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Association of sexually transmitted infections and human papillomavirus co-infection with abnormal cervical cytology among women in Saudi Arabia

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ABSTRACT

Human papillomavirus (HPV) is a causative agent of cervical and other cancers. Sexually transmitted Infections (STIs) may play a crucial role in HPV persistence, leading to serious complications, including cervical cancer. This study investigated the association of HPV/STI co-infection in cervical samples with cervical dysplasia among women in Saudi Arabia. HPV-positive cervical samples (n = 142) were obtained from previous studies and newly collected samples (n = 209) were obtained from women aged 19–83 years. For HPV detection and genotyping, PCR and Genoflow HPV assay kits were used. STIs were detected using a Genoflow STD array kit. Of 351 samples, 94 (27%) were positive for STIs. Among HPV-positive samples, 36 (25%) were positive for STIs; the most common pathogens were *Ureaplasma urealyticum*/*Ureaplasma parvu* (13%) and *Mycoplasma hominis* (6%). A global significant correlation was detected between HPV and STIs with progression of abnormal cervical cytology ($\chi^2 = 176$, $P < 0.0001$). Associations between cervical cytology diagnosis and HPV status, STI types (opportunistic and pathogenic), and the presence of *Ureaplasma* spp., and *Mycoplasma hominis* were significant ($P < 0.05$). Our results suggest that additional study in a larger population is warranted to determine the association between HPV/STI co-infection and cervical neoplasia in Saudi women.

1. Introduction

Sexually transmitted infections (STIs) are the most common infectious diseases worldwide. According to a 2016 World Health Organization study (Global health sector strategy on sexually transmitted infections 2016–2020), more than 1 million cases are reported every day. STIs are associated with serious reproductive tract health complications, such as ectopic pregnancy, pelvic inflammatory diseases, and infertility. They are also linked to several types of cancers, including cervical, anal, and oropharyngeal cancers (Caini et al., 2014). It is also well known that human papillomavirus (HPV), a double-strand DNA virus, is the causative agent of cervical cancer, the fourth most common cancer in women worldwide, with 530,000 new cases detected annually (Ferlay et al., 2015).

HPVs are classified according to their oncogenic potential into high risk (HR-HPV) and low risk groups. The high risk group, which currently includes HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82, has been associated with cervical intraepithelial lesions and may lead to the development of cervical cancer (Doorbar et al., 2012; de Villiers, 2013). The presence of HPV is important but not sufficient to cause cervical cancer, as the persistence of the virus plays an important role in cancer development (Kjaer et al., 2010; Tota et al., 2011). Indeed, HPV persistent

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infection has been closely associated with progression to carcinoma (Dalstein et al., 2003). There are many co-factors that help the virus become a persistent infection, and included in this list is infection with one or more other sexually transmitted disease that may increase the risk of having cervical neoplasia. An STI may facilitate the entry of multiple HR-HPVs as well as decrease the host's ability to resolve the HPV infection (Paba et al., 2008). Discovering and removing co-factors that are involved in HPV persistence and development into cervical dysplasia is critical (Kim et al., 2016). Because STIs play an important role as a co-factor in the development of cervical dysplasia and because they may lead to serious reproductive tract complications, any women who has tested positive for HPV should consider undergoing STI screening and needed treatment.

The association between STI and cervical abnormalities has been reported in many studies (Paba et al., 2008; Verteramo et al., 2009; Costa-Lira et al., 2017; Ghosh et al., 2017). For example, *Chlamydia trachomatis* has been correlated with the severity of abnormal cervical cytology and HPV infection (Luostarinen et al., 2004; Smith et al., 2004; Samoff et al., 2005). Herpes simplex virus infection has been reported with HPV infection, increasing the risk of invasive cervical cancer (Smith et al., 2002), and other STIs, including *Trichomonas vaginalis*, *Mycoplasma* spp., and *Ureaplasma* spp., have also been associated with HPV infection and cervical abnormal cytology (Lukic et al., 2006; Biernat-Sudolska et al., 2011; Donders et al., 2013; Lazenby et al., 2104; Ljubin-Sternak and Meštrović, 2014). Despite the results of those previous studies, the status of STI co-infections in Saudi communities is unknown. Therefore, the primary aim of the present study was to investigate whether an association exists between the presence of HPV/STI co-infection in cervical samples and the development of cervical dysplasia among Saudi women.

2. Methods

2.1. Ethical standards and study design

The presented retrospective study was approved by the office of Research Affairs at King Faisal Specialist Hospital and Research Centre (RAC #2180013) and was conducted in accordance with the Declaration of Helsinki. In total, 351 cervical samples were used, where 142 HPV-positive cervical samples were obtained from previous studies, and 209 additional new cervical samples were newly collected from women who attended the Primary Care Clinic at KFSHRC in Riyadh, Saudi Arabia. The inclusion criteria were women who were married, divorced, or widowed, and the exclusion criteria were women who were virgins or pregnant.

For each woman, cervical cytologic samples were collected using a brush (PreservCyt; ThinPrep Pap test Boxborough, MA, USA) for routine Papanicolaou testing and molecular detection experiments. The cytology of each collected sample was also determined and ranged from normal to various stages of abnormality. The stages of the abnormal cytology were identified using the Bethesda classification system in which lesion severity increases from negative for intraepithelial lesion (NIEL), to atypical squamous cells of undetermined significance (ASCUS), to low-grade squamous intraepithelial lesion (LGSIL), to high-grade squamous intraepithelial lesion (HGSIL), and finally to cervical cancer (Tabbara et al., 1992).

2.2. DNA extraction

A 1-ml aliquot of resuspended cells from the cervical sample was centrifuged, and DNA was extracted using a Gentra Puregene Cell Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The DNA concentration in each sample was estimated using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The quality of the extracted DNA was determined using β-globin primers. The amplified polymerase chain reaction (PCR) products were visualized using 1% agarose gels stained with ethidium bromide. The samples were then stored at −80 °C until required for further processing.

2.3. HPV detection and genotyping

Nested PCR was preformed to detect HPVs, using primers MY09/MY11 and GP5+/GP6+, which target a sequence located within the L1 region. The first round of PCR was performed using the MY09/MY11 primer set that targeted a 450-bp conserved sequence. The second round was performed using the GP5+/GP6+ primer set targeting a 150-base pair sequence within the 450-base pair product. The PCR conditions for the first round were as follows: 95 °C for 5 min; 35 cycles of 95 °C for 40 s, 54 °C for 40 s, and 72 °C for 40 s, and a final extension at 72 °C for 5 min. The PCR conditions for the second round were as follows: 95 °C for 5 min; 40 cycles of 95 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The positive controls were HeLa cells. UltraPure DNase/RNase-free water used as a negative control, and a β-globin control primer set was used as an internal control (Gravitt et al., 2000).

