Research Article

Does the frequency of ureaplasma increase in human papillomavirus positive women?

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Abstract

This study aimed to investigate the positive prevalence rate of Ureaplasma in Human papillomavirus (HPV) positive women. The role of sexually transmitted diseases (STD) in developing cervical cancer is evident. Clinical consequences can be prevented by screening and identifying risk factors. This retrospective cohort study was approved by the Ethics Committee of Sisli Memorial Hospital. Ureaplasma and HPV and PAP smear tests were performed for 526 sexually active women aged between 21 and 45 years (mean 31.66±4.91 years) visiting a Sisli Memorial Hospital for routine cervical screening. The association of HPV and Ureaplasma detection with cytological results were examined. The prevalence of Ureaplasma in asymptomatic women was 31%, high-risk HPV (HR-HPV) and Ureaplasma coincidence as 56.6% and the prevalence of Ureaplasma in HR-HPV was 91.5%. Most positive HPV was observed in the age range of 26-30 (50%). The most positive Ureaplasma was observed in the age range of 26-30 (51%). The highest simultaneous occurrence of two infections was observed in the age range of 26-30 (51%). Cervical screening abnormality was observed more in Ureaplasma-positive women. There was a statistically significant association between pap smear result and Ureaplasma (p-value < 0.001). There was a statistically significant association between HPV-positive and Ureaplasma (p-value < 0.001). There was a statistically significant association between HR-HPV and Ureaplasma (p-value < 0.001). Regarding the high prevalence of Ureaplasma in HR-HPV women, screening in women, especially in the age range of 26-30 years, is a breakthrough and can prevent adverse clinical consequences.

Keywords: human papillomavirus, ureaplasma, cervical cancer, sexually transmitted disease

1. Introduction

Based on epidemiological and laboratory studies all over the world, Human Papillomavirus (HPV) is the main factor of cervical cancer (1). The prevalence of persistent genital high risk-HPV (HR-HPV) in the cause of cervical cancer is approximately 99.7% (2). It is one of the most common causes of sexually transmitted diseases (STD) in women below 35 all over the world (3). Cervical cancer is the fourth most common cancer among women, and is almost found in poor and middle-income countries (4,5).

HPV infection is self-limiting, without symptoms, and associated with benign or malignant squamous mucosa proliferation. There are more than 190 types of this virus, which are classified into low-risk and high-risk groups. This infection also affects the quality of women's sexual activities (6-8).

Factors such as smoking, high parity, long-term use of oral contraceptives, other sexually transmitted infections, and co-infection affect the growth of cervical cancer in women with HPV. There is much evidence of a significant association between STD and the development of cervical cancer in HPV-positive women (9).

Ureaplasma is a common STD. Vaginal colonization with Ureaplasma spp. occurs in 40–80% of asymptomatic sexually active women (10). Ureaplasma is a frequent reason for vaginitis, cervicitis, spontaneous abortion, urethritis, and infertility (11). This infection is mainly transmitted through unprotected vaginal sex (12). Small and fastidious bacteria called Ureaplasma species often colonize the lower genitalia of asymptomatic hosts. Studies have shown that these bacteria cause neonatal infection, chorioamnionitis, urethritis, and surgical site infection in transplant recipients (13,14).

This study investigated the positive prevalence rate of Ureaplasma in HPV-positive women. In addition, the prevalence of these two infections was investigated separately and concurrently in different age groups. HR-HPV in women with Ureaplasma were also evaluated.

2. Materials and Methods

The Ethics Committee of Sisli Memorial Hospital approved this retrospective study (Decision no:6, Date:26.02.2021). Five hundred twenty-six asymptomatic healthy women who had Ureaplasma and pap smear tests simultaneously participated in this study from January 2016 - January 2021. The frequency of Ureaplasma infection accompanying abnormal and normal cytology is also planned to be

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controlled by dividing them into dysplasia subgroups. In addition, it is designed to investigate whether there is an increased incidence of infection in high-grade lesions.

