RESEARCH ARTICLE

Prevalence of Human Papillomavirus in Women from Saudi Arabia

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Abstract

Background: Human papillomavirus (HPV) infection is the main causes of cervical cancer in women worldwide. The goal of the present study was to determine the prevalence and distribution of HPV genotypes in women from Saudi Arabia. Recently, several HPV detection methods have been developed, each with different sensitivities and specificities. Methods: In this study, total forty cervical samples were subjected to polymerase chain reaction and hybridization to BioFilmChip microarray assessment. Results: Human papillomavirus (HPV) infections were found in 43% of the specimens. The most prevalent genotypes were HPV 16 (30%) HPV 18 (8.0%) followed by type HPV 45, occurring at 5.0%. Conclusion: Our finding showed the HPV infection and prevalence is increasing at alarming rate in women of Saudi Arabia. There was no low risk infection detected in the tested samples. The BioFilmChip microarray detection system is highly accurate and suitable for detection of single and multiple infections, allowing rapid detection with less time-consumption and easier performance as compared with other methods.

Keywords: Human papillomavirus (HPV) - cervical cancer - prevalence - diagnosis - Saudi Arabia

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Introduction

Cervical cancer is the second most prevalent disease worldwide occurrence with new cases every year and approximately 80% of them reported in developing countries (Munoz et al., 2003; Parkin and Bray, 2006; Lin et al., 2008; Chen et al., 2011 and 2012). Such type of cases appears mainly in between the age of 30 and 50. HPV belongs to family Papillomavirus which is non enveloped virus with 52-55 nm in size with circular double stranded circular DNA and a genome with 7,900 base pairs followed by eight overlapping open reading frames. The late genes (L1 and L2) and early genes (E6, E7, E1, E2, E4 and E5) that are expressed in more than 100 different HPV genotypes have been identified based on DNA sequence variations. They are classified according to various criteria such as their tissue tropism, potential of oncogenic and phylogenetic relationship using molecular biology techniques (Szostek et al., 2008).Human Papillomavirus (HPV) plays a very important role in cervical cancer and 99.7% of HPV DNA identified in invasive cervical carcinomas (Walboomers et al., 1999; Munoz et al., 2006). The prevalence of HPV infection varies substantially among countries and according to lifestyle, age, food and nutrition. The majority of HPV infections are asymptomatic and temporary in the adolescent population. It has been observed that up to 98% of cervical cancers in females are associated with HPV infection and more than 90% of new infections appear to induce high grade cervical neoplasia (Moscicki et al., 2006; Woodman et al., 2007). The most important risk factors include, drinking, smoking, education system, many partners, food, nutrition, use of long-term oral contraceptive and immune-suppression. Less involvement for testing is a probable cofactor that should be considered in an analysis of HPV in cervical female cancer. More than 100 HPV genotypes have been characterized at molecular level, and approximately 40 different types were identified to cause of genital tract infection. The World Health Organization has officially designated HPVs -16 and -18 as carcinogenic agents. According to earlier information, HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -69, -73, -59 and -82 were classified as "high-risk types" (Munoz et al., 2003; Bouvard et al., 2009), and associated with cervical cancer; while HPV-6, -11 -42, -43, -44 were classified as "low-risk types" (Middleton et al., 2003; Trottier and Burchell, 2009), which were found difficult for detection in cervical lesions but associated with anogenital warts.

Female individuals infected with low risk HPV types have a minimal possibility of developing cervical cancer.

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However, 10-20% of positive cases with HPV infection by several genotypes have been reported (Hwang et al., 2004; Meijer et al., 2006; Matsumoto 2007; Lie et al., 2008). HPV 16 is detectable in 21% of women with low grade squamous intraepithelial lesions (LSIL) and in more than 50% of women with cervical intraepithelial neoplasia grade 3 (CIN3). HPV 18 causes 10 15% of CIN 3 and also causes more than 35% of cervical adenocarcinomas, and it is complex to identify by current diagnostic tools (Bosch et al., 1995).

