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Molecular evaluation of common HPV genotypes for the patients attending thumbay hospitals, UAE

Abstract

HPV is a DNA virus comprising approximately 8,000 nucleotides surrounded by a protein capsid. It can infect the cervix, vagina, vulva or urethra in females and is an essential factor in the etiopathogenesis of cervical cancer. To date, more than 100 HPV genotypes have been identified. Some of the genotypes are highly associated with neo plastic transformation in cervix and about 40 of these can cause genital diseases. In this study, we have used a DNA-based dot blot assay to analyze the presence of HPV DNA signatures in liquid based cytology (LBC) cervical brush biopsy samples. Data based on LBC samples from 220 patients aged 18-55 years received at CABRI from July 2015 to September 2016 was analyzed. Ninety patients (41%) tested positive for human papillomavirus (HPV) high-risk genotypes. The results were thereafter correlated with the cytology findings following Pap smear using the Thin Prep method. Early molecular detection of HPV genotypes may enhance risk stratification, treatment and follow-up in infected patients. The improvement in diagnostic accuracy will help avoid unnecessary colposcopy in women demonstrating mild cellular atypia in their LBC Pap smears. In this paper, we have discussed the significance of these genotypes in relation to the currently available quadrivalent vaccine.

Keywords: human papillomavirus, DNA, pap smear

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Introduction

Human papillomavirus (HPV) testing was introduced to enhance the sensitivity and specificity of the Pap smear cytology often used as a diagnostic tool for borderline precancerous lesions.[1] Most HPV infections, caused by high-risk genotypes, are transient. Pap cytology screening shows that these coexist with normal cells in sexually active young women.[2–5] The UAE has a population of 1.82 million women aged 15years and above, who are at a risk of developing cervical cancer. According to current estimates, about 93women are diagnosed with the disease every year in the country, with 28 dying of it. Cervical cancer is the third most frequent cancer among women in the UAE. It is also the third most common form of cancer among women between 15 and 44years of age globally.[6–8] HPV is responsible for other diseases as well such as recurrent juvenile respiratory papillomatosis caused by HPV types 6 and 11.[9] The aim of this study was to determine the common HPV genotypes present in the LBC samples requested for HPV testing sent to CABRI, GMU, UAE. No similar study has been reported from the UAE so far.

Materials & methods

The sample used for HPV genotyping test was a cervical brush specimen collected in Thin-prep® Vials for the Pap test in PreservCyt® Solution using a specified endo-cervical brush. It contained cervical cells. HPV genotyping assay was done using the GenoFlow HPV array test kit (developed by Diagcor Bioscience Inc), which employs the polymerase chain reaction (PCR) amplification and "flow-through" hybridization technology. It covers 33 common genotypes classified into high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68b, 73 and 82) and low-risk types (6, 11, 26, 40, 42, 43, 44, 54, 55, 57, 61, 70, 71, 72, 81 and 84). DNA was extracted from the sample under sterile conditions, using the Genfind extraction kit, which gave high yield and ensured purity of DNA. Extraction was done according to the manufacturer (Genfind)'s recommendations and the DNA was eluted in 100µl of elution buffer.

The next step entailed conventional amplification of the extracted DNA using biotinylated primers. PCR was carried out in Veriti™ Thermal Cycler from Applied Biosystems. Under the third step, the amplicons were hybridized to capture specific probes via "flow-through" hybridization; this entails actively directing amplicons toward the probes to form duplexes. The "flow-through" technology has made hybridization an active channeling process (vis-à-vis passive previously), enabling recombination reactions to be completed in seconds. Hybridization was followed by stringent wash and signal development (Figure 1). For each sample, two control dots (amplification control and hybridization control) appeared, indicating the process was on the right track. In the fourth step, data was interpreted using the proprietary Diagcor software. It captures images of DNA analysis and converts them into data.

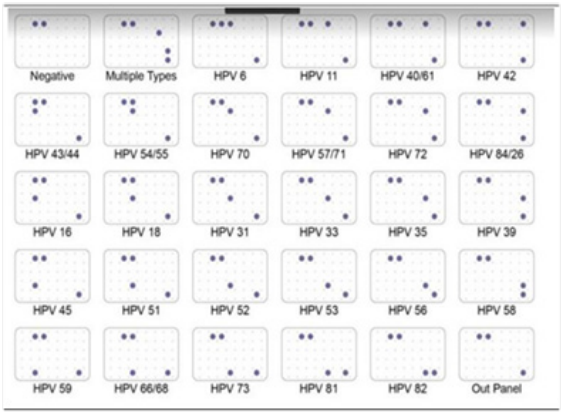

Figure 1 Visual interpretation of microarray patterns seen for different HPV subtypes.