For HPV genotyping, a GenoFlow HPV assay Kit (DiagoCor Bioscience Incorporation Limited, Hong Kong, China) was used. This kit enables the identification of HPV genotypes associated with cervical cancer. In total, 33 HPV high-risk and low-risk genotypes can be identified with this kit, including HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 66, 68, 70, 71, 72, 73, 81, 82, and 84. A universal probe was also included to detect rare HPV subtypes outside the 33-genotype panel. Target DNA was mixed with a biotin-labeled primer mix and DNA Taq polymerase provided with the kit and was followed by the PCR amplification step using the thermocycling conditions provided by the manufacturer. The PCR products were then denatured and hybridized to HPV-specific DNA, capturing probes via flow- through hybridization. The hybridized DNA was detected by colorimetric development (Wong et al., 2010).

2.4. STIs detection

For STI detection, a Genoflow STD array test kit (Hong Kong, China) was used that enables the identification of 11 sexually transmitted common pathogens, namely, *Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum*, herpes simplex virus types 1 and 2, human papillomavirus types 6 and 11. This test is based on PCR and hybridization technology. After amplification of the target DNA with specific primers, the amplicons were hybridized using flow-through hybridization according to manufacturer's instructions. The results were visualized by colorimetric detection.

2.5. Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS), version 9.4. Descriptive and inferential statistics were conducted between variables of interest by demographic and clinical data. Nonparametric tests such as chi-squared test, logistic regression, and Wilcoxon rank-sum of scores test were used to overcome the small sample size. Pearson's chi-squared test was conducted between variables to test for associations. Logistic models were created to test for a correlation between the categorical variables. A 2-sided $P < 0.05$ was considered statistically significant.

3. Results

Data from 351 participants were analyzed. The median age of the participants was 43 years (range from 19 to 83 years). The majority of the participants (85%) were married. The demographic and clinical characteristics of the participants are summarized in Table 1.

The overall prevalence of STIs in the 351 cervical samples was 26.78% (94 positives). The most common pathogens were *Ureplasma urealyticum* /*Ureplasma parvum* (21.1%; n = 7 4; $P < 0.05$) followed by *Mycoplasma hominis* (4.27%; n = 15; $P < 0.05$), and Human papillomavirus types 6 and 11 (1.9%; n = 7).

The general distribution of the cases by STI presence or absence is shown in Table 1. Of 351 women included in the study, 142 previously tested positive for HPV and thus were included in the following analysis. Of these, 70 women (49.2%) tested positive for HPV 16, 36 women (25.3%) tested positive for HPV 18, and 10 women (7.0%) tested positive for HPV 31. The most common HPV genotypes were HPV 16 followed by HPV 18.

The results of cytology testing showed that 256 of the cervical samples (72.9%) were normal, 6 samples (1.7%) were classified as ASCUS (Atypical Squamous Cells of Unknown Significance), 12 samples (3.4%) were LGSIL (Low-Grade Squamous Intraepithelial Lesion), 22 samples (6.2%) were HGSIL (High-grade squamous intraepithelial lesion), and 55 samples (15.6%) were cervical cancer. The HPV status for those patients is summarized in Table 2. A significant association between cytology grades and HPV status was found ($P < 0.005$).

Of the 142 HPV-positive samples, 36 (25.35%) also tested positive for an STI (odds ratio 25, $P < 0.001$). The most common STIs in the HPV-positive samples were *Ureplasma urealyticum* /*Ureplasma parvu* (13.38%; n = 19, odds ratio 0.43, $P < 0.01$), followed by *Mycoplasma hominis* (6.3%; n = 9), and HPV types 6 and 11 (4.2%; n = 6) (Table 3, Fig. 1). It is worth noting that the STI panel used in this study detects only 2 type of HPVs, namely, HPV types 6 and 11, both of which are low-risk HPVs, whereas the GenoFlow HPV array kits detects 33 high- and low-risk HPVs.

The results of a chi-square test indicated that there was an association between STI and HPV.

The distribution of cases by cervical cytology grades and STI presence is shown in Table 2, Fig. 2, and Table 4. Abnormal cervical cytology grades were associated with detection of pathogenic STIs (odds ratio 6.3, $P < 0.001$). Most of the 351 samples were negative for HPV and had normal cytology. Seventy two samples (20.5%) were positive for STI and also had normal cytology.

Supplementary Table 1 shows the correlation model in predicting diagnosis by HPV status and STI infection. The age distribution by clinical data is shown in Table 5 and Fig. 3, the only significance of age was only found by HPV status, positive patients were mostly older than negative patients.

4. Discussion

The present study aimed to investigate the association of HPV/STI co-infection with cervical dysplasia. Co-infection with an STI has been suggested to be a cofactor in HPV persistence and neoplasia progression. These STIs include viral infections, such as herpes simplex virus type 2 and human papillomavirus, bacterial infections, such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae,* and *Mycoplasma* spp., and infection with the protozoan *Trichomonas vaginalis*. Many studies have recognized an association between STIs, HPV infection, and cervical dysplasia especially cervical cancer, showing that the HPV rate is significantly higher among women with STIs (Vriend et al., 2015; Kim et al., 2016, 2018). By contrast, a few studies have shown that STI has no association with HPV, abnormal cytology, or cervical cancer. The lack of an association may be attributable to the absence of some relevant information and inaccuracies in self-reported information. Some authors have suggested further studies with larger sample sizes to confirm the associations between pathogens (Zhang et al., 2017).

The role of an STI as an HPV cofactor is to facilitate entry and persistence of HPV through chronic cervical inflammation and ulceration in the cervical epithelium as well as through a reduction in host cell–mediated immunity caused by the STI (Verteramo et al., 2009).

The overall prevalence of STIs in the 351 samples examined in the present study was 27%, which is consider low in comparison with that from previous studies in other counties that have tested for the prevalence of STIs in healthy women, for example, in Mexico (57.7%) and Korea (49.2%) (Kim et al., 2014, Magana-Contreras et al., 2015). Overall, the kingdom lacks current national statistics of STI prevalence; the most recent retrospective study conducted

Table 1
Demographic and clinical data analysed by STI status.

