



Frequency of Human Papillomavirus among Women with High-Grade Squamous Intraepithelial Lesions and Invasive Cervical Cancer Attending Shahid Beheshti University of Medical Sciences Clinics, Tehran, Iran

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Abstract

Background: The previous studies reported some information about prevalence release of high-risk HPV types in HSIL or cervical cancer globally and in Iran, however, this information is not enough for final judgment about vaccination against HPV or any screening program. The aim of the present study was to assess the HPV type distribution in HSIL and ICC specimens of women attending Shahid Beheshti University of Medical Sciences teaching hospitals, Tehran, Iran for treatment during 10 years.

Methods: This retrospective- descriptive study evaluated the HPV type distribution of pathologic specimens of Iranian women with invasive cervical cancer (ICC) and high-grade squamous cell intraepithelial lesions (HSIL). Formalin-fixed tumor biopsies that were retrieved from women presenting with histological confirmation for ICC and 17 pathologic confirmation for HSIL specimens.

Results: The most frequently identified HPV type 16 among both groups, women with invasive cervical cancer (42.18%) and women with High Grade Squamous Intraepithelial Lesion (29.41%), followed by HPV18, HPV31 and 26. HPV16 and / or 18 accounted for 82.2% of all infected samples.

Conclusion: The dominance of HPV16 over other high-risk types might be even higher than in a region with low HPV exposure. However, there was no strong evidence for any judgment that show to the policy makers; which one is cost-effectiveness and feasibility for cervical cancer prevention in Iran, vaccination, screening or both? More population based study and national meta-analysis needed for better understanding of HPV prevalence and HPV DNA patterns in Iran.

Keywords: Human papillomavirus, Squamous intraepithelial lesions, Invasive cervical cancer, Iran

Introduction

Cervical cancer is the fourth most common cancer in women and the seventh overall worldwide with an estimated 528000 new cases and 26600 death

in 2012, about 7.5% of all female cancer related death (1, 2). Iran, a country in west Asia and EMRO region with 76 million populations

accounted the age-standardized cervical cancer incidence rate is 2.8% with 1.2% mortality, 5 year prevalence of 3.2% and the rank is 11 among all female cancers (1, 3, 4).

The rate of abnormal Pap smear is between 0.3%-4.1% in population base studies (5-7) and 4% among women who come to gynecology clinic for any disorder and had opportunity pap smear test (8). In general, most of women who develop cervical cancer tend to have one or more identifiable factors such as early sexual activity, immune system deficiency, multi paternity, cigarette smoking, hormonal that increases their risk for the disease, but some types of Human Papillomavirus, in particular HPV 16 and 18, are found in over 70-99 per cent of cervical cancers (9, 10). Human papillomavirus infection (HPV) is one of the most common sexually transmitted diseases in the world (11). Infection by some of the HPV types is determined, as a necessary factor for development of cervical cancer (12). The HPV prevalence is very different around the world. A global report finds highest rates of HPV in Caribbean with 35% and East African women with 33%, while West Asia has the lowest rates of women infected with HPV (2.2%) in the normal population (13, 14). There are more than 100 types of HPV more than 40 types of known as oncogenist and the rest of those are Non-oncogenic or low-risk HPV types, such as HPV 6 or 11 which can cause genital warts (15).

It is now recognized that virtually all cervical cancers (both the squamous and adenocarcinoma histological types) and their precursor lesions are causally related to cervical infections through at least 14 high risk or oncogenic genotypes of HPV [16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68] that mainly *diagnosed* by molecular *methods, like* PCR assay (16-18). However, only a minority of pre-cancerous lesions progress to cervical cancer, the HPV type is a mighty risk factor for differentiation progression (17).

Previous studies reported some data about prevalence release of high-risk HPV types in HSIL or cervical cancer worldwide and Iran, however, this information is variable and not

enough for final judgment about the HPV type in a country such as Iran with low rate of cervical cancer, for cervical cancer control program (7, 12, 19-21). National data on type fomentation in different provinces of Iran is essential for estimating the impact of vaccines on cervical cancer and for the development of screening programs.