HPV was tested using the digene1 HC2 HR-HPV DNA Test1 (QIAGEN, Gaithersburg, MD) with the Rapid Capture System, which is based on signal amplification using RNA probes to target the entire HR-HPV genome. All steps were performed according to the manufacturer's protocols. Simply, cervical brush samples collected in preserve cytological solution underwent a process that included denaturation, hybridization, capture, and amplification of chemiluminescent signal detection.

Cervical secretion specimens were tested for UU using the CT/NG/UU nucleic acid test kit (HybriBio Ltd, Chaozhou, China). A PCR-fluorescent probe method (48 copies/ box) was used to detect UU.

The inclusion criteria were: (1) the premenopausal women between the ages of 18 and 50. The exclusion criteria were: (1) pregnant women and women in the breastfeeding period; (2) absence of chronic disease, immune suppression and ; (3) women with cervical operation and history of malignities and treatments.

In this study, two groups were considered for comparison: the low-risk group and the high-risk group for HPV infection. Types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 are considered HR-HPV types.

The Kolmogorov-Smirnov test was performed to check the normality, and the nonparametric tests were performed given the non-normality of the groups before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, such as age. SPSS v20 was used for statistical analyses. A value of $p < 0.05$ was accepted as statistically significant.

When the sample size was calculated with the G Power 3.1 (http://www.gpower.hhu.de/) program, the difference between two independent proportions based on a chi-square tests measure with the effect size of 80%, power of 75% and 0.05 type 1 error, was found to be at least 532 patients (15).

3. Results

The study included five hundred twenty six women (mean age ± SD: 33.66 ± 4.91). The mean age in HPV(+) and Ureaplasma(+) was 31.11±4.81 and 31.80±4.48, respectively. Table 1 shows age information in groups.

As stated in Table 2, a chi-square test found a statistically significant association between HR-HPV and Ureaplasma (p-value < 0.001). There was a statistically significant association between cervical smear result and Ureaplasma (p-value < 0.001). Data in tables 2, 3 and 4 are presented as numbers (percentages).

Table 1. Age information in groups

Variable	N	Minimum	Maximum	Mean	SD
Age(yr)	526	21.00	45.00	31.66	4.91
Age(yr) in HPV (+)	526	22.00	45.00	31.11	4.81
Age(yr) Ureaplasma (+)	526	22.00	45.00	31.80	4.48

Table 2. The relationship between ureaplasma and tests results

Variable		Ureaplasma (-) (n=125) n(%)	Ureaplasma (+) (n=163) n(%)	P
HPV positive	Low-Risk	39(31.2)	14(8.5)	<0.001*
	High-Risk	86(68.8)	149(91.5)	
Cervical smear result	Normal	24(19.2)	8(4.9)	<0.001*
	AGUS	20(16)	28(17.2)	
	ASCUS	53(42.4)	68(41.7)	
	LGSIL	21(16.8)	39(23.9)	
	HGSIL	7(5.6)	12(7.4)	
	Servisit	0(0)	8(4.9)	

HPV: Human papilloma virus, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, CIN: Cervical intraepithelial neoplasia. (+), positive; (-), negative
*A Chi-square test

As stated in Table 3, a chi-square test found a statistically significant association between HPV and Ureaplasma (p-value < 0.001). Data in tables 2, 3 and 4 are presented as numbers (percentages).

Table 3. The relationship between HPV results and Ureaplasma

Variable		HPV (-) (n=238) n(%)	HPV (+) (n=288) n(%)	P
Ureaplasma	No	238(100)	125(43.4)	<0.001*
	Yes	0(0)	163(56.6)	

*A Chi-square test. (+), positive; (-), negative.

As stated in Table 4, the highest frequency of Pap smear results were ASCUS (38.6%), Normal (27.9%), LGSIL (11.8%), AGUS (11%), Servisit (7%) and HGSIL (3.6%). These results were similar in all three age groups.

