

## Original Article

# No Evidence for Human Papillomavirus in Patients with HIV in Iran

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### Abstract

**Background and Aims:** Human Papillomaviruses (HPVs) have an established role in the development of cervical cancer. However, the presence of underlying conditions such as HIV/AIDS is a factor for the development of this malignancy. This study was aimed to evaluate the prevalence of HPV DNA in plasma samples from HIV-positive patients in Tehran, Iran.

**Materials and Methods:** Plasma specimens from 95 patients diagnosed with HIV infection from Tehran's hospitals were examined for the presence of HPV DNA by means of a Real-Time polymerase chain reaction (PCR) assay with the amplification of a fragment of L1 region of the HPV genome. Furthermore, HIV viral load testing was performed for all patients using the COBAS TaqMan assay.

**Results:** Out of 95 patients, 59 (62%) and 36 (38%) of the cases were males and females, respectively. The mean age of the patients was  $37.42 \pm 11.25$  (range 2–69) years. The mean HIV viral load of all patients with HIV was 73010.754 copies per ml. None of the 95 HIV-positive patients tested had HPV DNA detected in their plasma by Real-Time PCR assay.

**Conclusions:** Previous studies have suggested that patients with HIV infection are at risk for acquiring HPV infection. However, we have shown no evidence of HPV DNA in plasma samples of patients with HIV.

**Keywords:** HPV, Human Papillomavirus, HIV, Human Immunodeficiency Virus, Iran

### Introduction

HPV infection has been associated with the development of cervical cancer and it also causes major disease in man, including genital warts, penile cancer, anal cancer and certain oropharyngeal cancers (1). HPV-related diseases occur at high rate in human immunodeficiency virus (HIV)-infected patients. The reason for this is an HIV-impaired immune system which helps persistent HPV infection, leading to an

increased risk for the development of HPV-related Malignancies (2, 3).

Papillomaviruses require proliferating basal layer cells and must access the basal cell layer of the epidermis or mucosa to establish infection (4, 5). Hence, the transmission of HPV is believed to be facilitated by the presence of abraded or macerated epithelial surfaces (6, 7). HIV infected individuals have been observed to get high incidence of both these malignancies, as well as increased rate of genital warts (8, 9). Immunosuppression condition is the main reason of the appearance of HPV infection. Recently, HIV infection is considered as a pandemic health problem, especially in developing countries (10).

HIV can infect CD4+ cells and reduce both number and function of them. In this way, HIV leads to increased risk of other infections such as HPV infection (11). HPV is a common

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sexually transmitted disease (STD) in the world that is the main etiologic cause for cervical and anal cancer. Also, HPV can be a cofactor in acquisition of HIV infection (12). HIV infection has increased the incidence of HPV infection, increasing the risk to cervical neoplasia. Some reports have shown that HIV-positive individuals have a higher incidence of infection with many types of HPV, than the HIV-negative persons (13-15). In general, incidence of HPV infection can be increased with progressive decline of CD4+ cells (16).

Several recent studies have shown that HPV DNA can be found in circulating blood, including peripheral blood mononuclear cells (PBMCs), sera, plasma and arterial cord blood (17-19). Yet, more than 100 various HPV genotypes have been discovered, of which more than 40 have been identified in the anogenital area. Usually detecting of HPV infection is almost entirely based on molecular techniques, which are generally PCR-based. Universal PCR primers have been developed that detect a wide range of HPV genotypes in a single PCR (20, 21). In present study, we aimed to evaluate the presence DNA of most common HPV genotypes in plasma specimens from Iranian patients with HIV by Real-Time PCR assay.

### Methods

**Study population and samples.** Between February 2015 and April 2016, 95 patients with HIV infection referred to the Tehran's hospitals (affiliated to Iran University of Medical Sciences, Tehran, Iran) were enrolled in this study. Exclusion criteria were patients who had been receiving anti-retroviral therapy. The study received ethical approval from the local research ethics committee of Iran University of Medical Sciences, Tehran, Iran. The participants of the current study were acquainted about all aspects of the current study, and informed consent was obtained from all of the participants prior to their enrollment. All whole blood samples were collected into sterile EDTA-containing vacutainer tubes and plasma samples were separated from whole

blood via centrifugation followed by freezing procedure at -20 °C until further analysis.

**DNA Extraction.** HPV DNA was extracted from plasma using a Viral Nucleic Acid Extraction Kit (Yekta Tajhiz Azma, Iran) according to the manufacturer's instructions. After extraction, DNA in solution was stored at -20°C for subsequent analysis.

