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# Molecular Detection of Sexually Transmitted Infections in Women with and without Human Papillomaviruses Infection Who Referred to Tehran West Hospitals in Iran

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## Abstract

***Background:*** According to the studies, many pathogens function as cofactors interacting with Human papillomavirus in the development of pre-cancer or cancer of the cervix. The aim of this study was to investigate the prevalence rate of Sexually Transmitted Infections (STIs) pathogens including *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, and *Streptococcus agalactiae* in people with HPV and without HPV infection, and frequency rate of these pathogens in high and low risk of HPV.

***Methods:*** Cervical samples of 280 women who referred to Tehran west hospitals in Iran, between 2019 and 2020, were collected. After DNA extraction of samples, identification of HPV and genotyping was performed, and then, to detect each microorganism, the PCR was carried out with specific primers. Finally, the results were analyzed using descriptive statistics tests.

***Results:*** The mean age of patients was 37 years. Two groups of patients were identified based on positivity or negativity of HPV. In HPV-positive group (118 cases), the prevalence of *U. urealyticum*, *M. hominis*, *N. gonorrhoeae*, *G. vaginalis*, and *S. agalactiae* was 38 (13%), 7 (62%), 5.93%, 19.49%, 0.84% respectively. In HPV-negative group (162 cases), rate of infection with *U. urealyticum*, *M. hominis*, *N. gonorrhoeae*, *G. vaginalis*, and *S. agalactiae* was 29.62%, 6.17%, 3.08%, 16.04%, 0.61% respectively. Among the two groups, there was only 1 patient with *C. trachomatis* (0.84%), seen in HPV-positive group.

***Conclusions:*** In this study no significant association was found between HPV and bacteria such as G. vaginalis and S. agalactiae, and it was found that C. trachomatis, and especially N. gonorrhoeae are strongly associated with HPV infection.

**Keywords:** HPV, Sexually transmitted infections, PCR.

## Introduction

Human papillomaviruse infection is very common and human papillomaviruses are causative agents which have small double-stranded DNA belonging to the family Papillomaviridae. They infect squamous epithelia and develop proliferative lesions and

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skin warts. Almost 130 types of HPV have been identified that, depending on their oncogenic potential, are divided into high-risk and low-risk groups (1). High-risk group is oncogenic and low-risk group is non-oncogenic genotypes. The low-risk HPV is associated with benign or less malignant genital warts, but high-risk HPV is the major risk factor in the development of ano-genital malignancies, specially cervical intraepithelial neoplasia and cervical cancer, if it is persistent (2-4). Human papillomaviruse infection also is one of the risk factors in sexually transmitted diseases which can be transmitted by sexual contacts between humans. Also, other genital microorganisms including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, group B streptococcus, Mycoplasmas, *Gardnerella vaginalis*, Herpes Simplex Virus (HSV), and Human Immunodeficiency Virus (HIV), can cause sexually transmitted infections (STIs). Infections with these microorganisms also can have serious consequences (5-8). According to the studies, many pathogens, including *C. trachomatis* and *Ureaplasma urealyticum*, serve as cofactors interacting with HPV in the development of pre-cancer or cancer of the cervix. These agents by producing metabolites or carcinogenic substances, or increasing the susceptibility of the inflamed epithelium, disturbance of normal cellular metabolism, and damage to DNA, are directly or indirectly responsible for the development and progression of cervical lesions (9, 10). *M. hominis* and *U. urealyticum* belong to the Mollicutes class and *Mycoplasmataceae* family which can be considered as commensal of the female genital tract or pathogen (11, 12). They are the smallest organisms, having free-life and lacking a cell wall (13). *M. hominis* is associated with bacterial vaginosis (BV), Pelvic inflammatory disease (PID), cervicitis and endometritis (11) and *U. urealyticum* is associated with pregnancy abnormalities and infertility (12). *C. trachomatis* and *N. gonorrhoeae* are gram-negative bacteria and intracellular pathogens. *N. gonorrhoeae* can lead to serious disease including gonorrhea, pelvic inflammatory disease, infertility, ectopic pregnancy and urethritis. *C. trachomatis* has several serovars; A-C, D-K and L1-L3 serovars are associated with trachoma, urogenital infection and invasive lymphoma granuloma venereum (LGV), respectively (14, 15). *Streptococcus agalactiae* is also involved in genital infections. These gram-positive diplococcic are being colonized in genital area or rectum and invasive infection with them causes substantial morbidity and mortality (16, 17). *G. vaginalis* is a gram-variable, small, and normal flora of women vagina associated with BV (18, 19).

