

# The Characteristics of Human Papillomavirus Infection in Women with Vaginitis and the Correlation with Vaginitis

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## Research Article

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# Abstract

**Background:** Human papillomavirus (HPV) infection is the main cause of cervical cancer in women and vaginitis may affect HPV infection. In order to determine the characteristics as well as the relationship between HPV Infection and vaginitis, we retrospectively collected and analyzed the data from our hospital.

**Methods:** We retrospectively collected the data of 1330 women who underwent twenty-three HPV DNA quantitative measurement and vaginal microecology examination at the same visit from May 2019 to April 2020 in our hospital.

**Results:** The vaginal discharge infection rate was 37.67% in all cases. The total HPV infection rate in women with vaginitis was 39.60% and HPV58, 52, and 16 had the higher prevalence among 23 HPV types, with infection rates of 10.34%, 6.32% and 6.11% respectively. Single-type infection (77.26%) was more common than multiple-type infection (32%). There appeared the same trend in HPV positive cases and vaginitis, reaching the peak in women aged 31–40 years. Among four types of vaginitis, the HPV infection rates were 45.83%, 40.54%, 37.04% and 30.43% in patients with trichomonas vaginitis(TV) , vulvovaginal candidiasis(VVC), intermediate bacterial vaginitis(IBV)and bacterial vaginitis(BV),respectively. Only26.17% women were infected with HPV in patients with normal vaginal microbiota. Significant differences were found in HPV infection rate between normal vaginal microbiota group and different vaginitis ( $P<0.05$ ). There was a correlation between HPV infection and vaginitis( $\chi^2=31.966,P<0.05$  $r=0.114, P<0.05$ ).Furthermore, patients with BV showed a higher correlation with HPV infection when compared with other groups ( $r=0.537, P<0.05$ ).

**Conclusions:** This retrospective study demonstrated that HPV58, 52, and 16 are the most common infection subtypes. Our data provide valuable information for HPV-based screening and prevention strategies for women in vaginitis. The single HPV infection was still very common. There was a correlation between HPV infection and vaginitis and patients with BV showed a higher correlation with HPV infection.

## Background

Human papillomavirus (HPV) is a non-enveloped virus with double-stranded circular DNA that belongs to the papillomavirus family [1]. It is transmitted by skin or mucosal contact and the risk of being infected at least once in both men and women is 50 % [2, 3]. There are over 200 different HPV genotypes that mainly cause benign lesions and common anal or genital warts, as well as other non-skin diseases [4]. The role of HPV in cancer has been extensively studied, mainly in cervical cancer, as well as in other types of cancer [5]. Persistent HPV infection, especially high-risk human papillomavirus (HR-HPV) infection, is a major cause of precancerous lesions and cervical cancer [6]. About 70% of cervical cancer cases worldwide is associated with HR-HPV types 16 and 18 [7] and there is also many other types of HR-HPV circulating around the world, which vary widely both within countries and within China. A full

understanding of the epidemiology of HPV infection will benefit vaccine development and cervical cancer prevention. Besides, there are many other factors affecting HPV infection, such as smoking, oral contraceptives, HIV infection and vaginal dysbiosis [8]. Normal vaginal microbiota are dominated by one or more lactobacillus species which break down glycogen to produce lactic acid so as to maintain a normal weak acid environment. However, in a state of vaginal dysbiosis, there is a reduction of lactobacillus, an increase of bacterial diversity and a high number of some anaerobic bacteria, which will lead to vaginitis in some patients. Bacterial vaginitis(BV), vulvovaginal candidiasis(VVC) and trichomonas vaginitis(TV) are the most three common causes of vaginitis, causing 40–50% of cases, 20–25% of cases and 15–20% of cases respectively [9]. The vaginal dysbiosis will be exacerbated when patients with vaginitis are infected with HPV. Therefore, it is very important to investigate the characteristics of HPV infection in patients with vaginitis and the association between HPV infection and vaginitis. Some studies have focused on the vaginal microbiome in women with HR-HPV infection and cervical cancer [10–12], however, few studies have concentrated on the characteristics of HPV infection in women with vaginitis and it is still not very clear that whether HPV infection is associated with vaginitis.

In this study, all participants underwent vaginal discharge test and HPV genotyping test at the same visit. We aim to determine the characteristics of HPV infection in women with vaginitis and investigate the correlation between HPV infection and vaginitis.

