Specific Human Papilloma Virus Prevalence and Distribution in Women with Abnormal Cervical Cytology: A Single Centre Retrospective Observational Study in the Emirate of Abu Dhabi

Krishi Gowdara Revannasiddappa, Sreekala Sreehari & Shubha Shankari Manjunath

RESEARCH ARTICLE

Type Specific Human Papilloma Virus Prevalence and Distribution in Women with Abnormal Cervical Cytology: A Single Centre Retrospective Observational Study in the Emirate of Abu Dhabi

Krishi Gowdra Revannasiddappa, Sreekala Sreehari and Shubha Shankari Manjunath

1Department of Obstetrics and Gynaecology, NMC Speciality Hospital, Abu Dhabi, UAE
2Centralised Histopathology Laboratory, NMC Group of Hospitals, Abu Dhabi, UAE

Abstract:
Background: Human papillomavirus (HPV) infection is a major cause of cervical cancer and premalignant dysplasia. The prevalence and distribution of different high-risk HPV genotypes can affect HPV vaccination strategies and the design of cervical cancer prevention programs.

Objective: Here, we aimed to determine the prevalence of different HPV genotypes in women with abnormal Cervical cytology in the United Arab Emirates (UAE), evaluate correlations between HPV genotypes and the degree of cervical dysplasia, and observe distributions of HPV genotypes across age groups.

Methods: The study included 442 women who underwent HPV genotyping at our institution between January 2018 and September 2019. A retrospective chart review was conducted for all 442 cases, and data were collected from hospital records.

Results: The overall HPV positivity rate was 56.1%. The prevalence of high-risk HPV was 48%; 35.4% of patients had multiple high-risk HPV strains, whereas 64.6% had a single high-risk HPV strain. The most common high-risk HPV genotype was HPV-16 (15.2%), followed by HPV-31 (8.9%), HPV-53 (8.9%), HPV-66 (8.6%), and HPV-51 (8.3%). The prevalence of HPV-18 was only 3.8%. The high-risk HPV positivity rate increased from 39% in women with Atypical Squamous Cells of Undetermined Significance (ASCUS) to 81% in women with Low-grade Squamous Intraepithelial Lesions (LSILs), 81.3% in women with atypical squamous cells cannot rule out High-Frade Lesions, and 80% in women with High-Grade Squamous Intraepithelial Lesions (HSILs). The multiple high-risk HPV strain positivity rate increased from 32.4% in ASCUS to 44.7% in LSIL and 62.5% in HSIL. Increased HPV positivity rates and prevalence of HPV-16 were noted with increasing severity of cervical dysplasia. Decreased HPV positivity was observed with increasing age.

Conclusion: Overall, because the prevalence and distribution of different high-risk HPV genotypes affect HPV vaccination strategies, our findings may be useful for the design of cervical cancer prevention programs.

Keywords: Human papilloma virus, Cervical cytology, Genotype, Cancer vaccine, Cervical dysplasia, Cervical cancer.

1. INTRODUCTION

Cervical cancer is the fourth most common cancer in women worldwide [1]. According to the World Health Organisation, in 2018, an estimated 570,000 women were diagnosed with cervical cancer worldwide and about 311,000 women died from the disease [1, 2]. Additionally, according to the Department of Health Abu Dhabi, cervical cancer is the second most common cancer and the seventh leading cause of cancer-related death for women in the United Arab Emirates (UAE) [3]. The central role of human papillomavirus (HPV) infection as an etiological factor of cervical cancer and premalignant dysplasia is well established [4, 5]. In patients with cervical cancer, the prevalence of HPV DNA is as high as 99.7% [6]. Over 200 types of HPV have been identified on the
basis of DNA sequence, and approximately 30 types primarily infect the cervix, vagina, vulva, and anus [7]. Those with oncogenic potential are classified as high risk, whereas low-risk HPV types do not exhibit a causal association with cancer but can cause genital warts [7]. HPV-16 and -18 contribute to over 70% of all cervical cancers worldwide [8]. After HPV-16 and -18, the six most common HPV types (-31, -33, -35, -45, -52, and -58) account for 20% of cervical cancers worldwide. However, geographic variation has been noted in the prevalence and distribution of different HPV genotypes [9].

Cervical cancers can be effectively prevented by vaccination against HPV and routine screening with Pap smear and HPV testing. Knowledge of the prevalence and distribution of different HPV genotypes is vital to effectively plan primary and secondary cervical cancer prevention programmes.

