Prevalence, genotype distribution and temporal dynamics of human papillomavirus infection in a population in southern Italy

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SUMMARY

Human papillomavirus (HPV) is considered the most important risk factor for the development of ano-genital region cancer in both women and men. Whereas low-risk genotypes are responsible for cutaneous and genital lesions, high-risk genotypes are associated with ano-genital cancer. The aim of this study was to retrospectively analyse the prevalence, genotype distribution and temporal dynamics of HPV infection in 2312 specimens from 2312 subjects (2149 women and 163 men) who attended the laboratory of molecular biology, UOC Microbiology and Virology, Azienda Ospedaliera-Universitaria Policlinico of Bari, Italy. HPV DNA detection and genotyping was performed using a multiplex real-time PCR assay. In all, 1123/2312 subjects (48.57%) resulted positive for HPV DNA. In particular, HPV DNA was detected in (1056) 49.14% of females and (67) 41.10% of males. HPV co-infections were detected in 565 (24.44%) patients. High-risk and low-risk HPV genotypes were detected in 887 (38.37%) and 600 (25.95%) patients, respectively. The most prevalent HPV genotypes were HPV-42 (10.29%), HPV-16 (8.56%), HPV-31 (7.40%) and HPV-53 (7.14%). Statistically significant differences between female and male patients were not detected. Moreover, HPV prevalence remained constant in time while HPV-16, but not HPV-6, 11 and 18, showed a decreasing trend from 2013 (11.24%) to 2016 (6.67%). Other HPV genotypes showed some complex and different patterns. Our data showed an unusually high frequency of HPV-42 and a high prevalence of HPV infection in the patients analysed. Although evidence of a decreasing trend of HPV-16 could be a consequence of anti-HPV vaccination, corroboration from further studies will be needed. Moreover, the small number of studied males and the similarity to females in terms of HPV prevalence suggest that more active HPV screening and anti-HPV vaccination in the male population should be considered important tools to eliminate HPV sexual transmission.

Keywords: epidemiology, human papillomavirus, multiplex real-time polymerase chain reaction, HPV genotyping, temporal trend.

INTRODUCTION

Human papillomavirus (HPV) is one of the most frequent sexually transmitted agents [1]. Up to 80% of sexually active women are infected at some point in their lives [2]. The majority of these infections are transient and spontaneously resolve in less than one year. In some cases, HPV persists playing a role in the development of cervical cancer (CC) [3-6]. According to WHO, CC was the fourth most common cancer affecting women and it was worldwide responsible for approximately 266,000 deaths in 2012 with an incidence peak among women aged 55-59 [7]. Reported risk factors for cervical cancer development in-
clude smoking, sexually transmitted pathogens, oral contraceptives, and socioeconomic status [8]. Moreover, some studies have suggested a possible role of altered vaginal microbiota in the development of CIN and cervical cancer. In particular, an association between bacterial vaginosis and CIN has been reported by Gillet et al. [9]. Several biologically and behaviourally based risk factors have been related to both HPV acquisition and cervical cancer development [10]. However, also inflammatory status may favour the entry of other microorganisms, which can act as cofactors in the pathogenesis of cervical disease. In fact, several sexually transmitted pathogens have been correlated to HPV infection [11]. In general, there is an association between chronic inflammation, persistent infection and cancer. In fact, oncogenic process is mediated by autocrine and paracrine signals, causing changes in somatic cells under the influence of the microbial genome or of epi-
genetic factors [11, 12].

HPV genotypes are classified into high-risk (HR) and low risk (LR) types [13, 14]. Globally, the most common HR-HPV genotypes are 16 and 18 that account for 70% of CC, whereas 31, 33, 35, 52, 58 and other HR-HPV genotypes recur with a lower frequency, accounting for 20% [15-18].

Implementation of cervical cytology based on cervical screening programmes has successfully reduced the incidence and mortality in developed countries [17]. However, cervical cytology is affected by either a low sensitivity or reproducibility in detecting cervical intraepithelial neoplasia [19]. For this reason, HPV testing has been proposed as a useful tool for primary cervical screening [20, 21]. Currently, molecular methods for the detection of HPV DNA have been introduced into clinical practice with the aim of identifying women who are infected by HR-HPV, being at higher risk of developing precancerous and cancerous lesions [22, 23]. The purpose of this study was retrospectively to evaluate the prevalence, genotypes and trend of HPV infection in clinical samples collected from hospitalized and non-hospitalized patients from southern Italy.