Although for detection of these bacteria, the culture is golden standard, but it is difficult, and requires special culture media and a long time. Among methods of diagnosis, Polymerase Chain Reaction (PCR) is fast, sensitive, specific, easy for specimen transport and able to differentiate among various species (20, 21).

The purpose of this study was to investigate the prevalence rate of STI pathogens including *M. hominis*, *U. urealyticum*, *C. trachomatis*, *N. gonorrhoeae*, *G. vaginalis* and *S. agalactiae* in people with HPV and without HPV, and frequency rate of these pathogens in high risk and low risk of HPV.

## Materials and Methods

The study population consisted of 280 women at the age of 18-59 years (mean age was 37) who referred to Tehran west hospitals in Iran, between 2019 and 2020. Written informed consent was obtained, according to the protocol approved by the Ethics Committee for Student Research Committee of Bam University of Medical Sciences (Code of Ethic: IR.MUBAM.REC.1398.004). All participants completed the questionnaire that included items on age, smoking, marital status, and history of pregnancies. Cervical brush samples were collected by a gynecologist and each of the samples was placed in phosphate buffer solution (PBS), transported to laboratory, and immediately placed in the freezer at -80 °C. DNA of samples were extracted according to kit manufacturer instructions (Qiagen, Germany) and stored at –20 ˚C.



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Based on the study of Finan et al., immunohistochemistry of cervical smears was used to subdivide the specimen into two groups: HPV-positive group and HPV-negative group. Also, to confirm HPV identification, PCR using the MY09/MY11 primer system was applied as the gold standard (22).

Then HPV genotyping from samples also was performed using an HPV genotyping Test Kit (Master Diagnóstica, Granada, Spain). This kit can determine 36 HPV types including High risk HPV: (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82), and low risk HPV: (6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84, and 89).

To detect each microorganism in HPV-positive and HPV-negative specimens, the PCR was performed with specific primers. The sequences of primers for each microorganism are listed in Table 1. Amplification reaction was performed in a volume of 25 µl and contained 12 ml master mix (Ampliqon Co, Skovlunde, Denmark), 0.5 ml (20 pmol) of each forward and reverse primer, 10 ml sterile distilled water, and 2 µl extracted DNA. The PCR reactions for these microorganisms were run by PCR system (BIO-RAD C1000 Thermal Cycler) under the following condition: an initial cycle for pre denaturation at 95 °C for 10 min, followed by n cycles (cycles for each microorganism listed in Table 1) of denaturation at 95 °C for 30 s, annealing (Tm for each microorganism listed in Table 1) 30 s, and elongation at 72 °C for 1 minute, with a final cycle at 72 °C for 5 min. The PCR products were visualized and photographed under UV light after electrophoresis for 45 min at 100 V through a 1% agarose gel (Fig. 1). Finally, the results were analyzed using descriptive statistics tests.

**Table 1.** The DNA sequence of PCR primer, Tm, cycles, and Length.

| Name of bactery | Nucleotide sequence (5'-3') | Tm | Cycles | Length (bp) | Reference |
|---|---|---|---|---|---|
| *(U. urealyticum)* | F 5`- TGGAGTTAAGTCGTAACAAG-3`<br>R 5`- CTGAGATGTTTCACTTCACC-3` | 56 | 30 | 559 | (23) |
| *M. hominis*)( | F: 5′-CAATGGCTAATGCCGGATAC -3′<br>R: 5′-GGTACCGTCAGTCTGCAAT -3′ | 56 | 30 | 335 | (24) |
| *C. trachomatis)* | F: 5′-CTAGGCGTTTGTACTCCGTCA-3′<br>R: 5′-TCCTCAGGAGTTTATGCACT-3′ | 56 | 40 | 200 | (25) |
| (*N. gonorrhoeae*) | F: 5′-GCCTCGCGGCTTGGCTA -3′<br>R: 5′-GGCGCAGACGGTTACTTAAGCAGGA-3′ | 57 | 35 | 694 | (26) |
| (*G. vaginalis*) | F: 5′-TTACTGGTGTATCACTGTAA -3′<br>R: 5′-CCGTCACAGGCTGAACAGT-3′ | 55 | 30 | 330 | (27) |
| (*S. agalactiae*) | F: 5′- TTTCACCAGCTGTATTAGAAGTA -3′<br>R: 5′- GTTCCCTGAACATTATCTTTGAT-3′ | 57 | 30 | 153 | (28) |