Currently, the Department of Health Abu Dhabi (DoH) recommends a Pap testing every 3 years for women between the ages of 25 and 29 years and a Pap test and HPV test (co-recommends a Pap testing every 3 years for women between 25 and 52), and -58) account for 20% of cervical cancers worldwide. After HPV-16 and -18, the six most common HPV types (-31, -33, -35, -45, -52, and -58) account for 20% of cervical cancers worldwide. However, geographic variation has been noted in the prevalence and distribution of different HPV genotypes [9]. Cervical cancers can be effectively prevented by vaccination against HPV and routine screening with Pap smear and HPV testing. Knowledge of the prevalence and distribution of different HPV genotypes is vital to effectively plan primary and secondary cervical cancer prevention programmes.

Currently, the Department of Health Abu Dhabi (DoH) recommends a Pap testing every 3 years for women between the ages of 25 and 29 years and a Pap test and HPV test (co-testing) every 5 years for women aged 30 to 65 years [10]. Additionally, the DoH recommends HPV vaccination for the prevention of cervical cancer for women 15–26 years of age [11]. Currently, in the UAE, two vaccines are provided to protect against HPV infection, the bivalent vaccine Cervarix, which targets HPV-16 and -18, and the quadrivalent vaccine Gardasil, which targets HPV-6, -11, -16, and -18 [11]. A nonavalent vaccine protecting against HPV-6, -11, -16, -18, -31, -33, -45, -52, and -58 was approved by the United States Food and Drug Administration in 2014 and is currently being used in the United States of America (USA) and Europe [12]. This geographic variation observed in previous studies should be further investigated to elucidate the prevalence of different HPV genotypes, particularly those not covered by vaccines [8, 9, 13, 14].

In this study, we aimed to determine the prevalence of different HPV genotypes in women with abnormal Pap smears in the UAE. We also evaluated correlations between HPV genotype and the degree of cervical dysplasia and observed the distributions of HPV genotypes across age groups.

2. MATERIALS AND METHODS

2.1. Study Population

The study was conducted after taking approval by the Research and Ethics Committee of NMC Specialty Hospital, a private multi-specialty referral hospital in the Emirate of Abu Dhabi. NMC Specialty hospital is a 104 bedded hospital with the Department of Obstetrics and Gynecology attending to about 150 outpatients per day on an average.

All women with cervical epithelial cell abnormalities on pap smear among the women whose pap smears were evaluated by NMC cytology laboratory between January 2018 and September 2019 were offered HPV testing and typing and women who gave consent and underwent HPV testing and typing were included in the study. The exclusion criteria were women with abnormal cervical cytology in the specified time period who did not consent for the HPV testing and typing, Post Hysterectomy vault smears, women with a history of radiotherapy and/or chemotherapy for genital tract malignancy.

A total of 442 women who satisfied these criteria were included in the study.

Women with abnormal cervical cytology and/or high-risk HPV positivity were offered colposcopy examination and cervical biopsy. A cervical biopsy was performed in 126 cases. A retrospective chart review was performed for all 442 cases, and data including demographics, results of cervical cytology, HPV testing, typing and cervical biopsy, HPV vaccination history were collected from hospital records.

2.2. Cytology

Pap smears were performed using the Surepath liquid-based Pap test. Pathologists reported abnormal cytology according to the Bethesda system. Cervical epithelial cell abnormality was classified as atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells cannot exclude HSIL (ASC-H), squamous cell carcinoma (SCC), atypical glandular cells not otherwise specified (AGC-NOS), atypical glandular cells suspicious of adenocarcinoma in situ or cancer (AGC neoplastic).

For the purpose of analysis, ASCUS and LSIL were categorised as low-risk lesions, and HSIL and ASC-H were categorised as high-risk lesions. The remaining categories were studied independently, owing to their low counts.

2.3. HPV DNA Testing

HPV DNA testing was done in NMC Royal Hospital and NRL Lab, Abu Dhabi.

Method of testing: Seegene Anyplex II HPV28 (‘Anyplex’) is a semi-quantitative DNA PCR assay divided into set A, comprising 14 high risk (hr)HPV types; and set B, comprising 5 possibly hrHPV types and 9 low risk (lr)HPV types. Based on Seegene’s proprietary DPO™ and TOCE™ technologies, this multiplex assay performs on real-time PCR instruments and provides high sensitivity and specificity genotyping information of infected 28 HPV types in a single reaction. Anyplex is an HPV DNA genotyping test that can simultaneously detect, differentiate, and semi-quantify 28 HPV genotypes (19 hrHPV types and 9 lrHPV types) in only two PCR wells per sample. The test is divided into two sets: set A comprises 14 hrHPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), and set B comprises 5 phrHPV types (HPV26, 53, 69, 73, and 82) and 9 lrHPV types (HPV 6, 11, 40, 42, 43, 44, 54, 61, and 70). The test is a multiplex real-time PCR assay utilizing dual priming oligonucleotide and tagging oligonucleotide cleavage and extension technology. Country of kit manufacture: Seoul, South Korea.

