original article

Prevalence of high-risk human papillomavirus infections in healthy Saudi women attending gynecologic clinics in the western region of Saudi Arabia

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BACKGROUND AND OBJECTIVES: Infection with human papillomavirus (HPV) is the major cause of cervical cancer. There is little published data on the prevalence of HPV infection among Saudi women. The aim of this study was to determine the prevalence of HPV in a group of women in the western region of Saudi Arabia.

DESIGN AND SETTING: A prospective study of Saudi women seeking gynecologic care at King Abdulaziz University Hospital from March 2010 to January 2011.

PATIENTS AND METHODS: Four hundred eighty-five Saudi women of different age groups attending gynecology clinic were tested for high-risk HPV DNA. HPV DNA was detected in cervical scrapes using Hybrid Capture 2 (HC2) high-risk HPV DNA test. The prevalence of HPV DNA positivity in different age groups was calculated.

RESULTS: Out of the 485 specimens, 27 (5.6%) were positive for the high-risk HPV. The highest percentage was among women aged 60 years and older. Patients in the age group 40-49 years were more likely to accept HPV testing with a total of 188 patients.

CONCLUSION: The prevalence of HPV in this group of Saudi women is similar to what was reported in some Arab countries and lower than that reported in developed countries. This information could be used to help in establishing a primary screening program using HPV DNA testing in Saudi Arabia.

Cervical cancer is the third most common cancer affecting females and the fourth leading cause of cancer death in females worldwide, accounting for 9% (529 800) of the total newly diagnosed cancer cases and 8% (275 100) of the total cancer deaths among females in the year 2008. More than 85% of these cases and deaths occur in developing countries.[1]

The incidence of cervical cancer is low in Saudi women. According to the 2007 Saudi cancer registry report, cervical cancer is the thirteenth most frequent cancer in Saudi women. The incidence rate in Saudi Arabia is one of the lowest in the world at 1.9 cases per 100 000 women, accounting for 2.2% of diagnosed cases of cancer in Saudi women.[2] Although cervical cancer is both preventable and curable, most women in Saudi Arabia present at advanced stages that require extensive chemoradiation therapy.[3,4] This is due to the lack of a proper screening program.[5] Cervical cancer is caused by sexual exposure to an oncogenic type of the human papillomavirus (HPV), usually types 16 and 18.[6-9]

The FDA has approved the Digene Hybrid Capture 2 High-Risk HPV DNA Test as a cervical screening test for HPV infection.[10] There are clear benefits for the use of HPV DNA testing in the triage of equivocal smears, low-grade smears in older women and in the post-treatment surveillance of women after treatment for cervical intraepithelial neoplasia. However, there are still issues regarding how best to test in primary screening.[11] The most resourceful and cost-effective screening techniques include visual inspection of the cervix after applying acetic acid or Lugol iodine and DNA testing for human HPV DNA in cervical cell samples.[12] A

recent clinical trial from India in a low-resource area concluded that a single set of HPV DNA testing was associated with a 50% reduction in the risk of developing advanced cervical cancer and associated deaths.[13] Currently, little is known about the prevalence and type of distribution of HPV in Saudi Arabia. Introduction of appropriate screening for cervical cancer in Saudi Arabia requires extensive work to find whether HPV infection is a significant healthcare problem. In addition, baseline information on HPV prevalence and genotype distribution is highly desirable to evaluate the impact of prophylactic HPV vaccines in the near future. This study aimed to evaluate the feasibility of using HPV testing as a primary screening for cervical cancer by determining the prevalence in a group of Saudi women and assessing the prevalence among different age groups to evaluate the feasibility of using HPV DNA testing as a primary screening test for cervical cancer.

PATIENTS AND METHODS

This was a prospective cohort hospital-based study of all Saudi women attending gynecology clinics at King Abdulaziz University Hospital from March 2010 to January 2011 who fulfilled the inclusion criteria of being sexually active and of childbearing or postmenopausal age. Exclusion criteria included virginity, pregnancy, known cases of HPV, cervical precancerous lesions or cervical cancer, positive cytology on Pap smear, patient refusal to participate in the study and nonSaudi nationality. Women who were eligible for the study consented to participation after being counseled by one of the gynecologist in the clinics and given the liberty to participate or decline; only women who signed the consent form were included in the study. The women then had the traditional Pap smear using the wet mount technique and the Hybrid Capture 2 (HC2) reagents and materials for HPV detection (Digene Corporation, USA). Pap smear results were reported according to the Bethesda system for reporting of cervical cytology.[14] Smears with no abnormalities or one with reactive changes were considered normal while all smears of atypical squamous cell of undetermined significance (ASC-US) or higher were considered as abnormal.