PATIENTS AND METHODS

From January 2013 to November 2016, 2312 consecutive samples including 143 cervical swabs, 206 vaginal swabs from 2149 females and 163 urethral swabs from 163 males (female to male ratio=13.18), were collected. Median age of female and male patients was 36.30 years (Interquantile range [IQR]: 28.98-44.68) and 33.49 years (27.57-41.57), respectively. Specimens were transferred to the laboratory of Molecular Biology, U.O.C. Microbiology and Virology, Azienda Universitaria-Ospedaliera, Policlinico of Bari, where they were analyzed.

All procedures performed in studies involving human participants were in accordance with the 305 ethical standards of the institutional and / or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Sample information (date of sampling, ward, type of specimen, testing results) together with the data of patients for whom molecular testing was performed (i.e., age and sex) were recorded in an anonymous database by changing sensitive data into alphanumeric codes. No clinical data associated with these specimens were available. As a retrospective study, formal consent is not required.

Treatment of specimens

2 mL of phosphate-buffered saline (pH 7.4) (Sigma-Aldrich, Milano, Italy) were added to vaginal and cervical swabs, collected by a rigid cotton-tipped swab applicator (Nuova Aptaca, Cannelli, Italy), and vortexed. 1 mL of phosphate buffered saline (Sigma) was added to urethral swabs and vortexed. Finally, all samples were transferred to microcentrifuge tubes and they were stored at -20°C until processing.

DNA isolation and multiplex real-time PCR

To extract viral nucleic acids, microcentrifuge tubes were centrifuged at rcf=15,700 g for 15 minutes at 7°C. The majority of supernatant was discarded but 200 µl of supernatant was retained to resuspend the pellet. Viral nucleic acids were extracted from the resuspended pellet using the automated MagNa Pure 96 system (Roche Diagnostics GmbH, Mennheim Germany) according to the manufacturer’s instructions. DNA extraction was carried out starting from 200 µl of sample. DNA was eluted in a final volume of 100 µl. Extracted DNA samples were subject to multiplex real-time PCR (mRT-PCR) by Anyplex™ II HPV 28 Detection System
(Seegene, Seoul, Korea) performed on CFX96 Real-Time PCR (Bio-Rad, Hercules, CA, USA).

**Statistical analysis**

Independence of categorical and continuous variables was assessed by Fisher’s exact test, Chi-squared test and Mann-Whitney test, respectively, as appropriate. Strength of associations was assessed by odds ratio and 95% Confidence Interval.

Median, interquartile range, differences of age and crude-age specific HPV prevalence were evaluated on 1914 female and 144 male patients, respectively, because of the unavailability of some birth dates. Effect size of difference of ages and difference of number of HPV genotypes in multiple infections were evaluated by Cohen’s d.

To evaluate differences in median age between females and males, random replicates of 144 samples selected from the 1914 female patients were extracted without replacement. For each replicate, the Mann-Whitney test was evaluated. After 100,000 replicates, p value was calculated as 1-[(p values<0.05)/(number of replicates+1)].

To evaluate differences in HPV prevalence between females and males, random replicates of 163 samples selected from the 2149 female patients were extracted without replacement. For each replicate, a Fisher’s exact test was evaluated. After 100,000 replicates, p value was calculated as 1-[(p values<0.05)/(number of replicates+1)].

Differences in prevalence of each HPV genotype, HR and LR HPV genotypes were again evaluated by 100,000 random replicates. For each replicate, statistical differences in prevalence for each HPV genotype and HR and LR HPV genotypes were evaluated by Fisher’s exact test and Benjamini and Hochberg’s (BH) procedure with False Discovery Rate (FDR)<5% [24]. P values were calculated as 1-[(BH adjusted p values<FDR)/(number of replicates+1)].

Differences in HPV prevalence between females and males in the same age group were evaluated by Fisher’s exact test. Differences in prevalence in females and males patients ≤34 and >34, respectively, and differences in prevalence of HPV-16, HPV-18, HPV-6 and HPV-11 between both females and males ≤20 and >20 years old were evaluated by Fisher’s exact test. P values were then corrected by BH’s procedure with FDR<5%.

Exploratory analysis of time trends of HPV genotypes by Lowess smoothing (Locally weighted scatterplot smoothing) was performed on 6-month periods prevalences. Smoother span value was 1.