2.4. Histology

All biopsy specimens were submitted for histological assessment and graded as CIN1, CIN2, or CIN3.
2.5. Statistical Methods
Categorical variables are represented as frequencies (percentages), and continuous variables are presented as means ± standard deviations. HPV positivity and variables were evaluated using chi-square tests or Fisher’s tests. All tests were performed with the a-level set to 0.05 (two-tailed). All analyses were performed using IBM SPSS Statistics for Windows (ver. 26.0; IBM Corp., Armonk, NY, USA).

3. RESULTS
In total, 819 women within the age range of 17–76 years (average age: 37 ± 9.4 years) had abnormal cervical cytology reports during the specified time period. Of these, 442 women consented to undergo HPV testing typing, and satisfied the inclusion, exclusion criteria. None of the participants in the study were vaccinated against HPV. There were no known HIV-positive women in the study group.

3.1. Prevalence and Type-specific Distribution of HPV in Women with Abnormal Cervical Cytology.
Out of the 442 women with epithelial cell abnormalities on pap-smear, 248 tested positive for HPV. The overall HPV positivity rate in women with abnormal Pap smears (both low- and high-risk HPV) was 56.1%. The prevalence of high-risk HPV was 48%.

The most common high-risk HPV genotype was HPV-16 (15.2%), followed by HPV-31 (11.7%), -53 (8.9%), -66 (8.6%), and -51 (8.3%). The prevalence of HPV-18 was only 3.8% (Fig. 1). Moreover, among high-risk lesions, including ASC-H and HSIL, HPV-16 was the most common genotype and was observed in 57.1% of women. This was followed by HPV-35, -33, -52, -58, -18, and -31, each contributing to 9.5% of infections. Among the low-risk lesions, including ASCUS and LSIL, the most common strain was HPV-16, accounting for 19% of infections, followed by HPV-53 (14.8%) and -66 (13.2%).

3.2. High-Risk HPV Prevalence and Type-Specific Distribution in Different Age Groups
Analysis of the age group distribution showed that the highest HPV positivity rate (69.5%) was in patients less than 30 years old, followed by that in patients 31–50 years old (51.3%) and that in patients greater than 50 years old (41%; $P = 0.0001$) (Fig. 2). In patients less than 30 years old, the most common HPV genotype was HPV-16 (12.2%), followed by HPV-31 (9.9%) and -51 (8.4%). In women, 31–50 years old, the most common was HPV-16 (17.6%), followed by HPV-31 (9.1%), -53 (8.1%), and -66 (8.1%). Finally, in patients over 50 years old, the most common was HPV-31 (24%), followed by HPV-18 and -53 (12%).

3.3. Correlation Between High-Risk HPV Infection and Cervical Cytology
The distribution of abnormal Pap smears showed ASCUS in 81%, LSIL in 13.2%, ASC-H in 3.6%, and HSIL in 2.3% of cases.

The high-risk HPV positivity rate increased from 39% in cases with ASCUS to 81%, 81.3%, and 80% in women with LSIL, ASC-H, and HSIL, respectively ($p < 0.00001$) (Fig. 3). A statistically significant increase in the high-risk HPV positivity rate was observed in women with LSIL, ASC-H, and HSIL compared with that in women with ASCUS.
3.4. Correlation Between High-Risk HPV Infection and Cervical Intraepithelial Neoplasia on Histology

Of the 126 women who underwent colposcopy cervical biopsy, 65.1% had CIN1. The HPV positivity rate was 95.1%. The most common strains in women with CIN1 were HPV-16 (13.8%), followed by HPV-31 (9.2%) and -51(9.2%). CIN2 was noted in 20.1% of women with an HPV positivity rate of 96.2%. The most common strain was HPV-16 (17.1%), followed by HPV-52 (11.4%), -31, and -53 (8.6%). CIN3 was noted in 11.9% of women with an HPV positivity rate of 100%. The most common strain was HPV-16 (36%), followed by HPV-31 (12%). Thus, overall, increased HPV positivity rates and prevalence of HPV-16 were noted with increasing severity of cervical dysplasia. Because we only determined the percentages of the top three strains within each group of cervical biopsy results, the statistical significance of these findings is still unclear.

3.5. Prevalence of Multiple High-Risk HPV Infection and its Correlation to Cervical Cytology

Out of the 48% of women who had high-risk HPV infection, 35.4% had infection with multiple high-risk HPV strains, 64.6% had a single high-risk HPV strain. The multiple high-risk HPV positivity rate increased from 32.4% in ASCUS to 45% in LSIL and 62.5% in HSIL. Infection by multiple high-risk HPV strains was noted in 23.1% of ASC-H cases ($p = 0.125412$) (Fig. 4). Although not statistically significant, the increased rate of infection by multiple high-risk HPV strains was observed with increasing severity of cervical lesions, except in ASC-H.
4. DISCUSSION

In this study, we evaluated the prevalence and genotype distribution of HPV in women with abnormal cervical cytology. The high-risk HPV prevalence in women with abnormal Pap smears was 48%, which was higher than that (18.4%) observed in a study in Qatar, possibly because of the conservative socio-cultural norms in this country, and lower than that (76.4%) observed in a study in Kazakhstan [15, 16]. Similar to previous studies, we observed increased rates of high-risk HPV positivity as the severity of the cervical lesions increased [17 - 19]. These data supported the need for cotesting during screening for cervical cancer and precancerous lesions.

