HPV at the time of vaccine: has screening reached its goal?

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Summary

Introduction: The human papillomavirus (HPV) prevalence recognized a geographic distribution of genotypes but, in the last years, the change of sexual behaviours, the increase number of sex partners, and the reduction of geographic distances have changed its prevalence and distribution. *Objective:* To determine the prevalence of HPV types among females in the Molise region and its evolution in 24 months. *Materials and Methods:* The authors, from February to August 2008, used a representative sample of a female population (n = 299) aged 17 to 64 years who were interviewed and submitted cervico-vaginal swab specimens. Swabs were analyzed for cytologic screening and HPV detection and typing. The patients with a positive cytology were submitted to colposcopy and eventually biopsy. Cytological and colposcopic follow up was performed in 24 months. *Results:* The overall HPV prevalence was 30.1% and the prevalence of high- and low-risk HPV types was 22.41% and 18.06%, respectively. The prevalence of HPV vaccine types was relatively low for HPV-6-11-18. Only HPV-16 is well-represented in Molise, but recognizes a strictly geographic distribution. *Conclusion:* This study is one of the largest assessments of HPV genotypes to date in Italy. It is clear that several HPV-types are involved in cervical lesions, therefore the vaccine is profitable but limited by great number of types implicated in the pathogenesis of cancer and by their dishomogeneous distribution. Currently, a good campaign of screening is still necessary. In the future, second generation polyvalent HPV vaccines my be proposed for a wider and complete vaccine coverage.

Key words: HPV Prevalence; Risk factors; Vaccine; Screening.

Introduction

Human papilloma virus (HPV) is included in the family of Papillomaviridae, which now contains 29 genera formed by 189 papillomavirus types isolated from humans (120 types), in non-human mammals (64 types), in birds (3 types), and reptiles (2 types) [1]. HPV is actually estimated to be the most common sexually-transmitted infection in the world and its prevalence has been found to be the highest among young women within the first few years from sexual debut [2]. Genital HPV types are categorized according to their epidemiological association with cervical cancer in low-risk (LR-HPV) and high-risk (HR-HPV) HPV types. HR-HPV are detected in 99% of cervical cancers and approximately 70% of cervical cancers worldwide are due to HPV-16 (50%) and HPV-18 (20%) [2]. Although HPV infection is very common and has a high infective rate (66% of sexual partners), several studies suggest that approximately 90% of infections clear within two years [3, 4]. HPV prevalence recognized a geographic distribution of genotypes [5], but in the last years, changing of sexual behaviours, the increased number of sex partners, and the reduction of geographic distances have changed the prevalence and distribution of this virus [6]. Actually, there are two types of vaccines available to prevent HPV-infection and related diseases: both types contain hollow immunological virus-like particles (VLPs) assembled from recombinant HPV (16/18) coat proteins. One of these is tetravalent and also targeted for HPV-6 and HPV-11 which together currently cause about 90% of all cases of genital warts and 20%-30% of low-grade squamous intraepithelial lesions (L-SIL). Both types of vaccines require three doses (0.5 ml) given as intramuscular injections over six months (at times: 0; 2; 6). From 2008 also in Italy, the Health Office recommends the use of a prophylactic vaccine against HPV types 6, 11, 16, and 18 for routine use in females aged 11 to 12 years. The objective of vaccination is the progressive immunization of young adult female population exposed to the risk of infection reducing the incidence of precancerous lesions at brief-mid-term and of cervical cancer (almost 61%) at long-term. Such an immunization strategy involves a direct additional charge at the expense of the SSN National Healthcare System) to guarantee immunization to cover 90% of the target (in 2008 only, i.e., there was an outlay of Euro 60.000.000) [7]. Efficacy is based on completion of three doses of vaccine, which probably occurs in no more than 75% of females who initiate vaccination [8]. In Italy, i.e., only 53.1% of young adolescents born in 1997 had taken three doses of the vaccine and completed the immunization program by December 31, 2009 [9]. Different criticisms have led to a wide immunization program: available evidences are limited at this time and are small in comparison to the target of teenagers. The duration of protection and the requirement for booster doses are not known; the available information regarding a possible cruciform or widened protection to other genotypes induced by the vaccine are limited, neither it is possible to know if the