The aim of the present study was to assess the HPV type distribution among women who had a pathological diagnosed for HSIL and ICC, attendant to Shahid Beheshti University of Medical Sciences clinics for treatment during 10 years.

Materials and Methods

This retrospective-descriptive study evaluated the HPV type distribution of pathologic specimens of Iranian women with invasive cervical cancer (ICC) and high-grade squamous intraepithelial lesions (HSIL). Although we claim that this study with no research intervention do not need Ethics Committee approval, however the study was approved by the Cancer Research Center of Shahid Beheshti University, according the consent form that were signed by all study samples who already came to the clinics and mentioned that they take permission for any future evaluation. The first group was 64 formalin-fixed tumor biopsies that were retrieved from women presenting with histological confirmation for Invasive Cervical Cancer (ICC) and the second group was 17 pathologic confirmations for High-grade Squamous Intraepithelial Lesion (HSIL) specimens between 2002-2012, both to the Shahid Beheshti University of Medical Sciences teaching hospitals, all in Tehran, Iran. Formalin –fixed biopsies of the ICC and 17 HSIL specimens were evaluated. We used the PCR-based technique that is highly sensitive, specific, and widely used, for HPV DNA extraction. HPV DNA was extracted from cervical formaldehyde-fixed, paraffin-embedded tissue of ICC specimens and HSIL samples. One or more sections representing 1 centimeter (cm²) of the tissue were pre-digested with proteinase K, after which DNA was

extracted using magnetic beads. Beta-globin PCR analysis was performed first on all specimens to assess the quality of the DNA to be submitted to HPV PCR. A general primer GP5/6-mediated PCR was used for the detection of 44 mucosal HPV types (22). Subsequent HPV genotyping was performed by reverse-line blot hybridization of GP5/6-PCR products (23, 24).

Results

The median age of included women with ICC was 51 years (range: 26–84 years) and the median age of HSIL group was 43 years (range 26-72 years). We could not have access to demographic data of ICC group. All women in HSIL group were married, with history of one partner, except one of divorced individual who remain single and was multi partners and two women who have divorced or widow and get remarried, (usually according Iranian culture and religious believes, Iranian women start to have sexual relation after getting married). The mean of pregnancy was 3.4 and the number of children was 2.3. Except two women who had elementary education and one no educate all women finished high school or university graduation.

HPV16 was confirmed as the most common type among women with ICC (42.18%) with confidence interval (60.0%, 95% CI: 34.3–74.3), followed by HPV18 (22.2%, 95% CI: 11.2–37.1) and HPV31 (8.9%, 95% CI: 2.5–21.2) (Table 1).

The number of HPV positive cases in HSIL group was 8 (47.05%) and HPV type 16 was detected in 5 cases of HSIL group (29.41%), HPV type 18 detected in 2 cases (11.78%) and 31 was detected in one case (5.88%), the rest of HSIL samples were negative for any HPV types (Table 1).

Discussion

The result of this study shows that the HPV prevalence in women with HSIL was (47.05%) and in the ICC group was (71.87%), comparable with many formerly studies that the number of re-

searchers have studied HPV prevalence among women with cervical cancer in Iran (25, 26).

In a recent study HPV-DNA was detected in (79.59%) of ICC patients (27). The prevalence of HPV positive of the women in South of Iran with cervical cancer was 87.1%, of those HPV-16 genotypes was detected in 26.7%, but HPV type 18 did not find in the specimen of this study (28). In northern Iran, HPV-16 is the most prevalent type in the cervical cancer lesions too, HPV-18, and HPV-33 are also frequent types (29). While global report shown that HPV type 16 found in 54.4% of invasive cervical cancer, we found HPV-16 DNA in (42.18%) of ICC group and (29.41%) of HSIL group. HPV-18 DNA has been second frequently in both groups (15.62%) in the ICC and (11.78%) in HSIL group, very closed to other Muslim courtiers with similar culture, such as Egypt that HPV DNA was detected in 85.7% patients with CIN and in patients with ICC, 93.3% were positive for HPV DNA. HPV 16 was detected in the majority of both groups. The researcher came into conclusion that the high prevalence of HPV genotypes 16, 18, and 45 in Egypt deserves attention as it has important implications for the usefulness of vaccine in prevention of a significant proportion of cervical cancer (30).

