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3 (61) 2021 : 001-07 • ORIGINAL RESEARCH

The High-risk Human Papilloma Virus (Hr-HPV) in abnormal pap smears

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection · **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

Background: The Human papilloma virus (HPV) is the commonest sexually transmitted disease (STD) in low- and middle-income countries and it is the most powerful carcinogens implicated in cervical cancer.

Objectives: To detect types of Hr-HPV in abnormal Pap smears and cervical cytology abnormalities associated with Hr-HPV infection.

Methods: Women eligible for Pap smears and HPV-co-testing according to hospital protocol were included in this study. Pap smears were done using the liquid-based cytology (LBC) and evaluated according to the Bethesda system. HPV-DNA co-testing was done using the Anyplex-II. Collected data were statistically analyzed to detect types of Hr-HPV in abnormal Pap smears, and cervical cytology abnormalities associated with Hr-HPV infection.

Results: The prevalence of Hr-HPV in abnormal Pap smears was 48.2% (106/220). The Hr-HPV detected in abnormal Pap smears were HPV-16 (36.8% (39/106)), HPV-31 (12.3% (13/106)), HPV-58 (11.3% (12/106)), HPV-66 (10.4% (11/106)), HPV-51 (9.4% (10/106)), HPV-18 (7.5% (8/106)), while multiple Hr-HPVs-35, -33, -52, and 58 were detected in 12.3% (13/106) of abnormal Pap smears.

The Hr- HPV detected in 39.6% (42/106) of ASCUS, in 80.2% (85/106) of LSIL, in 81.1% (86/106) of ASC-H, and in 83.01 (88/106) of HSIL. The Hr-HPV detection rate was significantly higher in LSIL, ASC-H, and HSIL compared to ASCUS (P=0.002, 0.001 and 0.001, respectively).

Conclusion: The prevalence of Hr-HPV in abnormal Pap smears was 48.2%. The low prevalence of HPV-18 in this study confirms the variations in genotype distribution of Hr-HPV.

The Hr-HPV detection rate was significantly higher in LSIL, ASC-H, and HSIL compared to ASCUS. This finding supports the recommendation of HPV co-testing with routine Pap smear in women aged 30-65 years.

Keywords: High-Risk; HPV; Cervical; Pap; Cytology

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Word count: 1674 **Tables:** 2 **Figures:** 0 **References:** 24

Received: 20.08.2021
Accepted: 06.09.2021
Published: 28.09.2021

INTRODUCTION

Cancer cervix is an ideal malignancy for screening and prevention because it can be diagnosed and treated at the pre-cancerous lesions before development of invasive disease [1-3].

Most cervical cancers develop from infected cells with high-risk human papilloma virus (Hr-HPV), originated from the squamo-columnar junction of the cervix [4].

The HPV is the commonest sexually transmitted disease (STD) in low- and middle-income countries and it is the most powerful carcinogens implicated in cervical cancer [5]. Since the causal link between cervical cancer and Hr-HPV infection was established, much effort has been devoted for diagnosis and prevention of HPV infection [6,7].

The liquid-based cytology (LBC) approved by the FDA in 1996 as an alternative to conventional cervical smear (CCS) [8,9]. The polymerase chain reaction (PCR) based methods for detection of HPV deoxyribonucleic acid (DNA) is highly sensitive and specific, and it has the additional advantages of detecting small viral concentrations [10].

Initially, the Hr-HPV co-testing was recommended for ASC-US [11]. Based on the recommendations from National Institute of Health (NIH), American Society for Colposcopy and Cervical Pathology (ASCCP), and American Cancer Society (ACS) [12], the FDA in 2014 approved the Hr-HPV co-testing as primary screening of cervical cancer in women ≥25 years at intervals ≥3 years [13].