**Real-Time PCR.** After DNA extraction, Real-Time PCR was performed with the QIAGEN's Real-Time PCR cycler (Rotor-Gene Q 2plex Platform, QIAGEN Co, Germany) instrument. We used SYBR-Green PCR master mix (Maxima® SYBR Green qPCR Master Mix (2X), Applied Fermentas, EU). MY09 (5'CGT CCM AAR GGA WAC TGA TC3') and MY11 (5'GCM CAG GGW CAT AAY AAT GG3') oligonucleotides with a annealing temperature of 55 °C, which flanks a sequence of about 450 pb as a general primers were used to amplify the L1 gene to evaluate the presence of any HPV DNA in the. Primer sequences and amplification conditions were selected according to the previous study (5). The MY09-MY11 primer set is the most frequently used amplification systems that capable of amplifying a wide range of HPV types. We used two genotypes 16 and 18 as positive controls in this study.

**HIV-1 viral load quantification.** Quantification of HIV-1 RNA was performed by COBAS TaqMan (Roche Diagnostics, USA), according to the manufacture's recommendation. This technique is based on the use of dual-labeled hybridization probe in Real-Time PCR assay, which targets the highly conserved region of HIV-1 gag gene.

**Statistical analysis.** All demographic and experimental data were entered and analyzed using Statistical Package for Social Sciences software version 18.0 (SPSS Inc., Chicago, IL, USA). The frequency of qualitative variables and difference between groups were compared by the Fisher's exact and independent T tests respectively. For all comparisons, p-values lower than 0.05 ( $P \leq 0.05$ ) were considered statistically significant.

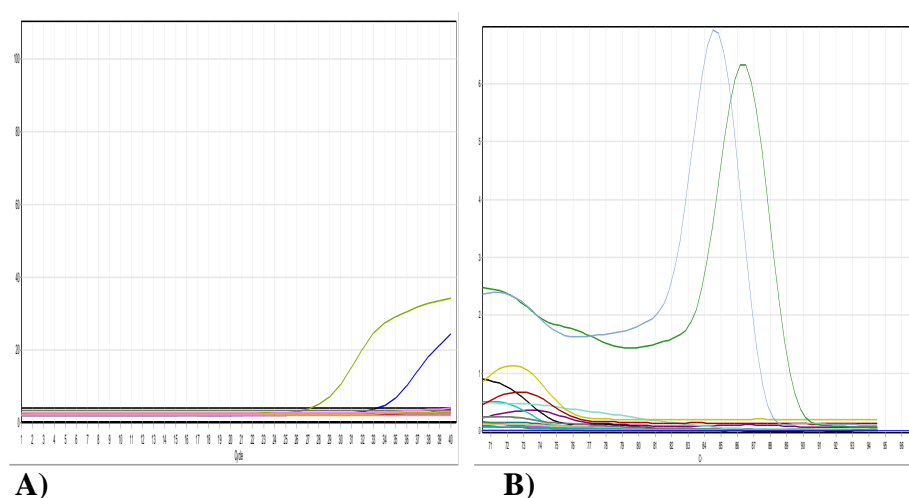
## Results

A total of 95 patients with HIV infection were enrolled in the study. The demographic data of the patients showed that 59 (62%) of the cases were males and 36 (38%) were females (Table 1). The mean age of the patients was  $37.42 \pm$

11.25 years, with a wide range (2-69 years) of age distribution. The mean HIV viral load of all patients with HIV was 73010.754 copies per ml. Results showed that none of the 95 HIV-positive patients tested had HPV-DNA detected in their plasma by Real-Time PCR assay (Fig 1).

**Table 1:** Demographic and viral load characteristics of the 95 subjects included in the study

Age Groups	Gender	n	Viral Load (Copies/ml)	Total (Copies/ml)
Less than 20 year	Male	6	39426.83	33801.14
	Female	1	47	
20-30 year	Male	15	59458.26	41979.49
	Female	7	4525	
31-40 year	Male	18	41852.72	39656.53
	Female	17	37331.17	
41-50 year	Male	12	49183.66	78782.71
	Female	6	137980.83	
51-60 year	Male	6	479746	319846.33
	Female	3	47	
Upper than 60 year	Male	2	8526.5	22795
	Female	2	37063.5	
Total		95		73010.754



**Fig. 1.** A: Graph showing cycle threshold at 32th and 38th cycles for positive controls and cycle threshold of negative samples. B: Melting curve analysis of the SYBR Green real-time PCR products after 40 amplification cycles for HPV. Graph showing dissociation curve peak at 78 °C. The shallow peaks before 70 °C represent primer dimer. No peaks of amplification products from negative samples were observe. We used two genotypes 16 and 18 as positive controls in this study.

