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The Frequency of High-Risk and Low-Risk Human Papillomavirus Genotypes in Different Grades of Cervical Lesions in Shiraz, South-West of Iran

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ABSTRACT

Background and Aim: Determination of HPV genotypes in cervical lesions is essential in screening programs and the impact of current HPV vaccines. Therefore, we investigated the frequency of various HPV genotypes among different grades of cervical lesions.

Materials and Methods: In total, 101 biopsy samples of different grades of cervical lesions were collected. Extracted DNA was subjected to PCR amplification of beta-globin to assess sample integrity. Duplex Taq-Man Real-Time PCR was performed to identify HPV types 16 and 18. Nested PCR following sequencing was done on those samples with HPV16/18 negative results.

Results: The mean age of participants was 43±1. Of 101 samples, 91 (90%) were infected with HPVs. In the LSIL group, HPV genotypes 16, 18, and 51; in the HSIL group, genotypes 6, 16, 18, 31, and 53; in the SCC group, genotypes 6, 16, 18, and 3; and in the AD group, HPV genotypes 16 and 18 were detected. Also, 49 (61%) samples had co-infection with HPV16 and HPV18.

Conclusion: Our results showed that HPV 16 and 18 were the most prevalent genotypes among different grades of lesions. The presence of low-risk HPV types, including genotypes 6 and 53, might need more attention in HPV screening and prevention programs.

Keywords: Human papillomavirus, Cervical lesion, Genotype, Iran

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1. Introduction

Epidemiological studies try to find the association between different genotypes of human papillomavirus (HPV) infections and the development of cervical cancer **(1)**. Studies have demonstrated that the association between HPV and cervical cancer is stronger for some oncogenic HPV types called high-risk types, including genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 **(1-3)**.

HPV is a small double-stranded DNA virus belonging to the papillomaviridae family **(4)**. The mechanism of carcinogenicity of HPV depends on virus integration into host DNA and expression of the oncoproteins E6

and E7, which disrupt the functions of the tumor suppressor proteins p53 and pRb, respectively **(5-7)**. HPV infection with high-risk types such as HPV16 and HPV18 has been reported in almost 70% of cervical lesions, from the low-grade squamous intraepithelial lesion (LSIL) to squamous cell carcinoma (SCC) **(8)**. Cervical cancer is the fourth most prevalent malignancy, with an incidence rate of 526 cases per 10000 people **(9)**. In Iran, cervical cancer is the twelfth most common cause of cancer, with an incidence rate of 2.5 cases per 100,000 individuals **(10)**.

The frequency of HPV genotypes varies in different regions as HPV-16 is the most prevalent genotype, followed by HPV18, 45, 31, and 33 worldwide. Also, in Iran, the most frequent high-risk and low-risk genotypes were HPV-16 and 51, HPV-6 and 11, respectively **(11)**. In another research in Iran, HPV 16 was determined as the most common type, followed by HPV 18 in all grades of cervical lesions **(12)**.

Moreover, in the USA and Europe, the most prevalent genotype responsible for cervical cancer are types 16, 18, 31, and 45 **(13)**. Furthermore, it has been reported that among the population of south China, types 52, 16, 58, 68, and 33 have the greatest prevalence **(14)**. Besides, HPV co-infection by multiple genotypes has been reported to occur in 10% to 20% of cases **(15)**. Investigating HPV genotype prevalence would be beneficial in setting up a proper screening program and developing prophylactic vaccines based on the prevalent genotypes **(16)**.

Highly efficient and lifesaving prophylactic HPV vaccines are available against cervical cancer, including Cervarix (HPV 16 and 18), Gardasil quadrivalent **(6, 11, 16, and 18)** and Gardasil nonavalent (6, 11, 16, 18, 31, 33, 45, 52, and 58) **(17)**. However, the frequency of high-risk HPV types varies in different geographical regions; therefore, it can influence the potential impact of HPV vaccines in preventing cervical cancer. Accordingly, in this study, we investigated the frequency of HPV genotypes among women with cervical high and low-grade cervical lesions as well as squamous/adeno cell carcinoma biopsies. The findings of This study could be helpful for health policy in future HPV screening and vaccination programs.

