High Risk Human Papilloma Virus Genotypes in Kurdistan Region in Patients with Vaginal Discharge

Nawfal R Hussein1*, Amer A Balatay2, Mahde S Assafi3*, Tamara Abdulezel Al-Mufty4, Amira S Khalil5

Abstract

**Background**: The human papilloma virus (HPV) is considered as the major risk factor for the development of cervical cancer. This virus is of different genotypes and generally can be classified into high and low risk types. **Objective**: To determine the rate of high risk HPV genotypes in women with vaginal discharge and lower abdominal pain in Kurdistan region, Iraq. **Materials and Methods**: Cervical swabs were taken from 104 women. DNA was extracted and the polymerase chain reaction (PCR) technique was used to determine the presence of high risk genotypes. **Results**: It was found that 13/104 (12.5%) of the samples were positive for high risk HPV genotypes. Amongst those who were positive, 4/13 (30.7%) were typed as genotype 16 and 7/13 (53.8%) showed mixed genotyping. On the other hand, genotypes 53 and 56 were found in only one sample each. **Conclusions**: High risk HPV genotypes are not uncommon and further community based study is needed to determine the prevalence of HPV and its genotypes and plan for prevention of infection.

Keywords: HPV - high risk group prevalence - vaginal discharge - cervical cancer - Iraq, Kurdistan Region

Introduction

Cervical cancer is the fourth most common cancer in women with more than half a million new cases in 2012. Africa is regarded as a high risk region with ASR over 30 per 100000 while eastern Asia including Iraq is within the low risk region (GLOBOCAN, 2012). HPV is considered a major risk factor for the development of cervical cancer. This virus is of different genotypes and according to the associations with neoplasms, HPV can be classified into low risk genotypes such as HPV 6, 11, 42, 43 and 44 that are associated with benign genital warts and high risk types such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70 that are associated with the development of cancer (Woodman et al., 2007). High risk HPV genotypes nucleic acid has been found in the vast majority of squamous cell carcinomas and adenocarcinomas of the cervix. Additionally, different studies showed HPV DNA in cervical intraepithelial neoplasia (CIN) (Kuhn et al., 2000). Amongst the high risk group, HPV-16 and HPV-18 are the most prevalent. While HPV type 16 is more frequently found in squamous cell carcinoma, type 18 is more commonly associated with adenocarcinoma (Franco et al., 1999). There is a great variation in the prevalence of HPV and the prevalent genotypes depending upon patient age, cytology stage, and geographic region.

The prevalence of cervical cancer in industrialized countries is less than that found in developing countries. One explanation is the well-established screening programs in the developed world (GLOBOCAN, 2012). Although a close relationship has been found between HPV-16/18 genotypes and cervical cancer, such genotypes are not always the most common types amongst subjects with cervical cancer. It has been postulated that HPV types may differ in different countries (Ault, 2006). Therefore, studying circulating HPV types in specific population is essential to tailor the screening and vaccination program. The aim of this study was to evaluate the rate of high-risk human papillomavirus in cervical samples from 104 women with vaginal discharge in Kurdistan region, Iraq.

Materials and Methods

**Patients**

Samples were collected from women referred to infectious disease unit in Azadi teaching hospital, Duhok and Maternity hospital, Hawler, Kurdistan region, Iraq. All patients referred for difficult infection not responding to a prior course of antibiotics. All patients presented with lower abdominal pain and vaginal discharge. Cervical swabs were taken with Cervex-Brush cervical cell sampler then dipped into in 20 mL transport medium (Cytec...
Corr, Boxborough, MA) according to the manufacturer’s instructions. Then, samples kept frozen and stored at -80°C until analysis performed.

**DNA extraction and HPV genotyping**

An aliquot of 400 μl sample was treated with proteinase K and then DNA was extracted using Qiagen Blood & Cell Culture DNA Mini-Kit following manufacturer’s instructions. As per manufacturer’s instructions, PCR was used to study the HPV types (Sacace, Italy). The test determined 19 human Papillomavirus subtypes of medium-high risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73 and 82.

**Data analysis**

All data analysis was performed using Excel spreadsheet. Mann-Whitney test was used to compare the age of different groups. P ≤0.05 was considered statistically significant.

**Ethics Statement**

Consent was obtained from patients recruited in the report. This research and method of attaining consent were approved by the scientific committee of the department of sciences, University of Zakho, Kurdistan Region, Iraq.

**Results**

During the period of study, 54 samples were collected from patients referred to infectious disease clinic in Azadi teaching hospital, Duhok and 50 samples were collected in Maternity hospital in Hawler city, Kurdistan Region, Iraq. All patients complained of vaginal discharge and lower abdominal pain and received a course of antibiotics but without benefit. The average age of our patients was 33.9±8.4.