In our study, the most common high-risk HPV strains were found to be HPV-16, -31, -53, -66, -51, and -52. HPV-18 was detected in only 3.8% of the study population. These findings are inconsistent with the findings of the ARTISTIC trial carried out in the United Kingdom, in which the most common high-risk HPV types were HPV-16, -18, -31, -51, and -52 [17]. A study in China showed the most common high-risk HPV genotype was HPV-18, followed by HPV-52, -16, -58, -33, and -53 [14]. Variations in the genotype distribution were also noted in studies conducted in South Korea [18, 19]. The low prevalence of HPV-18 was also observed in studies performed in South Korea and Qatar [15, 18].

Currently available bivalent and quadrivalent vaccines target high-risk HPV genotypes, i.e., HPV-16 and -18, which contributed to only 15.2% and 3.8% of high-risk HPV infections, respectively, in this study. The nonavalent vaccine Gardasil 9, which is available in Europe and the USA, targets the high-risk HPV genotypes HPV-16, -18, -31, -33, -45, -52, and -58, which cumulatively accounted for 47.8% of infections in the current study. Thus, genotypes not covered by vaccines contributed to a considerable proportion of high-risk HPV infections. This finding has important implications for prophylactic HPV vaccination programs and supports the need for further studies of potential cross-protection against non-vaccine genotypes and for trials of vaccines that cover more genotypes.

In our study, we observed a statistically significant decline in HPV positivity rates as age increased, with a different genotype distribution, consistent with the findings of previous studies [15]. This finding could help inform HPV vaccination programs. Because the group of patients aged 31–50 years also had a 51.3% high-risk HPV positivity rate, the role of catch-up vaccination in older patients should be considered. Moreover, studies examining immunogenicity in this age group would also be beneficial.

Multiple high-risk positivity rate in our study was 35.4% which is lower than the 44.7% observed from a study from Brazil [20] and 38.2% in a study from Houston [21]. In our study, a statistically insignificant increase was noted in HPV positivity rate with an increase in severity of cervical lesion except in ASC-H. In ASC-H, the multiple high-risk HPV positivity rate was only 23.1%. In the study from Houston, a synergistic effect was not observed for high-risk cervical lesions in multiple HPV infections [21]. The authors have explained this could be due to the concurrent infection of multiple HPV genotypes collectively inducing a more effective local or humoral immune response than that triggered by a single infection, with an overall stronger immunity against HPV infection through antibody cross-reactions. The study from Brazil shows that women with multiple high-risk HPV infection tend to have high-grade or persistent low-grade intraepithelial lesions of the cervix [20]. So larger studies with a follow-up for the progression of cervical dysplasia would be required to clarify the role of multiple HPV infections in cervical dysplasia and cancer.

Our study had some limitations. It is a single-centre observational study with a relatively small study population. Among the 819 women who had abnormal Pap smears, only 442 women consented to undergo HPV testing. Barriers to conducting HPV testing typing resulted in a lower number of women being included in the study and, therefore, a smaller than desired study population. Ethnic heterogeneity in the study population is both a strength and a limitation. The other limitations were that data regarding the history of other immunocompromised states was not collected and there was no
long-term follow up. Multicentric studies involving a larger study population with long-term follow-up will be required to confirm the findings of our study.

CONCLUSION
In conclusion, the most common high-risk HPV genotypes were HPV-16, HPV-31, HPV-53, HPV-66, and HPV-51. Geographic variations were noted in the genotype distribution, and changes in prevalence were observed in different age groups compared to studies from other geographic regions. Moreover, a significant proportion of infections was caused by HPV genotypes not covered by currently available vaccines. Because the prevalence and distribution of different high-risk HPV genotypes have an impact on HPV vaccination strategy, these results may be useful for the design of cervical cancer prevention programs.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Approval was taken from the Research and Ethics Committee of NMC Healthcare, Abu Dhabi (Ref num: 002/hmcsf-med-dir/2020).

HUMAN AND ANIMAL RIGHTS
No Animals were used in this research. All human research procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION
Informed consent was taken from all participants.

FUNDING
None.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
We would like to thank Rai Anagha (Research Associate, NMC Healthcare) and Dusane Rohit (Biostatistician, NMC Healthcare) for their contributions to the study.

REFERENCES