## Methods

### Patient enrollment and requirements

We retrospectively collected the data of 1330 women who underwent HPV DNA quantitative measurement and vaginal microecology examination at the same visit from May 2019 to April 2020 in our hospital. All participants met the following inclusion criteria and exclusion criteria. Inclusion criteria were patients with sexual life history, regular menstruation, no antimicrobial therapy within one month, no vaginal medication history within 48 h, no sexual intercourse within 24 h, no bath or vaginal flushing and without HPV vaccination history. Exclusion criteria were pregnancy and lactation, menstrual period or vaginal bleeding and with a history of surgery that month, such as abortion, hysteroscopy and laparoscopic surgery. The participants' identities were hidden to protect their confidentiality and we have applied for exemption of informed consent. And the study was approved by the Ethics Committee of the third Hospital of Xinxiang Medical University (K2021-019-01).

### The microecology tests of vaginal discharge

For sampling, after the insertion of a vaginal speculum, a standard sterile swab was placed onto the upper wall of the vagina, waited for a while and got as much secretion as possible to ensure sufficient sample. Vaginal microecology tests were performed by experienced inspection experts at the laboratory

of our hospital. Briefly, the vaginal discharge was spread evenly on a clean glass slide, dried naturally, fixed and Gram staining was performed. The remaining sample was diluted with 0.5ml normal saline and observed under a microscope immediately. BV and intermediate bacterial vaginitis (IBV) were diagnosed according to Nugent score [13]. VVC and TV could be diagnosed by visualization of yeast hyphae and motile, flagellated protozoa respectively in a woman with symptoms on saline microscopy [14].

## HPV genotyping testing

Sample collection for HPV typing test were as follows: the special sampling brush for HPV detection was inserted into the cervix. Rotated it clockwise 3 times, waited for a while, put the brush into the preservation liquid, labeled it, and sent it to the Laboratory immediately. HPV isolates were genotyped using the HPV Genotyping Real-time PCR kit (Yapei Biotech, China), which can identify 17 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and 6 LR-HPV types (6, 11, 42, 43, 44 and 81). Briefly, HPV DNA was extracted, amplified, and genotyped according to the manufacturers' protocol. The  $\beta$ -globin DNA was also detected to evaluate sample quality and PCR inhibitors. Furthermore, sterile water and specimens with known HPV genotypes were used as the negative and positive controls respectively.

## Statistical analysis

Statistical analysis was performed by using SPSS25.0 (IBM Corporation, NY, USA). Data were presented as mean  $\pm$  standard deviation or frequency and percentage for numerical or categorical variables, respectively. The  $\chi^2$  test and the Fisher's exact test were used for categorical variables. The correlation between HPV infection and vaginitis was analyzed. A p-value  $<0.05$  was considered statistically significant.

## Results

### The overall distribution of vaginal discharge infection

In our study, 1330 participants underwent vaginal microecology test. Among them, 856 individuals were thought to be with normal vaginal microecology (64.36%, 856/1330), 69 patients were diagnosed with BV (5.19%, 69/1330), 270 patients were diagnosed with IBV (20.30%, 270/1330), 111 patients were diagnosed with VVC (8.35%, 111/1330) and 24 patients were diagnosed with TV (1.80%, 24/1330). The overall vaginal discharge infection rate was 37.67% (Figure 1).

### The prevalence of HPV infection in women with vaginitis

The total HPV infection rate in 474 patients with vaginitis was 39.60% and HPV58, 52, and 16 had the higher prevalence among 23 HPV types, with infection rates of 10.34%, 6.32% and 6.11% respectively. HPV18 had a prevalence of 2.91% and was ranked eighth (Figure 2). In single infection, the three most prevalence types were also HPV58 (10.98%, 29/264), HPV 52 (5.68%), and HPV16 (4.92%). In multiple infections, the HPV 58 subtype was also the most common (13.99%, 20/143), followed by HPV16 (11.19%) and HPV52 (10.49%) (Figure3). Among HPV positive cases in women with vaginitis, 68% were infected with one HPV type, 19% were infected with two types, 9% were infected with three types, 3% were infected with four types and only 1% were infected with five types (Figure 4). In our data single-type HPV infection had a significantly higher prevalence than multiple-type infection in women with vaginitis.