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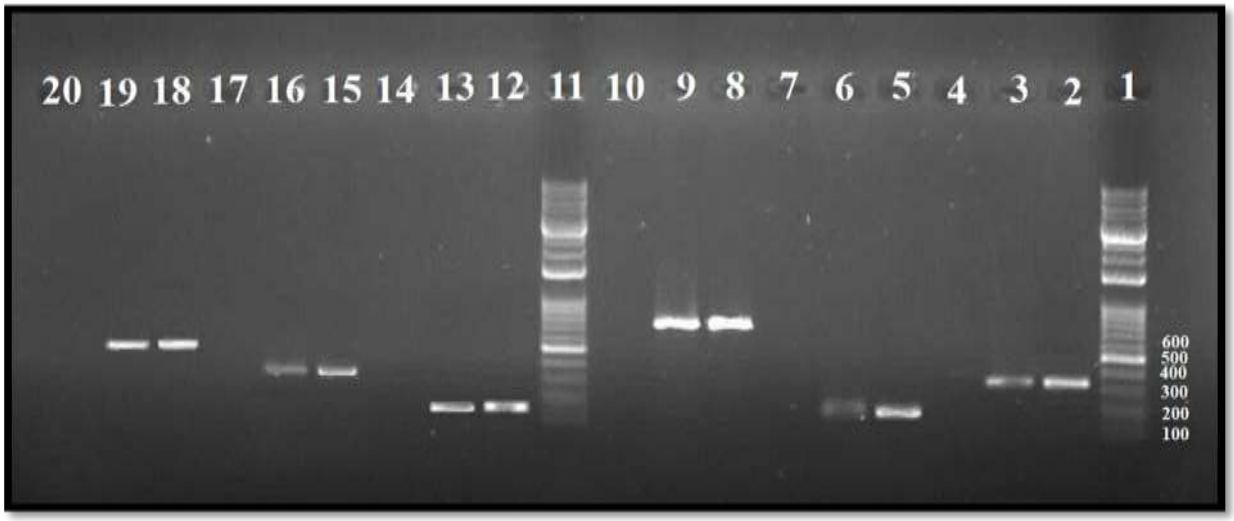


**Fig. 1.** Agarose gel electrophoresis of PCR amplified products.

Lane 1 & 11: 100 bp ladder marker.
Lane 2: positive control for *G. vaginalis* (330 bp), Lane 3: positive sample for *G. vaginalis* (330 bp).
Lane 4: negative control for *G. vaginalis* (distilled water).
Lane 5: positive control for *C. trachomatis* (200 bp), Lane 6: positive sample for *C. trachomatis* (200 bp).
Lane 7: negative control for *C. trachomatis* (distilled water).
Lane 8 positive control for *N. gonorrhoeae* (694 bp), Lane 9: positive sample for *N. gonorrhoeae* (694 bp).
Lane 10: negative control for *N. gonorrhoeae* (distilled water).
Lane 12: positive control for *S. agalactiae* (153 bp), Lane 13: positive sample for *S. agalactiae* (153 bp).
Lane 14: negative control for *S. agalactiae* (distilled water).
Lane 15: positive control for *M. hominis* (335 bp), Lane 16: positive sample for *M. hominis* (335 bp).
Lane 17: negative control for *M. hominis* (distilled water).
Lane 18 positive control for *U. urealyticum* (559 bp), Lane 19: positive sample for *U. urealyticum* (559 bp).
Lane 20: negative control for *U. urealyticum* (distilled water).

## Results

In this investigation, 280 women were recruited. Two groups of patients were identified based on positivity or negativity of HPV (118 HPV positive and 162 HPV negative subjects). According to the genotyping kit, 3 groups were identified: High-risk (n= 31, 26.2%), low-risk (n= 52, 44%), and High-risk + low-risk (n= 35, 29.6%). In HPV-positive and HPV-negative women, the mean age was 37 years, and most people were in the age range of 30-35 years, so there was no difference between the two groups with respect to age.

On the other hand, no significant difference was observed in history of pregnancies, marital status, and smoking in women with and without HPV, respectively (Table 2).