The joint recommendation released in 2012 advocates HPV co-testing in conjunction with routine Pap smear in women aged 30-65 years [14]. The co-testing increases the detection of cervical-intraepithelial lesion-2 (CIN2) or greater lesions at baseline screening, and significantly decreases the detection of CIN2/CIN3 lesions at subsequent screening compared to cytology alone [15]. Variation in the genotype distribution of Hr-HPV was reported in previous studies [16-18]. Therefore, this study design to detect types of Hr-HPV in abnormal Pap smears and cervical cytology abnormalities associated with Hr-HPV infection.

OBJECTIVES

To detect types of Hr-HPV in abnormal Pap smears and cervical smear abnormalities associated with Hr-HPV infection.

MATERIALS AND METHODS

Women eligible for Pap smears and HPV-co-testing according to hospitals protocol were included in this prospective study which was conducted over two years from January 2019 till January 2021, after informed consents in accordance with Helsinki declaration to detect the types of Hr-HPV in abnormal Pap smears and cervical smear abnormalities associated with Hr-HPV infection.

Eligibility to Pap smear and HPV-co-testing include sexually active women, between 25-65 years old. Women received HPV vaccine; previous abnormal Pap smear, previous hysterectomy, previous genital malignancy, women under treatment for genital tract malignancy (i.e., either chemo and/or radiotherapy) or women refused to participate and/or to give consent were excluded from this study.

Pap smears were done using the LBC and evaluated according to the Bethesda system. HPV-DNA testing was done using the Anyplex-II (Anyplex, Seoul, South Korea) which is a semi-quantitative real-time PCR test utilizing dual priming oligonucleotide and tagging oligonucleotide cleavage.

Anyplex-II provides sensitive and specific genotyping information of 28 HPV types (19 Hr-HPV types and 9 low-risks HPV (Lr-HPV)) in single reaction.

The abnormal cervical cytology was classified as atypical squamous cells of undetermined significance (ASCUS), low-grade and high-grade squamous intraepithelial lesion (LSIL and 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 or in situ carcinoma (AGC neoplastic) [1].

Women with abnormal Pap smears and/or Hr-HPV positivity were offered colposcopy examination and cervical biopsy. The biopsy specimens were examined and graded as CIN1, CIN2, CIN3, in situ carcinoma or invasive cancer. Collected data were statistically analyzed to detect types of Hr-HPV in abnormal Pap smears (primary outcome) and cervical cytology abnormalities associated with Hr-HPV infection (secondary outcome).

Sample Size

The required sample size calculated using G Power software version 3.1.9.7 for sample size calculation, setting α -error probability at 0.05, power (1-β error probability) at 0.95%, and effective sample size (w) at 0.5. An effective sample ≥220 Pap smears was needed to produce a statistically accepted acceptable figure.

Statistical Analysis

Categorical variables will be presented as number and percentage. Chi-square test (x2) was used for analysis of qualitative variables to detect types of Hr-HPV in abnormal Pap smears (primary outcome) and cervical cytology abnormalities associated with Hr-HPV infection (secondary outcome). P<0.05 was considered significant.

RESULTS

Prevalence of HPV in abnormal Pap smears: Four hundred and thirty (430) cervical cytology specimens were collected from January 2019 to January 2021 for Pap smears and HPV co-testing, 51.2% (220/430) of the collected cervical cytology specimens had abnormal Pap smear results, and 55.5% (122/220) of the abnormal Pap smears were positive for HPV (Hr-HPV and Lr-HPV). The prevalence of Hr-HPV in abnormal Pap smears was 48.2% (106/220).

Types of Hr-HPV in abnormal Pap smears: The Hr-HPV detected in abnormal Pap smears were HPV-16 (36.8% (39/106)), HPV-31 (12.3% (13/106)), HPV-58 (11.3% (12/106)), HPV-66 (10.4% (11/106)), HPV-51 (9.4% (10/106)), HPV-18 (7.5% (8/106)), while multiple Hr-HPVs-35, -33, -52, and 58 were detected in 12.3% (13/106) of abnormal Pap smears (Tab. 1.).

Lr-HPV detected in abnormal Pap smears include HPV-6 (37.5% (6/16)), HPV-40 (25% (4/16)), HPV-44 (18.75% (3/16)), and HPV-61 (18.75% (3/16)) (Tab. 1.).