## HPV infections of women with vaginitis in different age groups

The women were 17–65 years old (mean age  $36.1 \pm 9.5$  years) and were divided into six groups according to their ages. As shown in Figure 5, the total cases of vaginitis exhibited an ascending trend in the  $\leq 30$ -year-old age group and attained the peak of 186 cases in the 31- to 40-year-old age group, then dropped to the lowest point of 5 cases in the  $\geq 60$ -year-old age group. Similarly, the positive cases of HPV infection also showed the same age distribution, with a peak of 73 cases in the 31- to 40-year-old age group and the detection of the lowest point of 2 cases in the  $\leq 20$ -year-old age group and  $\geq 60$ -year-old age group.

## The correlation between HPV infection and vaginitis

As shown in table 1 and figure 6, the HPV infection rate was 26.17% in normal patients, 45.83% in patients with TV, 40.54% in patients with VVC, 37.04% in patients with IBV, and 30.43% in patients with BV. Statistical significant differences in HPV infection rate were found between normal and TV, normal and VVC, normal and IBV, normal and BV ( $P < 0.05$ ). The correlation between HPV infection and vaginitis was analyzed, exhibiting that there was a link between HPV infection and vaginitis ( $\chi^2 = 31.966, P < 0.05, r = 0.114, P < 0.05$ ). Furthermore, patients with BV showed a higher correlation with HPV infection when compared with other groups ( $r = 0.537, P < 0.05$ ). In other words, cases in BV group had a higher likelihood with HPV infection.

Table 1

HPV prevalence in different vaginal discharge

HPV types	vaginal discharge					subtotal
	normal	TV	VVC	IBV	BV	
subtotal	856	24	111	270	69	1330
HPV-	634	13	66	170	48	931
HPV+	224	11	45	100	21	399
$\chi^2$ value		7.088	16.125	12.365	113.065	
r value		0.096	0.140	0.106	0.537	
Note: Compared with the normal group, $P < 0.05$						

## Discussion

Vaginitis is the most common gynecologic diagnosis in medical center, which is characterized by the symptoms of abnormal vaginal discharge, odor, irritation, itching or burning [14]. Diagnosis with vaginitis is often made by using a combination of symptoms, physical examination findings and laboratory testing. And the Nugent score is one of the most common laboratory tests, which demands for sophisticated experience. In our study, all 1130 patients underwent vaginal discharge test from experienced technician and a 37.67% infection rate was observed, in which bacterial vaginitis was overwhelmingly dominant followed by VVC and TV groups. This distribution data is consistent with most previously published data, although prevalence rate varies in different geographic regions and ethnicities [15].

To further investigate the relationship between vaginitis and HPV infection, 447 patients with vaginitis were chosen for HPV genotyping results, showing that the total HPV infection rate was 39.60% in women with vaginitis. Previous studies have focused on the prevalence of HPV infection in different countries [16, 17] and within China, and the prevalence varies from 9.9 to 31.9% in different areas in China [18–20]. In our study, the infection rate was 39.60%, indicating a higher frequency in women with vaginitis. Considering this high prevalence, it is indispensable to know the HPV genotype distribution for vaccine development in women with vaginitis. Because vaccination remains an important means of preventing cervical cancer, though there appears low vaccine acceptability in some countries recently [21]. Among 23 HPV subtypes examined in our study, HPV58, 52, and 16 had the highest prevalence in overall, single-type and multiple-type infected participants, which is slightly different from some data for screening [17]. The study also showed that the most common infection was with HPV58, which was detected in 10.34% of the women with vaginitis. HPV52 was found in 6.32% of the cases and the frequency of HPV16 was 6.11%. These results are all higher than data published by Liang et al [22]. In our study, the single HPV

infection rate was also higher than multiple infection, which is consistent with data from the general population [23].

In different age groups, the peak cases of infection was noted in the 31–40 years group, with age  $\leq 20$  years and age  $\geq 60$  years having the lowest cases, which is significantly different from the bimodal phenomenon in different age groups of the HPV screening population[20]. HPV can be sexually transmitted and most HPV infections can be cleared or suppressed within 1 or 2 years in 70–91% of cases [24].

Therefore, the higher frequency in 31–40 years group may be associated with active sexual behavior and late marriage. Besides, women in this group also have a higher prevalence of vaginitis. A consistent trend can be seen between vaginitis and HPV infection in women with vaginitis. This phenomenon may indicate that there is a possible relationship between vaginitis and HPV infection.