**Table 4.** Distribution of age by PAP smear results

Cervical smear result	Total N= 526	18-25 N= 84 (16%)	26-30 N= 100 (19%)	31-45 N= 342 (65%)
Normal	147(27.9)	32(38.1)	21(21.0)	94(27.5)
AGUS	58(11.0)	10(11.9)	15(15.0)	33(9.6)
ASCUS	203(38.6)	29(34.5)	40(40.0)	134(39.2)
LGSIL	62(11.8)	9(10.7)	18(18.0)	35(10.2)
HGSIL	19(3.6)	1(1.2)	4(4.0)	14(4.1)
Servisit	37(7.0)	3(3.6)	2(2.0)	32(9.4)

Table 5 shows distribution of age by infection of HPV and Ureaplasma. Most HPV and Ureaplasma positives were observed in the age group between 26-30. In the age group of 18-25, HPV and Ureaplasma positives had the lowest frequency. The age group over 30 years was similar to the total frequency in cases HPV and Ureaplasma positives.

Table 5. Distribution of age by infection of HPV and Ureaplasma

Infection	Total N= 526	18-25 N= 84 (16%)	26-30 N= 100 (19%)	31-45 N= 342 (65%)
HPV (+)	288(54.7)	50(59.5)	67(67)	171(50)
HPV (-)	238(45.3)	34(40.5)	33(33)	171(50)
Ureaplasma (+)	163(31)	12(14.3)	51(51)	100(29.3)
Ureaplasma (-)	363(69)	72(85.7)	49(49)	242(70.7)
HPV (+) Ureaplasma (+)	163(31)	12(14.3)	51(51)	100(29.3)
HPV (+) Ureaplasma (-)	125(23.7)	38(45.2)	16(16)	71(20.7)
HPV (-) Ureaplasma (+)	122(23.3)	3(3.5)	16(16)	103(30.1)
HPV (-) Ureaplasma (-)	116(22)	31(37)	17(17)	68(19.9)

(+), positive; (-), negative.
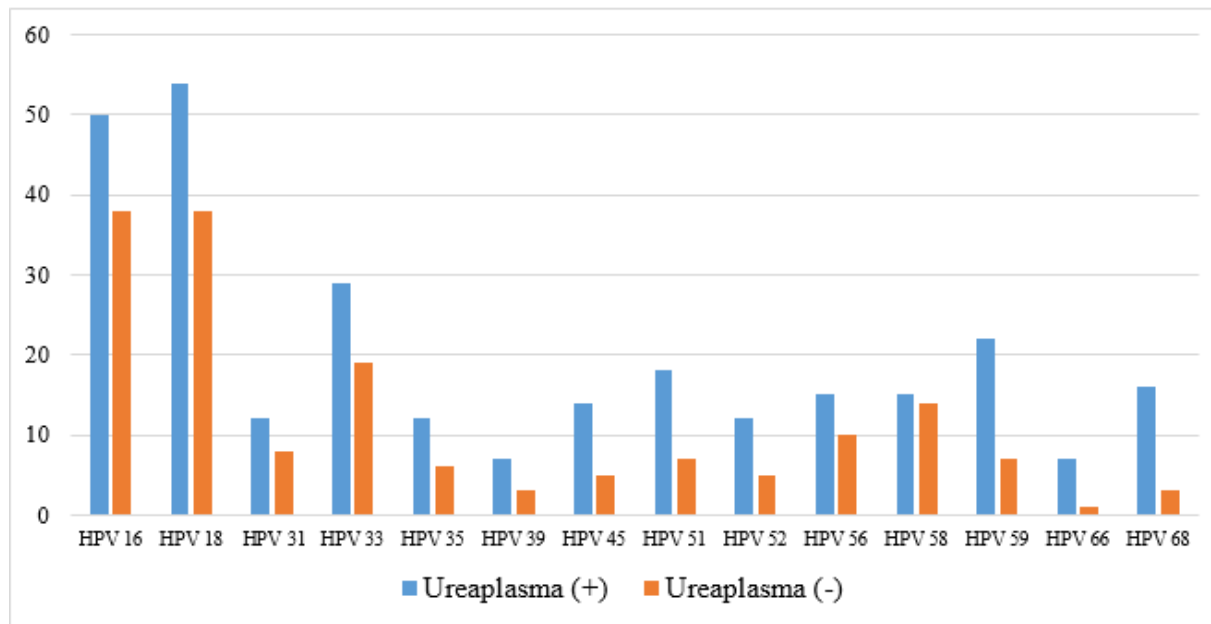
Fig. 1. Comparison of HR- HPV prevalence between ureaplasma positive and ureaplasma negative