The cervical cytology abnormalities associated with Hr-HPV infection: The Hr- HPV detected in 39.6% (42/106) of ASCUS, in 80.2% (85/106) of LSIL, in 81.1% (86/106) of ASC-H, and in 83.01 (88/106) of HSIL. The Hr-HPV detection rate was significantly higher in LSIL,

Tab. 1. Types of Hr-HPV and Lr-HPV detected in abnormal Pap smears.

Type of HPV	Number and (%)
Hr-HPV	
HPV-16	36.8% (39/106)
HPV-31	12.3% (13/106)
HPV-58	11.3% (12/106)
HPV-66	10.4% (11/106)
HPV-51	9.4% (10/106)
HPV-18	7.5% (8/106)
Multiple Hr-HPV-35, -33, -52, and 58	12.3% (13/106)
Lr-HPV	
HPV-6	37.5% (6/16)
HPV-40	25% (4/16)
HPV-44	18.75% (3/16)
HPV-61	18.75% (3/16)

ASC-H, and HSIL compared to ASCUS (P=0.002, 0.001 and 0.001, respectively (Tab. 2.).

Correlation between abnormal Pap smears and CIN: Women with abnormal Pap smears and/or Hr-HPV positivity were offered colposcopy examination and cervical biopsy. Out of 122 colposcopy examinations, the CIN1 was diagnosed in 36.9% (45/122) and CIN2 was diagnosed in 21.3% (26/122) of cases.

DISCUSSION

The LBC approved by the FDA in 1996 as an alternative to CSS. A systematic review showed that the LBC had an equivalent performance to CCS [9]. The detection of HPV-DNA using PCR based techniques are highly sensitive and specific, and it has the additional advantages of detecting small viral concentrations [10]. Variation in the genotype distribution of Hr-HPV was reported in previous studies [16-18]. Therefore, this study designed to detect types of Hr-HPV in abnormal Pap smears and cervical smear abnormalities associated with Hr-HPV infection.

The prevalence of Hr-HPV in abnormal Pap smears was 48.2% (106/220), which was higher than that (18.4%) observed in Qatar, and lower than that (76.4%) observed in Kazakhstan [16,19].

This can explained by the conservative socio-cultural norms in Qatar, and by method of HPV-DNA testing used in this study (Anyplex-II), which is highly sensitive and specific semi-quantitative real-time PCR method for detection of HPV-DNA at small viral concentrations [10].

Like other studies [20-22], this study found that the Hr-HPV detection rate was significantly higher in LSIL, ASC-H, and HSIL compared to ASCUS (P=0.002, 0.001 and 0.001, respectively), which support the need for HPV co-testing during cervical cancer screening.

The ATHENA trial found that about 10% of HPV-16 and HPV-18 positive women had high-grade CINs (≥CIN2) [21].

Initially, the Hr-HPV co-testing test recommended for ASC-US [11]. Based on the recommendations from the NIH, ASCCP, and ACS [12], the FDA in 2014 approved the Hr-HPV co-testing as primary screening of cervical cancer in women ≥25 years at intervals ≥3 years [13].

Although, the ARTISTIC trial reported HPV-16, -18, -31, -51, and -52 as the most common Hr-HPV in UK [20].

A Chinese study reported HPV-18, followed by HPV-52, -16, -58, -33, and -53 as the most common Hr-HPV in China [22]. In addition, this study, found the most common Hr-HPV in Kuwait were HPV-16 (36.8%), HPV-31 (12.3%), HPV-58 (11.3%), HPV-66 (10.4%), HPV-51 (9.4%), and HPV-18 (7.5%).

The low prevalence of HPV-18 was not only observed in this study, but also observed in studies performed in Qatar and South Korea [16-18]. Variations in the genotype distribution of Hr-HPV were also reported in South Korea studies [17,18].