The relationship between HPV infection and cervical cancer had given great impetus to the development of prophylactic vaccines against the most common HR-HPV types. Worldwide, HPV16 and 18 resulted for more thn 70% of cervical cancer cases (Munoz et al., 2003). Currently very few HPV vaccines; Gardasil (HPV6/11/16/18) (Villa et al., 2005) and Cervarix (HPV16/18) (Harper et al., 2004) were developed against these two major cancer-causing HPV types (Yoshikawa, 2009). Based on the meta-analysis reported worldwide, HPV type 16 and 18 were the most common while in (de Sanjose et al., 2007; Bruni et al., 2010), HPV type 45, 31 and 33, were in Europe and Africa. HPV 52 and 58 presented a more frequent prevalence in Asia (Bao et al., 2008; Sun et al., 2010; Chen et al., 2012).

Early detection can be difficult for most cervical infections since HPV is asymptomatic. Various diagnostic programs are available for HPV identification in different countries such as by using the traditional detection based on the Papanicolaou smear discovered in 1943. However, false negatives (between 15-50% for cervical premalignant lesions and cervical cancer) and false positives (30%) should be considered during screening by using that cytological technique (Jacobs et al., 1999; Cuzick et al., 2003).Cytology examinations have limitations with regards to specificity and low predictive value for high grade pathology (Shafi 1994; Raffle et al., 1995). Highly sensitive methods have been developed to detect HPV such as: polymerase chain reaction (PCR) method; and is considered the most sensitive HPV detection technique worldwide (Young et al., 1989). PCR is based on the use of primers such as the MY09/MY11 primer set (MY PCR), which is the most frequently used amplification system for the detection of the virus in clinical samples. This was synthesized with multiple nucleotides in each primer and mixed with 25 primers including HMB01M, which detect a large spectrum of HPV types? This has been used in North and South America as well as in Asia (Wheeler et al., 1993; Hildesheim et al., 1994; Ley et al., 1994; Liaw et al., 1995; Lazcano et al., 2001; Thomas et al., 2004).

On the basis of published information, this study was design and conducted to detect and determine the frequency and prevalence of HPV genotypes infection in Saudi Arabian population suffering from cervical cancer at KAUH and to establish a correlation between HPV genotypes involved in malignancies and the high risk type by using BiofilmChip detection method.

Materials and Methods

Study population and specimen collection

Fresh samples were collected from January 2011 to January 2012 at king Abdulaziz university hospital. A woman was eligible for study subject if she was a gynecological outpatient with genital tract disease related symptom, was not presently pregnant; had not undergone a total uterus or cervix resection; and was willing to undergo HPV testing and also consent to participate in the present study. The study was carried out with the approval of the hospital ethical committee, and patients consent was obtained for the collection of cervical cells. Total forty tissue biopsies were freshly collected from cervical cancer cases. Examination performed by gynaecological practitioners in above-mentioned hospital. The ages of the patients enrolled in this study were between 36-80 years. The control group comprised specimens with normal cytology and HPV DNA negative by PCR.

DNA isolation

DNA extraction was performed by using by Qiamp DNA mini kit (QIAGEN, Valencia, CA) with following manufacturer's instructions. After extraction, all specimens were subjected to PCR amplification of the β-globin gene to serve as an internal control as described previously (Shadrina et al., 2007) and stored subsequently at –20°C until tested.

HPV detection and genotyping

Total DNA was isolated by using Kit (QIAGEN, Valencia, CA) followed by manufacturer instructions and multiplex PCR was performed of the targeted DNA followed by primer extension detection. Positive samples for HPV DNA by multiplex PCR were selected for BiofilmChip hybridization assay. The hybridization and detection were performed using autogenomics INFINITI Analyzer (Vista, CA) according to the manufacturer's instructions. The BiofilmChip contained specific DNA probes of 26 HPVs (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The specific primer amplified only for the respective target and unamplified for other unrelated targets was chosen.

