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ORIGINAL ARTICLE

# Human papillomavirus infection and its association with lung cancer: a case-control study (2011–2019)

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## ABSTRACT

**Background and Objectives:** Human papillomavirus (HPV) causes about 4.5% of all new human cancers. The purpose of this study was to look into the prevalence of various HPV types in patients with lung cancer.

**Materials and Methods:** The study included a cohort of 61 individuals who had received treatment at Imam Khomeini Hospital in Ahvaz between 2011 and 2019. Paraffin-embedded tissues were used for molecular analysis. The primary goal was to assess the differences in HPV prevalence between lung cancer patients and a control group, using a Nested PCR assay followed by sequencing.

**Results:** Among the lung cancer patients, HPV DNA was detected in 10 individuals, while three individuals in the control group were also positive (16.3% versus 12.0%, P=0.65). Notably, every detected HPV variant was classified as the high-risk type 16. Additionally, the researchers investigated potential associations between age, sex, smoking habits, and lung cancer in both HPV-positive and negative patients. The study findings revealed that age, sex, and smoking habits did not show statistically significant associations with the presence of HPV (P>0.05). Moreover, Lung cancer incidence was not significantly correlated with HPV infection (P>0.05).

**Conclusion:** Therefore, according to the study's findings, smoking, HPV infection, and lung cancer prevalence were not significantly correlated in the population under investigation.

**Keywords:** Human papillomaviruses; Lung neoplasms; Polymerase chain reaction; Tissues; Iran

## INTRODUCTION

One common and extremely deadly type of cancer is lung cancer. In 2020, It was the most common cause of death and the second most common cancer in terms of prevalence (1). Globally, Lung cancer is predicted to cause 2.1 million new cases and 1.8 million deaths in 2018, making it the leading cause of cancer-related mortality (1). Small-cell lung cancer, squamous cell lung cancer, adenocarcinoma, and large-cell lung cancer are the four primary forms of lung cancer. Small-cell lung cancer and squamous cell lung cancer are more commonly observed in men, while adenocarcinoma is more prevalent in non-smoking women (2).

A number of variables, including the use of tobacco and infectious agents like *Mycobacterium tuberculosis* and possibly human papillomavirus (HPV), are linked to an elevated risk of lung cancer (3, 4). According to a comprehensive meta-analysis con-

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ducted in 2009, 24.5 percent of cases of lung cancer worldwide had HPV (5). However, a more recent systematic review and meta-analysis from 2023 revealed a lower 16.4 percent HPV DNA prevalence in primary lung cancer. It is noteworthy that the specific pathological subtype and geographic location have a substantial impact on the detection of HPV DNA in lung cancer (6). Asian nations have the highest rates of HPV-positive lung cancer cases, with some studies showing a prevalence of over 78% (5).

The Papillomaviridae family comprises over 450 types, approximately 200 of which infect humans (7, 8). Among these, the HPV is the most common cause of STDs worldwide, and it is a major contributing factor to a number of human cancers. Analysis of the global cancer burden reveals that approximately 4.5% of all newly diagnosed cancer cases worldwide are caused by HPV, equating to roughly 630,000 cases annually. This attributable fraction exhibits a substantial gender disparity, representing 8.6% of all cancer cases in women as opposed to 0.8% in men.

The spectrum of HPV-related cancers primarily encompasses the cervix, but also comprises a significant percentage of cancers of the head and neck area and the anogenital tract, which includes the vulva, vagina, penis, and anus (9, 10). Human papillomaviruses are divided into two groups based on their cancer-causing potential: high-risk (HR) and low-risk (LR), with approximately 14 types falling into the HR category. Cervical, anal, penile, and oropharyngeal cancers are just a few of the many cancers that HR-HPVs can cause (8, 11). While many HPV infections are asymptomatic and resolve spontaneously, detection and screening are crucial due to their association with a broad spectrum of cancers, especially cervical cancer, which accounts for approximately 99% of cases (12-14). Among the HR-HPVs, HPV 18 and HPV 16 have the strongest association with the development of cancer. These two types account for about 70% of cervical cancer cases (15) and have an even higher prevalence in other HPV-related cancers. For example, in individuals with oropharyngeal cancer, the prevalence of these two viruses can be as high as 90% (16). In a 2021 meta-analysis conducted by Christian Schulz in Germany, out of 9,385 lung cancer patients, 1,268 cases were found to be HPV-positive, indicating a positivity rate of 13.5% (17).