Results

In total, 220 cases were received at CABRI from July 2015 to September 2016. The patients examined aged 18-55years. Ninety of them (41%) tested positive for HPV genotypes. About 38% were infected by only one genotype of the virus. The rest 62% showed multiple genotype infections (Table 1). The most common genotypes detected in our study were 66/68(17%), 51(16%), 6(15%), 43/44(11%), 16(11%) and 33(5%); details are given in (Table 2). The remaining 130 patients (59%) tested negative for the 33 genotypes covered in our assay. Of the 90 positive HPV cases, Thin Prep Pap smear was performed on 37. Of these, the results were negative for 31, while there were two cases of atypical squamous cells of undetermined significance (ASCUS), three of low-grade squamous intraepithelial lesion (LSIL) and one of condylomata accuminatum (Table 3).

Table 1 Overview of dot blot data

Prominent findings
90 cases positive out of 220
25 different genotypes
56 had mixed infections

Table 2 Genotype spread among the 90 cases examined images were transferred to a server For storage, analysis and reporting

HPV genotypes	% Cases
66/68	17%
51	16%
6	15%
16	11%
43/44	6%
33	5%
52	4%
31, 33, 53, 42, 31, 56	3% each
18, 11, 45	2%
Others (26/84, 35, 39, 40/61,54/55, 57/71, 58, 59, 68, 72, 81)	1% each
Total	100%

Table 3 Incidence of mixed infections

Number of viruses co infecting	Number of cases	%cases
1	34	38%
2	19	21%
3	13	14%
4	14	16%
5	6	7%
6	2	2%
7	1	1%
10	1	1%
Total	90	100%

Discussion

Supplementing Pap smear test with HPV testing improves the risk prognostication for cervical cancer—the false-negative rate for the two tests combined is lower than that for Pap testing alone. A woman with normal Pap smear results but positive HPV test will usually be advised by the doctor to return in a year for repeat screening to see if the HPV infection has persisted and whether any changes in cells have happened that warrant further follow-up testing. Alternatively, the woman may undergo another HPV test to specifically detect HPV 16 and 18, the two types that cause most cervical cancers. If a woman's Pap test result indicates abnormality but her HPV test is negative (normal), the follow-up tests will depend on the results of the Pap smear. If the Pap test indicates ASCUS and the HPV genotyping is also positive, the diagnosis will change to LSIL. If the patient is less than 30 years old, repeat reflex testing after one year is recommended. If the patient is above 30 years, colposcopy is advised (ASCP and ACGO guidelines). If the Pap test result is abnormal with a positive HPV test indicating any high-risk HPV type, the doctor will usually have the woman receive follow-up testing with colposcopy.

Burd[10] reviewed the HPV genotypes associated with cervical intraepithelial neoplasia and cervical carcinoma and found 6, 11, 16 and 18 to be the most important. Zuna et al.[11] studied 282 HPV cases in Tanzania and found HPV 58, 16, 35, 52, 66 and 73 to be the most common. A study by Franceschi et al.[4] in Hyderabad, India,[12] indicated the prevalence of several HPV types: 16(66.7%), 18(19.4%), 33(5.6%), 35(5.6%), 45(5.6%), 52(2.8%), 58(2.8%), 59(2.8%) and 73(2.8%). Our study on the UAE suggests that types 66/68(17%), 51(16%), 6(15%), 43/44(11%), 16(11%) and 33(5%) are the most prevalent in the country.[13–16] The quadrivalent vaccine available in the UAE protects against HPV 16 and 18 and low-risk genotypes 6 and 11. Thus, it safeguards against just one prevalent genotype - HPV 16 - which is responsible for only 9% of the cases that were tested.

Conclusion

The LBC-based Pap smear has been widely applied for cervical cancer screening, with sensitivity ranging from 40-80% reported in high-grade cervical intraepithelial neoplasia. Due to the shortcomings associated with the technique (frequency of false-negative results and sampling error, especially when abnormal cells are not recovered on the smear), it needs to be supplemented with molecular DNA testing using PCR, a highly sensitive and specific method for HPV DNA detection. This makes the detection process more robust as it includes HPV genotyping, which is vital for early prognostication. Data regarding HPV subtypes detected in patients from the UAE is rare, and this is the first paper in this regard. Also, efficacy of the current quadrivalent vaccine should be reevaluated to assess its relevance for the country. A large study in this regard has been proposed.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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