Overall, 104 samples were collected from subjects suffering from lower abdominal pain and vaginal discharge. All of the patients were married and denied illegitimate sex. In Duhok city, 8/54 (14.8%) of the samples were typed positive for high risk HPV genotypes. Amongst positive samples in Duhok, 2/8 (25%) were typed as genotype 16 while 4/8 (50%) carried mixed genotypes. Genotypes 53 and 56 were found in one sample for each. In Hawler city, 5/50 (10%) were typed positive for high risk genotypes amongst them 2/5 (40%) carried genotype 16 and the rest carried mixed genotypes. Overall, it was found that 13/104 (12.5%) of the samples were positive for high risk HPV genotypes. Amongst those who were positive, 4/13 (30.7%) typed as genotype 16 and 7/13 (53.8%) carried mixed genotypes. On the other hand, genotypes 53 and 56 were found in one sample for each. The average age of patients with positive samples was 33.6±9.1 while the average age of negative samples patients was 33.9±8.3 (Mann-Whitney test p=0.37).

**Discussion**

Although cervical cancer can be preventable and curable, the prevalence of such cancer is much higher in developing countries than that found in industrialized countries. This variation can be explained in part by the vaccination and screening program found in developed countries.1, 3, 5 Baseline information about the prevalence of cervical cancer and circulating HPV genotypes should be available for a better planning of prevention. Previous studies have shown that the prevalence of HPV and its genotypes varies from region to region with an estimate HPV prevalence ranging from 1% to more than 25% in women with normal cytology (Giuliano et al., 2002, Ault, 2006, Woodman et al., 2007). A survey conducted by international agency for research on cancer showed a prevalence of 9.6%, of which 5.4% were high-risk types and found that HPV genotype 16 was twofold as frequent as any other high risk genotype in all regions (Smith et al., 2004, Cancer, 2007). In this study, high risk genotypes were found in 12.5% of the samples. This is slightly lower than that found in a previous report from Iraq where they found about 17% of the samples were positive for HPV (Ali et al., 2012). Amongst those who were positive, 30.7% were genotype 16 and 53.8% carried mixed genotypes. One the other hand, genotypes 53 and 56 were found in one sample for each. In a previous study from Iraq, HPV 16 was the most commonly detected high risk HPV genotype amongst the positive cases (28.4%). In agreement with other studies, most of the infected subjects were in their third decade of life (Sawaya et al., 2015). When we compared between the age of subjects who were positive for high risk HPV and those who were negative, no significant difference was found. In a study conducted in Turkey recruiting 403 women, 23% of the samples tested positive for HPV DNA (Dursun et al., 2009). The most common HPV genotypes were HPV 16 (36%), HPV 6 (22%) and HPV 18 (13%). The rate of multiple HPV infections in women with normal Pap test was 2.2%. In another study conducted in Iran, HPV was tested in 50 women with abnormal cytology in their Pap smears and 77 women with normal Pap smear. In the normal group, HPV DNA was found in 13% of the samples, while HPV DNA was found in 60% in the abnormal samples. The most prevalent HPV genotypes amongst the recruited samples were HPV 16 (76%) and HPV18 (12.7%) (Farjadian et al., 2003). In a study conducted in Saudi where 485 patients were recruited, high risk HPV was found in 5.6% of the samples. In the same study, the HPV genotypes were not determined (Bondagji et al., 2013). However, in another study conducted in Saudi, it was shown that the most prevalent genotype was 68 followed by genotypes 18 and 16 (AlObaid et al., 2014). In a screening program in Morocco, the prevalence of HPV was 16% and genotypes 16 and 18 were the most common genotypes (Alhamamy et al., 2010). In Egypt, it was found that 40% of samples were positive for HPV. Amongst those who were positive, 21%, 16% and 63% of samples were of low risk, high risk genotype and mixed infection, respectively. HPV 16, 18 and 31 were the most prevalent high risk genotypes, constituting 42%, 29% and 12.9 %, respectively (Youssef et al., 2016). In another study conducted in Jordan recruiting subjects with cervical pathology, high risk HPV positivity was found in more than 70% of the samples and genotypes 16 and 18 as the most prevalent types (Obeidat et al., 2013).
Our study has limitations. Firstly, our sample size was small due to high expenses of consumables used in the experiments. The government should support and encourage such practice changing research for a better prevention planning. Also, it seemed that HPV was not a priority for gynaecologists in the region due to the misbelief that HPV is uncommon. Furthermore, patients were reluctant to participate due to lack of appropriate education. Secondly, this rate of high risk genotypes may not reflect the prevalence of such genotypes in the community because the study recruited symptomatic subjects only. Population based study is needed to study the prevalence of HPV and its genotypes in the society.

References