Annual trends of overall HPV, HR, LR, HR except HPV-18, HPV-16, HPV-6 and HPV-11 were evaluated by Chi-squared test for trend and BH’s procedure with FDR<5%.

Calculations were performed by open source statistical environment R (version 3.2.4) [25]. A p value <0.05 was considered statistically significant.

**RESULTS**

Globally, 1123/2312 (48.57%) samples were positive for HPV DNA. Overall the prevalence of HPV infection in females was 49.14% (1056) while in males it was 41.10% (67). Among women, the prevalence of HPV from vaginal swabs (48.9%, 979) and from cervical swabs (53.8%, 77) was not statistically different (Chi-squared test p value=0.280). The difference in prevalence between females and males was not statistically significant (Fisher’s exact test p value=0.0511, P100,000=0.809, Odds ratio [OR]=0.722, 95% CI: 0.514-1.001). The age difference between female and male patients was not statistically significant (p value=0.0214, P100,000=0.682, Cohen’s d=0.206).

In 565 samples (24.44%) a HPV co-infection was detected. The prevalence of co-infections was 25.36% (545) and 12.27% (20) in females and males, respectively (p value<0.001, P100,000=0.809, Odds ratio [OR]=0.722, 95% CI: 0.514-1.001). The median number of co-infection was 2 (2-3). The median number of HPV co-infections was 2 (2-3) and 2.5 (2-3) in females and males, respectively. The difference in the median number of HPV co-infections between females and males was not statistically significant (p value=0.949, P100,000=0.998, Cohen’s d=0.093).

The prevalence of HR and LR genotypes was 38.37% (887) and 25.95% (600), respectively. HR and LR genotypes were not significantly different in prevalence between females and males (39.18% Vs 27.61%, p value=0.0034, P100,000=0.938, OR=1.689, 95% CI: 1.174-2.464 and 26.29% Vs 21.47%, p value=0.195, P100,000=1, OR=1.304, 95% CI: 0.879-1.978, respectively).

The most prevalent HPV genotypes were HPV-42 (10.29%, 238), HPV-16 (8.56%, 198), HPV-31
(7.40%, 171), HPV-53 (7.14%, 165), HPV-54 (6.10%, 141) and HPV-6 (5.84%, 135). Among females, HPV-42 (10.70%, 230), HPV-16 (8.89%, 191), HPV-31 (7.63%, 164) and HPV-53 (7.35%, 158) were the most prevalent viral genotypes. On the other hand, the most prevalent genotypes in males were HPV-6 (7.98%, 13), HPV-42 (4.91%, 8), HPV-16 (4.29%, 7) and HPV-31 (4.29%, 7). Differences in prevalence between females and males for each viral genotype were not statistically significant (Table 1).

In females, the prevalence rates among different group ages ranged from 56.15% to 40.31%. Moreover, overall HPV prevalence in females ≤34 and >34 was statistically different (57.75% Vs 42.10%) (Table 2).

Table 1 - Overall prevalence of HPV genotypes detected and their prevalence in female and male patients.