Results

The analysis confirms the positive samples by the BiofilmChip hybridization assay and all the fourty samples were selected for testing. Applying the assay, total three HPV genotypes (HPV 16, 18, and 45) were identified covering high-risk.

Detection and prevalence of HPV genotypes

The most common HR-HPV types observed in this study were HPV 16, HPV 18 and HPV 45. Detection of HPV genotypes by BiofilmChip hybridization assay showed that the highest prevalence HPV 16 infection was found in the 35-76 year old age group and decreased slowly in older age groups in Saudi Arabian population and no other genotypes like HPV 31, 33, 35/68, 39/56, 51/59, 52/58, 6/11 were detected in all the screened samples (Table 1).

An age-specific prevalence was observed in overall HPV genotypes (Figure 1). Outpatients the age of 35-76

Table 1. Distribution and Age Specific Prevalence of HPV Genotypes in Women from Saudi Arabia

HPV genotypes	Total No. of positive cases	Age groups	Prevalence (%)	HR-HPV (%)	LR-HPV (%)
beta-globin	35/40	35-82	87.50%		
HPV16	12/40	35-76	30%	30%	0
HPV18	3/40	45-64	7.50%	7.50%	0
HPV31	0/40	0	0	0	0
HPV33	0/40	0	0	0	0
HPV45	2/40	41-45	5%	5%	0
HPV35/68	0/40	0	0	0	0
HPV39/56	0/40	0	0	0	0
HPV51/59	0/40	0	0	0	0
HPV52/58	0/40	0	0	0	0
HPV6/11	40	0	0	0	0

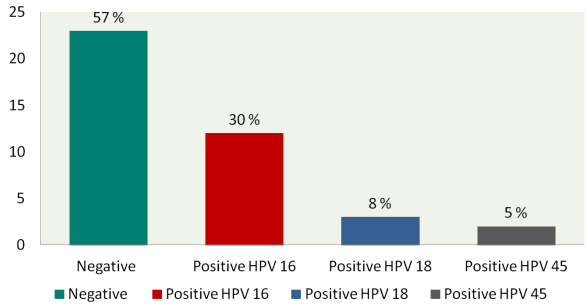

Figure 1. Prevalence of HPV Genotypes in Women from Saudi Arabia

years had the highest HPV prevalence (30.0%), which was significantly higher than that of other age groups. Very less HPV infection (8%) was found in women 45-64 years of age which was significantly higher than that of middle aged groups age ranged from 41 to 45 years. The LR-HPV genotypes were not detected in any samples.

Discussion

This study reports the detection and prevalence of HPV genotypes in Saudi Arabian population by BiofilmChip hybridization assay. The HPV 16 genotype was detected at the highest prevalence rate (30%) in Saudi Arabian population followed by HPV 18 and HPV 45. The Low risk genotype was not detected in any samples. In women, about 50% of new infections of most low-grade squamous intraepithelial lesions may disappear spontaneously within 12 months whereas the clearance rate in older women is lower (Chumworathayi et al., 2010). Approximately 1% of low-grade squamous intraepithelial lesions and 12% of high-grade squamous intraepithelial lesions may progress to invasive cervical cancer (Clifford et al., 2005) and more than 90% of HR-HPV genotypes pose an increased risk for progression to cervical cancer. Thus, HR-HPV genotypes identification will be helpful in prevention of cervical cancer development and is important for developing suitable HPV vaccines.