On account of this phenomenon, we then explored the prevalence of HPV infection in women with different vaginitis and did compare with normal vaginal flora. In our study, the HPV infection rate in different vaginitis groups were all higher than the normal group, and the HPV prevalence in IBV group together with BV group was 67.47%, which is higher than the other vaginitis. The healthy vaginal flora are usually populated by *Lactobacillus spp* which can ensure a low pH, providing the first-line of defense against pathogenic agents [25]. Once this healthy state being disturbed, vaginal dysbiosis will come in and BV is one of the best studied vaginal dysbiosis. Furthermore, BV is associated with increased rate of sexual transmitted infections including HPV [26]. Some immune mechanisms also play an important role in BV regarding the development of HPV infection. Several BV and BV associated bacteria effect immune parameters within vagina have been found, such as cytokines/chemokines, antimicrobial proteins, proinflammatory responses and immune cell populations [27, 28]. Some clinical studies have also demonstrated immunosuppressive effects with lower levels of interferon gamma-induced protein 10 and soluble leukocyte protease inhibitor in women with BV[29, 30] These data can explain the high HPV prevalence in women with vaginitis especially with BV. As shown in our study there was a correlation between HPV infection and vaginitis and patients with BV showed a higher correlation with HPV infection, when compared with other groups. Therefore, timely HPV screening should be performed in women with BV, and early BV-related treatment should be taken to reduce HPV infection.

However, HPV infection has an impact on the host's immune defenses

and the mucosal metabolism with an adverse effect on the community structure of the vaginal microbiota[31], so it is difficult to define the cause-and-effect. Similarly, we only analyzed the relationship between HPV infection and vaginitis, the causality and detailed mechanisms still need to be studied between them. And our study was a single center, there need more samples to be detected.

## Conclusions

In summary, this retrospective study demonstrated that the three most prevalent HPVs were HPV58, 52, and 16. Our data provide valuable information for HPV-based screening and prevention strategies for women in vaginitis. The single HPV infection was still very common. There was a correlation between HPV infection and vaginitis and patients with BV showed a higher correlation with HPV infection. Therefore, timely HPV screening should be performed in women with BV, and early BV-related treatment should be taken to reduce HPV infection.

## **Declarations**

## **Ethics approval and consent to participate**

The study design was approved by the Ethics Committee of the third Hospital of Xinxiang Medical University (2021-019-01). And we have applied for the exemption of informed consent.

## **Consent for publication**

Not applicable.

## **Availability of data and material**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## **Competing interests**

The authors declare that they have no competing interests.

## **Funding**

None.

## **Authors' contributions**

FZ (Feng Zhang) and XZY (Xiuzhen Yang) conceived and designed the study, having full access to all of the data in the study and taking responsibility for the content of the manuscript. FZ (Feng Zhang) and HZ (Hui Zhu) analyzed the data, took responsibility for the accuracy of the data analysis and wrote the first draft of the manuscript. YXZ (Yongxin Zhao) and Tiehan Zhang (THZ) contributed to the collection and inputs of the data for microecology tests of vaginal discharge. Yanyan Wang (YYW) and Wenjie Pan (WJP) are responsible for the collection and inputs of the data for HPV genotyping. All authors were

involved in the revision of the manuscript for important intellectual content and approved the final version to be published.

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Not applicable.