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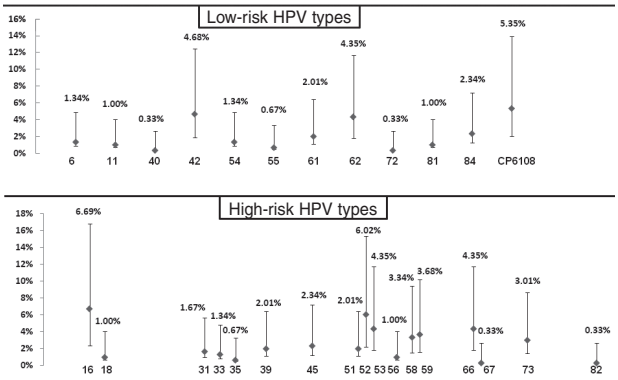

Figure 1. — Prevalence of HPV genotypes in Molise.

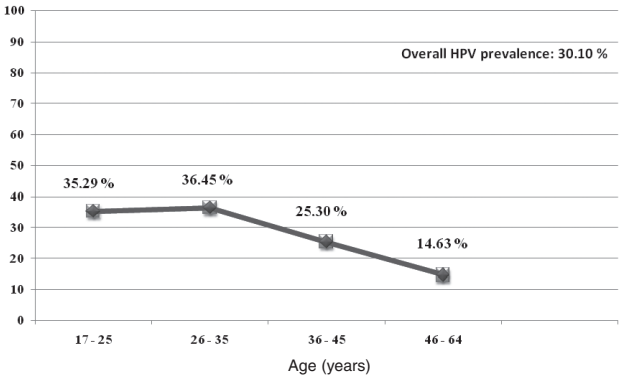

Figure 2. — Prevalence of HPV among females aged 17 to 64 years in Molise.

possible risk of ecological genotyping replacement can reduce the effectiveness of the vaccine. Doubts have finally been lifted with respect to the elevated costs of every immunization program [7]. Therefore the question remains: is it really necessary to use an extensive vaccine? Do we know the exact and real distribution of circulating HPV genotypes in our countries? Do vaccine genotypes agree with these circulating genotypes? Has screening reached its goal?

Materials and Methods

The University of Molise in association with the ASREM (Health Authority of Molise Region), from February to August 2008 used a representative sample of the female population (n = 299) aged 17 to 64 years who were interviewed and submitted cervico-vaginal swab specimens. Swabs were analyzed for a cytologic screening (thinPrepSystem) and for HPV-DNA by polymerase-chain-reaction (PCR) followed by type specific hybridization. HPV detection and typing was performed by using the Roche Linear Array HPV Genotyping Test and it included probes for 37 HPV types. The patients with positive cytology were submitted to colposcopy and eventually biopsy. Cytological follow up of HPV positive patients was performed in 24 months.

Study population and design

The representative sample is obtained by using a complex stratified multistage probability sample design with unequal probabilities of selection to obtain a regionally-representative sample. All females aged 17 to 64 years were eligible for participation in the study. All patients were informed about the methods and objectives of this research and signed an informed consent form (a parental permission was obtained for minors). The project was approved by the ethical committee of the University of Molise on September 26th, 2007.

Demographic and behavioural data

Nationality, race, and ethnicity were reported. Grade of instruction, job/profession, marital status, smoke, drugs, and use of alcohol were also included. Age at first sexual intercourse, lifetime number of sexual partners, sexual behaviour, use of contraception (condom, IUD, oral-contraceptives), past history of pregnancy, abortion, sexually-transmitted infections, past cytological, and colposcopical alterations were reported. Stature, weight, and body mass index (BMI) were evaluated.

Specimen collection and processing

Cervico-vaginal swab specimens were performed in the structures of ASREM in Campobasso, Isernia, and Termoli. A collection device was utilized, which was a small cervical sampler on a plastic handle packaged in an individual re-closable, non-sterile plastic sleeve for single use only (Cervex-Brush). The swabs were stored in 20 ml of a PreservCyt LBC media (ThinPrep-liquid-PAPvial; Cytyc Corporation, USA); a methanol-based transport medium and preservative for cytologic samples at room temperature were also used and then sent to laboratories of the University of Molise. The swabs were analyzed for a thin-Prep Pap test and for the presence of HPV-DNA by Linear Array HPV genotyping test (Roche-Molecular-Systems, Inc. Branchburg, NJ, USA) – a qualitative in vitro test for the determination of 37 HPV-DNA genotypes. The authors considered as LR-HPV types: 6; 11; 40; 42; 54; 55; 61; 62; 64; 71; 72; 81; 83; 84; CP6108 and as HR-HPV types: 16; 18; 26; 31; 33; 35; 39; 45; 51; 52; 53; 56; 58; 59; 66; 67; 68; 69; 70; 73; 82; IS39.