The specimen collection was done via cells taken from the cervix with the Digene cervical sampler kit, then placed into the Digene liquid collection medium. The specimen collection was performed by a gynecologist after taking a detailed history and performing a physical examination including pelvic examination. The Digene HPV HC2 test used in the study detects the high/intermediate risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). The target DNA hybridizes with a specific high-risk HPV RNA probe-forming RNA/DNA hybrids which are captured onto the surface of a microplate well coated with antibodies specific for RNA/DNA hybrids. Fixed hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for the RNA/DNA hybrids, which are then identified with a chemiluminescent substrate, where several alkaline phosphatase molecules unite to each antibody. Multiple united antibodies bind to each captured hybrid give considerable signal amplification. Light is released when the substrate is broken by the bound alkaline phosphatase, then measured as relative light units (RLUs) on a luminometer and its strength indicates the presence or absence of target DNA in the specimen. The interpretations of the test results were carried out according to the manufacturer's instruction. The women were classified into groups based on their age (19-29 years), (30-39), (40-49), (50-59) and (60 years and older). Data were collected and analyzed using SPSS statistical package version 16. The institutional human ethics committee for King AbdulAziz University Hospital approved the study protocol based on the international recommendations on human subject research and according to principles of the Helsinki declaration.

RESULTS

During the study period, 6585 women were seen in the gynecology clinics for different clinical complaints. The majority of cases had menstrual cycle abnormalities followed by pelvic pain, vaginal discharge, urinary incontinence and dyspareunia in order of frequency. Applying the inclusion criteria, 1649 patients were eligible. Out of 1649 eligible women, 1164 were excluded from the study for the following reasons: 704 refused to participate in the study after counseling, 429 were pregnant or seen for pregnancy complications, and 16 had technical difficulties in collecting or processing the sample according to the study protocol, 15 for previous positive HPV, cervical carcinoma or cervical precancerous lesions. The commonest cause for refusal to participate was the psychological fear of the impact of positive test on the patient's physical and social life.

Four hundred and eighty-five women participated in the study. The age range was 19 to 91 years with a mean age of 44.7 years. The majority of women were multiparous, 403 (83%). The parity ranged from 1 to 9 with a mean of 3.5. Four hundred and seventeen women (86%) were married, 44 (9%) divorced and 24 (5%) were widows. The Pap smear was abnormal in 118 (24.3%) women and normal in 367 (75.7%).

Of 485 patients, 458 (94.4%) were negative for

HPV infection while 27 (5.6%) were positive (**Table 1**). The detection rate of HPV, DNA varied according to age showing the highest rate among women age 60 years and older. Furthermore the study showed that 16 women of 334 patients in the reproductive age group (19-49 years) tested positive for HPV DNA with a detection rate of 4.8% compared to 11 of 151 postmenopausal women (50 years and older) with a detection rate of 7.3%. The highest number of tested patient was reported in the group 40-49 years old with 188 women (32% of the collected samples).

Table 1. Result of negative and positive HPV DNA in cervical specimens by Hybrid Capture 2.

Age group (years)	n	Number of negative patients (%)	Number of positive patients (%)
19-29	54	53 (98.1)	1 (1.9)
30-39	92	87 (94.6)	5 (5.4)
40-49	188	178 (94.7)	10 (5.3)
50-59	108	101 (93.5)	7 (6.5)
≥60	43	39 (90.7)	4 (9.3)
19-91	485	458 (94.4)	27 (5.6)