**Table 2.** Characteristics of studied population.

| Characteristics | Smoking | | Marital Status | | History of Pregnancies | | Mean Age | |
|---|---|---|---|---|---|---|---|---|
| Frequency | Hpv + (n= 118) | Hpv – (n= 162) | Hpv + (n= 118) | Hpv – (n= 162) | Hpv + (n= 118) | Hpv – (n= 162) | Hpv + | Hpv – |
| | 5(4.23%) | 7(4.32%) | 83(70.33%) | 129(79.62%) | 51(43.22%) | 72(44.4%) | 37 | 37 |

In HPV-positive group (118 cases), prevalence of *U. urealyticum*, *M. hominis*, *N. gonorrhoeae*, *G. vaginalis*, and *S. agalactiae* was 45 (38.13%), 9 (7.62%), 7 (5.93%), 23 (19.49%), 1 (0.84%) respectively. Among the two groups of low-risk and High-risk, the


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highest frequency of *N. gonorrhoeae* and *G. vaginalis* was observed in the low-risk group, and the highest frequency of *U. urealyticum* and *M. hominis* was observed in High-risk + low-risk, and High-risk group respectively.

In HPV-negative group (162 cases), the rate of infection with *U. urealyticum*, *M. hominis*, *N. gonorrhoeae*, *G. vaginalis*, and *S. agalactiae* was 48 (29.62%), 10 (6.17%), 5 (3.08%), 26 (16.04%), 1 (0.61%) respectively. Among the two groups, there was only 1 infection with *C. trachomatis* (0.84%), was seen in HPV-positive group (High-risk) (Table 3).

According to Table 4, the highest frequency of infection with *U. urealyticum*, *M. hominis* and *G. vaginalis* in both groups was seen in the 31–40 years' age category, while the lowest was seen in the older age range (up 50 years) and under 20 years. The highest infection with *N. gonorrhoeae* was observed in the group with and without HPV in 31-35 and 36-40 years, respectively. Also, the most coinfections were observed in both positive and negative HPV groups between *U. urealyticum* and *G. vaginalis* where the highest prevalence belonged to the negative HPV group (Table 5).

**Table 3.** Distribution of of bacteria in HPV negatives and positives.

| Name of bacteria | HPV negatives (n= 162) | HPV positive (n= 118) | | | |
|---|---|---|---|---|---|
| | | HR (n= 31) | LR (n= 52) | HR+LR (n= 35) | Total (n= 118) |
| ***U. urealyticum*** | 48 (29.62%) | 8(6.77%) | 18(15.25%) | 19(16.10%) | **45(38.13%)** |
| ***M. hominis*** | 10 (6.17%) | 4(3.38%) | 2(1.69%) | 3(2.54%) | **9(7.62%)** |
| ***C. trachomatis*** | 0 (0%) | 1(0.84%) | 0 (0%) | 0 (0%) | **1(0.84%)** |
| ***N. gonorrhoeae*** | 5 (3.08%) | 2(1.69%) | 4(3.38%) | 1(0.84%) | **7(5.93%)** |
| ***G. vaginalis*** | 26(16.04%) | 5(4.23%) | 13(11.01%) | 5(4.23%) | **23(19.49%)** |
| ***S. agalactiae*** | 1 (0.61%) | 0(0%) | 1(0.84%) | 0(0%) | **1(0.84%)** |
| **Uu+Mh** | 2(1.23%) | 1(0.84%) | 0(0%) | 0(0%) | **1(0.84%)** |
| **Uu+Ng** | 1 (0.61%) | 1(0.84%) | 1(0.84%) | 1(0.84%) | **3(2.54%)** |
| **Uu+Gv** | 13(8.02%) | 2(1.69%) | 2(1.69%) | 3(2.54%) | **8(6.77%)** |
| **Ng+Gv** | 0(0%) | 0 (0%) | 2(1.69%) | 0(0%) | **2(1.69%)** |
| **Mh+Gv** | 2(1.23%) | 0(0%) | 0(0%) | 1(0.84%) | **1(0.84%)** |
| **Mh+Ng** | 1(0.61%) | 0(0%) | 0(0%) | 0(0%) | **0(0%)** |
| **Uu+mh+gv** | 1(0.61%) | 1(0.84%) | 2(1.69%) | 0(0%) | **3(2.54%)** |
| **Uu+mh+ng** | 1(0.61%) | 0(0%) | 0(0%) | 0(0%) | **0(0%)** |
| **Uu+gv+ng** | 1(0.61%) | 0(0%) | 0(0%) | 0(0%) | **0(0%)** |
| **Ct+Mh+Gv** | 0(0%) | 1(0.84%) | 0(0%) | 0(0%) | **1(0.84%)** |