Age, years	STI positive (n = 94) % (26.78) (N) %	STI Negative (n = 257) % (76.86) (N) %	Total (n = 351) (N) %	Chi-square (P value)
Age < 20 (n = 1)	NA	0.3 (1)	0.3 (1)	2.70 (0.61)
21–30 (n = 43)	4.3 (15)	8.0 (28)	12.3 (43)	
31–40 (n = 108)	8.3 (29)	22.5(79)	30.8 (108)	
41–50 (n = 78)	6.3 (22)	26.5 (93)	22.2 (78)	
Age > 50 (n = 121)	8 (28)	26.5 (93)	34.5 (121)	
Marital Status				
Married (n = 298)	24.5 (86)	60.4 (212)	84.9 (298)	4.79(0.091)
Divorced (n = 26)	1.4 (5)	6.0 (21)	7.4 (26)	
Widowed (n = 27)	0.9 (3)	6.8 (24)	7.7 (27)	
Nationality				
Saudi (n = 293)	23.1 (81)	60.4 (212)	83.5 (293)	0.67(0.41)
Non-Saudi (n = 58)	3.7 (13)	12.8 (45)	16.5 (58)	
Religion				
Muslim (n = 318)	24.8 (87)	65.8 (231)	90.6 (318)	0.813(0.66)
Christian (32)	2.0 (7)	7.1 (25)	9.1 (32)	
Other (n = 1)	NA	0.3 (1)	0.3 (1)	
Smoking Status				
Smoker (n = 21)	1.9 (5)	6.2 (16)	8.1 (21)	0.33(0.56)
Non-Smoker (n = 238)	27.4 (71)	64.5 (167)	91.9 (238)	
UNK (n = 92)				

Table 2
Association between STIs and clinical diagnosis[a] by cytology grades, test of association was conducted, and the P value is reported.

Variables N (%)	Normal	ASCUS	LGSIL	HGSIL	Cervical cancer	Chi Test P Value
HPV						
Positive (142,40.5%)	52(14.8%)	2(0.6%)	11(3.1%)	21(6%)	54(15.4%)	<0.001**
Negative (209,59.5%)	204(58.1%)	4(1.2%)	1(0.3%)	1(0.3%)	1(0.3%)	
STI Panel[b]						
Positive (94,26.8%)	72(20.5%)	3(0.9%)	5(1.4%)	3(0.9%)	11(3.1%)	0.17
Negative (257,73.2%)	184(52.4%)	3(0.9%)	7(2.0%)	19(5.4%)	44(12.5%)	
STI Type[c]						0.005**
Pathogenic (12,12.8%)	5(5.3%)	2(2.1%)	3(3.2%)	1(1.1%)	2(2.1%)	
Opportunistic (82,87.2%)	67(71.3%)	1(1.1%)	2(2.1%)	2(2.1%)	9(9.6%)	
UU/UP						
Positive (74,21.1%)	63(18%)	2(0.6%)	1(0.3%)	1(0.3%)	7(2.0%)	0.049*
Negative (277,78.9%)	193(55%)	4(1.1%)	11(3.1%)	21(6.0%)	48(13.7%)	
TV						NA
Positive (1,0.3%)	1(0.3%)	NA	NA	NA	NA	
Negative (350,99.7%)	255(72.7%)	6(1.7%)	12(3.4%)	22(6.3%)	55(0.7%)	
MH						
Positive (15,4.3%)	8(2.3%)	1(0.3%)	3(0.9%)	1(0.3%)	2(0.6%)	0.003**
Negative (336,95.7%)	248(70.7%)	5(1.4%)	9(2.7%)	21(6.0%)	53(15.1%)	
CT						
Positive (7,2%)	5(1.4%)	NA	2(0.57%)	NA	NA	0.005**
Negative (344,98%)	251(71.5%)	6(1.7%)	10(2.9%)	22(6.3%)	55(15.7%)	
NG						
Positive (1,0.3%)	1(0.3%)	NA	NA	NA	NA	NA
Negative (350,99.7%)	255(72.7%)	6(1.7%)	12(3.4%)	22(6.3%)	55(0.7%)	

[a] Cervical cytology grades was assigned by histology lab (ASCUS = Atypical Squamous Cells of Unknown Significance, LGSIL = Low-grade squamous intraepithelial lesion, HGSIL = High-grade squamous intraepithelial lesion).
[b] Sexually transmitted infections (STIs) detect by *DiagCor* GenoFlow hybridization STD assay.
[c] sexually transmitted infections (STIs)Types (**opportunistic** organisms include Ureaplasma (Urealyticum (UU), Parvum (UP)) and mycoplasmas (Genitalium (MG) and Hominis (MH)), while the the **pathogenic** organisms include Trichomonas Vaginalis (TV) , Chlamydia Trachomatis (CT), and Neisseria Gonorrhoeae (NG).
* P value is less than 0.01.
** P Value is less than 0.001.

Table 3
Association between clinical diagnosis and HPV status.

Variable N (%)	HPV Positive	hpv Negative	Chi-Square P Value	OR (95% CI)	(OR) P Value
STI Panel[a]					
Positive (94,26.8%)	36(10.3%)	58(16.5%)	0.62	0.884 (0.5–1.4)	0.62
Negative (257,73.2%)	106(30.2%)	151(43.0%)		1	
STI Type[b]					
Pathogenic (12,12.8%)	11(11.7%)	1(1.1%)	<0.0001***	25 (3.1–204.9)	<0.0001***
Opportunistic (82,87.2%)	25(26.6%)	57(60.6%)		1	
UU/UP					
Positive (74,21.1%)	19(5.4%)	55(15.7%)	0.004**	0.4(0.2–0.8)	0.003**
Negative (277,78.9%)	123(35.0%)	154(43.9%)		1	
TV					
Positive (1,0.3%)	1(0.3%)	NA	NA	NA	NA
Negative (350,99.7%)	141(40.2%)	209(59.6%)			
MH					
Positive (15,4.3%)	9(2.6%)	6(1.7%)	0.12	2.28(0.8–6.6)	0.12
Negative (336,95.7%)	133(37.9%)	203(57.8%)		1	
CT					
Positive (7,2.0%)	2(0.6%)	140(39.9%)	0.22	0.58(0.1–3.0)	0.51
Negative (344,98%)	140(39.89%)	204(58.1%)			
NG					
Positive (1,0.3%)	1(0.3%)	NA	NA	NA	NA
Negative (350,99.7%)	141(40.2%)	209(59.5%)			

*P value is less than 0.01.
[a] Sexually transmitted infections (STIs) detected by *DiagCor* GenoFlow hybridization STD assay.
[b] STI types are **opportunistic** organisms, including *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), *Mycoplasma genitalium* (MG), and *Mycoplasma hominis* (MH), as well as **pathogenic** organisms, including *Trichomonas vaginalis* (TV), *Chlamydia trachomatis* (CT), and *Neisseria gonorrhoeae* (NG)..
** P Value is less than 0.001.