DISCUSSION

The unduly high burden of cervical cancer in developing countries is mostly due to a lack of screening programs that allows detection of precancerous and early stage cervical cancer.[15,16] Out of the 30 to 40 known HPV genotypes that infect the mucosa of the female genital tract, eight types (16, 18, 45, 31, 33, 52, 58, and 35) are accountable for 95% of cervical cancers and two genotypes (16 and 18) are responsible for 70 percent of the cervical cancer cases.[17] The HPV vaccine protects against the most common strains of HPV infections (HPV types 16 and 18). Effective utilization of the available vaccine depends on the prevalence and the genotype of HPV in the targeted population. The estimated global HPV prevalence was 11.7%. It was estimated to be 24.0% in Sub-Saharan Africa, 21.4% in Eastern Europe, and 16.1% in Latin America. The age-specific HPV prevalence distribution showed a first peak at younger ages (<25 years) in Latin America and older ages (≥45 years) in North America and Africa.[18]

In two recent studies from Saudi Arabia on the HPV genotype associated with cervical cancer, Alsbeih et al[19] showed that 81% of cervical cancers specimens tested in their institution in the central part Saudi Arabia were associated with HPV infection, the majority 78.7% (70/89) of HPV-positive tumors were infected with HPV-16/18. Al-Badawi et al[20] reported similar finding with 95.5% detection of HPV in cervical cancer specimens, the most common HPV genotype detected being HPV-16 (63.4%), followed by HPV-18. These two studies clearly show that the most prevalent HPV genotype in Saudi women with cervical cancer were 16 and 18 which is no different than was reported globally.[17]

The role of high-risk HPV DNA testing is growing and HPV DNA testing, either alone or in combination with cervical cytology, has been shown in many studies to be more sensitive than cervical cytology alone in detecting low- or high-grade cervical lesions.[21-23] In addition, HPV DNA testing has been proposed both as a primary screening method (either as an adjunct or instead of Pap smear) and as a method to triage Pap smear results that are equivocal.[24-26]

Many studies have documented the use of HPV DNA testing as a primary screening tool. In a Canadian randomized control trial on 10 154 women, Mayrand et al[27] compared HPV DNA testing with conventional Pap smear and concluded that HPV testing has greater sensitivity for the detection of cervical intraepithelial neoplasia than the conventional Pap smear. Ronco et al,[28] in a large randomized controlled Italian trial that included two groups of women, 47,001 were assigned to the cytology group and 47,369 to the HPV testing group. They concluded that HPV-based screening is more effective than cytology in preventing invasive cervical cancer, by detecting persistent high-grade lesions earlier.

In a low-resource setting, a single round of HPV DNA testing was associated with a significant reduction in the numbers of advanced cervical cancers and deaths from cervical cancer. This was clearly demonstrated in a randomized trial of 131 746 women aged 30 to 59 years in rural India that compared a single lifetime screening with one of three screening modalities with standard care; the screening modalities were HPV testing using the Hybrid Capture HC2, cervical cytology, or visual inspection of the cervix with acetic acid.[29]

The current study reported a prevalence of 5.6% of the high/intermediate-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) in Saudi women attending gynecology clinics for different complains. The only other reports from Saudi Arabia identified through a PubMed search were by Al-Muammar et al[30] and Gazzaz.[31] In a small number of patients attending family medicine clinics in Riyadh, Saudi Arabia, Muammar et al[30] reported a high prevalence of HPV infection, reaching 31.6% with the majority of cases be-

ing HPV-16 followed by 18. The age distribution was not mentioned in that study.

The prevalence in the current study (5.6%) is similar to that reported by Gazzaz[31] (5%) and much lower than the one reported by Al-Muammar et al[30] (31.6%). The current study showed a prevalence peak on relatively older women (>60 years), which might be explained by the lack of screening program and earlier testing in those women. A report from Egypt on 5453 women[32] showed a prevalence of 4.0% for HPV among Egyptian women included in the study. These figures are in accordance with our figure, but the age distribution for HPV positivity was younger in the Egyptian women compared to our results. The prevalence of HPV IN 1026 Lebanese women aged 18-76 years[33] was 4.9% with 3% for high risk HPV type 16 DNA with peak at 60-69 years of age, which was similar to our results.