However, previous findings have been controversial, particularly due to geographical variations and the use of different methods. Therefore, this research aimed to determine whether HPV was present in samples of lung cancer patients from Imam Khomeini Hospital in Ahvaz between 2011 and 2019. Paraffin-embedded lung cancer tissue and control samples were collected, examined, and then subjected to Nested-PCR testing. The extracted DNA was subsequently analyzed by sequencing. Furthermore, viral types were identified using online databases. It is crucial to remember that our research only looked at a small portion of the Iranian population, a demographic that has not received much research attention in relation to this specific subject. As a result, our research advances knowledge about the prevalence of HPV in lung cancer in this specific population.

## MATERIALS AND METHODS

**Inclusion and exclusion criteria.** We have chosen paraffin-embedded tissues of excellent quality that have been diagnosed with small cell lung cancer, squamous cell lung cancer, adenocarcinoma of the lung, and large cell lung cancer for the purpose of molecular analysis. However, we excluded certain samples due to inadequate volumes of tissue and the absence of patient information in their corresponding records.

**Sample and ethics approval.** This case-control study was approved by the Ethics Committee of AJUMS (IR.AJUMS.MEDICINE.REC.1399.004). From February 2011 to March 2019, a total of 86 paraffin-fixed lung tissue samples were chosen from the Imam Khomeini Hospital in Ahvaz's pathology department archive. Among the samples, 61 were lung cancer samples of various types, and 25 were healthy samples (negative for lung cancer). The lung cancer samples consisted of 8 small-cell lung cancer samples, 12 squamous cell lung cancer samples, 4 adenocarcinoma samples, and 1 large-cell lung cancer sample. The remaining 36 samples that did not fit into any of these categories were classified separately. For deparaffinization and rehydration, the tissue samples were divided into 5-micrometer sections and put in microtubes.

**Deparaffinization and rehydration.** The deparaffinization and rehydration protocol was according to the approach published by Kumar et al. (2020),

with minor modifications to optimize for our laboratory conditions (18). The samples were subjected to deparaffinization and rehydration procedures using xylene and graded ethanol solutions (98%, 90%, and 70%, respectively). Each 1 ml microtube received approximately 300-400 µl of xylene, which was then gently mixed for 5 minutes before being centrifuged at 2000 rpm using the Labnet Prism R Refrigerated Micro-Centrifuge C2500-R. Following that, for the rehydration process, the samples were consecutively exposed to ethanol solutions with various concentrations (98%, 90%, and 70%) for 5 minutes each.

**Tissue digestion.** The tissue samples were digested using a lysis buffer and proteinase K (Sina Clone MO5421-Proteinase K). While lysis buffers generally have a similar composition across different sources, there might be variations in details depending on the specific tissue, desired volume, and objective of use (18-20). The following components were employed in this experiment: 10 mM Tris-HCl (pH 8.0), 100 mM EDTA (pH 8.0), 50 mM NaCl, 0.5% SDS, and proteinase K.

The lysis buffer and proteinase K were added to the samples at the same time. Subsequently, For one hour, the samples were incubated at 60°C. Following that, the samples were placed in a shaking incubator (LLSI-3016R LABTECH) overnight. The samples were inspected for tissue digestion, and if all desired tissues were digested, they were prepared for further steps. An extra 10 µl of proteinase K was added to the samples, and they were left in a shaking incubator for the entire night if any discernible tissue remnants remained.

**DNA extraction.** The samples' DNA was extracted using a pH 8 phenol-chloroform-isoamyl alcohol solution (21). After a few minutes of gentle shaking, the samples were centrifuged for 20 minutes at 20,000 rpm. The upper phase was carefully transferred to separate microcentrifuge tubes. In order to aid in the precipitation and sedimentation of nucleic acids (19, 22), an equal volume of absolute ethanol was added to the upper phase, followed by the addition of 10 µl of 1 M NaCl. The samples were then stored overnight at -20°C in a refrigerator. Subsequently, the samples were centrifuged at 20,000 rpm for 30 minutes. The upper liquid was removed, and to remove any remaining alcohol, the microcentrifuge tubes holding the precipitated DNA were left to air dry. Finally, 10 µl of distilled water was added to each sample.

To ensure the quality of the isolated DNA, several quality control methods were employed. Thermo Fisher Scientific's NanoDrop One spectrophotometer was used to assess the amount and purity of the extracted DNA. In particular, UV absorbance at 260 nm was used to measure DNA concentration. In order to identify possible contaminants, such as proteins or organic compounds, purity was evaluated by computing the absorbance ratios A260/A280 and A260/A230. Gel electrophoresis was employed to evaluate DNA integrity and detect impurities. DNA from each sample was stored at -70°C.