Although in Saudi Arabia cervical cancer incidence is low, suggesting a low prevalence to HPV infection due to environmental, cultural and genetic differences, the prevalence of HPV genotypes among Saudi Arabian women with cervical cancer is comparable to the international rates, whereas HPV genotype detection among cervical cancer samples is 95.5% and the most common HPV genotype is HPV-16 (63.4%), followed by HPV-18 (11.1%), HPV-45 detected in (4.5%) of cases (31, 32).

In Karachi, Pakistan study a very low burden of HPV infection in the general female population of Karachi was disclosed (2.8%), considerably lower than Tehran, the capital city of Iran and western countries (7, 33). HPV 16 was also the predominant HPV type (75.8%) in the ICC, followed by HPV18 (6.6%) and 45 in (4.4%) of cases (34). Overall HPV16 and 18 accounted for over 58% of ICC in this study, confirmed the high preva-

lence of HPV16 observed in women with cervical cancer in other national studies (27, 28) and world region (21, 35, 36). Regarding HSIL group HPV was found in 47.05% of samples with 29.41% HPV 16 and 11.78% HPV 18. It is lower than the worldwide prevalence among HSIL samples that HPV found in 82.5% of HSIL cases, while prevalence of HPV16 was 46.5% and the prevalence of HPV18 was 8.9% (35).

Previous studies showed a different pattern for HPV types in other part of Iran demonstrated HPV type 16 as the main inorganic type of HPV associated with cervical cancer while other HPV types of HPV reported at lower frequency in ICC and HSIL samples (25-29, 37, 38). The result of our study would suggest that the predominance of HPV16 over other high-risk types might be even higher than in settings of low HPV exposure. However, there was no strong evidence for any judgment that show to policy makers; which one is cost-effectiveness and feasibility for cervical cancer prevention in Iran vaccination, screening or both? More population based study and national meta-analysis needed for better understanding of HPV prevalence and DNA patterns in Iran, a country that cervical cancer is not seen among 10 of common women's cancers (1, 4). The limita-

tion of this study was the lack of complete demographic data and small samples for assessing the risk factors of HPV infection, ICC and HSIL among Iranian women and also different DNA detection techniques for ICC and HSIL samples.

Conclusion

While it is well accepted that HPV is an important causal factor for the cervical cancer development, our study confirmed a high prevalence of HPV infection among women with either cervical cancer or high-grade squamous intraepithelial lesions. It is the time that organized cervical cancer screening, availability for HPV DNA testing technology, or vaccination come into account for further cervical cancer control policy and provides patients with appropriate counseling about HPV-related concerns.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Table 1: Frequency of HPV types in 81 women (17 with High-grade Squamous Intraepithelial Lesions and 64 with Invasive Cervical Cancer) in Tehran, Iran

HPV Type High risk/Low risk	HSIL n:17(100%)	ICC n:64(100%)	All 81(100%)
16	5(29.41)	27 (42.18)	32(39.5)
18	2(11.78)	10(15.62)	12(14.81)
31	1(5.88)	4(6.25)	5 (6.17)
45	0	2(3.12)	2(2.46)
58	0	2(3.12)	2(2.46)
26 (low risk)	0	1(1.5)	1(1.23)
All HPV +	8(47.05)	46(71.87)	54(66.6)