2. Materials and Methods

Sample Selection and Tumor Classification

In this study, 101 formalin-fixed paraffin-embedded biopsy samples of different grades of the cervical lesion, including LSIL, high-grade squamous intraepithelial cell lesion (HSIL), SCC and Adenocarcinoma (ADC), were collected from archives of the department of pathology, Motahari Clinic of Shiraz University of Medical Sciences, Shiraz, Iran, during 2014-2015. Hematoxylin and eosin slides were retrieved, and the classification of different histologic subtypes of the cervix, including cervical intraepithelial neoplasia (CIN) I, II, III, and SCC, was reconfirmed by the pathologist according to the WHO Classification of Tumors of the Female Genital Tract classification system **(18)**.

Sample Preparation and DNA Extraction

Ten µm-thick sections of Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were subjected to deparaffinization as described previously **(19)**. In brief, xylene was added to microtubes containing the samples, vortexed vigorously, incubated, and centrifuged (two times). Then the pellet was washed twice with 96% ethanol, vortexed, and centrifuged. The tubes were incubated at 42°C for evaporation of residual ethanol. After deparaffinization, genomic DNA was extracted by using the QIAamp FFPE Tissue Kit (QIAGEN-Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was stored at -20°C until analysis.

Evaluation of the quality of extracted DNA

After DNA extraction, to check the quality of extracted DNA, a PCR test was carried out to detect the 111 bp product of the human β-globin as described previously **(7)**.

Duplex Taq-Man Real-time PCR for Detection of HPV 16 and 18

Positive β-globin samples were processed to identify high-prevalence HPV genotypes, including HPV 16/18, using an in-house duplex real-time PCR described previously **(20)**. Positive β-globin samples were processed to identify high-prevalence HPV genotypes, including HPV 16/18, using an in-house duplex real-time PCR, as described previously. Briefly, real-time PCR was performed using Ampliqon real Q Plus 2x Master Mix for the probe and each HPV genotype probe and primer. In each run, HPV16 and HPV18 standard curve uses a plasmid containing the HPV16 or HPV18 *E7* gene, with 10-fold serial dilutions (ranging from 3×10^7 to 30 copies for HPV16 and 3×10^6 to 30 copies of HPV18) used.

Nested PCR Assays and Sequencing

An in-house nested PCR assay using HPV general MY09/11 primers followed by GP5+/6+ primers was done on negative samples for HPV16/18 by duplex real-time assay. PCR amplification was performed as previously described **(7)**. Briefly, PCR amplification was performed in a reaction containing 100-200 ng of DNA template, 2.5 mM of MgCl2, 10 pmol of each primer (GP5+/6+), and 50 mM of each dNTP and 2 U of Taq DNA polymerase. The PCR amplification cycles

were included as follows: initial 3-min denaturation at 94°C, followed by 45 cycles of 94°C for 30 s, 50°C for 45 s, and 72°C for 1 min, and a final extension period of 5 min at 72°C. The PCR products were run on a 1.5% agarose gel. In order to determine the genotype, a 150 bp PCR product indicating the partial L1 gene fragment amplification was introduced into bidirectional sanger sequencing with the help of GP5+ and GP6+ primers

Genotype Determination by Sequence Analysis

The sequenced fragments from samples were aligned with confirmed HPV reference sequences that were available in Gene Bank databases, and their similarity was investigated by using the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/blast/).

Statistical Analysis

The results were analyzed using SPSS 16 (SPSS Inc., Chicago, IL., USA) software. Fisher's exact test was used for data analysis, and a P-value less than 0.05 was considered statistically significant.

3. Results

Sample Selection and Tumor Classification

Out of 101 samples, 32.6% (n=33) were confirmed to be LSIL grade, 41.5% (n=42) were HSIL grade, 22.7% (n=23) were SCC, and 2.9% (n=3) were adenocarcinoma. The mean age of patients was 42±12 years. The mean age of LSIL, HSIL, SCC, and adenocarcinoma were 40±13, 38.5±12, 50±12, and 67±7, respectively. A statistically significant difference was observed between older age and higher lesion grades (P<0.001).

