

Type-specific prevalence of human papillomavirus among women with abnormal cytology in Jordan

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Summary

Background: Infection with human papillomavirus (HPV) is the most common sexually transmitted infection in women worldwide. Infection with high-risk HPV (HR-HPV) is the main cause of cervical cancer. However, little is known about its prevalence in the female population in Jordan. The aim of this study was to discover HPV type-specific prevalence in women living in Jordan. Data regarding HPV type-specific prevalence for a particular country are crucial for providing baseline information to estimate the effectiveness of implementing HPV-based cervical cancer prevention. **Materials and Methods:** Between 2008 and 2014, cervical epithelium samples were collected by conventional Papanicolaou (Pap) smear; all abnormal smears had reflex HPV testing from apparently healthy and non-healthy women. HPV prevalence and its genotype distribution were examined in these smears. DNA samples were extracted and HPV genotyping was performed. The results were retrospectively analyzed. **Results:** A total of 209 abnormal Pap smears were identified and reflex HPV testing was performed on these cases; 153 (73.2%) of the tested cases were HPV positive. The mean age of the women included was 38.3 ± 10.2 (range: 20-70) years and the mean age of HPV-positive women was 38.5 ± 10.3 (range: 21-70) years. There was a significant increase in the incidence of HPV infection over the study period. HPV 6 and 11 infections were very common in the HPV positive women; 38.6% and 47%, respectively. HPV 16 was the single most common (57.5%) HPV infection detected. HPV 30's (31, 33, 34, 35, and 39) as a group were the most common HPV infections recorded at 59.5%. HPV 50's (51, 52, 53, 56, 58, and 59) were detected in 56.9% of the cases. HPV type 68 was recorded in one case (0.6%). Of notice, the vast majority of cases had co-infection of multiple genotypes (89%) and only 11% were single genotype infection. **Conclusion:** The results of the study showed a relatively high prevalence (73.2%) of HPV infection and a higher incidence of co-infection with multiple high risk genotypes (89%) in comparison with other studies from the present region. This study suggests that there is sufficient evidence to warrant further population-based studies and further interventions. HPV vaccination should be considered for Jordanian girls as a preventative measure.

Key words: HPV; Pap smear; Cervical cancer; Vaccination; Jordan.

Introduction

Cervical cancer continues to be a major killer in the developing world. Introduction of cervical screening program and early treatment of cervical intraepithelia neoplasia (CIN) have managed to reduce the incidence [1]. Still, 83% of cervical cancer cases are in the developing world [2]. Human papillomavirus (HPV) is an essential causative agent for cervical cancer [1]. HPV belongs to the most prevalent sexually transmitted infections worldwide [3]. More than 200 types have been isolated, 40 of which are transmitted sexually and 15 of these are carcinogenic and classified as high risk HPV (HR-HPV) types. HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are the main cause of cervical cancer [2, 3].

Genotypes 16 and 18 are responsible for 68-82% of cervical cancer cases worldwide [4]. Infection with HPV and subsequent event can lead to HPV genome integration and

unregulated expression of viral oncogenes E6 and E7. This promotes malignant transformation and immortalization of cervical cells [1].

In addition, the HR-HPV genotypes have been associated with other types of cancers such as anogenital and head and neck cancers [5]. Low-risk HPV (LR-HPV) can cause genital warts and epithelial growth over the vocal cords [6]. HPV infection is responsible for almost all cervical cancer-related deaths, 80% of these cases occur in the developing countries [2].

Regular gynecological screening using conventional Papanicolaou (Pap) smear examination has been effective in preventing cervical cancer. The incidence of cervical cancer in countries where the majority of women receive regular screening of the cervical epithelium has decreased by more than 70% [7-9].

However, regular gynecological screening programs are difficult to implement in low-resources countries such as

Table 1. — Results of HPV testing in women with abnormal cytology.

Group	n (%)
Total number of samples	209
HPV negative	56 (26.8%)
HPV positive	153 (73.2%)
Infection with a single HPV genotype	23 (11%)
Co-infection with multiple HPV genotypes	186 (89%)

Jordan. Among ever-married Jordanian women, the lifetime prevalence of Pap smear testing was 27.8% in 2012 [10]. In addition, Pap smear testing has low sensitivity for high-grade cervical dysplasia lesions and the diagnostic classifications are complex and subjective [11, 12].

HPV vaccines have been licensed in many countries. Bivalent (Cervarix) targets HPV 16 and 18, quadrivalent vaccine (Gardasil) targets HPV 6, 11, 16, and 18 and the most recent nonavalent vaccine (Gardasil 9) targets high risk HPV types 16, 18, 31, 33, 45, 52 and 58, as well as the low risk types 6 and 11 which cause 90 % of genital warts [2]. Thus, the identification of HPV prevalence and genotype distribution among women in Jordan is crucial to determine the health economic calculations and effectiveness of implementing HPV vaccination program. Although HPV 16 and 18 are the most common types of high risk HPV, there are geographical variations. The International Agency for Research on Cancer established that some genotypes were particularly prevalent in different continents (e.g., HPV 31, 33, and 45 in America; HPV 33 and 31 in Europe, HPV 45 and 33 in Africa and HPV 52 and 58 in Asia) [3].