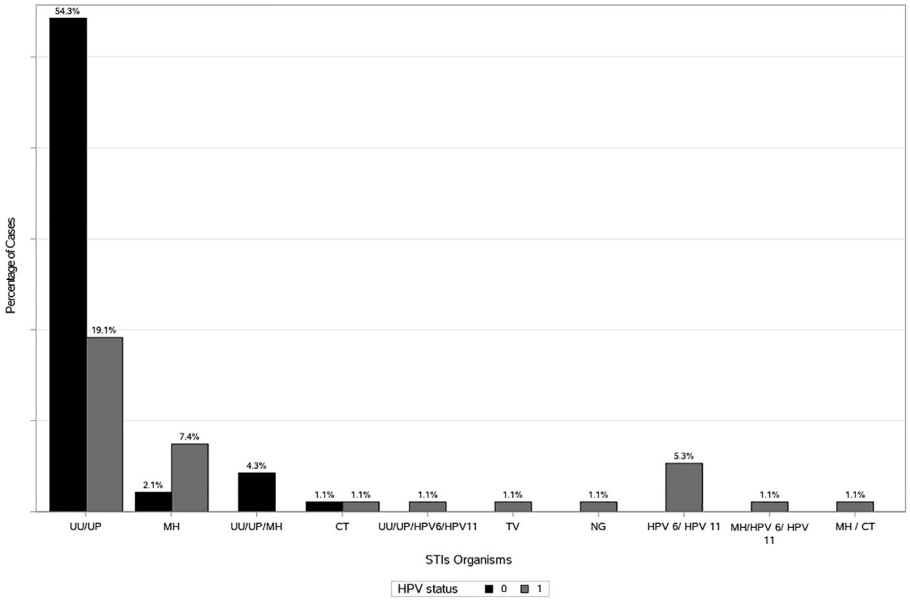

Fig. 1. Distribution of sexually transmitted infections (STIs) by HPV status. The STIs were detected by the DiagCor GenoFlow sexually transmitted disease panel. The kit detects 11 types of STI pathogens, including HPV-6, HPV-11, TV, CT, NG, UU, UP, MG, MH, HSV-1, and HSV-2. The highest detected organism is UU/UP, followed by MG, and HPV 6 and 11.

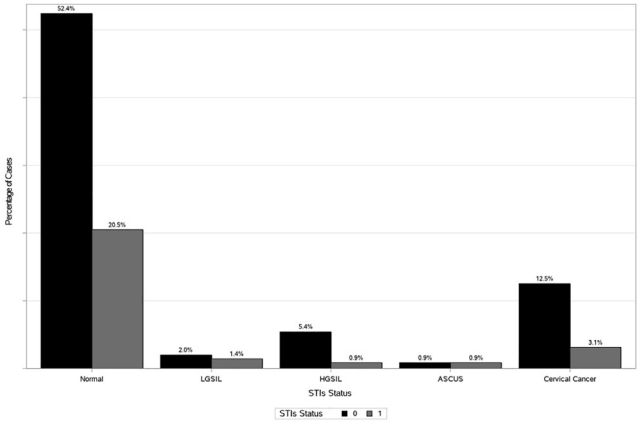

Fig. 2. Distribution of cases by cervical cytology grade and the presence of sexually transmitted infection (STI). Most of the cases are normal, while 20.5% have a normal cytology grade and are positive for an STI. Of the cervical cancer cases, 12.5% have no STI, and 3.1% are positive for an STI.

in Saudi Arabia in 2015 analyzed the annual number of STI cases from 2005 to 2012. The data for that study were obtained from the Ministry of Health in Saudi Arabia, and the main finding was that STIs in Saudi Arabia remain low, with an annual incidence of 92.1 cases per 100,000 persons (Memish et al., 2016).

The present study results were inconsistent with those from a previous prospective case-control study that aimed to measure the prevalence of STIs in fallopian tubes collected from 135 Saudi women with ectopic pregnancy (Ashshi et al., 2015). That study observed that STIs were detected in the upper genital tract in 31.8% of the Saudi women. The difference between the present study and that study is the site of sample collection. The present study targeted the lower genital tract, whereas the previous study targeted the upper genital tract. In addition, the sampling technique differed between that study and the present study. In the present study, the most commonly detected pathogens were *Ureplasma urealyticum*/*Ureplasma parvom*, *Mycoplasma hominis*, and HPV types 6 and 11. This finding is consistent with that of a previous study showing that the *Mycoplasmataceae* family, including the two genera *Mycoplasma* spp. and *Ureaplasma* spp., were the most common reported STI pathogens. These two bacteria are important opportunistic pathogens of the female lower genital tract (Verteramo et al., 2009). The role of these pathogens as a risk factor in cervical dysplasia progression has not been completely studied. The presence of these infections may play a role in initiating cellular anomalies and in viral persistence (28). Indeed, some authors (Verteramo et al., 2009; Kim et al., 2018) have suggested that the presence of a high density of *U. urealyticum,* greater than 10^4 CCU/mL, may be a risk factor for HPV infection in asymptomatic women.

In the present study, the most common HPV genotypes detected were HPV 16 (49.2%) followed by HPV 18 (25.3%). This result is similar to two previous studies in Saudi Arabia showing that the most prevalent types included HPV 18, 16, 31, 58, 56, and 42 (33, 34). As we anticipated, we found a strong association between HR-HPV and abnormal cervical cytology, which agrees with previous studies (Trottier et al., 2006; Jacobs et al., 2000).

The present study found that HPV infection was more frequent among women with older age, and patients diagnosed with cervical cancer had the oldest age followed by patients with HGSIL and ASCUS. Kjaer and colleagues found that women 30 years of age and younger have a better ability to clear HPV infection than those infected after the age of 30 (Parthenis et al., 2018). As a result, women older than 30 are more likely to develop a persistent HPV infection and then to develop cervical cancer (Kjaer et al., 2006). Bondagji et al. (2013) showed that the prevalence of HPV is high in older women. The lack of a screening program in Saudi Arabia plays a role in this finding because HPV infection may be asymptomatic.