Comparing our positive results (5.6%) with those from other countries such as the United States[34] (26.8%) and China[21,35] (13.5%-17.6%) show that the prevalence of cervical HPV infection among females in Saudi Arabia is relatively low. In addition, the age distribution shows a marked difference. The current study shows a prevalence peak in women in the age group 60 years and older compared to a prevalence peak in the age group of 20 to 24 years in the United States.[34]

The current study shows a low detection rate (1 case, 1.9%) of HPV DNA positivity in women under the age of 30 years. Our findings concurs with the findings of Kjaer et al[36] in their study to determine the absolute risk of cervical abnormalities in women with normal cytology and a positive high-risk Hybrid Capture 2 (HC2) test, which showed that the rate of development of a cervical lesion to be 17.7% in younger women compared to 24.5% in older women. In another study by Khan et al[37] from the United States to explore the risk of cervical precancerous lesions in women with normal cytology and positive testing for HPV DNA type 16 and 18 showed that women 30 years of age and older had a higher risk of developing cervical lesions than younger women.

Datta et al[38] measured the Pap test results and high-risk HPV prevalence by Hybrid Capture 2 assay in 9657 women age 14 to 65 years receiving routine cervical screening and concluded that high-risk HPV was widespread among women receiving cervical screening in the United States. They suggested that many women 30 years of age or older with normal Pap tests would need follow-up if Hybrid Capture 2 testing is added to cytology screening.

In the present study HPV infection among females in a Saudi community was done on a larger number than that was done by Gazzaz[31] and Muammar et al.[30] The current study provides a unique opportunity to gather an idea about baseline data on cervical HPV prevalence among females in the western region of Saudi Arabia. The protocol and methodology applied in the current study was successful and could be used in a larger nationwide research.

The prevalence of HPV in this group of Saudi women in the western region of Saudi Arabia is similar to what is reported in some other Arab countries and lower than what is reported in developed countries and some parts of Asia. This information can be used in establishing a proposal for using HPV testing by hybrid capture as a primary screening for cervical cancer in Saudi Arabia. Multicenter population prevalence data for HPV on a larger scale in women in Saudi Arabia is required before the implementation of routine HPV vaccination in this country.