HPV Genotyping

The Duplex real-time PCR assay revealed that out of 101 samples, 24 (23.8%) and 10 (9.9%) specimens were positive for HPV16 and 18. Also, 49 (48.5%) of the samples had a co-infection of HPV16/18. Moreover, nested PCR assays following sequence analysis showed that two samples were infected with HPV type 6, 3 with type 31, one with type 51, 2 with type 53 (low-risk HPV), and 3 with type 16. Out of 33 LSIL samples, 26 (78%) were infected with HPV genotype 16, 18, or 51, and out of 42 HSIL, 39 (93%) were infected with genotype 16, 18, 6, 31, or 53. All 23 SCC samples were infected with HPV 16, 18, 6, or 31, and all 3 ADC samples had HPV16/18 co-infection. Totally, 7 LSIL and 3 HISL samples were negative for HPV (Table 1). This analysis also showed that while 97.2 % of samples were infected with high-risk HPV types (16, 18, 31, and 51), the low-risk HPV types (type 6 and 53) were detected only in 2.9% of samples.

Table 1. Frequency of HPV genotypes in each grade of cervical lesion

HPV genotypes	LSIL	HSIL	SCC	AD	Total	P-value
6	0/33 (0%)	1/42(2.4%)	1/23(4.3)	0/3(0%)	2/101(1.98%)	0.623
16	5/33(15.2%)	13/42(31%)	6/23(26%)	0/3(0%)	24/101(23.7%)	0.503
18	4/33(12.1%)	3/42(7.1%)	3/23(13%)	0/3(0%)	10/101(9.9%)	0.813
31	0/33(0%)	2/42(4.8%)	1/23(4.3%)	0/3(0%)	3/101(2.97%)	0.649
51	1/33(3%)	0/42(0%)	0/23(0%)	0/3(0%)	1/101(0.99%)	0.568
53	0/33(0%)	2/42(4.8%)	0/23(0%)	0/3(0%)	2/101(1.98%)	0.434
16 and 18 co-infection	16/33(48.5%)	18/42(42.9)	12/23(52.2%)	3/3(100%)	49/101 (48.5%)	0.787
HPV negative	7/33(21.2%)	3/42(7.1%)	0/23(0%)	0/3(0%)	10/101(9.9%)	0.093
Total	33(100%)	42(100%)	23(100%)	3(100%)	101(100%)	

4. Discussion

Results of molecular epidemiology of HPV genotypes are necessary to devise the appropriate preventive or diagnostic strategies for cervical cancer in different areas. This study determined the distribution of different HPV genotypes among patients with different grades of cervical lesions of LSIL, HSIL, SCC, and ADC in southwest Iran.

The results showed that overall HPV prevalence among patients with LSIL, HSIL, SCC, and ADC is 90%. Following this study's results, HPV DNA has been reported in 100% of the premalignant and malignant lesions in Brazil **(21)**, 95% in the United States **(22)**, 94% in Zambia **(23)** and 88% in Pakistan **(24)**. Moreover, in Iran, HPV DNA was detected in 87.1% of cervical cancer samples **(25)**. Also, in Shiraz, Iran, in two separate

studies, 79.5% (26) and 73% **(27)** of CINs and SCC samples were infected with different types of HPV.

We also found that with the progression and severity of cervix lesions, the frequency of HPV increases from 78% in LSIL to 93% in HSIL and 100% in SCC/ADC. In agreement with our results, Heydari *et al.* **(28)** reported the prevalence of HPV was 44.5% in LSIL, 92.3% in HSIL, and 98.2% in invasive cervical cancer. Moreover, Jalilvand *et al.* reported that HPV prevalence in Iranian women with LSIL, HSIL, and invasive cervical cancer was 65.3%, 71.8%, and 77.4%, respectively **(29)**. The lower prevalence of HPV in all grades of their study might be due to the lower sensitivity of the molecular method used for HPV detection.

The results of our study also showed that HPV 16 with 72 % (24% mono-infection and 48.5% co-infections with HPV18) frequency was the most prevalent HPV genotype in all grades of the cervical lesion. The frequency of HPV 16 in LSIL was 63.7% (15.2% mono-infection and 48.5% co-infection with HPV18),74% in HSIL patients (31% mono-infection and 42.9% co-infection with HPV18),78.3% in SCC (26.1% mono-infection and 52.2% co-infection with HPV 18) and 100% in ADC patients (all have HPV 16 and 18 co-infection). The second rank in frequency was HPV 18 with 10% mono-infection and 48.5 co-infections with HPV16. Research done by Ciapponi *et al.* **(30)** revealed that 46.5% and 8.9% of HSIL cases harbored HPV 16 and HPV18, and 53.2% and 13.2% of invasive cervical lesions harbored HPV16 and HPV18.