In this study, multiple Hr-HPVs infection (HPV-35, -33, -52, and 58) were detected in 12.3% of abnormal Pap smears which is lower than the 44.7% observed in Brazil [23] and 38.2% in Houston [24]. This could be explained by the concurrent Hr-HPV infection which induces effective local or humoral immune response than that triggered by a single infection, with an overall stronger immunity against other Hr-HPV infection.

The study from Brazil shows that women with multiple high-risk HPV infection tend to have high-grade or persistent CIN lesions [23]. So larger future studies are needed to confirm the association between multiple HPV infection and cervical pre-cancerous and/or cancerous lesions.

This study found that the prevalence of Hr-HPV in abnormal Pap smears was 48.2% (106/220). The most common Hr-HPV detected in this study was HPV-16 (36.8%), HPV-31 (12.3%), HPV-58 (11.3%), HPV-66 (10.4%), HPV-51 (9.4%), and HPV-18 (7.5%). The Hr-HPV detected in 39.6% of ASCUS, in 80.2% of LSIL, in 81.1% (86/106) of ASC-H, and in 83.01 (88/106) of HSIL. The Hr-HPV detection rate was significantly higher in LSIL, ASC-H, and HSIL compared to ASCUS.

This study was the first prospective study conducted in Kuwait and confirms the variations in genotype distribution of Hr-HPV because of the low prevalence of HPV-18 observed in this study.

This study was also the first prospective study conducted in Kuwait to detect types of Hr-HPV in abnormal Pap smears using the Anyplex-II which is highly sensitive and specific PCR method for detection of HPV-DNA at small viral concentrations.

Tab. 2. The cervical cytology abnormalities associated with Hr-HPV infection.

	Hr-HPV in ASCUS	Hr-HPV in ASCUS	Hr-HPV in ASCUS
	39.6 (42/106)	39.6 (42/106)	39.6 (42/106)
Hr-HPV LSIL	80.2% (85/106)	-	-
Hr-HPV in ASC-H	-	81.1% (86/106)	-
Hr-HPV in HSIL	-	-	83.01% (88/106)
P value (X^2 test)	0.002	0.001	0.001

ASC-H: Atypical squamous cells cannot exclude HSIL.
ASCUS: Atypical squamous cells of undetermined significance.
Chi-square (X^2) test used for statistical analysis
Data presented as number and percentage (%)
Hr-HPV: High-risk Human papilloma virus
HSIL: High-grade squamous intraepithelial lesion.
LSIL: Low-grade squamous intraepithelial lesion.

Women refused to participate and/or give consent was the only limitation faced during this study. Larger future studies are needed to confirm the association between multiple HPV infection and cervical pre-cancerous and/or cancerous lesions.

CONCLUSION

The prevalence of Hr-HPV in abnormal Pap smears was 48.2%. The low prevalence of HPV-18 in this study confirms the variations in genotype distribution of Hr-HPV.

The Hr-HPV detection rate was significantly higher in LSIL, ASC-H, and HSIL compared to ASCUS. This finding supports the recommendation of HPV co-testing with routine Pap smear in women aged 30-65 years.

CONFLICT OF INTERESTS

Authors declare no conflict of interests related to this study.

FINANCIAL SUPPORT

Nil.

ETHICS APPROVAL

Studied women were included in this study after written consent in accordance with Helsinki declaration and according to hospital protocol.