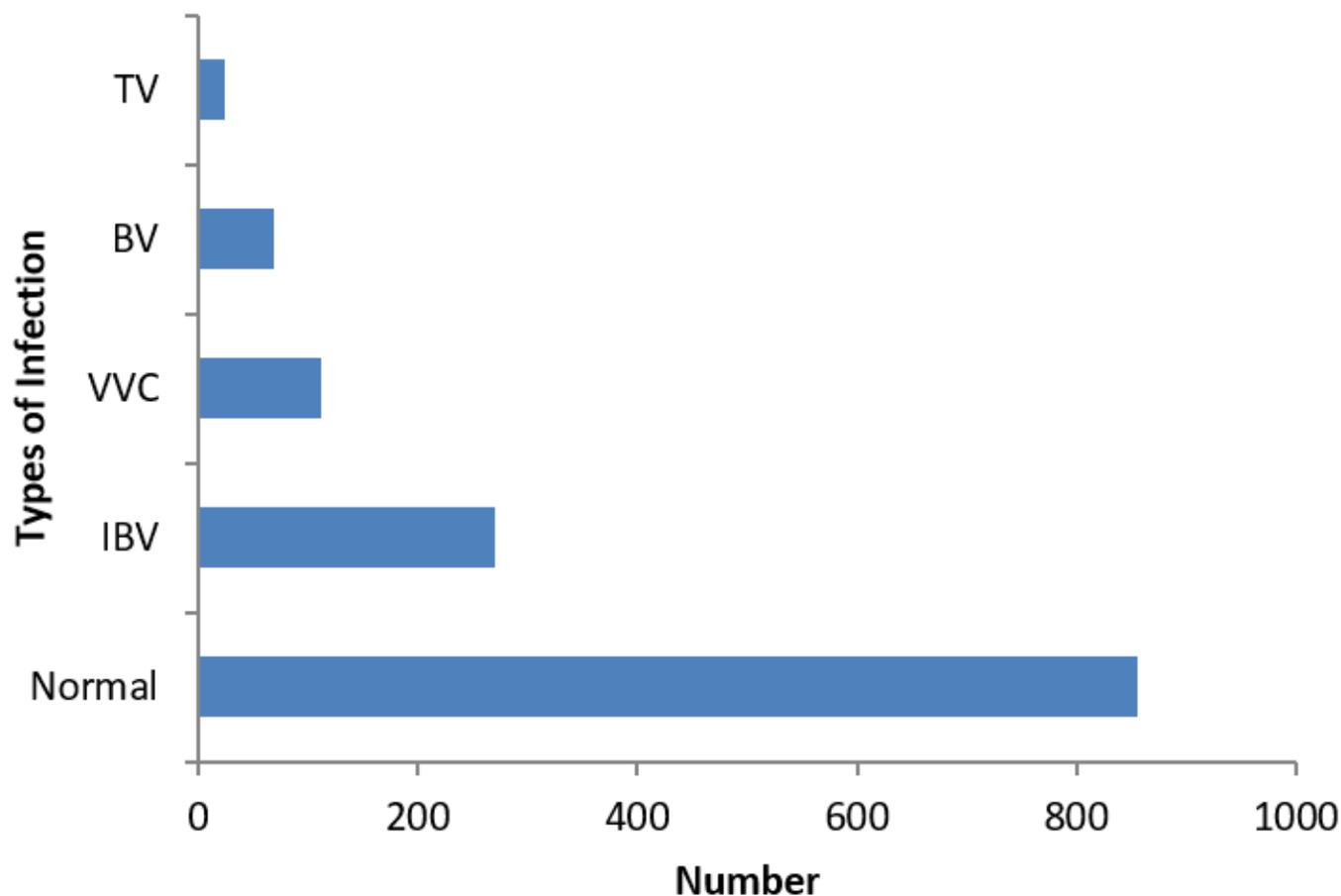
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## Figures