<table>
<thead>
<tr>
<th>HPV Genotypes</th>
<th>HPV total prevalence (95%CI)</th>
<th>HPV prevalence in females (95%CI)</th>
<th>HPV prevalence in males (95%CI)</th>
<th>P value</th>
<th>100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 42</td>
<td>10.29 (9.10-11.62)</td>
<td>10.70 (9.44-12.11)</td>
<td>4.91 (2.30-9.77)</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>HPV 16</td>
<td>8.56 (7.47-9.80)</td>
<td>8.89 (7.74-10.19)</td>
<td>4.29 (1.89-8.99)</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>HPV 31</td>
<td>7.40 (6.38-8.56)</td>
<td>7.63 (6.56-8.86)</td>
<td>4.29 (1.89-8.99)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HPV 53</td>
<td>7.14 (6.14-8.28)</td>
<td>7.35 (6.30-8.56)</td>
<td>4.29 (1.89-8.99)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HPV 54</td>
<td>6.10 (5.17-7.17)</td>
<td>6.28 (5.31-7.41)</td>
<td>3.68 (1.51-8.20)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HPV 6</td>
<td>5.84 (4.93-6.89)</td>
<td>5.68 (4.75-6.76)</td>
<td>7.98 (4.49-13.53)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 66</td>
<td>4.63 (3.82-5.59)</td>
<td>4.84 (3.99-5.86)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 51</td>
<td>3.94 (3.20-4.83)</td>
<td>4.09 (3.31-5.04)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 58</td>
<td>3.72 (3.00-4.59)</td>
<td>3.77 (3.02-4.69)</td>
<td>3.07 (1.13-7.39)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 73</td>
<td>3.46 (2.77-4.31)</td>
<td>3.49 (2.77-4.38)</td>
<td>3.07 (1.13-7.39)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 56</td>
<td>3.37 (2.69-4.21)</td>
<td>3.44 (2.73-4.33)</td>
<td>2.45 (0.79-6.56)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 61</td>
<td>3.29 (2.62-4.12)</td>
<td>3.44 (2.73-4.33)</td>
<td>1.23 (0.21-4.82)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 68</td>
<td>2.94 (2.31-3.74)</td>
<td>3.12 (2.44-3.97)</td>
<td>0.61 (0.03-3.89)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 18</td>
<td>2.85 (2.23-3.64)</td>
<td>2.93 (2.28-3.76)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 52</td>
<td>2.85 (2.23-3.64)</td>
<td>3.07 (2.40-3.91)</td>
<td>0.00 (0.00-2.87)</td>
<td>0.98</td>
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</tr>
<tr>
<td>HPV 44</td>
<td>2.60 (2.00-3.35)</td>
<td>2.61 (1.99-3.39)</td>
<td>2.45 (0.79-6.56)</td>
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<td></td>
</tr>
<tr>
<td>HPV 59</td>
<td>2.55 (1.96-3.30)</td>
<td>2.65 (2.03-3.45)</td>
<td>1.23 (0.21-4.82)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 39</td>
<td>2.38 (1.81-3.11)</td>
<td>2.51 (1.91-3.29)</td>
<td>0.61 (0.03-3.89)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 33</td>
<td>1.82 (1.33-2.47)</td>
<td>1.86 (1.35-2.55)</td>
<td>1.23 (0.21-4.82)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 40</td>
<td>1.77 (1.29-2.42)</td>
<td>1.77 (1.27-2.44)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 70</td>
<td>1.77 (1.29-2.42)</td>
<td>1.77 (1.27-2.44)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 43</td>
<td>1.56 (1.11-2.17)</td>
<td>1.54 (1.08-2.18)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 35</td>
<td>1.30 (0.89-1.87)</td>
<td>1.40 (0.96-2.01)</td>
<td>0.00 (0.00-2.87)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 45</td>
<td>1.25 (0.86-1.82)</td>
<td>1.21 (0.81-1.79)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 11</td>
<td>0.95 (0.61-1.46)</td>
<td>0.74 (0.44-1.23)</td>
<td>3.68 (1.51-8.20)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 82</td>
<td>0.87 (0.54-1.36)</td>
<td>0.79 (0.48-1.29)</td>
<td>1.84 (0.48-5.71)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 26</td>
<td>0.13 (0.03-0.41)</td>
<td>0.14 (0.04-0.44)</td>
<td>0.00 (0.00-2.87)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HPV 69</td>
<td>0.04 (0.00-0.28)</td>
<td>0.05 (0.00-0.30)</td>
<td>0.00 (0.00-2.87)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>High risk HPV</td>
<td>38.37 (36.38-40.39)</td>
<td>39.18 (37.12-41.29)</td>
<td>27.61 (21.04-35.25)</td>
<td>0.938</td>
<td></td>
</tr>
</tbody>
</table>

HPV genotypes are ordered by their overall prevalence. Differences in prevalence of HPV genotypes between male and female patients are not statistically significant.
Prevalence values among different group ages ranged from 44.26% to 26.09% in males. No significant differences in HPV prevalence in males ≤34 were detected. Moreover, statistically significant differences in HPV-6, HPV-11, HPV-16 and HPV-18 prevalence in male patients ≤20 and >20, respectively, were not detected (Table 3).

Exploratory analysis of HPV type specific infection rates showed different and complex patterns (Figure 1). In particular, HPV-16 decreased over time until reaching a slight stabilization towards the end of the study period. Among HR genotypes, HPV-52 and HPV-66 showed a slight increase with time. Among LR genotypes HPV-6 and HPV-44 revealed an increase while HPV-11 and HPV-61 decreased with time. In particular, HPV-42 showed an increase until halfway through 2014 and then it stabilized.