For STIs, women who have ASCUS and HGSIL were younger than women with LGSIL and cervical cancer. In our study, STIs were more prevalent among women older than 40 years and thus is inconsistent with previous studies that have suggested that STIs are associated with young, sexually active women. These results can be attributable to religious and cultural beliefs that prevent sexual relations before marriage (Franceschi et al., 2007; Kim et al., 2016).

Table 4
Association between cytology results (Abnormal includes: ASCUS, LGSIL, HGSIL, and cervical cancer) and STIs.

Variable N (%)	Abnormal cytology	Normal cytology	Chi-Square P Value	OR (95% CI)	OR P Value
STI Panel[a]					
Positive (94,26.8%)	22(6.3%)	72(20.5%)	0.35	0.77(0.44–1.33)	0.35
Negative (257,73.2%)	73(20.8%)	184(52.4%)		1	
STI Type[b]					
Pathogenic (12,12.8%)	7(7.6%)	5(5.3%)	0.002**	6.3(1.7–22.4)	0.0048**
Opportunistic (82,87.2%)	15(15.9%)	67(71.3%)		1	
UU/UP					
Positive (74,21.1%)	11(3.1%)	63(18%)	0.008**	0.4(0.2–0.8)	0.005**
Negative (277,78.9%)	84(23.9%)	193(55%)		1	
TV					
Positive (1,0.3%)	NA	1(0.3%)	NA	NA	NA
Negative (350,99.7%)	95(27.1%)	255(72.7%)			
MH					
Positive (15,4.3%)	7(1.99%)	8(2.3%)	0.08	2.46 (0.86–6.99)	0.01
Negative (336,95.7%)	88(25.1%)	248(70.7%)		1	
CT					
Positive (7,2.0%)	2(0.57%)	5(1.42%)	0.93	1.1(0.21–5.67)	0.93
Negative (344,98%)	93(26.5%)	251(71.5%)			
NG					
Positive (1,0.3%)	1(0.3%)	NA	NA	NA	NA
Negative (350,99.7%)	95(27.1%)	255(72.7%)			
HPV Status					
Positive (142,40.5%)	5(1.4%)	52(14.8%)	<0.0001***	70.6(27.3–182.7)	<0.0001***
Negative (209,59.5%)	90(25.6%)	204(58.1%)		1	

*P value is less than 0.01.
[a] Sexually transmitted infections (STIs) detected by *DiagCor* GenoFlow hybridization STD assay.
[b] STI types are **opportunistic** organisms, including *Ureaplasma urealyticum* (UU), *Ureaplasma parvum* (UP), *Mycoplasma genitalium* (MG), and *Mycoplasma hominis* (MH), as well as **pathogenic** organisms, including *Trichomonas vaginalis* (TV), *Chlamydia trachomatis* (CT), and *Neisseria gonorrhoeae* (NG).
** P Value is less than 0.001.

Table 5
Age distribution (mean & standard deviation) by clinical data. Wilcoxon Sum of Scores Test was conducted to see if age was significantly different in the clinical data.

Variables N (%)	Mean age (SD)	Wilconxin sum of scores test (P value)
STI Panel		
Positive	43.65(13.35)	0.143
Negative	46.23(14.01)	
STI Type		
Pathogenic	39.1(10.12)	0.257
Opportunistic	44.32(13.68)	
HPV Status		
Positive	47.93(13.84)	0.006**
Negative	43.92(13.79)	
UU/UP		
Positive	44.67(13.79)	0.512
Negative	45.77(13.90)	
TV		
Positive	25(NA)	NA
Negative	45.60(13.84)	
MH		
Positive	40.66(12.63)	0.20
Negative	45.76(13.89)	
CT		
Positive	37.71(10.85)	0.141
Negative	45.70(13.88)	
NG		
Positive	43(NA)	NA
Negative	45.5(13.86)	

However, the main finding of the present study was that there was a significant association between STI (especially *Ureplasma* spp. and *Mycoplasma hominis*), HR-HPV, and abnormal cervical cytology. The overall percentage of STIs in the HPV-positive samples was 25.35%, and 3.1% of the samples with cervical cancer were positive for STIs. These findings support a number of previous studies that have found correlations among STIs, HR-HPV, and cervical dysplasia. For example, a cross-sectional study in Korea of 800 women who underwent liquid-based cervical cytology analysis shows an association not only between STIs and HR-HPV infection but also between abnormal cervical cytology and STIs, suggesting that cytological changes diagnosed as ASCUS in women without HR-HPV could be false-positives due to inflammation and changes of cervical epithelial cells induced by the STIs because some STIs can change the size and shape of cervical epithelial cells. However, the present study did not support an association between cervical cytology and STIs (Kim et al., 2016). An epidemiology study in Greece that enrolled 345 women who visited a gynecology clinic for routine cervical screening found an association between *Ureaplasma* spp. and HR-HPV infection (Parthenis et al., 2018). By contrast, an epidemiological study in China (Zhang et al., 2017) that included 1218 married women did not support any association between presence of HR-HPV and *T. vaginalis*, *C. trachomatis*, and *U. urealyticum*. Another study conducted in Mexico showed that there was no meaningful association between STIs and HR-HPV infection (Magana-Contreras et al., 2015).

Although several studies have shown an association between *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* and the presence of HPV as well as the development of cervical dysplasia (Arnheim et al., 2011; Vriend et al., 2015; de Abreu et al., 2016), the present study results did not support that association; however, the low number of positive cases affecting the results made it difficult to find statistical significance.

Our study found a significant association between cervical cytology diagnosis and HPV status, STI type (opportunistic and pathogenic), the presence of *Ureaplasma* spp., and *Mycoplasma hominis* ($P < 0.05$). It is important to note that the presence of an STI is recognized as one of the risk factors associated with cervical cancer, and it is important to conduct STI screening tests on HPV-