*Uu: *U. urealyticum* Mh: *M. hominis* Ng: *N. gonorrhoeae* Gv: *G. vaginalis* Ct: *C. trachomatis*

**Table 4.** Frequency of bacteria in different age groups (in women with and without HPV infection).

| Age groups | *U.urealyticum* | | *M. hominis* | | *C. trachomatis* | | *N. gonorrhoeae* | | *G. vaginalis* | | *S. agalactiae* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hpv+ | Hpv- | Hpv+ | Hpv- | Hpv+ | Hpv- | Hpv+ | Hpv- | Hpv+ | Hpv- | Hpv+ | Hpv- |
| **Under 20** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| **20-30** | 14 | 11 | 3 | 2 | 0 | 0 | 3 | 1 | 5 | 4 | 1 | 0 |
| **31-40** | 26 | 31 | 5 | 8 | 1 | 0 | 3 | 3 | 15 | 21 | 0 | 1 |
| **41-50** | 5 | 5 | 1 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 0 |
| **Up 50** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |



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**Table 5.** Frequency of common infections in different age groups.

| Age groups | 20-30 | | 31-40 | | 41-50 | | Total | |
|---|---|---|---|---|---|---|---|---|
| Common infection | Hpv+ | Hpv- | Hpv+ | Hpv- | Hpv+ | Hpv- | Hpv+ | Hpv- |
| **Uu+Mh** | 1 | | | 2 | | | 1 | 2 |
| **Uu+Ng** | | | 3 | | | | 3 | 0 |
| **Uu+Gv** | 3 | 5 | 3 | 7 | 1 | 1 | 7 | 13 |
| **Ng+Gv** | 2 | | | | | | 2 | 0 |
| **Mh+Gv** | 1 | | | 2 | | | 1 | 2 |
| **Mh+Ng** | | 1 | | | | | 0 | 0 |
| **Uu+mh+gv** | 1 | 1 | 2 | | | | 3 | 1 |
| **Uu+mh+ng** | | | | 1 | | | 0 | 1 |
| **Uu+gv+ng** | | | | 1 | | | 0 | 1 |
| **Ct+Mh+Gv** | | | 1 | | | | 1 | 0 |

* In this study, no common infections were observed in age groups under 20 and over 50 years

## Discussion

Genital microorganisms can be transmitted through human sexual contacts and cause sexually transmitted infections (STIs) and serious consequences. One of them is HPV that, depending on their oncogenic potential, is divided into high-risk and low-risk groups. Many pathogens act as cofactors interacting with HPV in the development of pre-cancer or cancer of the cervix and can directly or indirectly result in the development and progression of cervical lesions. To detect these microorganisms, Polymerase Chain Reaction (PCR) was selected which is fast, sensitive, specific, and easy. In this study, the correlation between infection of *M. hominis*, *U. urealyticum*, *C. trachomatis*, *N. gonorrhoeae*, *G. vaginalis* and *S. agalactiae* in people with HPV and without HPV was evaluated by PCR. According to the results, the prevalence rate of *U. urealyticum*, *M. hominis*, *G. vaginalis* and *S. agalactiae*, in both groups (with and without HPV) was similar, with no significant difference. In contrast, infection with *C. trachomatis* was present only in the HPV-positive group. Also, a higher prevalence of *N. gonorrhoeae* was seen in HPV-positive women compared to HPV-negative women. So, there was a correlation between HPV-positive, especially high-risk, with *C. trachomatis* and *N. gonorrhoeae*.

In addition, common infections (triple and double infections) in groups with and without HPV were relatively high (19 (16.1%) and 22 (13.5%) respectively), and the highest prevalence of double infection was *U. urealyticum* and *G. vaginalis*, (8(6.77%) 13(8.02%) respectively) and the highest prevalence of triple infection was in women with HPV between *U. urealyticum*, *M. hominis* and *G. vaginalis* (3(2.54%)).

On the other hand, no significant correlation between women with and without HPV, and these microorganisms' infection and the factors including smoking, marital status, and history of pregnancies, was found.