REFERENCES

1. **Bal MS, Goyal R, Suri AK, et al.** Detection of abnormal cervical cytology in Papanicolaou smears. *J Cytol.* 2012;29(1):45-7.
2. **Verma K**. Early diagnosis of cancer cervix- epidemiology and incidence. *J Cytol.* 2001;18:73-89.
3. **Afrakhteh M, Khodakarami N, Moradi A, et al.** A study of 13315 Papanicolaou smear diagnoses in Sohada hospital. *J Fam Reprod Health.* 2007;1:75-9.
4. **Mitteldorf CATS.** Cervical cancer screening from Pap smear to future strategies. *J Bras Patol Med Lab.* 2016;52(4):238-245.
5. **Crosbie EJ, Einstein MH, Franceschi S, et al.** Human papillomavirus and cervical cancer. *Lancet.* 2013;382(9895):889-99.
6. **Tota JE, Chevarie-Davis M, Richardson LA, et al.** Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med.* 2011;53:S12-21.
7. **Saslow D, Castle PE, Cox JT, et al.** Gynecologic Cancer Advisory Group, Garcia F. American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. *CA Cancer J Clin.* 2007;57(1):7-28.
8. **Saslow D, Solomon D, Lawson HW, et al.** ACS-ASCCP-ASCP Cervical Cancer Guideline Committee. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA Cancer J Clin.* 2012;62(3):147-72.
9. **Davey E, Barratt A, Irwig L, et al.** Effect of study design and quality on unsatisfactory rates, cytology classifications, and accuracy in liquid-based versus conventional cervical cytology: a systematic review. *Lancet.* 2006;367(9505):122-32.
10. **Abreu AL, Souza RP, Gimenes F, et al.** A review of methods for detect human Papillomavirus infection. *Virol J.* 2012; 9:262.
11. **Solomon D, Schiffman M, Tarone R, et al.** Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst.* 2001;93(4):293-9.
12. **Wright TC Jr, Schiffman M, Solomon D, et al.** Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol.* 2004;103(2):304-9.
13. **Abraham J, Stenger M. Cobas** HPV test for first-line screening for cervical cancer. *J Community Support Oncol.* 2014;12(5):156-7.
14. **Committee on Practice Bulletins-Gynecology.** ACOG Practice Bulletin Number 131: Screening for cervical cancer. *Obstet Gynecol.* 2012;120(5):1222-38.
15. **Bouchard-Fortier G, Hajifathalian K, McKnight MD, et al.** Co-testing for detection of high-grade cervical intraepithelial neoplasia and cancer compared with cytology alone: a meta-analysis of randomized controlled trials. *J Public Health (Oxf).* 2014;36(1):46-55.
16. **Bansal D, Elmi AA, Skariah S, et al.** Molecular epidemiology and genotype distribution of Human Papillomavirus (HPV) among Arab women in the State of Qatar. *J Transl Med.* 2014;12:300.
17. **So KA, Hong JH, Lee JK.** Human Papillomavirus Prevalence and Type Distribution Among 968 Women in South Korea. *J Cancer Prev.* 2016;21(2):104-9.
18. **Ouh YT, Min KJ, Cho HW, et al.** Prevalence of human papillomavirus genotypes and precancerous cervical lesions in a screening population in the Republic of Korea, 2014-2016. *J Gynecol Oncol.* 2018;29(1):e14.
19. **Aimagambetova G, Azizan A.** Epidemiology of HPV Infection and HPV-Related Cancers in Kazakhstan: a Review. *Asian Pac J Cancer Prev*. 2018;19(5):1175-80.
20. **Sargent A, Bailey A, Almonte M, et al.** ARTISTIC Study Group. Prevalence of type-specific HPV infection by age and grade of cervical cytology: data from the ARTISTIC trial. *Br J Cancer.* 2008;98(10):1704-9.
21. **Wright TC Jr, Stoler MH, Sharma A, et al.** Evaluation of HPV-16 and HPV-18 genotyping for the triage of women with high-risk HPV+ cytology-negative results. *Am J Clin Pathol.* 2011;136(4):578-86.
22. **Wang X, Zeng Y, Huang X, et al.** Prevalence and Genotype Distribution of Human Papillomavirus in Invasive Cervical Cancer, Cervical Intraepithelial Neoplasia, and Asymptomatic Women in Southeast China. *Biomed Res Int.* 2018;2018:2897937.
23. **De Brot L, Pellegrini B, Moretti ST, et al.** Infections with multiple high-risk HPV types are associated with high-grade and persistent low-grade intraepithelial lesions of the cervix. *Cancer Cytopathol.* 2017;125(2):138-143.
24. **Salazar KL, Zhou HS, Xu J, et al.** Multiple Human Papilloma Virus Infections and Their Impact on the Development of High-Risk Cervical Lesions. *Acta Cytol.* 2015;59(5):391-8.