**Figure 1**

The overall distribution of vaginal discharge infection

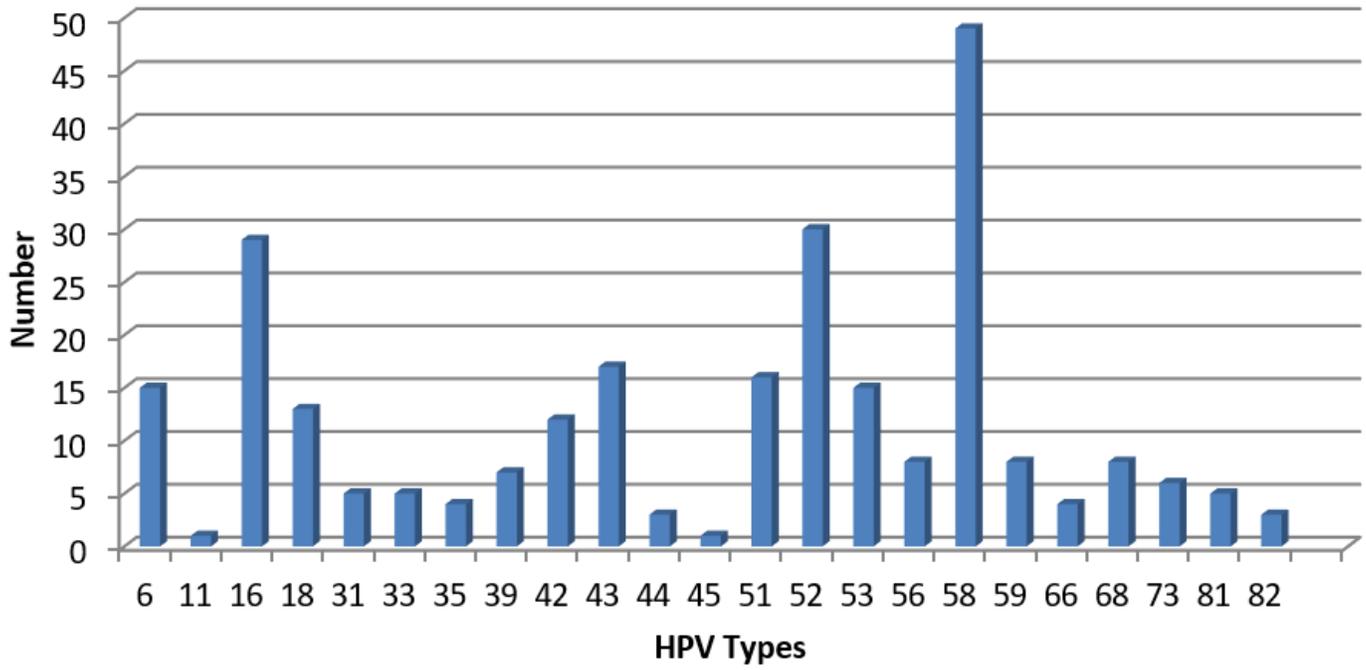


Figure 2

The overall infection number of different HPV types