**Nested-PCR procedure.** To carry out a sensitive and precise diagnostic test to identify different types of high-risk and low-risk HPV, we used the Nested-PCR test with two pairs of primers: MY09/11 (MY09: CGTCCMARRGGAWACTGATC, MY11: GCMCAGGGWCATAAYAATGG) and GP5/6+ (GP5+: TTTGTTACTGTGGTAGATAC, GP6+: GAAAAATAAACTGTAAATCA). This test aimed to identify the hyper-variable L1 capsid region (23-29).

For the initial PCR, Taq 2X Master Mix Red, 400 ng of DNA sample, deionized water, 1.5 mM MgCl2, and MY09/11 primers were all included in the reaction mixture. Thermal cycling (PeqLab PeqSTAR, Germany) was carried out for ten minutes at 95°C, one minute at 95°C, one minute at 55°C, and one minute at 72°C for extension. After 35 cycles of these procedures, a final extension step was performed for five minutes at 72°C.

Ampliqon Taq 2X Master Mix Red, 1.5 mM MgCl2, deionized water, GP5/6 primers, and 1 or 2 µl of the first run's products made up the reaction mixture for the second PCR cycle. Initial denaturation at 95°C for five minutes, denaturation at 95°C for one minute, annealing at 40°C for one minute, and extension at 72°C for one minute were the thermal cycling conditions. Following a final extension step at 72°C for five minutes, these procedures were repeated 35 times.

PCR amplification and Sanger sequencing were conducted to confirm the presence of specific DNA targets. The resulting PCR products were observed on a 2-D agarose gel for the determination of a range of approximately 150 bp, which indicates HPV DNA. An NTC control (non-template control) was used to prevent contamination in the PCR process. Positive samples were then sequenced using the Sanger se-

quencing method (Niagen Noor Laboratory).

The obtained results and data were assessed and validated using Chromas version 2.5.0, SPSS version 25, and the BLAST software accessible at https://blast.ncbi.nlm.nih.gov/. After alignment of the sequence, it was observed that the 13 HPV16-positive samples shared 100% sequence identity. Two genes were consequently added to the GenBank database. To perform phylogenetic analysis, two Ahvaz strains of the HPV16 L1 gene, with accession numbers OR901497 and OR901498, were employed. With the use of MEGA version 7.0 software, the phylogenetic tree was constructed using the maximum likelihood method.

## RESULTS

This study included a total of 86 participants, who were stratified into a lung cancer group (n=61) and a healthy control group (n=25). The mean age of the entire cohort was 58.63 ± 19.56 years, with a majority of participants (48/86, 55.81%) aged over 60 years. The study population was predominantly male (61/86, 70.93%). Overall, HPV DNA was detected in 15.12% (13/86) of all samples. All 13 positive samples were identified as high-risk HPV-16. A prevalence of 16.3% (10/61) was found within the lung cancer group, while the prevalence in the control group was 12.0% (3/25). Table 1 presents the differences in means and relationships between the variables of age, sex, smoking status, and lung cancer. The analysis's findings showed that smoking and an individual's risk of developing lung cancer were correlated ($p < 0.05$, $r = 0.48$).

The distribution of HPV-16 according to age, sex, smoking status, and lung cancer status was examined in two groups of patients: those with and without the virus. The study's findings demonstrated that the demographic variables of age, sex, and smoking were not significantly related to the presence of the virus in the patients ($p > 0.05$). Additionally, there was no significant correlation ($p > 0.05$) between the virus's presence and the patients' prevalence of lung cancer.

This study also examined the types of cancer in two groups of patients: those with and without the virus. The findings showed that there was no significant correlation ($p > 0.05$) between the presence of the virus and various cancer types. Table 2 displays the analysis's findings.

## DISCUSSION

Given the high prevalence and mortality rate of lung cancer, it is essential to identify the underlying causes in order to enhance preventive measures and treatment strategies. Despite the fact that tobacco use has long been known to be a major risk factor, the number of reported cases among nonsmokers has significantly increased in recent years (30-32). In this study, HPV-16 was detected in 10 of 61 lung cancer samples and in 3 of 25 controls. Nevertheless, there was no significant correlation between the two groups according to the statistical analysis (P value $> 0.05$, $P = 0.65$). Therefore, it is imperative to investigate alternative factors, such as infectious agents.