There are about 14 HR-HPV types including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. In this study, a comparison was made between the Ureaplasma positive and negative in terms of the prevalence high-risk HPV types (Fig. 1). All high-risk types of HPV were more in the Ureaplasma positive

4. Discussion
The relationship between HR-HPV and cervical cancer is clear. There is evidence that facilitating factors such as sexually transmitted diseases increase the risk of cervical cancer. This study investigated the frequency of Ureaplasma as a sexually transmitted disease in women with HPV infection. In the current study, the Ureaplasma positivity rate was 56.6% in HPV-positive women. The prevalence of Ureaplasma in asymptomatic women was 31%, and the prevalence of Ureaplasma in HR-HPV was 91.5%. This study examined the frequency of Ureaplasma infection in both low and high-risk HPV groups; it was observed that the frequency of Ureaplasma infection is higher in women with HR-HPV infection. By studying different age ranges, women between 26-30 years old were more to have HPV and Ureaplasma. After this group, women between 30-45 years old were more

to have Ureaplasma and HPV infection than women under 25. A significant difference was found when the HPV type and cervical smear results were compared in terms of the Ureaplasma result. This study indicated that HR-HPV infection was significantly associated with Ureaplasma, meaning that Ureaplasma may serve as a cofactor in developing Cervical cancer.

Previous studies have shown that Ureaplasma infection increases the risk of HPV infection. In their study on 1200 married Chinese women, Zhang et al. (11)showed that the prevalence of Uroplasma was 35.5%, and the coexistence of HPV and Ureaplasma was 8.6%. Xiaolei et al. (16) reported the prevalence of HPV and Ureaplasma coincidence as 53.2% in 233 Chinese women. Kim et al. (17) reported the prevalence of HPV as 33.1%, Ureaplasma (U>104 CFU/ml) as 36%, and HPV and Ureaplasma coincidence as 19.5% in 264 Chinese asymptomatic women. Lv et al. (18) reported the prevalence of HR-HPV as 30.7%, Ureaplasma as 11.9%, and HPV and Ureaplasma coincidence as 15.4% in 826 asymptomatic Chinese women. Parthenis et al. (19) noted the prevalence of Ureaplasma as 18.2%, and HR-HPV and Ureaplasma coincidence as 25.4% in 345 asymptomatic

Greek women. Lopez et al. (20) reported the prevalence of HR-HPV as 17.1%, different types of Ureaplasma between 0.08% to 32.9%, and HR-HPV and Ureaplasma coincidence as 16.7% in 258 asymptomatic Mexican women. Tantengco et al. (21) noted the prevalence of different types of HPV as 75%, and HR-HPV and Ureaplasma coincidence as 22.7% in 44 asymptomatic Filipino women.

Zhang et al. (11) reported a significant relationship between Ureaplasma infection and HR-HPV infection. Camporiondo et al. (22), Kim et al. (17), and Xiaolei C et al. (16) reported a significant relationship between Ureaplasma and HPV. In contrast, Tantengco et al. (21) reported no a significant relationship between these two infections by examining 44 Filipino women. Also, by studying 37 Turkish women, Biyik et al. (23) concluded that there is no significant relationship between Ureaplasma and HPV. The sample size is very small in these two studies, where no significant relationship has been reported.

In conclusion, a high prevalence of Ureaplasma was observed in HR-HPV women, which indicates that Ureaplasma was a risk factor in HR-HPV. These results showed the importance of simultaneous diagnosis of sexually transmitted infections in the genital tract as an essential strategy to prevent adverse clinical outcomes of uterine cancer. Screening for the simultaneous diagnosis of Ureaplasma and HPV infection in asymptomatic women is recommended, especially between 26-30 years of age. It is hoped that this study will attract more attention to the common sexually transmitted diseases, that these diseases will be included in the routine screening, and will inform the patients thus helping reduce cervical cancer a little.

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