The evaluation of annual prevalence rates is reported in Figure 2. In particular, HPV-16 showed a decreasing trend from 11.24% in 2013 to 6.67% in 2016 (Chi-squared for trend p value=0.001, significant after BH’s correction). On the other hand, HPV-18 peaked in 2015 (4.00%) from the minimum value in 2013 (2.09%) and then decreasing in 2016 (2.39%) (p value=0.568). HPV-6 showed an increasing but non-significant pattern from 4.67% in 2013 to 6.67% in 2016 (p value=0.101). HPV-11 peaked in 2014 (0.80%) and then decrease (p value=0.256). On the other hand, HPV prevalence remained nearly constant from 50.72% in 2013 to 43.93% in 2016 (p value=0.029, non-significant after BH’s correction), as did low risk HPV (p value=0.594) and high risk HPV except HPV-16 (p value=0.105). On the contrary, high risk HPV prevalence decreased from 40.58% in 2013 to 33.16% in 2016 (p value=0.011, significant after BH’s correction).

**DISCUSSION**

Our study shows a high prevalence of HPV infection in women and men living in Apulia. Moreover,
the prevalence of 49.14% in females is higher than in other Italian studies [11,26,27]. The prevalence of HPV infection in males was quite high (41.10%). Such a result is in agreement with other studies in which a prevalence of about 50% was observed [28-30]. Moreover, the prevalence of HPV infection was not statistically different between younger and older men as reported elsewhere [31]. Such data confirm the need of a strict screening of the male population as a viral reservoir and because of the risk of virus-related pathologies such as genital warts, penile intraepithelial neoplasia and penile carcinomas [32]. In fact, HPV in males is considered highly frequent and one of the most common sexually transmitted infections. Moreover, a high prevalence of HPV infection in male partners of HPV-infected women has been reported [30]. Interestingly, in this population sub-clinic lesions have been detected in 18.75% of patients at peniscopy while in 20% of patients HPV was detected without associated lesions [30].

A high prevalence of HR-HPV infections (38.37%) was detected. In line with other epidemiological studies, HR-HPV 16/31/53 group types had the highest frequency among the population analyzed [26,33-39]. Moreover, HPV-6 was confirmed as the most prevalent genotype in men [40-42]. HPV-42 was observed both in female and male subjects (10.70% and 4.91%, respectively). In particular, HPV-42 was the most prevalent genotype in females while in males it was the second most prevalent. Such data were not confirmed in other Italian studies except in the studies of Ammatuna et al. and Ronco et al. [38, 43-48]. Despite the differences in age of the screened population, some studies performing HPV diagnosis by Anyplex-TM II HPV 28 did not report HPV-42 as the most prevalent genotype [11, 49, 50]. On the contrary, Estrade et al. reported a high prevalence of HPV-42 [51]. In particular, a significant difference in the detection of HPV-42 between Anyplex™ II HPV 28 and PGMY-CHUV PCR assay was reported. This was explained by with the low sensitivity of PGMY primers in the presence of low viral loads in multiple infections, as confirmed by the presence of a significant decreasing trend for discor-

