nucleotide sequences of 4 HPV isolates.

Isolated strains	EL1-R (GQ799958)	EL2-R (GQ799959)	M3-MF (GQ452049)	M49-MF (GQ452050)
EL1-R (GQ799958)	-	-	-	-
EL2-R (GQ799959)	2%	-	-	-
M3-MF (GQ452049)	1%	1%	-	-
M49-MF (GQ452050)	5%	5%	4%	-

Table 2. The similarity report of the isolate EL1-R (GQ179958) obtained from NCBI GenBank.

Alignment	DB-ID (Transact-SQL)	Source	Length	Score	Identity (%)
1	EU911303	Human papillomavirus isolate 06JAN_PHL_MY099_08 L1 capsid protein (L1) gene, partial cds.	409	658	97
2	S73503	L1 [human papillomaviruse type 6 HPV6, Genomic, 449 nt].	449	649	96
3	EU779746	Human papillomavirus type 6 strain SRB198AK L1 protein (L1) gene, partial cds.	419	649	96
4	EU911508	Human papillomavirus isolate 06JAN_PHL_MY164_03 truncated L1 capsid protein (L1) gene, partial cds.	411	649	96
5	EU911529	Human papillomavirus isolate 06JAN_PHL_MY168_02 L1 capsid protein (L1) gene, partial cds.	411	649	96
6	EU911319	Human papillomavirus isolate 06JAN_PHL_MY106_03 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
7	EU911507	Human papillomavirus isolate 06JAN_PHL_MY164_01 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
8	EU911510	Human papillomavirus isolate 06JAN_PHL_MY164_05 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
9	EU911511	Human papillomavirus isolate 06JAN_PHL_MY164_06 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
10	EU911516	Human papillomavirus isolate 06JAN_PHL_MY164_15 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96

Table 3. The similarity report of the isolate EL2-R (GQ179959) obtained from NCBI GenBank.

Alignment	DB-ID (Transact-SQL)	Source	Length	Score	Identity (%)
1	EU911303	Human papillomavirus isolate 06JAN_PHL_MY099_08 L1 capsid protein (L1) gene, partial cds.	409	658	97
2	S73503	L1 [human papillomaviruse type 6 HPV6, Genomic, 449 nt].	449	649	96
3	EU779746	Human papillomavirus type 6 strain SRB198AK L1 protein (L1) gene, partial cds.	419	649	96
4	EU911508	Human papillomavirus isolate 06JAN_PHL_MY164_03 truncated L1 capsid protein (L1) gene, partial cds.	411	649	96
5	EU911529	Human papillomavirus isolate 06JAN_PHL_MY168_02 L1 capsid protein (L1) gene, partial cds.	411	649	96
6	EU911319	Human papillomavirus isolate 06JAN_PHL_MY106_03 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
7	EU911507	Human papillomavirus isolate 06JAN_PHL_MY164_01 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
8	EU911510	Human papillomavirus isolate 06JAN_PHL_MY164_05 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
9	EU911511	Human papillomavirus isolate 06JAN_PHL_MY164_06 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
10	EU911516	Human papillomavirus isolate 06JAN_PHL_MY164_15 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96

Table 4. The similarity report of the isolate M3-MF (GQ452049) obtained from NCBI GenBank.

Alignment	DB-ID (Transact-SQL)	Source	Length	Score	Identity (%)
1	EU911469	Human papillomavirus isolate 06JAN_PHL_MY152_02 L1 capsid protein (L1) gene, partial cds.	413	229	75
2	EU911449	Human papillomavirus isolate 06JAN_PHL_MY145_12 truncated L1 capsid protein (L1) gene, partial cds.	410	230	84
3	GQ179958	Human papillomavirus isolate Isfahan EL1R L1 protein gene, partial cds.	139	419	93
4	GQ179959	Human papillomavirus isolate Isfahan EL2R L1 protein gene, partial cds.	139	419	93
5	DQ312263	Human papillomavirus type 6 isolate 36 L1 capsid protein gene, partial cds.	101	419	93
6	S73503	L1 [human papillomaviruse type 6 HPV6, Genomic, 449 nt].	449	401	91
7	EU779746	Human papillomavirus type 6 strain SRB198AK L1 protein (L1) gene, partial cds.	419	401	91
8	EU911322	Human papillomavirus isolate 06JAN_PHL_MY106_06 truncated L1 capsid protein (L1) gene, partial cds.	412	401	91
9	EU911508	Human papillomavirus isolate 06JAN_PHL_MY164_03 truncated L1 capsid protein (L1) gene, partial cds.	411	401	91
10	EU911529	Human papillomavirus isolate 06JAN_PHL_MY168_02 L1 capsid protein (L1) gene, partial cds.	411	401	91

Table 5. The similarity report of the isolate M49-MF (GQ452050) obtained from NCBI GenBank.