According to other studies, diagnosis based on cytology showed various multiple infections in 7% to 23% of patient samples (Rousseau et al., 2003). The infection shows a peak in young women aged below 25 and decreases slightly to less than 4% above the age of 40 (Peto et al., 2004). The variations depend on characteristics of the population studied and detection methods.Geographical, clinical factors and demographics contribute to various extents to multiple HPV infections (Trottier et al., 2006). Variable results have been achieved by PCR detection for different genotypes. For multiple genotypes identification efficiently, a combination of typing methods and cytology is more suitable for risk assessment but expensive for routine diagnosis (Galan-Sanchez et al., 2009). Multiple infections, which increase the risk of invasive cervical cancer compared with single infection, are usually found in low-grade squamous intraepithelial lesions patients (Tucker et al., 1993).

In this study, highly sensitive and time saving BiofilmChip hybridization assay was used for HPV detection. Upon testing with DNA chip, the highest prevalence of HPV infection was found in the 35-76 year age groups and decreased slightly. Single HPV infection was more prevalent than multiple HPV infections. HPV 16 is the most prevalent genotype in cervical cancer (30.0%) and the second most predominant genotypes HPV 18 (8.0%) respectively which correlated with other findings that HPV16 is the most prevalent genotype and HPV 18 is the second in the CA group, nearly the world average (15.0%) (Munoz et al., 2003; Clifford et al., 2005). In addition, the third most prevalent genotype HPV45 (5.0%) was detected in Saudi Arabian followed by Yemini and Somalian population. These results have shown the diversity of HPV genotypes which provide information with regard to the design of multivalent prophylactic vaccines suitable for each geographical area.

Interestingly, a high incidence of HPV58 has been found in Asia, Africa and other areas while it is not common worldwide especially in Europe and North America. According to a study conducted in Korea and Japan, HPV58 is highly prevalent in high-grade squamous intraepithelial lesions and squamous cell carcinoma (Miura et al., 2006). The evolution and spread of HPV 58 have been spread earlier. The original source of ancient HPV58 may have been in West Africa and Southeast Asia may be a subsequent "relay center" (Chan et al., 2002; Li et al., 2009). However, the sequencing method applied has the capacity to detect these genotypes and others which are uncommon such as HPV 67, HPV 70 and HPV 84. HR-HPV testing can be applied to primary screening and management. For this reason, clinical laboratories should determine advantages and disadvantages of each method for HPV typing. All methods produced highly similar results. In the previous study, the prevalence of probably high-risk, low-risk and unclassified-risk was high in low-grade squamous intraepithelial lesions grade and normal cytology such as HPV 66, HPV 11 and HPV 71, respectively (Chansaenroj et al., 2010). Thus, multiple HPV infections still need to be identified.

The size of the overall system and the time required for examination is less in comparison to sequencing and the other commercial kits. Finally, the operation of HPV genotyping assays depends on their primer sets. The differences in HPV prevalence depend on many factors such as sample size, measurement and detection method. Combinations of highly concordant HPV genotyping methods for primary screening are recommended.

Significance of HPV prevalence in any area should be considered for vaccination program.

In conclusion, the BiofilmChip hybridization assay is more suitable to identify both single and multiple infections of HR-HPV genotypes than direct sequencing. It can reduce size of the overall system and time required for examination compared to sequencing and the other commercial kits. This study showed that the significance of viral genotypes of HPV and others should be considered for vaccination programs. Finally, combinations of highly concordant HPV genotyping methods are recommended for primary screening. It is reported that cervical cancer is a major health problem with increasing frequencies in recent years. Our study showed that HPV infection is one of the major causes of cervical cancer in the screened group of patients. HPV type 16, 18 and 45 were detected in 43% of the cases confirming the international data about involvement of HPV in cervical cancer. Managing costs associated with increased detection of positive HPV patients is needed in our population. Autogenomics infinity analyzer can be used in clinical labs for fast and reliable method for detection of many genetic diseases. The load and go capability of the machine, makes it one of the few technologies available today that are considered to be the new generation of analyzers with full automation in genetic testing. Our finding demonstrated that the HPV infection and prevalence is increasing at alarming rate in Saudi Arabia. There was no any low risk infection was detected in tested samples.

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