HPV, known to be implicated in various cancers, may also play a role in lung cancer development. Assessing the presence of HPV in cancerous tissues provides valuable insights for targeted prevention and treatment. However, it is crucial to highlight that the virus's existence in tissues does not necessarily imply a direct association with cancer, as indicated by this study and related investigations (33, 34). Due to the challenges in obtaining samples from healthy individuals or the limited availability of healthy samples, most studies conducted in this field are not case-control studies. Many case-control studies have identified high-risk HPVs in the tissues of healthy individuals (35, 36).

Viral infections display diverse characteristics that directly or indirectly contribute to carcinogenesis. These characteristics include latency, long-term and chronic infections, widespread transmission, potential for reinfection, and the "hit-and-run" hypothesis. The "hit-and-run" hypothesis postulates that viruses initiate cancer development. However, over time, it is thought that the viral genome is completely removed from the host cell, either by an immune response or by multiple mutations being accumulated. If this scenario transpires, it is feasible that many types of cancer, not traditionally linked to viral causes, might have been triggered by a virus, despite the subsequent loss of viral DNA. Even with the utilization of highly sensitive molecular techniques, like nested PCR, it is not possible to trace viral genomes. Although this study detected viral DNA in some samples, the potential role of mechanisms such as 'Hit and Run' in the negative samples cannot be entirely ruled out.

As shown in Fig. 1, the tree demonstrates that

**Table 1.** Frequency and significance of relationships among demographic variables, lung cancer, and types of lung cancer.

| Variables | | Total | Lung Cancer | P | Cancer type | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N (%) | | | Type 1 | Type 2 | Type 3 | Type 4 | Others [a] |
| Mean ± SD | | 58.63 ± 19.56 | 60.1 ± 15.63 | 0.42* | 54.63 ± 20.84 | 58.33 ± 13.16 | 67 ± 13.09 | 53 ± 0 | 60.97 ± 16.24 |
| Age | <40 | 14 (16.28%) | 7 (50%) | | 2 (28.6%) | 1 (14.3%) | 0 (0.0%) | 0 (0.0%) | 4 (57.1%) |
| | 40-60 | 24 (27.91%) | 20 (83.3%) | 0.15** | 3 (15.8%) | 5 (26.3%) | 1 (5.3%) | 1 (5.3%) | 9 (47.3%) |
| | >60 | 48 (55.81%) | 34 (70.83%) | | 3 (9.4%) | 6 (18.8%) | 3 (9.4%) | 0 (0.0%) | 20 (62.4%) |
| Sex | Male | 61 (70.93%) | 43 (70.49%) | | 5 (12.5%) | 8 (20.0%) | 3 (7.5%) | 1 (2.5%) | 23 (57.5%) |
| | Female | 25 (29.07%) | 18 (72.0%) | 0.84** | 3 (16.7%) | 4 (22.2%) | 1 (5.6%) | 0 (0.0%) | 10 (55.6%) |
| Smoker [b] | Yes | 9 (42.86%) | 9 (100.0%) | | 1 (11.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 8 (88.9%) |
| | No | 12 (57.14%) | 6 (50.0%) | 0.01** | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (14.3%) | 6 (85.7%) |

Note: a=3 missing data in cancer type (others), b= The data of only 21 people about smoking or non-smoking were available in the files of patients; p value for *: Independent Samples T-Test **: $\chi 2$ test; type1: small cell lung cancer, type2: squam cell lung cancer, type3: adeno cell lung cancer, type4: large cell lung cancer

**Table 2.** The frequency of the HPV among all individual samples and the studied demographic

| Variables | | Total | Negative | Positive | P-value |
|---|---|---|---|---|---|
| | | N=86 (100%) | N=73 (84.88%) | N=13 (15.12%) | |
| Age | Total | 58.63 ± 19.56 | 58.85 ± 20.2 | 57.25 ± 15.69 | 0.47* |
| | <40 | 14 (16.28%) | 12 (85.7%) | 2 (14.3%) | 0.97** |
| | 40-60 | 24 (27.91%) | 20 (83.3%) | 4 (16.7%) | |
| | >60 | 48 (55.81%) | 41 (85.4%) | 7 (14.6%) | |
| Sex | Male | 61 (70.93%) | 49 (80.3%) | 12 (19.7%) | 0.06** |
| | Female | 25 (29.07%) | 24 (96.0%) | 1 (4.0%) | |
| Smoker [a] | Yes | 9 (42.86%) | 6 (66.7%) | 3 (33.3%) | 0.15** |
| | No | 12 (57.14%) | 11 (91.7%) | 1 (8.3%) | |
| Lung Cancer | Positive | 61 (70.9%) | 51 (83.7%) | 10 (16.3%) | 0.65** |
| | Negative | 25 (29.O%) | 22 (88%) | 3 (12 %) | |
| Cancer type | Type 1 | 8 (13.79%) | 6 (75.0%) | 2 (25.0%) | 0.53*** |
| | Type 2 | 12 (20.69%) | 11 (91.7%) | 1 (8.3%) | |
| | Type 3 | 4 (6.9%) | 4 (100.0%) | 0 (0.0%) | |
| | Type 4 | 1 (1.72%) | 0 (0.0%) | 1 (100.0%) | |
| | Others [b] | 33 (56.9%) | 30 (90.9%) | 3 (9.1%) | |