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Reference

1. Bray F, Ren JS, Masuyer E, Ferlay J (2013). Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int J Cancer*, 132:1133-45.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F (2013). Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer*, 49:1374-403.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, 127:2893-917.
4. Ministry of Health (2009-2010) Iran Cancer Registration Report 2008-2009. In: *Iran Center for Disease Control & Prevention* pp. 434.
5. Nokiani FA, Akbari H, Rezaei M, Madani H, Ale Agha ME (2008). Cost-effectiveness of pap smear in Kermanshah, Iran. *Asian Pac J Cancer Prev*, 9:107-10.
6. Maryam Afrakhteh, Nahid Khodakarami, Afshin Moradi, Ehsan Alavi, Farshad Hosseini Shirazi F (2007). A study of 13315 Papanicolaou Smear Diagnoses in Shohada Hospital. *Journal of Family and Reproductive Health*, 1, (2), 75-79.
7. Khodakarami N, Clifford GM, Yavari P et al. (2012). Human papillomavirus infection in women with and without cervical cancer in Tehran, Iran. *Int J Cancer*, 131:E156-61.
8. Khodakarami N, Farzaneh F, Aslani F, Alizadeh K (2011). Comparison of Pap smear, visual inspection with acetic acid, and digital cervicography as cervical screening strategies. *Arch Gynecol Obstet*, 284:1247-52.
9. Castle PE, Sideri M, Jeronimo J, Solomon D, Schiffman M (2008). Risk assessment to guide the prevention of cervical cancer. *J Low Genit Tract Dis*, 12:1-7.
10. Franceschi S, Plummer M, Clifford G, de Sanjose S, Bosch X, Herrero R, Munoz N, Vaccarella S, International Agency for Research on Cancer Multicentric Cervical Cancer Study G, International Agency for Research on Cancer Human Papillomavirus Prevalence Surveys Study G (2009). Differences in the risk of cervical cancer and human papillomavirus infection by education level. *Br J Cancer*, 101:865-70.
11. Vinodhini K, Shanmughapriya S, Das BC, Natarajaseenivasan K (2012). Prevalence and risk factors of HPV infection among women from various provinces of the world. *Arch Gynecol Obstet*, 285:771-7.
12. Kjaer SK, Munk C, Junge J, Iftner T (2014). Carcinogenic HPV prevalence and age-specific type distribution in 40,382 women with normal cervical cytology, ASCUS/LSIL, HSIL, or cervical cancer: what is the potential for prevention. *Cancer Causes and Control*, 25(2):179-89.
13. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, Vignat J, Ferlay J, Bray F, Plummer M, Franceschi S (2012). Global burden of human papillomavirus and related diseases. *Vaccine*, 30 Suppl 5:F12-23.
14. Vaccarella S, Bruni L, Seoud M (2013). Burden of human papillomavirus infections and related diseases in the extended middle east and North Africa Region. *Vaccine*, 31:G32-G44.
15. Thomsen LT (2014). High-Risk and Low-Risk Human Papillomavirus and the Absolute Risk of Cervical Intraepithelial Neoplasia. *Obstet Gynecol*, 123.
16. DeCew AE, Hadler JL, Daley AM, Nicolai L (2013). The prevalence of HPV associated cervical intraepithelial neoplasia in women under age 21: who will be missed under the new cervical cancer screening guidelines? *J Pediatr Adolesc Gynecol*, 26:346-9.
17. No authors listed (2013). [HPV and cervical cancer]. *J Midwifery Womens Health*, 58:245-6.
18. Halec G, Schmitt M, Dondog B et al. (2013). Biological activity of probable/possible high-risk human papillomavirus types in cervical cancer. *Int J Cancer*, 132:63-71.
19. Tzenov YR, Andrews PG, Voisey K, Popadiuk P, Xiong J, Popadiuk C, Kao KR (2013). Human papilloma virus (HPV) E7-mediated attenuation of retinoblastoma (Rb) induces hPygopus2 expression via Elf-1 in cervical cancer. *Mol Cancer Res*, 11:19-30.
20. Amaro-Filho SM, Golub JE, Nuovo GJ et al. (2013). A comparative analysis of clinical and molecular factors with the stage of cervical cancer in a Brazilian cohort. *PLoS One*, 8:e57810.