**Figure 1** - Evaluation of temporal patterns from 2013 to 2016 of type-specific HPV infections by Lowess smoothing with smoothing span value 1. HPV prevalences by HPV type are calculated as the number of positive infections per total number of people analyzed within a 6-month period.
dance in the presence of increasing viral loads (p value = 0.0010) [51].
HPV co-infections have been detected in 24.44% patients studied. In particular, they accounted for 25.36% of females and 12.27% of males, respectively. Other Italian studies reported prevalence rates of HPV co-infections ranging from 30.9% to 49.7% in female population [36, 38, 52, 53]. Lorenzon et al. reported HPV co-infections in 18.2% of males in stable relationship [54]. In particular, the genital HPV co-infections prevalence in human papillomavirus infection in men (HIM) study was 25.7% [31]. Nyitray et al. reported HPV co-infections in 23.8% of men having sex with men, 37.3% of men having sex with women and men and 29.3% of men having sex with women [55].
Rousseau et al. reported HPV co-infections were not independent to each other and HPV combinations 6/11-18, 16-52 and 16-68 occurred with a frequency less than expected [56]. Competition between HPV-16 and HPV-52 was also confirmed by Nie et al. [57]. Moreover, Dickson et al. reported that 57 combinations of different HPV genotypes occurred more frequently than expected while 8 combinations occurred less frequently than expected [58]. However, the impact of HPV co-infections is not clear. Some studies reported an increased risk of development or progression of CC [59-62]. Other studies failed to detect an increased risk of precancerous lesions or invasive cancer [64, 65].
Primary prevention of HPV infections and HPV-related cancers has become more appealing because of the introduction of specific vaccines. To date, two types of prophylactic anti-HPV vaccines have been routinely used in clinical settings in Italy: a bivalent against HPV-16 and HPV-18 and a quadrivalent against HPV-16, HPV-18, HPV-6 and HPV-11 [66, 67]. In Apulia, anti-HPV vaccination was approved in 2007 for the 1997 birth cohort and it has been subsequently extended to males [67]. Complete vaccinal coverage reached 83.31% of the 1997 birth cohort [68]. Moreover, total CC screening in 2012-2015 only reached 70.5% of women aged 25-64 while national Italian coverage was 79.2% [69]. Martinelli et al. and Chironna
et al. detected HPV infection in 33% and 30.4% of female patients in Apulia, respectively [36,70]. In our study the overall HPV prevalence was 48.57% and in female patients it was 49.14%. This difference may be due to the different sensitivity of HPV diagnostic tests, the composition of analyzed populations, or an increased prevalence of HPV in Apulia over time.

The introduction of the HPV vaccination has modified the HPV prevalence. In a study performed after a vaccination campaign in a Sweden, several changes were observed [71]. HPV-6, HPV-11, HPV-16 and HPV-18 prevalence decreased. Moreover, the prevalence of HPV-6, HPV-16 and HPV-18 mostly decreased among women younger than 23 years [71]. Also Fischer et al. reported a decrease of HPV-16, HPV-18 and HPV-31 in female patients aged < 23 years [72]. After the introduction of four genotypes anti-HPV vaccination, HPV-6, HPV-11, HPV-16 and HPV-18 decreased in young females in their teens and 20s [73]. Such a trend was not observed in our study. In fact, a decrease in HPV-6 or HPV-11 or HPV-16 or HPV-18 prevalences has not been observed in female and male patients ≤ 20 years old. Despite the absence of the vaccinal status of patients and with the exception of the decreasing trend of HPV-16, our data failed to detect clues related to a positive effect of the anti-HPV vaccinal campaign. Therefore, other wider studies will be needed to better clarify the situation.

There are several limitations of this study. First, the reported data are not representative of the overall Apulia population and are not generalizable. Secondly, data regarding vaccination and sexual history are lacking. The vaccinal status would have been useful to better infer regarding the vaccine effect. In fact, the temporal change in prevalence does not always imply a casual relationship. Among HPV genotypes covered by vaccine, only HPV-16 showed a decreasing prevalence but it seemed to stabilize during 2016. Moreover, the prevalence of the four HPV genotypes in age groups with the highest likely uptake of anti-HPV vaccination was not different from other age groups. For this reason, it is possible that this phenomenon is not related to the vaccination campaign. Furthermore, clinical data were not available to assess risk factors connected with the presence of HPV. In particular, cytological data on the enrolled individuals were absent. For this reason we did not have the data of cervical lesions and it was not possible to correlate the presence of HPV with the cytological and/or histological findings. Moreover, the lack of behavioral information on the enrolled individuals did not permit to better characterize the analyzed population for the roles of such exposure variables in the acquisition of HPV and therefore the association between these factors and HPV prevalence could not be analyzed. The absence of individual-level data on background factors which may have changed over time did not permit to correlate changes in screening and in risk factors to HPV and to different HPV genotypes related trends. Moreover, the absence of some data regarding the age of patients has reduced the statistical power regarding the analysis of HPV distribution in different age groups.

In conclusion, our study shows either an unusually high presence of HPV-42 or a high prevalence of HPV infections in the analyzed patients. The higher presence of HR-HPV genotypes compared to low-risk genotypes underlines the need for genotyping to allow for the correct clinical management of HPV infections. Moreover, the involvement of the male population and the continuous spread of the anti-HPV vaccine campaign should be considered as important tools to eliminate HPV sexual transmission among people.

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**Ethical approval**
The study followed the local ethical guidelines of the Azienda Ospedaliero Universitaria Policlinico of Bari.

**Informed consent**
Not necessary

**Conflict of interest**
The authors declare that they have no conflict of interest to disclose.

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