Note: a= The data of only 21 people about smoking or non-smoking were available in the files of patients. b=3 missing data in cancer type (others)

P-value for *: Mann-Whitney Test, **: $\chi 2$ test, ***: Kruskal-Wallis Test

HPV16 exhibits closer relatedness to the HPV52 L1 gene sequence and the HPV31 L1 gene sequence compared to other HPV types. The tree bifurcates into two primary clades: one encompassing strains from Iran, the Philippines, and other countries, and another consisting of strains from the United States and other countries. The two Iranian strains are closely connected, implying a recent common ancestor. Additionally, the subtree containing the Iranian strains demonstrates close relatedness to a subtree containing strains from the Philippines and Iran, implying that the HPV16 in Ahvaz might have originated from this region. Nonetheless, the study group's 15.1% HPV type 16 prevalence indicates that the virus might be widespread in Ahvaz. To investigate the prevalence of HPV at the provincial level, more research is necessary. Other factors may also influence the role of HPV in cases of lung cancer, such

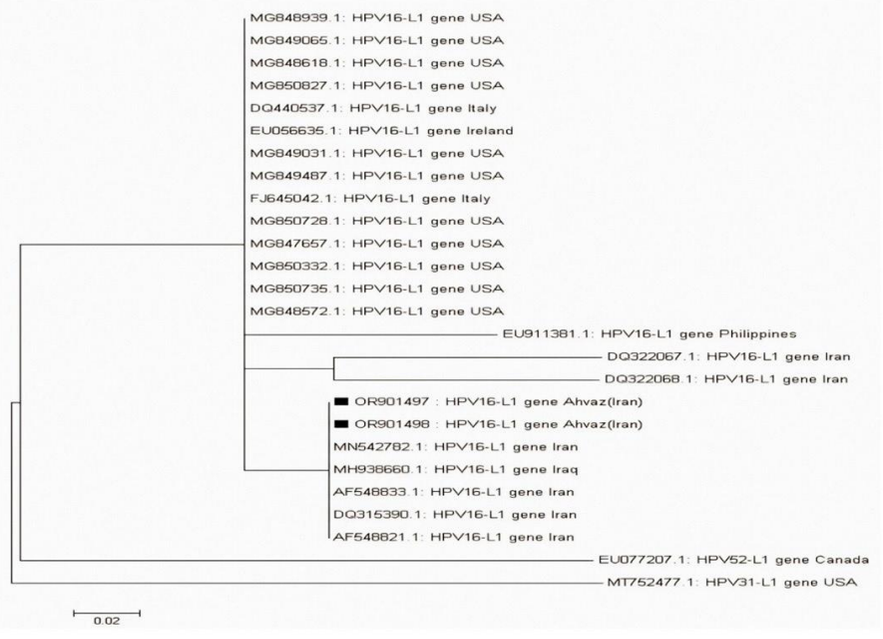


**Fig. 1.** Molecular phylogenetic analysis by Maximum Likelihood method
The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model. The tree displaying the highest log likelihood (-154.13) is presented. The initial trees for the heuristic search were automatically obtained by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The topology with the highest log likelihood value was selected. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 26 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were removed. There was a total of 40 positions in the final dataset. Evolutionary analyses were conducted in MEGA.

as genetic predisposition or the immune system's response to carcinogens.

The connection between HPV infection and lung cancer risk has been investigated in a number of studies. For instance, Chiou et al. (2003) discovered a link between lung cancer and HPV-16/18 infection in Taiwanese women who do not smoke (37). Crusz et al. (2020) suggested that HPV infections may contribute to 5% of all cases of human cancers thus, to highlight the potential impact of HPV on cancer development (38). Ragin et al. (2014) conducted meta-analyses and consistently revealed that the most prevalent genotypes in lung tumors globally are HPV16 and HPV18 (39). Though , Anantharaman et al. (2014) conducted over 50 case studies on HPV DNA presence in the tissues of lung tumors without proving a clear causal link between lung cancer and HPV infections (34). Karnosky et al. (2021) carried out a meta-analysis and systematic review that further support the hypothesis that high-risk oncogenic HPV infections considerably raise the risk of lung cancer, potentially paving the way for prevention through prophylactic vaccines (17). Nie et al. (2022) provide recent evidence suggesting that HPV infection may contribute to angiogenesis in lung cancer, further implicating its role in disease development (40).