21. Katki HA, Schiffman M, Castle PE et al. (2013). Five-year risks of CIN 3+ and cervical cancer among women with HPV testing of ASC-US Pap results. *J Low Genit Tract Dis*, 17:S36-42.
22. Jacobs MV, Walboomers JM, Snijders PJ, Voorhorst FJ, Verheijen RH, Franssen-Daalmeijer N, Meijer CJ (2000). Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. *Int J Cancer*, 87:221-7.
23. van den Brule AJ, Pol R, Franssen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ (2002). GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol*, 40:779-87.
24. Abreu AL, Souza RP, Gimenes F, Consolaro ME (2012). A review of methods for detect human Papillomavirus infection. *Virology*, 9:262.
25. Shamsiah R, Khademalhosseini M, Mehrdad N, Ramezani F, Nadji SA (2011). Human papillomavirus genotypes in Iranian patients with cervical cancer. *Pathol Res Pract*, 207:754-7.
26. Ghaffari SR, Sabokbar T, Mollahajian H, Dastan J, Ramezanzadeh F, Ensani F, Yarandi F, Mousavi-Jarrahi A, Mohagheghi MA, Moradi A (2006). Prevalence of human papillomavirus genotypes in women with normal and abnormal cervical cytology in Iran. *Asian Pac J Cancer Prev*, 7:529-32.
27. Haghshenas M, Golini-Moghaddam T, Rafiei A, Emadeian O, Shykhpour A, Ashrafi GH (2013). Prevalence and type distribution of high-risk human papillomavirus in patients with cervical cancer: a population-based study. *Infect Agent Cancer*, 8:20.
28. Farjadian S, Asadi E, Doroudchi M, Dehaghani AS, Tabei SZ, Kumar VP, Ghaderi A (2003). High risk HPV types in southern Iranian patients with cervical cancer. *Pathol Oncol Res*, 9:121-5.
29. Esmaeili M, Bonyadi M, Dastranj A, Alizadeh M, Melli MS, Shobeiri MJ (2008). HPV typing in women with cervical precancerous and cancerous lesions in northwestern Iran. *Gynecol Obstet Invest*, 66:68-72.
30. Abd El-Azim S, Lotfy M, Omer A (2011). Detection of human papillomavirus genotypes in cervical intraepithelial neoplasia and invasive cancer patients: Sharkia Governorate, Egypt. *Clin Lab*, 57:363-71.
31. Al-Badawi IA, Al-Suwaine A, Al-Aker M, Asaad L, Alaidan A, Tulbah A, Fe Bohol M, Munkarah AR (2011). Detection and genotyping of human papilloma virus in cervical cancer specimens from Saudi patients. *Int J Gynecol Cancer*, 21:907-10.
32. Alsbeih G, Al-Harbi N, El-Sebaie M, Al-Badawi I (2013). HPV prevalence and genetic predisposition to cervical cancer in Saudi Arabia. *Infect Agent Cancer*, 8:15.
33. De Vuyst H, Clifford G, Li N, Franceschi S (2009). HPV infection in Europe. *Eur J Cancer*, 45:2632-9.
34. Raza SA, Franceschi S, Pallardy S, Malik FR, Avan BI, Zafar A, Ali SH, Pervez S, Serajuddaula S, Snijders PJ, van Kemenade FJ, Meijer CJ, Shershah S, Clifford GM (2010). Human papillomavirus infection in women with and without cervical cancer in Karachi, Pakistan. *Br J Cancer*, 102:1657-60.
35. Ciapponi A, Bardach A, Glujovsky D, Gibbons L, Picconi MA (2011). Type-specific HPV prevalence in cervical cancer and high-grade lesions in Latin America and the Caribbean: systematic review and meta-analysis. *PLoS One*, 6:e25493.
36. Kirschner B, Junge J, Holl K, Rosenlund M, Collas de Souza S, Quint W, Molijn A, Jenkins D, Schledermann D (2013). HPV genotypes in invasive cervical cancer in Danish women. *Acta Obstet Gynecol Scand*, 92:1023-31.
37. Asadi-Amoli F, Heidari AB, Jahanzad I, Jabbarvand M (2011). Detection of human papillomavirus in squamous cell carcinoma of conjunctiva by nested PCR: a case control study in Iran. *Acta Med Iran*, 49:707-14.
38. Mortazavi S, Zali M, Raoufi M, Nadji M, Kowsarian P, Nowroozi A (2002). The Prevalence of Human Papillomavirus in Cervical Cancer in Iran. *Asian Pac J Cancer Prev*, 3:69-72.