The investigation of non-HPV16/18 presence in samples showed that HPV 31(3%), HPV 53(2%), HPV 6 (2%) and HPV 51(1%) were detected by sequencing. Jalilvand *et al.* **(29)** and Ghaffari *et al.* **(27)** Reported that HPV -16, 18, 6/11, 31, and 33 are the most frequent types in all grades of cervical lesions in Iran. Moreover, in another study from Iran, Ahmadi *et al.* showed that the most frequent HPV types in LSIL and HSIL specimens were HPV-18 and 6, but in SCC grade, HPV-16 was the most frequent genotype **(16)**. Furthermore, according to a meta-analysis, about 70% of invasive cervical cancer are caused by HPV 16 and/or 18 worldwide **(29)**.

In contrast to our results, a study in shiraz reported that HPV18 (41%), 16 (29.49%), 45 (24.36%), and 39(5.13%) were the most common HPV genotypes **(26)**. On the other hand, Jalilvand *et al.*, in a meta-analysis, reported that the frequency of HPV types in different grades of cervical lesions is low. They reported that 37.5% and 16.3% of LSIL cases, 47.6% and 16.9% of HSIL cases, and 50.1% and 14.4% of SCC cases were infected with HPV type 16 and 18 were lower than our findings. They assumed these types must be more prevalent than extrapolated from past reports based on the low sensitivity of molecular assays.

Regarding the HPVs co-infection, we found that 48.5% (49/101) of all positive HPV samples were infected with HPV 16 and 18, simultaneously. Similar to this finding, de Jesus *et al.* reported that 75.4% (43/57) of cervical biopsies had HPV 16/18 co-infection in Brazil **(21)**. Ghaffari *et al.* also reported this co-infection in 29% (2/7) of SCC samples **(27)**. The importance of HPV co-infections, particularly oncogenic types, is the increased risk of progressing to cervical cancer compared with mono-infection **(31)**. Besides, we see a higher prevalence of co-infections in all grades than mono infections. Therefore, in the single genotype detection PCR method, co-infections of high-risk HPV might be missed, samples with lower virus load might be neglected, and a false negative result may be reported.

Regarding low frequent HPV genotypes, two samples with HSIL lesions were infected with HPV53. In a study in Korea, Yung-Taek *et al.* found that HPV 53 was the most prevalent genotype in healthy women **(1)**. Halec *et al.* reported that mono-infection of HPV 53 in the cervical lesion could activate the same cellular pathways as carcinogenic HR-HPV types; therefore, those women infected with this genotype may finally progress to cervical cancer **(32)**. Also, we found that 2.4% of HSIL grade and 4.2% of SCC grade are infected with HPV type 6 as a low-risk HPV type. In this accordance, Heydari *et al.* reported that 1.9% of SCC grade were infected with HPV6 **(28)**.

5. Conclusion

Our results show that HPV 16 and 18 were the most prevalent genotype among different grades. HPV genotypes 31, 51, and 53 were also detected in a few cases, which might need more attention in HPV screening and prevention programs. Besides, our study demonstrated that more sensitive molecular methods could give more accurate results about HPV genotype prevalence, especially in multiple HPV infections. Furthermore, a higher frequency of co-infections in all grades may imply the importance of using duplex real-time PCR assay

Limitations

The limitations of our study were the relatively small sample size for different studied groups, particularly the ADC group, which can be overcome in future studies by considering a larger sample size.

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Conflict of Interest

The authors declared no conflict of interest.