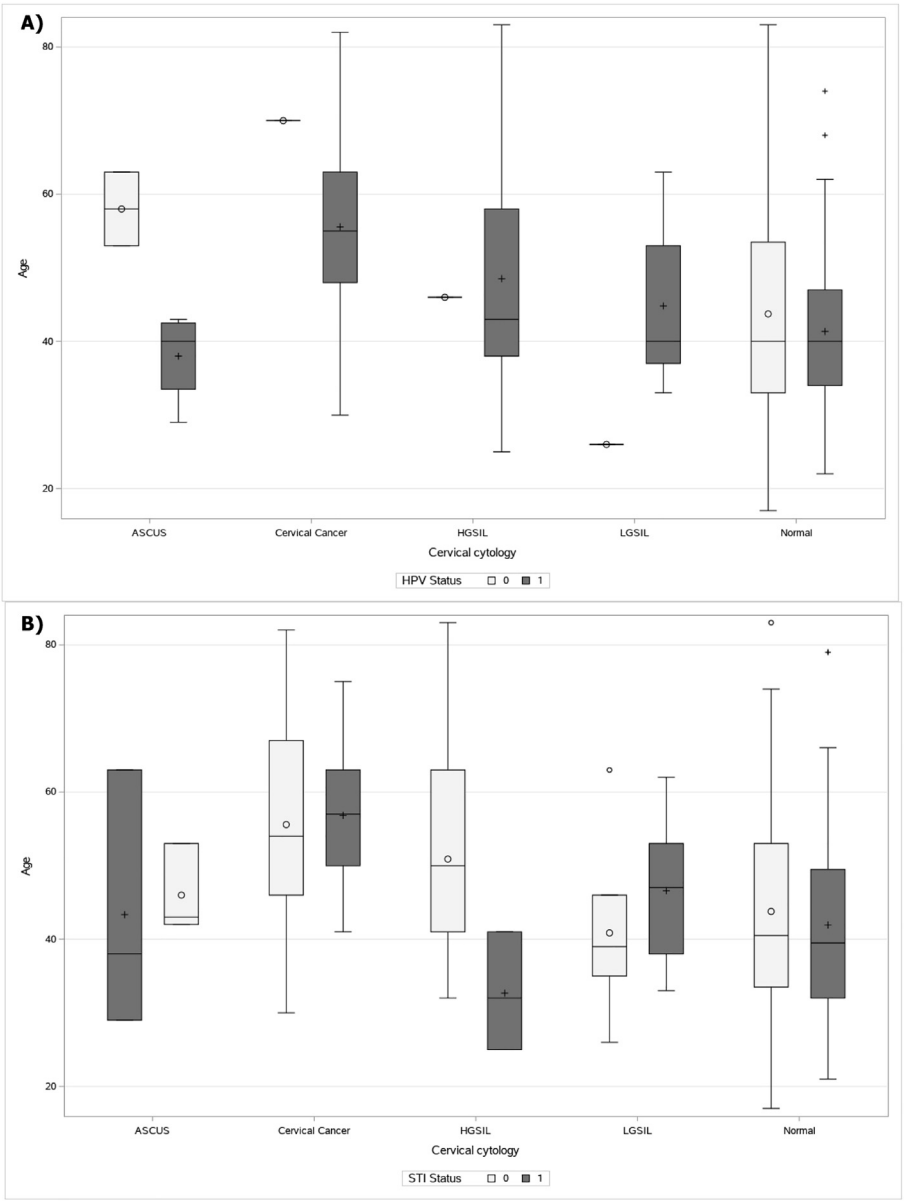

Fig. 3. (A) Distribution of age by HPV status and cytology grade. Women who are positive for HPV and have abnormal cytology grades are older than the other women. (B) Distribution of age by sexually transmitted infection (STI) status and cytology grade. Women with ASCUS or HGSIL who are positive for STIs are younger. For LGSIL and cervical cancer, most of the women who are positive for STIs are older than those in the group negative for STIs.

positive women. STIs have been associated with an inflammatory response and alteration in the epithelial cells of the cervix that help the entry of HPV. Moreover, STIs are associated with changes in the immunological response pathways and thus may have an impact on an individual's susceptibility to other pathogens; this may lead to a decreased ability to clear HPV from the cervix and help the persistence of the infection.

5. Conclusion

The present study found an association between HR-HPV/STI co-infection and abnormal cervical cytology. However, we suggest that further investigation is needed with a larger sample size obtained over a longer period of time and from a more widespread Saudi population. In Saudi Arabia, the reported cases of STIs are low due to the conservative culture and perceived shame of visiting a healthcare professional for such a disorder. Although sexual behaviors and societal norms may reduce individual exposure to STIs, awareness and prevention of STI remains important to avoid future complications.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Author contributions

Dr. Alhamlan designed and led the study. H. Alotabi is a graduate student and this is part of her work. D. Obaid, F. Almajhdi and A. Alsaleh contributed to the study design and data analysis. H. Khayat conducted the GeneFlow molecular assays. T. Al-Muammer is an OB-GYN consultant who provided cervical specimens. A. Tulba is a pathologist who ran the Pap tests and interpreted the clinical results. M. Alfageeh and M. Al-Ahdal are senior consultants in microbiology who contributed to the study design and obtained fund. All authors contributed to and approved the final version of the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Arnheim, Dahlström L., Andersson, K., Luostarinen, T., Thoresen, S., Ögmundsdottír, H., Tryggvadottír, L., Wiklund, F., Skare, G.B., Eklund, C., Sjölin, K., Jellum, E., Koskela, P., Wadell, G., Lehtinen, M., Dillner, J., 2011. Prospective seroepidemiologic study of human papillomavirus and other risk factors in cervical cancer. Cancer Epidemiol. Biomarkers Prev. 20, 2541–2550. https://doi.org/10.1158/1055-9965.EPI-11-0761.

Ashshi, A.M., Batwa, S.A., Kutbi, S.Y., Malibary, F.A., Batwa, M., Refaat, B., 2015. Prevalence of 7 sexually transmitted organisms by multiplex real-time PCR in fallopian tube specimens collected from Saudi women with and without ectopic pregnancy. BMC Infect. Dis. 15, 569. https://doi.org/10.1186/s12879-015-1313-1.

Biernat-Sudolska, M., Szostek, S., Rojek-Zakrzewska, D., Klimek, M., Kosz-Vnenchak, M., 2011. Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. Adv. Med. Sci. 56, 299–303. https://doi.org/10.2478/v10039-011-0028-9.

Bondagji, N.S., Gazzaz, F.S., Sait, K., Abdullah, L., 2013. Prevalence of high-risk human papillomavirus infections in healthy Saudi women attending gynecologic clinics in the western region of Saudi Arabia. Ann. Saudi Med. 33, 13–17. https://doi.org/10.5144/0256-4947.2013.13.

Caini, S., Gandini, S., Dudas, M., Bremer, V., Severi, E., Gherasim, A., 2014. Sexually transmitted infections and prostate cancer risk: a systematic review and meta-analysis. Cancer Epidemiol. 38, 329–338. https://doi.org/10.1016/j.canep.2014.06.002.