On the other hand, studies such as Argyri et al. (2017) declared that there was no correlation between lung cancer and HPV status when using mRNA tests as the most reliable method for assessing the connection between malignant transformation and HPV (41). He et al. (2020) found no evidence of a link between primary lung cancer and HPV infection in the Chinese population (33). Similarly, Kapeu et al. (2010) found no association between HPV16 or HPV18 infections and lung cancer in women (42). Furthermore, a meta-analysis by Sirera et al. (2022) emphasized how HPV prevalence varies greatly across the globe in primary lung cancer specimens and noted that it is still debatable whether HPV infection causes lung cancer (43). In addition, a separate analysis carried out by Park et al. (2007) and Badillo Almaráz et al. (2013) It found that HPV infection prevalence in patients with non-small cell lung cancer varies from

region to region, such as Korea and Mexico, giving information about possible differences in HPV prevalence among various lung cancer populations (44, 45).

These conflicting findings highlight the complexity of the association between HPV infection and lung cancer. While some studies indicate a potential connection, others do not find a significant association. These disparities may be attributed to factors such as regional variations, sample sizes, and methodological differences across studies. the study's outcomes highlight a moderate correlation between smoking and the prevalence of lung cancer (Table 1). Nevertheless, there was no discernible link found between lung cancer and other demographic factors. Furthermore, no correlation was found between demographic variables and HPV infection. Conducting a larger population study with a more substantial number of participants could yield more comprehensive and pertinent results.

It is important to acknowledge that the accuracy and validity of molecular research results may be influenced by the limitations of working with paraffin-embedded tissues. Consequently, these limitations should be considered, and measures should be taken to mitigate their impact. Not incorporating housekeeping genes in the analysis can lead to misleading outcomes, thereby advocating for their inclusion in future molecular research. Although this study obtained high-quality paraffin samples of lung cancer from Imam Khomeini Hospital in Ahvaz throughout the years, it is advisable to collect larger samples from multiple reference hospitals across the country, if feasible. This approach would ensure that the findings are representative of a diverse population and diminish potential biases associated with a single hospital sample.

## CONCLUSION

The association between HPV infection and the risk of lung cancer is still the subject of active debate in academia. Although some studies have suggested a possible association, conflicting results and inconclusive data justify further extensive research efforts to determine the exact role of HPV in the pathogenesis of lung cancer.

In conclusion, some studies suggest a possible association between HPV infection and lung cancer, but the evidence is still inconclusive. Further research is needed to clarify the role of HPV in the development of lung cancer and to identify preventive measures such as prophylactic vaccines.

Lung cancer and HPV-16 infection were not found to be significantly correlated in this case-control study conducted in Ahvaz, Iran. Although a strong link between smoking and lung cancer was confirmed, the etiological role of HPV remains unclear and warrants further extensive investigation in this population. The detection of a high prevalence of HPV-16 underscores the complex and potentially indirect nature of this relationship.

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## REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; 68: 394-424.
2. Brambilla E, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. *Eur Respir J* 2001; 18: 1059-1068.
3. Fol M, Koziński P, Kulesza J, Białecki P, Druszczyńska M. Dual Nature of Relationship between Mycobacteria and Cancer. *Int J Mol Sci* 2021; 22: 8332.
4. Prabhu PR, Jayalekshmi D, Pillai MR. Lung cancer and human papilloma viruses (HPVs): examining the molecular evidence. *J Oncol* 2012; 2012: 750270.
5. Klein F, Amin Kotb WF, Petersen I. Incidence of human papilloma virus in lung cancer. *Lung Cancer* 2009; 65: 13-18.
6. Li X, Ling Y, Hu L, Zhang L, Lin S, Zhang X, et al. Detection of Human papillomavirus DNA, E6/E7 messenger RNA, and p16INK4a in lung cancer: A Systematic review and Meta-analysis. *J Infect Dis* 2023; 228:

1137-1145.
7. McBride AA. Human papillomaviruses: diversity, infection and host interactions. *Nat Rev Microbiol* 2022; 20: 95-108.
8. Mehta K, Laimins L. High-Risk Human Papillomaviruses and DNA Repair. *Recent Results Cancer Res* 2021; 217: 141-155.
9. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. *Int J Cancer* 2017; 141: 664-670.
10. Shapshak P, Sinnott JT, Somboonwit C, Kuhn JH (2015). Global Virology I - Identifying and Investigating Viral Diseases. New York: Springer.
11. Ferrari FA, Magni F, Bosco M, Biancotto G, Zorzato PC, Laganà AS, et al. The role of micronutrients in Human Papillomavirus infection, Cervical Dysplasia, and Neoplasm. *Healthcare (Basel)* 2023; 11: 1652.
12. Rai R, Nahar M, Jat D, Gupta N, Mishra SK. A systematic assessment of stress insomnia as the high-risk factor for cervical cancer and interplay of cervicovaginal microbiome. *Front Cell Infect Microbiol* 2022; 12: 1042663.
13. Dunne EF, Park IU. HPV and HPV-Associated Diseases. *Infect Dis Clin North Am* 2013; 27: 765-778.
14. Yu YQ, Hao JQ, Mendez MJG, Mohamed SB, Fu SL, Zhao FH, et al. The Prevalence of cervical HPV infection and genotype distribution in 856,535 Chinese women with normal and abnormal Cervical Lesions: A Systemic Review. *J Cytol* 2022; 39: 137-147.
15. Ebrahimi N, Yousefi Z, Khosravi G, Malayeri FE, Golabi M, Askarzadeh M, et al. Human papillomavirus vaccination in low- and middle-income countries: progression, barriers, and future prospective. *Front Immunol* 2023; 14: 1150238.
16. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological agents. *IARC Monogr Eval Carcinog Risks Hum* 2012; 100: 1-441.
17. Karnosky J, Dietmaier W, Knuettel H, Freigang V, Koch M, Koll F, et al. HPV and lung cancer: A systematic review and meta-analysis. *Cancer Rep (Hoboken)* 2021; 4(4): e1350.
18. Kontham SS, Walter CEJ, Raju M, Mohanapriya CD, Karuppasamy N, Shankaran ZS, et al. An Effective and viable DNA extraction protocol for FFPE tissues and its effect on downstream molecular application. *Am J Biochem Biotechnol* 2020; 16: 148-161.
19. Hearn RP, Arblaster KE. DNA extraction techniques for use in education. *Biochem Mol Biol Educ* 2010; 38: 161-166.
20. Pikor LA, Enfield KS, Cameron H, Lam WL. DNA Extraction from paraffin embedded material for genetic and epigenetic analyses. *J Vis Exp* 2011; (49): 2763.
21. Liu AW, Villar-Briones A, Luscombe NM, Plessy C. Automated phenol-chloroform extraction of high molecular weight genomic DNA for use in long-read single-molecule sequencing. *F1000Res* 2022; 11: 240.
22. Seiler C, Sharpe A, Barrett JC, Harrington EA, Jones EV, Marshall GB. Nucleic acid extraction from formalin-fixed paraffin-embedded cancer cell line samples: a tradeoff between quantity and quality? *BMC Clin Pathol* 2016; 16: 17.
23. Depuydt CE, Boulet GA, Horvath CA, Benoy IH, Vereecken AJ, Bogers JJ. Comparison of MY09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types. *J Cell Mol Med* 2007; 11: 881-891.
24. Nilyanimit P, Chansaenroj J, Poomipak W, Praianantathavorn K, Payungporn S, Poovorawan Y. Comparison of four human Papillomavirus Genotyping methods: Next-generation Sequencing, INNO-LiPA, Electrochemical DNA Chip, and Nested-PCR. *Ann Lab Med* 2018; 38: 139-146.
25. Lim YK, Choi JH, Park S, Kweon OJ, Park AJ. Comparison of three different commercial kits for the Human Papilloma Virus Genotyping. *J Clin Lab Anal* 2016; 30: 1110-1115.
26. Clifford GM, Vaccarella S, Franceschi S, Tenet V, Umulisa MC, Tshomo U, et al. Comparison of Two Widely Used Human Papillomavirus Detection and Genotyping Methods, GP5+/6+-Based PCR Followed by Reverse Line Blot Hybridization and Multiplex Type-Specific E7-Based PCR. *J Clin Microbiol* 2016; 54: 2031-2038.
27. Dastjerd NT, Abbasian L, Mohebbi SR, Banaem LM, Ashtiani MF, Rostamafshar Z, et al. Prevalence and distribution of human papillomavirus genotypes in women living with HIV: a cross-sectional study in Iran. *BMC Infect Dis* 2025; 25: 1471.
28. Bzhalava D, Eklund C, Dillner J. International standardization and classification of human papillomavirus types. *Virology* 2015; 476: 341-344.
29. Nakagawa T, Shigehara K, Kato Y, Kawaguchi S, Nakata H, Nakano T, et al. Are bladder washing samples suitable for investigation of HPV infection in urinary bladder? Comparison in HPV prevalence between urine and washing samples. *J Med Virol* 2023; 95(1): e28110.
30. Jiang YJ, Chao CC, Chang AC, Chen PC, Cheng FJ, Liu JF, et al. Cigarette smoke-promoted increases in osteopontin expression attract mesenchymal stem cell recruitment and facilitate lung cancer metastasis. *J Adv Res* 2022; 41: 77-87.
31. Li YJ, Tsai YC, Chen YC, Christiani DC. Human papilloma virus and female lung adenocarcinoma. *Semin Oncol* 2009; 36: 542-552.
32. Li M, Xu Y, Zhang Z, He Z, Yu X, Deng Z, et al. Association between human papillomavirus and EGFR mutations in advanced lung adenocarcinoma. *Oncol Lett*