### Discussion

Our study is the first report of molecular prevalence of HPV in plasma samples collected from HIV-infected patients in Tehran, Iran. Results of the present survey have shown no evidence of HPV DNA in plasma samples of patients with HIV. According to several studies, HIV-infected women can be considered as high risk group for HPV infection. However, the prevalence and genotype distribution varies in different regions of the world (2, 22). The transmission of HPV is similar to HIV, through sexual contact. These facts suggest a need for more studies on HIV infected risk groups who can be co- or superinfected with HPV. Oncogenic HPVs have a well-established role in the development of all invasive cervical cancers (23).

Patients with HIV have a higher risk for anal cancer compared to the general population, and the risk is higher for HIV-positive homosexual males. Identification of genital HPV infection in men is difficult to evaluate because it depends on the anatomical site, sampling methods, sample, and HPV DNA diagnostic methods. The most prevalence of HPV infection in males is detected in anogenital areas and in men who have sex with men. The presence of HPV infection has furthermore been associated with the acquisition of HIV infection. The oral HPV infection has not been detected. The rate of oral HPV differs from 0 to 70% in normal oral mucosa, ranges from 0 to 85% in potentially malignant oral syndromes and rates from 0 to 100% in oral malignancies. This difference is due to changes like the sampling variation and limitations of the molecular techniques used in different studies (24, 25). The results of other studies revealed the presence of HPV infection in cancers that occur at sites other than the site of initial infection such as colorectal, lung and breast cancer (26). Presence of HPV infection in non-genital cancers leads to the question that how HPV got to the site of cancer. This is due to the possibility that HPV can enter the bloodstream and spread to internal organs as a

possible route of infection (27). In support this possibility, HPV DNA has been found in peripheral blood mononuclear cells (PBMCs), sera and plasma. The binding ability of HPVs to a wide range of cells from different species has been previously reported. HPV appears to be attached to the PBMCs by a cell surface protein (17-19).

The prevalence of HPV DNA in the circulation of patients with cervical cancer was controversial in previous studies. In Thailand, Pornthanakasem et al. reported that only 6 out of 50 (12%) of HPV positive patients were demonstrated to have circulating HPV DNA in plasma by PCR amplification (28). On the other hand, Emmanuelle Jeannot et al. reported a high frequency (87%) of circulating HPV DNA in plasma of cervical cancer patients (19). The observed difference can be resulted from the different populations, different sample types, and different detection methods used. In study by Dong et al., quantitative PCR identified HPV-16 or HPV-18 E7 DNA in 11 out of 175 (6.9%) of plasma samples in patients with invasive cervical cancer (29). Until now, it remains unclear what the source of HPV DNA in plasma. However, the presence and quantity of HPV DNA in plasma may be a reflection of tumor load or metastasis (19).

### Conflicts of interest

All contributing authors declare no conflicts of interest.

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### References

1. Lee CH, Lee SH, Lee S, Cho H, Kim K-H, Lee JE, et al. Anal Human Papillomavirus Infection among HIV-Infected Men in Korea. *PloS one*. 2016;11(8):e0161460.
2. Martins AES, Lucena-Silva N, Garcia RG, Welkovic S, Barboza A, Menezes MLB, et al. Prevalence of human papillomavirus infection, distribution of viral types and risk factors in

cervical samples from human immunodeficiency virus-positive women attending three human immunodeficiency virus-acquired immune deficiency syndrome reference centres in northeastern Brazil. *Mem Inst Oswaldo Cruz*. 2014;109(6):738-47.

3. Park IU, Palefsky JM. Evaluation and management of anal intraepithelial neoplasia in HIV-negative and HIV-positive men who have sex with men. *Curr Infect Dis Rep*. 2010;12(2):126-33.

4. Ghasemian E, Monavari S, Irajian GR, Jalali Nodoshan M, Roudsari RV, Yahyapour Y. Evaluation of human papillomavirus infections in prostatic disease: a cross-sectional study in Iran. *Asian Pac J Cancer Prev*. 2013;14(5):3305-8.

5. Yahyapour Y, Shamsi-Shahrabadi M, Mahmoudi M, Motevallian A, Siadati S, Shefaii S, et al. High-risk and low-risk human papillomavirus in esophageal squamous cell carcinoma at Mazandaran, Northern Iran. *Pathol Oncol Res*. 2013;19(3):385-91.

6. Salehi-Vaziri M, Sadeghi F, Alamsi-Hashiani A, Haeri H, Monavari SH, Keyvani H. Merkel cell polyomavirus and human papillomavirus infections in cervical disease in Iranian women. *Arch Virol*. 2015;160(5):1181-7.

7. Salehi-Vaziri M, Sadeghi F, Hashemi FS, Haeri H, Bokharai-Salim F, Monavari SH, et al. Distribution of human papillomavirus genotypes in Iranian women according to the severity of the cervical lesion. *Iran Red Crescent Med J*. 2016;18(4).