Cervical cancer ranks 15th among other cancers in Jordan. However, this reflects HPV infection 10-15 year earlier. It was noticed from the present authors' previous work that the incidence of abnormal cervical smear is on the increase, so as the incidence of HPV infections. The aim of the current study was to determine HPV genotype distribution in cervical epithelium samples among apparently healthy and non-healthy women in Jordan.

Materials and Methods

Between April 2008 and November 2014, 209 women were enrolled in the current study during their regular visits to private gynecology clinics in Amman, Jordan. All tests were done at First Medical Laboratories by a single accredited American Board certified cytopathologist (HAF). Women had their cervical smear as an opportunistic screening or if there was a clinical indication. Only women with positive cytology were then offered reflex HPV testing. Pregnant women, menstruating women, those with hysterectomy were excluded.

Pap smears were examined and classified in accordance with Bethesda system (2001). The results were subdivided into normal, atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS), low-grade squamous intraepithelial lesions (LSILs), including changes induced by HPV infection, high-grade squamous intraepithelial lesions (HSILs), and carcinoma. All Pap smear

cases that have ASCUS abnormality and the aforementioned were tested for HPV genotyping. Samples were collected with cotton swabs and suspended in 2 ml saline.

DNA was extracted and the HPV screening kit was used on cervical swabs for HPV differentiation into LR and HR types. The assay reportedly enables detection of 24 HPV types: six LR (LR HPV 6, 11, 40, 42, 43, and 44) and 18 HR (HR HPV 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) types. HPV screening is a test based on amplification of a fragment of a highly conserved region of the HPV L1 gene using biotin-labeled primers. The reaction product is subsequently used for reverse hybridization with HPV specific, HR HPV specific and LR HPV specific probes firmly anchored to membrane strips. The amplification control gene is the GAPDH gene. The PCR product specifically bound to a specific probe is detected through biotin-streptavidin-conjugated antibody and subsequent color reaction.

This study was approved by the Institute Review Board (IRB) of Ibn Al-Haytham Hospital and registered with Scientific Research Committee, Faculty of Medicine, University of Jordan, which conforms to the World Medical Association Declaration of Helsinki.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 20.0. Descriptive statistics were presented as numbers, percentages, and means \pm standard deviations.

Results

Between 2008 and 2014, a total of 209 abnormal Pap smears were identified and reflex HPV testing were performed on these cases. The results were retrospectively analyzed. The mean age of the women included was 38.3 \pm 10.2 (range: 20-70) years; 153 (73%) of tested cases were HPV positive and their mean age was 38.5 \pm 10.3 (range: 21-70) years (Table 1). There was a significant increase in the incidence of HPV infection over the study period (Figure 1). HPV 16 was the single most common HPV isolated (57.5%). HPV 30's (31, 33, 34, 35, and 39) as a group were the most common isolate at 59.5%. HPV 50's (51, 52, 53, 56, 58, and 59) were isolated in 56.9% of the cases (Figure 2). HPV 6 and HPV 11 infections were recorded in 38.6% and 47% of the women; respectively. HPV type 68 was isolated in one case (0.6%). Of notice, the vast majority of cases had co-infection of multiple genotypes (89%) and only 11% were single genotype infection (Figure 3).

Discussion

HPV is the most common sexually transmitted disease worldwide [3]. This is the first study in Jordan to assess the distribution of different HPV genotypes as an adjunct to cytology in cervical cancer screening. With the advent of HPV genotype testing, it is now possible to identify the magnitude of HPV burden in Jordan and compare it to other countries in the region and to other regions [1, 13]. Such data if available will assist planning of health services and introduce appropriate measures based on current prevalence of HPV genotypes. There are indications that HPV is on the increase in the Extended Middle East and North Africa (EMENA) region [14].