Reference

1. Ouh YT, Min KJ, Cho HW, Ki M, Oh JK, Shin SY, et al. Prevalence of human papillomavirus genotypes and precancerous cervical lesions in a screening population in the Republic of Korea, 2014-2016. J Gynecol Oncol. 2018;29(1):e14. [DOI:10.3802/jgo.2018.29.e14] [PMID] [PMCID]
2. Humans IWGotEoCRt, Meeting IWGotEoCRtH, Organization WH, Cancer IAfRo. Human papillomaviruses: World Health Organization; 2007.
3. Grahovac M, Račić I, Hadžisejdić I, Dorić A, Grahovac B. Prevalence of human papillomavirus among Croatian women attending regular gynecological visit. Coll Antropol. 2007;31(2):73-7.
4. Aziz H, Iqbal H, Mahmood H, Fatima S, Faheem M, Sattar AA, et al. Human papillomavirus infection in females with normal cervical cytology: Genotyping and phylogenetic analysis among women in Punjab, Pakistan. Int J Infect Dis. 2018;66:83-9. [DOI:10.1016/j.ijid.2017.11.009] [PMID]
5. Münger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, et al. Mechanisms of human papillomavirus-induced oncogenesis. J Virol. 2004; 78(21):11451-60. [PMID] [PMCID] [DOI:10.1128/JVI.78.21.11451-11460.2004]
6. Mahmoudvand S, Safaei A, Erfani N, Sarvari J. Presence of Human Papillomavirus DNA in Colorectal Cancer Tissues in Shiraz, Southwest Iran. Asian Pac J Cancer Prev. 2015;16(17):7883-7. [DOI:10.7314/APJCP.2015.16.17.7883] [PMID]
7. Bakhtiyrizadeh S, Hosseini SY, Yaghobi R, Safaei A, Sarvari J. Almost Complete Lack of Human Cytomegalovirus and Human papillomaviruses Genome in Benign and Malignant Breast Lesions in Shiraz, Southwest of Iran. Asian Pac J Cancer Prev. 2017;18(12):3319-24.
8. Eghbali SS, Amirinejad R, Obeidi N, Mosadeghzadeh S, Vahdat K, Azizi F, et al. Oncogenic human papillomavirus genital infection in southern Iranian women: population-based study versus clinic-based data. Virol J. 2012; 9(1):194. [DOI:10.1186/1743-422X-9-194] [PMID] [PMCID]
9. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol. 2017;3(4):524-48. [DOI:10.1001/jamaoncol.2016.5688] [PMID] [PMCID]
10. Bruni L, Barrionuevo-Rosas L, Albero G, Aldea M, Serrano B, Valencia S, et al. Human papillomavirus and related diseases in the world. Summary report. 2015;20140822.
11. Sabet F, Mosavat A, Ghezeldasht SA, Basharkhah S, Shamsian SAA, Abbasnia S, et al. Prevalence, genotypes and phylogenetic analysis of human papillomaviruses (HPV) in northeast Iran. Int J Infect Dis. 2021;103:480-8. [DOI:10.1016/j.ijid.2020.12.015] [PMID]
12. Salavatiha Z, Farahmand M, Shoja Z, Jalilvand S. A meta-analysis of human papillomavirus prevalence and types among Iranian women with normal cervical cytology, premalignant lesions, and cervical cancer. J Med Virol. 2021;93(8):4647-58. [DOI:10.1002/jmv.26928] [PMID]
13. Harro CD, Pang Y-YS, Roden RB, Hildesheim A, Wang Z, Reynolds MJ, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. J Natl Cancer Inst. 2001;93(4):284-92. [DOI:10.1093/jnci/93.4.284] [PMID]
14. Taghizadeh E, Taheri F, Abdolkarimi H, Ghorbani Renani P, Gheibi Hayat SM. Distribution of Human Papillomavirus Genotypes among Women in Mashhad, Iran. Intervirology. 2017;60(1-2):38-42. [DOI:10.1159/000477848] [PMID]
15. Hwang H-S, Park M, Lee S-Y, Kwon K-H, Pang M-G. Distribution and prevalence of human papillomavirus genotypes in routine pap smear of 2,470 Korean women determined by DNA chip. Cancer Epidemiol Biomark Prev. 2004;13(12): 2153-6. [DOI:10.1158/1055-9965.2153.13.12]
16. Ahmadi S, Goudarzi H, Jalilvand A, Esmaeilzadeh A. Human Papilloma Virus Genotype Distribution in Cervical lesions in Zanjan, Iran. Asian Pac J Cancer Prev. 2017;18(12):3373-7.

17. Cuzick J. Gardasil 9 joins the fight against cervix cancer. Taylor & Francis; 2015. [DOI:10.1586/14760584.2015.1051470] [PMID]

18. Nayar R, Wilbur DC. The pap test and Bethesda 2014. J Virol. 2015;59(2):121-32. [DOI:10.1159/000381842] [PMID]

19. Mahmoodvan S, Zamani K, Safaei A, Khashei R, Motamedifar M, Azizi Z, et al. No detection of Streptococcus g allolyticus and Helicobacter pylori in colorectal cancer tissue samples in Shiraz, Iran. Iran J Cancer Prev. 2017;10(1):e6337. [DOI:10.17795/ijcp-6337]