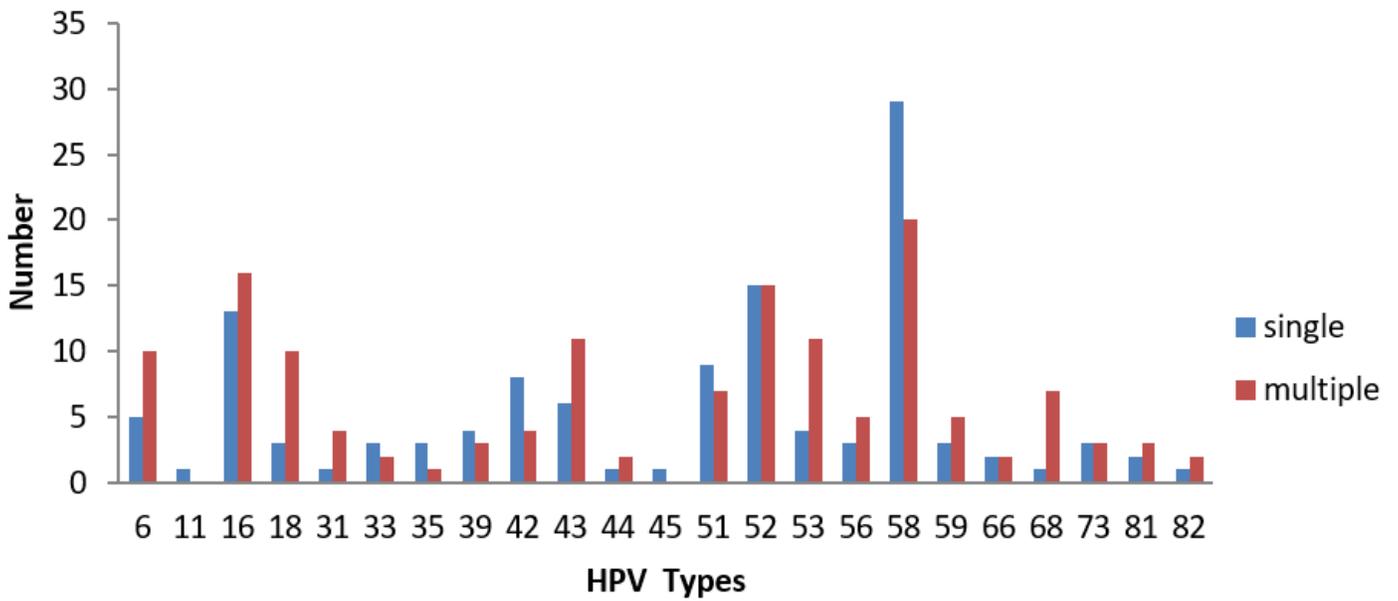


Figure 3

Single and multiple type infection number of different HPV types

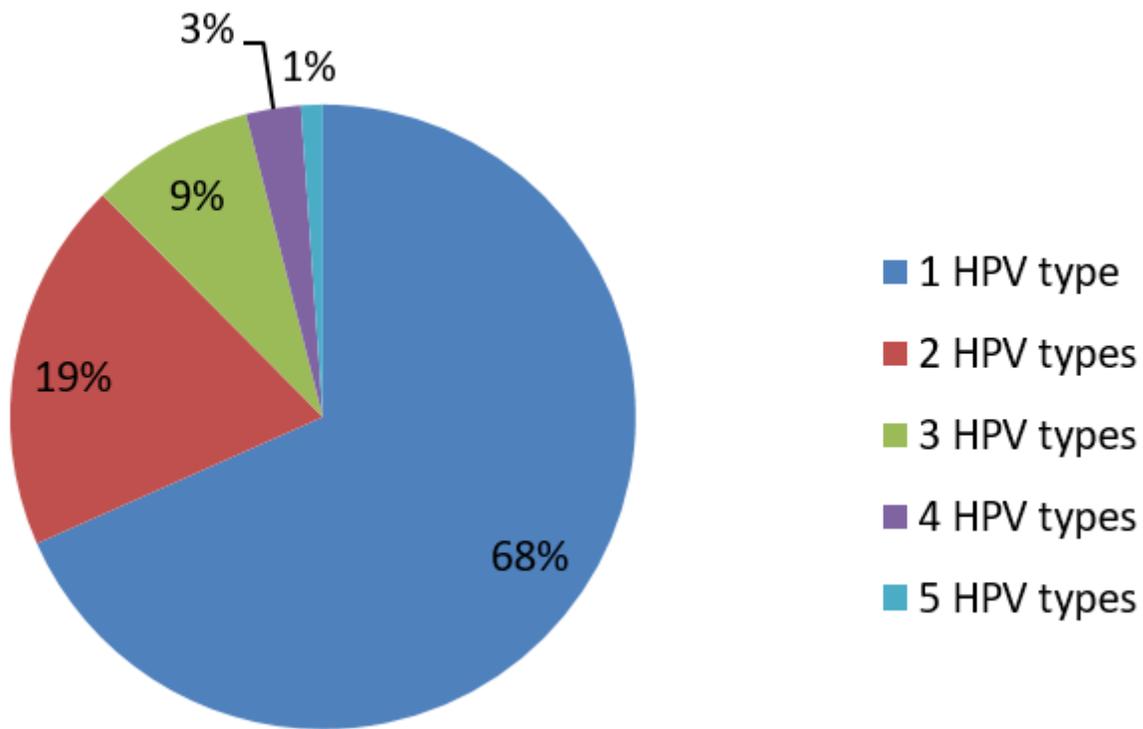


Figure 4

Single type and Multiple type HPV infection rate