8. Nasserli S, Monavari SH, Keyvani H, Nikkhoo B, Roudsari RV, Khazeni M. The prevalence of Human Papilloma Virus (HPV) infection in the oligospermic and azospermic men. *Med J Islam Repub Iran*. 2015;29:272.

9. Salehi-Vaziri M, Sadeghi F, Bokharai-Salim F, Younesi S, Alinaghi S, Monavari SH, et al. The prevalence and genotype distribution of human papillomavirus in the genital tract of males in Iran. *Jundishapur J Microbiol*. 2015;8(12).

10. Fedrizzi EN, Laureano JK, Schlup C, Campos MO, Menezes ME. Human papillomavirus (HPV) infection in HIV positive women of Florianopolis, state of Santa Catarina, Brazil. *J bras Doenças Sex Transm*. 2011;23(4):210-4.

11. Palefsky JM. Cervical human papillomavirus infection and cervical intraepithelial neoplasia in women positive for human immunodeficiency virus in the era of highly active antiretroviral therapy. *Curr Opin Oncol*. 2003;15(5):382-8.

12. Ferenczy A, Coutlée F, Franco E, Hankins C. Human papillomavirus and HIV coinfection and the risk of neoplasias of the lower genital tract: a

review of recent developments. *Can Med Assoc J*. 2003;169(5):431-4.

13. Centers for Disease Control and Prevention. AIDS-indicator conditions diagnosed in patients reported in 1996, by age group, United States. *HIV/AIDS Surveillance Rep*. 1997;8:18.

14. Ellerbrock TV, Chiasson MA, Bush TJ, Sun X-W, Sawo D, Brudney K, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *Jama*. 2000;283(8):1031-7.

15. Harris TG, Burk RD, Palefsky JM, Massad LS, Bang JY, Anastos K, et al. Incidence of cervical squamous intraepithelial lesions associated with HIV serostatus, CD4 cell counts, and human papillomavirus test results. *Jama*. 2005;293(12):1471-6.

16. Konopnicki D, Manigart Y, Gilles C, Barlow P, De Marchin J, Feoli F, et al. High-risk human papillomavirus infection in HIV-positive African women living in Europe. *J Int AIDS Soc*. 2013;16(1).

17. Bodaghi S, Wood LV, Roby G, Ryder C, Steinberg SM, Zheng Z-M. Could human papillomaviruses be spread through blood? *Journal of clinical microbiology*. 2005;43(11):5428-34.

18. Diaz Jr LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Microbiol*. 2014;32(6):579-86.

19. Jeannot E, Becette V, Campitelli M, Calméjane MA, Lappartient E, Ruff E, et al. Circulating human papillomavirus DNA detected using droplet digital PCR in the serum of patients diagnosed with early stage human papillomavirus-associated invasive carcinoma. *J Pathol*. 2016;2(4):201-9.

20. Coutlée F, Gravitt P, Kornegay J, Hankins C, Richardson H, Lapointe N, et al. Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. *J Clin Microbiol*. 2002;40(3):902-7.

21. Venceslau EM, Bezerra MM, Lopes ACM, Souza ÉV, Onofre ASC, Melo CMd, et al. HPV detection using primers MY09/MY11 and GP5+/GP6+ in patients with cytologic and/or colposcopic changes. *J Bras Patol Med Lab*. 2014;50(4):280-5.

22. da Silva L, Miranda A, Batalha R, Ferreira L, Santos M, Talhari S. High-risk human papillomavirus and cervical lesions among women living with HIV/AIDS in Brazilian Amazon, Brazil. *Braz J Infect Dis*. 2015;19(6):557-62.

23. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;2003(348):518-27.

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24. Gaester K, Fonseca LA, Luiz O, Assone T, Fontes AS, Costa F, et al. Human papillomavirus infection in oral fluids of HIV-1-positive men: prevalence and risk factors. *Sci Rep.* 2014;4.
25. Hoory T, Monie A, Gravitt P, Wu T-C. Molecular epidemiology of human papillomavirus. *J Formos Med Assoc.* 2008;107(3):198-217.
26. Burd EM. Human papillomavirus and cervical cancer. *Clin Microbiol Rev.* 2003;16(1):1-17.
27. Bosch FX, Lorincz A, Muñoz N, Meijer C, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol.* 2002;55(4):244-65.
28. Pornthanakasem W, Shotelersuk K, Termrungruanglert W, Voravud N, Niruthisard S, Mutirangura A. Human papillomavirus DNA in plasma of patients with cervical cancer. *BMC cancer.* 2001;1(1):2.
29. Dong SM, Pai SI, Rha S-H, Hildesheim A, Kurman RJ, Schwartz PE, et al. Detection and quantitation of human papillomavirus DNA in the plasma of patients with cervical carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2002;11(1):3-6.