REFERENCES

1. Jemal A,Bray F, Center M,Ferlay J, Ward E,Forman D.Global cancer statistics. CA CANCER J CLIN 2011; 61:69– 90.
2. Saudi Cancer Registry cancer incidence and survival reports Saudi Arabia 2007. National Saudi Cancer Registry. Riyadh (KSA): Ministry of Health. Available at: http://www.scr.org.sa/reports/SCR2007.pdf.
3. El Dosoky M, Ismail N, Dagastani M. Preinvasive cervical carcinoma in Saudi Arabia. Lancet 1995 Mar 11; 345(8950): 650.
4. Manji M. Cervical cancer screening program in Saudi Arabia: action is overdue. Ann Saudi Med 2000 Sep-Nov; 20(5-6): 355–357.
5. Kitchener HC, Symonds P. Detection of cervical intraepithelial neoplasia in developing countries. Lancet 1999 Mar 13; 353(9156): 856-857.
6. Zur Hausen H. Human papillomaviruses in the pathogenesis of anogenital cancer. Virology 1991 Sep; 184(1): 9–13.
7. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995 June 7; 87(11): 796–802.
8. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999 Sep; 189(1): 12–19.
9. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003 Feb 6; 348(6): 518–527.
10. US Food and Drug Administration. FDA News. FDA approves expanded use of HPV test. www.fda.gov/bbs/topics/news/2003/new00890.html. Accessed July 14, 2003.
11. Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH, Dillner J,et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine. 2008 Aug 19; 26 Suppl 10:K29-41.
12. Sherris J, Wittet S, Kleine A, et al. Evidence-based, alternative cervical cancer screening approaches in low-resource settings. Int Perspect Sex Reprod Health. 2009; 35:14 7-154.
13. Sankarana rayanan R, Nene BM, Shastri SS, et al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009; 360: 1385-13 94.
14. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002; 287:2114-2119.
15. Parkin DM, Almonte M, Bruni L, Clifford G, Curado MP, Pineros M. Burden and trends of type-specific human papil lomavirus infections and related diseases in the Latin America and Caribbean an region. Vaccine. 2008; 26(suppl 11):L1-L 15.
16. Mathew A, Geoge PS. Trends in incidence and mortality rates of squamous cell carcinoma and adenocarcinoma of cervix– worldwide. Asia n Pac J Cancer Prev. 2009; 10:645-6 50.
17. Kahn JA. HPV vaccination for the prevention of cervical intraepithelial neoplasia. N Engl J Med 2009; 361:271.
18. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S.Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010 Dec 15; 202(12):1789-99.
19. Alsbeih G, Ahmed R, Al-Harbi N, Venturina LA, Tulbah A, Balaraj K. Prevalence and genotypes' distribution of human papillomavirus in invasive cervical cancer in Saudi Arabia. Gynecol Oncol. 2011 Jun 1; 121(3):522-6.
20. Al-Badawi I, Al-Suwaine A, Al-Aker M, Asaad L, Alaidan A. Tulbah A,et al. Detection and Genotyping of Human Papilloma Virus in Cervical Cancer Specimens from Saudi Patients. Int J Gynaecol Cancer: 2011; 21: 907-910.
21. Wu RF, Dai M, Qiao YL, Clifford GM, Liu ZH, Arslan A, et al. Human Papillomavirus infection in women in Shenzhen City, People's Republic of China, a population typical of recent Chinese urbanisation. Int J Cancer 2007, 121:1306-1311.
22. ASCUS-LSIL Traige Study (ALTS) Group. A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. Am J Obstet Gynecol 2003; 188:1393.
23. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer 2006; 119:1095.
24. Kim JJ, Wright TC, Goldie SJ. Cost-effectiveness of human papillomavirus DNA testing in the United Kingdom, The Netherlands, France, and Italy. J Natl Cancer Inst 2005; 97:888.
25. Denny LA, Wright TC Jr. Human papillomavirus testing and screening. Best Pract Res Clin Obstet Gynaecol 2005; 19:501.
26. Koliopoulos G, Arbyn M, Martin-Hirsch P, et al. Diagnostic accuracy of human papillomavirus testing in primary cervical screening: a systematic review and meta-analysis of non-randomized studies. Gynecol Oncol 2007; 104:232.
27. Mayrand MH, Duarte-Franco E, Rodrigues I, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med 2007; 357:1579.
28. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomized controlled trial. Lancet Oncol. 2010 Mar; 11(3):249-57.
29. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM et al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009 Apr 2;360(14):1385-94.
30. Al-Muammar T, Al-Ahdal MN, Hassan A, Kessie G, Dela Cruz DM, Mohamed GE. Human papilloma virus-16/18 cervical infection among women attending a family medical clinic in Riyadh. Ann Saudi Med. 2007 Jan-Feb; 27(1):1-5.
31. Gazzaz FS. Molecular Testing of Human Papillomavirus (HPV) in Cervical Specimens. Saudi Med J 2007; Vol. 28 (12): 1810-1818.
32. El-All HS, Refaat A, Dandash K.Prevalence of cervical neoplastic lesions and Human Papilloma Virus infection in Egypt: National Cervical Cancer Screening. Infect Agent Cancer. 2007 Jul 4; 2:12.
33. Mroueh AM, Seoud MA, Kaspar HG, Zalloua PA.Prevalence of genital human papillomavirus among Lebanese women Eur J Gynaecol Oncol. 2002; 23(5):429-32.
34. Dunne EF, Sternberg M, McQuillan G, Swan DC, Patel SS, Markowitz LE. Prevalence of HPV infection among females in the United States. JAMA 2007 Feb 28; 297(8):813-819.
35. Li LK, Dai M, Clifford GM, Yao WQ, Arslan A, Li N, Shi JF,et al. Human Papillomavirus infection in Shenyang City, People's Republic of China: A population-based study. Br J Cancer 2006, 95:1593-1597.
36. Kjaer S, Høgdall E, Frederiksen K, Munk C, van den Brule A, Svare E, et al. The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. Cancer Res 2006; 66:10630.
37. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR et al. The elevated 10-year risk of cervical precancerous and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005; 97:1072.
38. Datta SD, Koutsky LA, Ratelle S, Unger ER, Shlay J, McClain T et al. Human papillomavirus infection and cervical cytology in women screened for cervical cancer in the United States, 2003-2005. Ann Intern Med. 2008 Apr 1; 148(7):493-500.