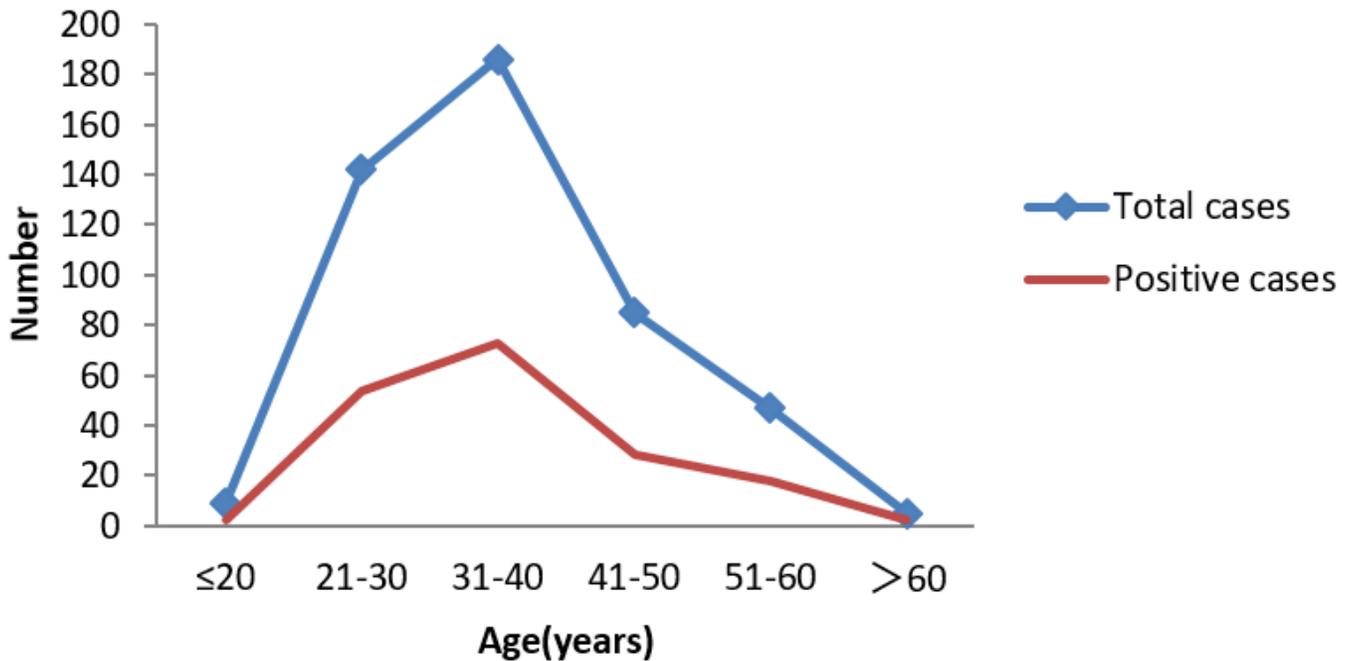
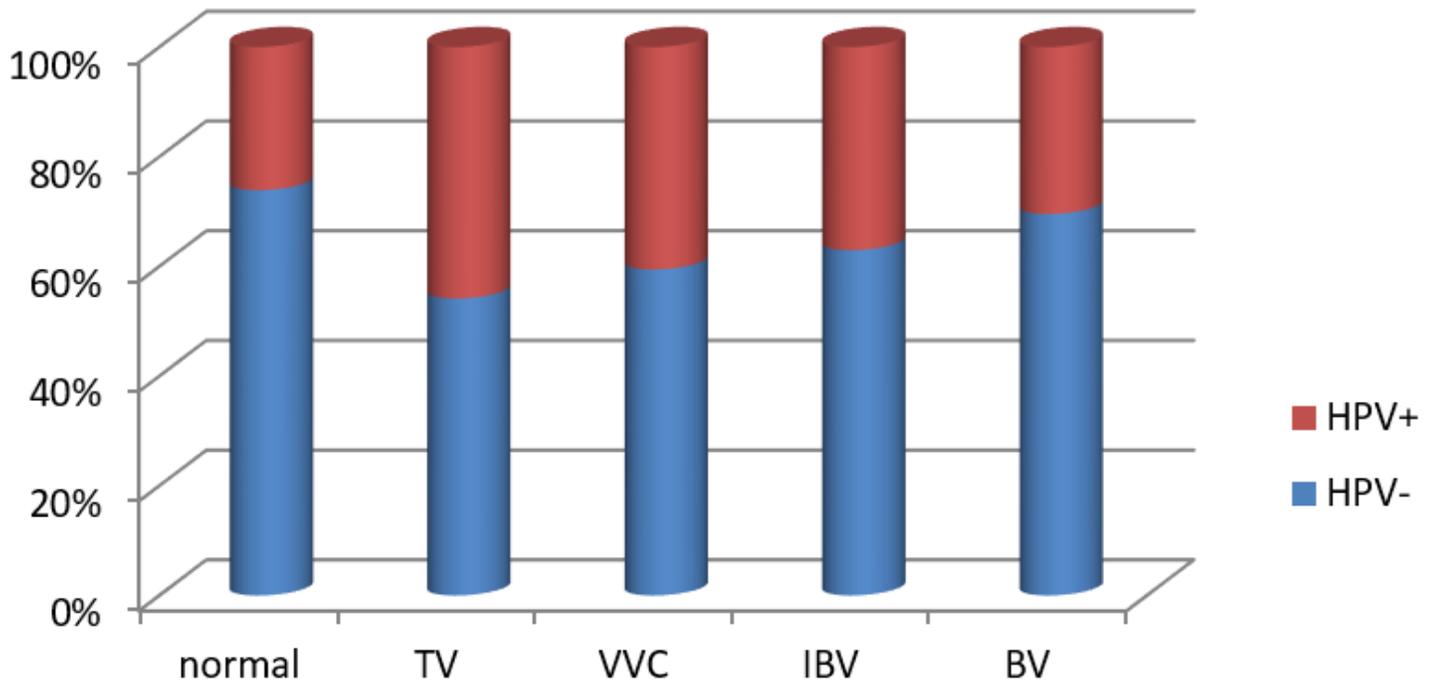


Figure 5

HPV infection in different age groups



**Figure 6**

HPV prevalence in different vaginal discharge