2016; 12: 1953-1958.
33. He F, Xiong W, Yu F, Xiao R, Ye H, Li W, et al. Human papillomavirus infection maybe not associated with primary lung cancer in the Fujian population of China. *Thorac Cancer* 2020; 11: 561-569.
34. Anantharaman D, Gheit T, Waterboer T, Halec G, Carreira C, Abedi-Ardekani B, et al. No causal association identified for human papillomavirus infections in lung cancer. *Cancer Res* 2014; 74: 3525-3534.
35. Carpagnano GE, Koutelou A, Natalicchio MI, Martinelli D, Ruggieri C, Di Taranto A, et al. HPV in exhaled breath condensate of lung cancer patients. *Br J Cancer* 2011; 105: 1183-1190.
36. Giuliani L, Jaxmar T, Casadio C, Gariglio M, Manna A, D'Antonio D, et al. Detection of oncogenic viruses SV40, BKV, JCV, HCMV, HPV and p53 codon 72 polymorphism in lung carcinoma. *Lung Cancer* 2007; 57: 273-281.
37. Chiou HL, Wu MF, Liaw YC, Cheng YW, Wong RH, Chen CY, et al. The presence of Human Papillomavirus type 16/18 dna in blood circulation may act as a risk marker of lung cancer in taiwan. *Cancer* 2003; 97: 1558-1563.
38. Crusz SM, El-Shakankery K, Miller RE. Targeting hpv in gynaecological cancers – current status, ongoing challenges and future directions. *Womens Health (Lond)* 2020; 16: 1745506520961709.
39. Ragin C, Obikoya-Malomo M, Kim S, Chen Z, Flores-Obando R, Gibbs D, et al. HPV-associated lung cancers: an international pooled analysis. *Carcinogenesis* 2014; 35: 1267-1275.
40. Nie Z, Zhang K, Li Z, Bing X, Jin S, Li M. Human papillomavirus 16 E6 promotes angiogenesis of lung cancer via SNHG1. *Cell Biochem Biophys* 2023; 81: 325-336.
41. Argyri E, Tsimplaki E, Marketos C, Politis G, Panotopoulou E. Investigating the role of human papillomavirus in lung cancer. *Papillomavirus Res* 2017; 7-10.
42. Simen-Kapeu A, Surcel HM, Koskela P, Pukkala E, Lehtinen M. Lack of association between human papillomavirus type 16 and 18 infections and female lung cancer. *Cancer Epidemiol Biomarkers Prev* 2010; 19: 1879-1881.
43. Sirera G, Videla S, Saludes V, Castellà E, Sanz C, Ariza A, et al. Prevalence of HPV-DNA and E6 mRNA in lung cancer of HIV-infected patients. *Sci Rep* 2022; 12: 13196.
44. Park MS, Chang YS, Shin JH, Kim DJ, Chung KY, Shin DH, et al. The prevalence of human papillomavirus infection in Korean non-small cell lung cancer patients. *Yonsei Med J* 2007; 48: 69-77.
45. Badillo-Almaraz I, Zapata-Benavides P, Saavedra-Alonso S, Zamora-Avila D, Reséndez-Pérez D, Tamez-Guerra R, et al. Human papillomavirus 16/18 infections in lung cancer patients in Mexico. *Intervirology* 2013; 56: 310-315.