Alignment	DB-ID (Transact-SQL)	Source	Length	Score	Identity (%)
1	EU911303	Human papillomavirus isolate 06JAN_PHL_MY099_08 L1 capsid protein (L1) gene, partial cds.	409	658	97
2	S73503	L1 [human papillomaviruse type 6 HPV6, Genomic, 449 nt].	449	649	96
3	EU779746	Human papillomavirus type 6 strain SRB198AK L1 protein (L1) gene, partial cds.	419	649	96
4	EU911508	Human papillomavirus isolate 06JAN_PHL_MY164_03 truncated L1 capsid protein (L1) gene, partial cds.	411	649	96
5	EU911529	Human papillomavirus isolate 06JAN_PHL_MY168_02 L1 capsid protein (L1) gene, partial cds.	411	649	96
6	EU911319	Human papillomavirus isolate 06JAN_PHL_MY106_03 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
7	EU911507	Human papillomavirus isolate 06JAN_PHL_MY164_01 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
8	EU911510	Human papillomavirus isolate 06JAN_PHL_MY164_05 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
9	EU911511	Human papillomavirus isolate 06JAN_PHL_MY164_06 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96
10	EU911516	Human papillomavirus isolate 06JAN_PHL_MY164_15 truncated L1 capsid protein (L1) gene, partial cds.	410	649	96

Discussion

HPV infections can be detected by identifying viral DNA, RNA, or proteins in infected tissues. Among these, viral DNA detection in lesions is the most common approach (9). In this study, DNA extraction was performed using the phenol-chloroform-isoamyl alcohol method. Despite recent advances in commercial DNA extraction kits for FFPE tissues, challenges such as DNA degradation, formalin-induced crosslinking, and poor amplification of long fragments persist. Several studies have shown that PCR success of FFPE samples is directly related to target fragment length, with fragments shorter than 200–300 bp showing the highest amplification success (10,11). Additionally, tissue type, formalin fixation duration, storage temperature, and paraffin block age affect extracted DNA quality (12,13). Therefore, the phenol-chloroform-isoamyl alcohol method used here is a logical, cost-effective approach for archival samples with limited tissue volume. In this study, two SCC samples were examined, and neither showed HPV infection. However, due to the small sample size (n=2), no definitive conclusion can be drawn regarding the role of HPV in SCC. Among 21 adenocarcinoma and 20 cervical dysplasia samples, 23.8% and

40% were HPV-positive, respectively in rates lower than those reported globally (14). Differences in HPV prevalence and type distribution across populations may reflect cultural and behavioral factors, age at first sexual intercourse, number of partners, host immunity, and access to screening and vaccination (15). Studies from the Middle East and North Africa report lower HPV prevalence than Europe and North America, though this may be underestimated due to underreporting, screening limitations, and study design differences (14). Host genetic factors and differences in innate and adaptive immune responses may also influence HPV clearance and cervical neoplasia (16). Therefore, population-specific studies, including in Iran, are needed to guide future research. Recent evidence indicates that HPV prevalence varies widely globally, depending on demographic, regional, and lesion type factors.

Conclusion

In this study, neither SCC sample showed HPV infection. However, due to the small sample size, no definitive conclusion can be drawn regarding HPV's role in SCC. HPV prevalence in adenocarcinoma and precancerous lesions was lower than international reports, likely due to environmental, behavioral, or genetic risk factors unique to the Iranian population, some of which remain unidentified. Conversely, a high percentage of cervicitis samples were HPV-positive, a finding that deviates from global statistics and may reflect different transmission dynamics or underlying factors in Iran. These findings underscore the importance of HPV screening in cervical inflammation and precancerous lesions and highlight the need for tailored prevention programs in Iran.

Conflicts of interest

The authors declare that there are no conflicts of interest associated with this manuscript.

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