The incidence of HPV infection was low at the beginning

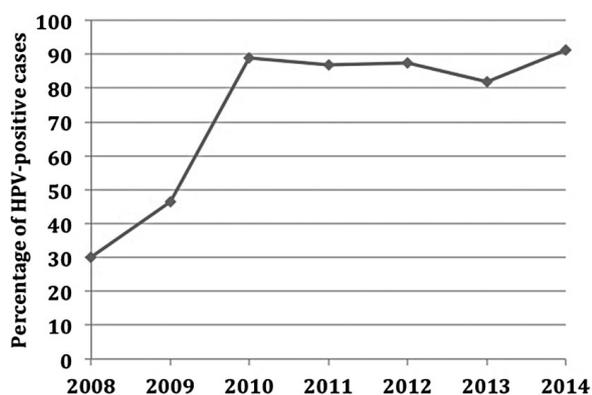


Figure 1. — Prevalence of HPV infection in women by year

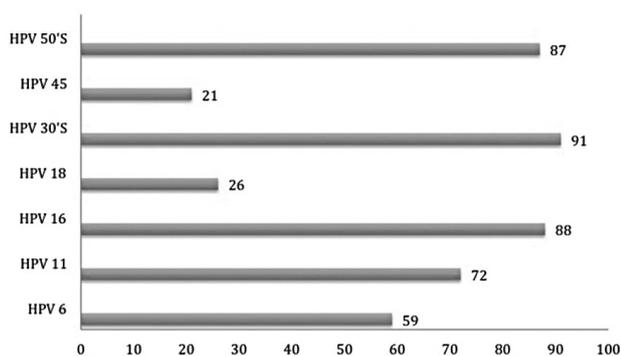


Figure 2. — Distribution of HPV genotypes showing the number of infected women.

Single versus mixed infections

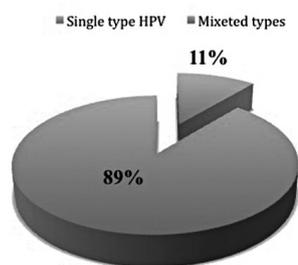


Figure 3. — Incidence of co-infection with multiple genotypes and single genotype infection.

of the study period in 2008 with approximately 30% of abnormal Pap smear (Figure 1), which is similar to the rate reported in Qatar in 2012 [15]. However the percentage increased to 90% in the years 2010 and beyond (Figure 1), although the present authors have been using the same kit from 2008. It is well know from their previous work that the incidence of abnormal smears (LSIL and higher) in-

creased from 2.7% in the years 2000-2004 to 12.3% in the years 2010- 2014). The area has been going through crises. Jordan population has increased dramatically over the last ten years. The increment was largely due to floods of migrant from nearby countries at war. Whether this has to do with the increase in HPV prevalence or due to globalization and change in perception of sexual behavior is not yet known.

Same as the global trends [16] the present authors documented a large HPV prevalence of 73.2% among women with cytological abnormalities. This percentage is much higher than what have been reported from other studies in the region [15]. However, it is within the same range of other studies in the same region [17]. The reason for this variation is not clear and may be explained by the difference in sensitivity of HPV detection kit used in this study. All cytological specimens were read by one person (American Board certified cytopathologist) which can explain partially the high prevalence rate.

The prevalence of HPV genotypes varies according to the geographical area. HPV genotypes 33 and 31 are more common in Europe, whereas HPV 52 and 58 are more common in Asia. In the present study, the distribution of HPV genotypes is somehow hybrid between Asia and Europe.

A previous study from Jordan showed that prevalence of HPV 16 was 53.8%, followed by HPV types 30's and 45. HPV testing in that study was carried out on paraffin embedded samples from invasive cancer and cervical intraepithelial neoplasia (CIN) cases [18]. In the current study HPV testing was performed on specially collected media aimed at identification of HPV genotypes.

The present study confirms the importance of HPV 16, 30's, and 45, but also raises the importance of HPV types 50's. Although many of these types were identified as co-infection of multiple HPV genotypes and not a single genotype. Previous studies have confirmed that HPV genotypes 52 and 58 have high contribution in Asia [19, 20]. HPV 16 was the most common single HR genotype isolated among women with cytological abnormalities (57.5%). HPV 16 is responsible for about 50% of cervical cancer worldwide [21].

Co-infection with multiple genotypes in the present study was very high (89%). Although infection with multiple genotypes can occur at random, Chaturvedi *et al.* suggested that co-infecting HPV genotypes lead to cervical disease independently. Multiple infections in his series were 42% [22]. While others believe that co-infection with multiple species are significantly associated with high grade lesions compared with single infection [22]. Jaisamrarn *et al.* found that co-infection with multiple species could increase the risk of progression to CIN, but also it increases the chances of clearance [23]. Others suggested that infections with multiple genotypes synergistically promote carcinogenesis [24]. Few studies from EMENA countries reported HPV prevalence in women with abnormal cytology to be up to 98% [25, 26]; in the present study the rate was 89.

Conclusions

In conclusion, the present study showed a relatively high prevalence (73.2%) of HPV infection and a higher incidence of co-infection with multiple HR genotype (89%) in comparison with other studies from the present region [15]. This study suggests that there is sufficient evidence to warrant further population based studies and further interventions. HPV vaccination should be considered for girls as a preventative measure considering the fact that only 7% of women had a smear in the last three years [10]. This is supported by a positive attitude among healthcare providers towards HPV vaccination [27]. Cost-effective studies have yet to be conducted. The type of vaccine should take into consideration the isolated types in the community. The data from this study suggests that nona-valent vaccine (Gardasil 9) has the potential to prevent infections in Jordanian women.

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