Based on Table 3, the highest frequency of HPV and the most frequent microorganisms in groups with and without HPV were in 20-40 years, and in women over the age of 40, there was seen less frequency of HPV and these bacteria, possibly due to a decrease in sexual activity in older women.

In numerous studies, the frequency of these microorganisms has been investigated, such as studies that reported 21.3% and 14.9% prevalence for *G. vaginalis* in HPV-positive group and HPV-negative group. Their investigation was carried out on intraepithelial



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neoplasia (CIN) grade I (low-grade squamous lesion), and the methodology in detection of HPV infection and *Gardnerella vaginalis* was based on the study of Schneider et al. and finding of clue cells respectively (29). Differences in the results of their investigation and those of our study may be due to their methodology and the samples used.

In other study, in samples obtained from vaginal canal and uterine cervix of female sex workers, *G. vaginalis* was observed to be 76.9% (10.13) and 79.6% (43.54), and *C. trachomatis* was reported to be 38.5% (5.13) and 22.2% (12.54), in the group with and without HPV (30). High rate of genital agents such as *G. vaginalis* and *C. trachomatis* in their study can be due to the characteristics of their studied population (sex workers) since they were constantly exposed to sexually transmitted agents.

Infection of *C. trachomatis* in women with abnormal cytology in the study by Finan showed a high rate: 21.44 in HPV-positive as compared to 11.77 in HPV-negative cases. There was higher prevalence of this bacterium in the 31–40 age group category (22). Since infection with *C. trachomatis* facilitates the development of invasive squamous cell carcinoma (22), high frequency of this microorganism in abnormal cytology in their study is reasonable. But in our study, normality or abnormality of samples was not determined. In the present study, only 1 case was positive for *C. trachomatis* which was in the age 31-40, similar to Finan's study.

In a study by Biernat-Sudolska M, frequency of *U. urealyticum* and *M. hominis* in HPV-positive women (n= 58) was 12(20.6%) and 1(1.72%) (10). Existence of *U. urealyticum* in the group with HPV in their study was higher than that in the control group, in contrast to the finding in our study which may be due to the type of their samples (abnormal cervix and cervical carcinomas).

Marianna Martinelli et al. also evaluated some of STI pathogens in women Pap smear with an abnormal cervical cytology and reported prevalence of 6(5.26%), 2(1.75%), 0(0%) and 0(0%) in *U. urealyticum*, *M. hominis*, *C. trachomatis* and *N. gonorrhoeae* respectively in high-risk HPVs (31). The prevalence of *U. urealyticum*, *M. hominis*, *C. trachomatis* and *N. gonorrhoeae* in our study was higher (25.8%, 12.9%, 3.2%, 6.4% respectively) in high-risk HPV group, as compared with the study conducted by Marianna Martinelli.

Another study in Poland detected 4 cases *U. urealyticum* in HPV-positive women (31 cases) two of whom were hemodialysed women and 2 other cases were in the control group (non-hemodialysis women) (32). In our investigation, higher frequency of *U. urealyticum* was detected that may be because of the bigger sample size in our study. On the other hand, their study was performed on hemodialysed women that may have affected the rate of *U. urealyticum*.

In a study by Prete et al., detection of STI pathogens was performed by multiplex Real-Time PCR in vaginal and cervical specimens showing that the prevalence of *U. urealyticum*, *M. hominis*, *C. trachomatis* and *N. gonorrhoeae* was 30 (2.36%), 15 (1.18%), 5 (0.39%), and 1 (0.08%) respectively. Also, coinfection prevalence of *U. urealyticum* + *M. hominis* in their study was 10 (0.79%) (33). Higher frequency of *U. urealyticum*, *M. hominis* and *N. gonorrhoeae*, and lower prevalence of *C. trachomatis* in the present study (negative HPV + positive HPV) compared with the study by Prete et al. may be associated with differences in the number and kind of samples, technique, or even race.

In this study no significant association was found between HPV and bacteria such as *G. vaginalis* and *S. agalactiae*, and the results indicated that some of analyzed bacteria (*U. urealyticum*, *M. hominis* and *G. vaginalis*) could be part of normal flora in Iranian women. On the other hand, the results demonstrated that *C. trachomatis* and especially *N. gonorrhoeae* are strongly associated with HPV infection, and HPV infection may increase susceptibility to other infections like *C. trachomatis* and especially *N. gonorrhoeae*. This investigation confirmed that screening for genital agents may be important to rapid treatment.



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The authors declare that they have no conflict of interest.

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