Costa-Lira, E., Jacinto, A.H.V.L., Silva, L.M., Napoleão, P.F.R., Barbosa-Filho, R.A.A., Cruz, G.J.S., Astolfi-Filho, S., Borborema-Santos, C.M., 2017. Prevalence of human papillomavirus, Chlamydia trachomatis, and Trichomonas vaginalis infections in Amazonian women with normal and abnormal cytology. Genet. Mol. Res. 16 (2). https://doi.org/10.4238/gmr16029626.

Dalstein, V., Riethmuller, D., Prétet, J.L., Le Bail Carval, K., Sautière, J.L., Carbillet, J.P., Kantelip, B., Schaal, J.P., Mougin, C., 2003. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int. J. Cancer. 106, 396–403. https://doi.org/10.1002/ijc.11222.

de Abreu, A.L., Malaguti, N., Souza, R.P., Uchimura, N.S., Ferreira, É.C., Pereira, M.W., Carvalho, M.D., Pelloso, S.M., Bonini, M.G., Gimenes, F., Consolaro, M., 2016. Association of human papillomavirus, Neisseria gonorrhoeae and Chlamydia trachomatis co-infections on the risk of high-grade squamous intraepithelial cervical lesion. Am. J. Cancer Res. 6, 1371–1383. eCollection 2016.

de Villiers, E.M., 2013. Cross-roads in the classification of papillomaviruses. Virology 445 (1-2), 2–10. https://doi.org/10.1016/j.virol.2013.04.023.

Donders, G.G., Depuydt, C.E., Bogers, J.P., Vereecken, A.J., 2013. Association of Trichomonas vaginalis and cytological abnormalities of the cervix in low risk women. PloS One. 8, (12). https://doi.org/10.1371/journal.pone.0086266 e86266.

Doorbar, J., Quint, W., Banks, L., Bravo, I.G., Stoler, M., Broker, T.R., Stanley, M.A., 2012. The biology and life-cycle of human papillomaviruses. Vaccine. 30 (Suppl 5), F55–F70. https://doi.org/10.1016/j.vaccine.2012.06.083.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F., 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer. 136, E359–E386. https://doi.org/10.1002/ijc.29210.

Franceschi, S., Smith, J.S., van den Brule, A., Herrero, R., Arslan, A., Anh, P.T., Bosch, F. X., Hieu, N.T., Matos, E., Posso, H., Qiao, Y.L., Shin, H.R., Sukvirach, S., Thomas, J. O., Snijders, P.J., Muñoz, N., Meijer, C.J., 2007. Cervical infection with Chlamydia trachomatis and Neisseria gonorrhoeae in women from ten areas in four continents. A cross-sectional study. Sex. Transm. Dis. 34, 563–569. https://doi.org/10.1097/01.olq.0000258417.66619.0e.

Ghosh, I., Muwonge, R., Mittal, S., Banerjee, D., Kundu, P., Mandal, R., Biswas, J., Basu, P., 2017. Association between high risk human papillomavirus infection and co-infection with Candida spp. and Trichomonas vaginalis in women with cervical premalignant and malignant lesions. J. Clin. Virol. 87, 43–48. https://doi.org/10.1016/j.jcv.2016.12.007.

Gravitt, P.E., Peyton, C.L., Alessi, T.Q., Wheeler, C.M., Coutlée, F., Hildesheim, A., Schiffman, M.H., Scott, D.R., Apple, R.J., 2000. Improved amplification of genital human papillomaviruses. J. Clin. Microbiol. 38, 357–361.

Jacobs, M.V., Walboomers, J.M., Snijders, P.J., Voorhorst, F.J., Verheijen, R.H., Fransen-Daalmeijer, N., Meijer, C.J., 2000. Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. Int. J. Cancer. 87, 221–227.

Kim, H.S., Kim, T.J., Lee, I.H., Hong, S.R., 2016. Associations between sexually transmitted infections, high-risk human papillomavirus infection, and abnormal cervical Pap smear results in OB/GYN outpatients. J. Gynecol. Oncol. 27,. https://doi.org/10.3802/jgo.2016.27.e49 e49.

Kim, S.I., Yoon, J.H., Park, D.C., Lee, D.S., Lee, S.J., Choe, H.S., Kim, J.H., Park, T.C., Lee, S. J., 2018. Co-infection Of Ureaplasma urealyticum And human papilloma virus in asymptomatic sexually active individuals. Int. J. Med. Sci. 15, 915–920. https://doi.org/10.7150/ijms.26523.

Kim, Y., Kim, J., Lee, K.A., 2014. Prevalence of sexually transmitted infections among healthy Korean women: implications of multiplex PCR pathogen detection on antibiotic therapy. J. Infect. Chemother. 20, 74–76. https://doi.org/10.1016/j.jiac.2013.08.005.

Kjaer, S., Høgdall, E., Frederiksen, K., Munk, C., van den Brule, A., Svare, E., Meijer, C., Lorincz, A., Iftner, T., 2006. The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. Cancer Res. 66, 10630–10636. https://doi.org/10.1158/0008-5472.CAN-06-1057.

Kjaer, S.K., Frederiksen, K., Munk, C., Iftner, T., 2010. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J. Natl. Cancer Inst. 102, 1478–1488. https://doi.org/10.1093/jnci/djq356.

Lazenby, G.B., Taylor, P.T., Badman, B.S., McHaki, E., Korte, J.E., Soper, D.E., Young, Pierce J., 2014. An association between Trichomonas vaginalis and high-risk human papillomavirus in rural Tanzanian women undergoing cervical cancer screening. Clin. Ther. 36, 38–45. https://doi.org/10.1016/j.clinthera.2013.11.009.

Ljubin-Sternak, S., Meštrović, T., 2014. Chlamydia trachomatis and genital mycoplasmas: pathogens with an impact on human reproductive health. J. Pathog. 2014,. https://doi.org/10.1155/2014/183167 183167.

Lukic, A., Canzio, C., Patella, A., Giovagnoli, M., Cipriani, P., Frega, A., Moscarini, M., 2006. Determination of cervicovaginal microorganisms in women with abnormal cervical cytology: the role of Ureaplasma urealyticum. Anticancer Res. 26, 4843–4849.

Luostarinen, T., Lehtinen, M., Bjørge, T., Abeler, V., Hakama, M., Hallmans, G., Jellum, E., Koskela, P., Lenner, P., Lie, A.K., Paavonen, J., Pukkala, E., Saikku, P., Sigstad, E., Thoresen, S., Youngman, L.D., Dillner, J., Hakulinen, T., 2004. Joint effects of different human papillomaviruses and Chlamydia trachomatis infections on risk of squamous cell carcinoma of the cervix uteri. Eur. J. Cancer. 40, 1058–1065. https://doi.org/10.1016/j.ejca.2003.11.032.