20. Joharinia N, Farhadi A, Hosseini SY, Safaei A, Sarvari J. Association of HPV16 and 18 genomic copies with histological grades of cervical lesions. Virusdisease. 2019;30(3):387-93. [PMID] [PMCID] [DOI:10.1007/s13337-019-00545-2]

21. De Jesus SP, Da Costa ACM, Barcellos RB, De Medeiros RM, Da Silva CMD, Rossetti MLJBJoM. A high prevalence of human papillomavirus 16 and 18 co-infections in cervical biopsies from southern Brazil. Braz J Microbiol. 2018;49(1):220-3. [DOI:10.1016/j.bjm.2018.04.003] [PMID] [PMCID]

22. Bryan JT, Taddeo F, Skulsky D, Jansen KU, Frain BM, Qadadri B, et al. Detection of specific human papillomavirus types in paraffin-embedded sections of cervical carcinomas. J Med Virol. 2006; 78(1):117-24. [DOI:10.1002/jmv.20512] [PMID]

23. Bateman AC, Katundu K, Polepole P, Shibemba A, Mwanahamuntu M, Dittmer DP, et al. Identification of human papillomaviruses from formalin-fixed, paraffin-embedded pre-cancer and invasive cervical cancer specimens in Zambia: a cross-sectional study. Virol J. 2015;12(1):2. [DOI:10.1186/s12985-014-0234-8] [PMID] [PMCID]

24. Gul S, Murad S, Javed A. Prevalence of High risk Human Papillomavirus in cervical dysplasia and cancer samples from twin cities in Pakistan. Int J Infect Dis. 2015;34:14-9. [DOI:10.1016/j.ijid.2015.02.018] [PMID]

25. Farjadian S, Asadi E, Doroudchi M, Dehaghani AS, Tabei S, Kumar V, et al. High risk HPV types in southern Iranian patients with cervical cancer. Pathol Oncol Res 2003;9(2):121-5. [DOI:10.1007/BF03033756] [PMID]

26. Haghshenas M, Golini Moghaddam T, Rafiei A, Emadeian O, Shykhpour A, Ashrafi GH. Prevalence and type distribution of high-risk human papillomavirus in patients with cervical cancer: a population-based study. Infect Agent Cancer. 2013;8(1):20. [DOI:10.1186/1750-9378-8-20] [PMID] [PMCID]

27. Ghaffari SR, Sabokbar T, Mollahajian H, Dastan J, Ramezanzadeh F, Ensani F, et al. Prevalence of human papillomavirus genotypes in women with normal and abnormal cervical cytology in Iran. Asian Pac J Cancer Prev. 2006;7(4):529-32.

28. Heydari N, Oskouee MA, Vaezi T, Shoja Z, Esmaeili HA, Hamkar R, et al. Type-specific human papillomavirus prevalence in cervical intraepithelial neoplasia and cancer in Iran. J Med Virol. 2018;90(1):172-6. [DOI:10.1002/jmv.24908] [PMID]

29. Jalilvand S, Shoja Z, Nourijelyani K, Tohidi HR, Hamkar R. Meta-analysis of type-specific human papillomavirus prevalence in Iranian women with normal cytology, precancerous cervical lesions and invasive cervical cancer: Implications for screening and vaccination. J Med Virol. 2015;87(2):287-95. [DOI:10.1002/jmv.24053] [PMID]

30. Ciapponi A, Bardach A, Glujovsky D, Gibbons L, Picconi MA. Type-specific HPV prevalence in cervical cancer and high-grade lesions in Latin America and the Caribbean: systematic review and meta-analysis. PLoS One. 2011;6(10):e25493. [DOI:10.1371/journal.pone.0025493] [PMID] [PMCID]

31. Schmitt M, Depuydt C, Benoy I, Bogers J, Antoine J, Arbyn M, et al. Multiple human papillomavirus infections with high viral loads are associated with cervical lesions but do not differentiate grades of cervical abnormalities. J Clin Microbiol. 2013;51(5): 1458-64. [DOI:10.1128/JCM.00087-13] [PMID] [PMCID]

32. Halec G, Alemany L, Lloveras B, Schmitt M, Alejo M, Bosch FX, et al. Pathogenic role of the eight probably/possibly carcinogenic HPV types 26, 53, 66, 67, 68, 70, 73 and 82 in cervical cancer. J Pathol. 2014;234(4):441-51. [DOI:10.1002/path.4405] [PMID]