Magana-Contreras, M., Contreras-Paredes, A., Chavez-Blanco, A., Lizano, M., DelaCruz-Hernandez, Y., DelaCruz-Hernandez, E., 2015. Prevalence of sexually transmitted pathogens associated with HPV infection in cervical samples in a Mexican population. J. Med. Virol. 87, 2098–2105. https://doi.org/10.1002/jmv.24278.

Memish, Z.A., Filemban, S.M., Al-Hakeem, R.F., Hassan, M.H., Al-Tawfiq, J.A., 2016. Sexually transmitted infections case notification rates in the Kingdom of Saudi Arabia, 2005–2012. J. Infect. Dev. Ctries. 10, 884–887. https://doi.org/10.3855/jidc.7020.

Paba, P., Bonifacio, D., Di Bonito, L., Ombres, D., Favalli, C., Syrjänen, K., Ciotti, M., 2008. Co-expression of HSV2 and Chlamydia trachomatis in HPV-positive cervical cancer and cervical intraepithelial neoplasia lesions is associated with aberrations in key intracellular pathways. Intervirology. 51, 230–234. https://doi.org/10.1159/000156481.

Parthenis, C., Panagopoulos, P., Margari, N., Kottaridi, C., Spathis, A., Pouliakis, A., Konstantoudakis, S., Chrelias, G., Chrelias, C., Papantoniou, N., Panayiotides, I.G., Tsiodras, S., 2018. The association between sexually transmitted infections, human papillomavirus, and cervical cytology abnormalities among women in Greece. Int. J. Infect. Dis. 73, 72–77. https://doi.org/10.1016/j.ijid.2018.06.001.

Samoff, E., Koumans, E.H., Markowitz, L.E., Sternberg, M., Sawyer, M.K., Swan, D., Papp, J.R., Black, C.M., Unger, E.R., 2005. Association of Chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. Am. J. Epidemiol. 162, 668–675. https://doi.org/10.1093/aje/kwi262.

Smith, J.S., Bosetti, C., Muñoz, N., Herrero, R., Bosch, F.X., Eluf-Neto, J., Meijer, C.J., Van Den Brule, A.J., Franceschi, S., Peeling, R.W., IARC multicentric case-control study, 2004. Chlamydia trachomatis and invasive cervical cancer: a pooled analysis of the IARC multicentric case-control study. Int. J. Cancer. 111, 431–439. doi: 10.1002/ijc.20257.

Smith, J.S., Herrero, R., Bosetti, C., Muñoz, N., Bosch, F.X., Eluf-Neto, J., Castellsagué, X., Meijer, C.J., Van den Brule, A.J., Franceschi, S., Ashley, R.; International Agency for Research on Cancer (IARC) Multicentric Cervical Cancer Study Group, 2002. Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. J. Natl. Cancer Inst. 94, 1604–1613. doi: 10.1093/jnci/94.21.1604.

Tabbara, S., Saleh, A.D., Andersen, W.A., Barber, S.R., Taylor, P.T., Crum, C.P., 1992. The Bethesda classification for squamous intraepithelial lesions: histologic, cytologic, and viral correlates. Obstet. Gynecol. 79, 338–346. https://doi.org/10.1097/00006250-199203000-00003.

Tota, J.E., Chevarie-Davis, M., Richardson, L.A., Devries, M., Franco, E.L., 2011. Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. Prev. Med. 53 (Suppl. 1), S12–S21. https://doi.org/10.1016/j.ypmed.2011.08.017.

Trottier, H., Mahmud, S., Costa, M.C., Sobrinho, J.P., Duarte-Franco, E., Rohan, T.E., Ferenczy, A., Villa, L.L., Franco, E.L., 2006. Human papillomavirus infections with multiple types and risk of cervical neoplasia. Cancer Epidemiol. Biomarkers Prev. 15, 1274–1280. https://doi.org/10.1158/1055-9965.EPI-06-0129.

Verteramo, R., Pierangeli, A., Mancini, E., Calzolari, E., Bucci, M., Osborn, J., Nicosia, R., Chiarini, F., Antonelli, G., Degener, A.M., 2009. Human Papillomaviruses and genital co-infections in gynaecological outpatients. BMC Infect. Dis. 9, 16. https://doi.org/10.1186/1471-2334-9-16.

Vriend, H.J., Bogaards, J.A., van Bergen, J.E., Brink, A.A., van den Broek, I.V., Hoebe, C. J., King, A.J., van der Sande, M.A., Wolffs, P.F., de Melker, H.E.; Medical Microbiological Laboratories and the CSI group, 2015. Incidence and persistence of carcinogenic genital human papillomavirus infections in young women with or without Chlamydia trachomatis co-infection. Cancer Med. 4, 1589–1598. doi:10.1002/cam4.496.

Wong, F.K., Ching, J.C., Chow, J.K., 2010. Comparison of the DiagCor GenoFlow Human Papillomavirus Array Test and Roche Linear Array HPV Genotyping Test. Open Virol. J. 4, 169–174. https://doi.org/10.2174/1874357901004010169.

World Health Organization. Global health sector strategy on sexually transmitted infections 2016–2021. https://www.who.int/reproductivehealth/publications/rtis/ghss-stis/en/ (accessed 22 September 2019).

Zhang, D., Li, T., Chen, L., Zhang, X., Zhao, G., Liu, Z., 2017. Epidemiological investigation of the relationship between common lower genital tract infections and high-risk human papillomavirus infections among women in Beijing, China. PLoS One. 12, (5) e0178033.

Further Reading

Alhamlan, F.S., Khayat, H.H., Ramisetty-Mikler, S., Al-Muammar, T.A., Tulbah, A.M., Al-Badawi, I.A., Kurdi, W.I., Tulbah, M.I., Alkhenizan, A.A., Hussain, A.N., Ahmed, M., Al-Ahdal, M.N., 2016. Sociodemographic characteristics and sexual behavior as risk factors for human papillomavirus infection in Saudi Arabia. Int. J. Infect. Dis. 46, 94–99. https://doi.org/10.1016/j.ijid.2016.04.004.

AlObaid, A., Al-Badawi, I., Al-Kadri, H., Gopala, K., Kandeil, W., Quint, W., Al-Aker, M., DeAntonio, R., 2014. Human papillomavirus prevalence and type distribution among women attending routine gynecological examinations in Saudi Arabia. BMC Infect. Dis. 14, 643. https://doi.org/10.1186/s12879-014-0643-8.