Statistical analysis

All females who were submitted to an adequate swab for HPV evaluation were included in the final analysis (299/299). HPV prevalence was estimated within the 95% confidence interval (CI). CI were calculated by using the SE of log transformation with the SE of the log prevalence. Statistical analysis samples deemed positive for high- and low-risk HPV were categorized as single or multiple infections. To explore the association with age and overall HPV prevalence, age was categorized into four year intervals (17-25, 26-35, 36-45, and 46-64). Data were analyzed by an SPSS-12.0 Statistical Package for Windows. Pearson's χ^2 test was used to evaluate the significance of differences between designated groups. All tests were two-sided; a p value of < 0.05 was considered statistically significant.

Results

The number of healthy women was 299, with a mean age of 34 years (range 17-64) entered in the study and 29/37 different HPV genotypes were identified. Overall, 206 viruses were detected in 90 women. Multiple HPV infections were observed in 55.64% (50/90) of HPV positive samples with a mean of three viruses per woman (range 2-8) (Table 1).

The overall HPV prevalence was 30.1% (95% CI, 25, 39-35, 28) among females of Molise aged 17 to 64 years

Table 1. — *Multiple HPV infections*.

Number of patients with two genotypes: 18 HPV types detected								
45/52 16/81	52/CP6108 62/66	72/CP6108 53/59	53/73 53/59	35/52 58/62	56/66 61/84	42/59 31/56	53/62 51/59	53/66 52/58
Number of patients with three genotypes: 13 HPV types detected								
18/53/66 31/33/84	6/18/39 16/42/CP1608	42/52/58 42/45/73	42/66/81 45/53/73	16/52/59 6/45/53	52/58/84 6/40/66	61/84/CP6108 —		
Number of patients with four genotypes: 11 HPV Types Detected								
39/42/58/66 16/52/59/CP6108	16/39/52/58 16/42/53/84	52/53/54/62 51/59/81/82	52/54/55/58 16/31/42/45	16/18/51/66 16/33/42/62	16/31/42/84 —			
Number of patients with five genotypes: 3 HPV types detected								
16/33/45/53/73	16/51/56/59/61	16/55/62/66/CP6108	—					
Number of patients with six genotypes: 4 HPV types detected								
39/42/45/53/54/CP6108	6/31/39/42/73/CP6108	52/58/59/62/66/73	52/58/59/62/66/73					
Number of patients with seven genotypes: 0 HPV types detected								
None								
Number of patients with eight genotypes: 1 HPV types detected								
11/16/35/52/54/58/62/73								

(n = 299) and the overall prevalence of HR-HPV and LR-HPV typess was 22.41% (95% CI, 18,14-27,35) and 18.06% (95% CI, 14,16-22,75), respectively. The prevalence of HR-HPV and LR-HPV is represented in Figure 1. The most common HR-HPV were: HPV-16: 6.69% (95% CI, 4.36-10.13), HPV-52: 6.02% (95% CI, 3.83-9.34), HPV-53: 4.35% (95% CI, 2.54-7.34), HPV-66: 4.35% (95% CI, 2.54-7.34), HPV-59: 3.68% (95% CI, 2.05-6.52), HPV-58: 3.34% (95% CI, 1.81-6.10), HPV-73: 3.01% (95% CI, 1.57-5.68), HPV-45: 2.34% (95% CI, 1.12-4.83), and HPV-39: 2.01% (95% CI, 0.90-4.39). Considering the LR-HPV, the most common types were HPV-CP6108: 5.35% (95% CI, 3.31-8.55), HPV-42: 4.68% (95% CI, 2.79-7.75), HPV-62: 4.35% (95% CI, 2.54-7.34), and HPV-84: 2.34% (95% CI, 1.12-4.83). The prevalence for other types of HPV was lower (≤ 2%). There was a statistically significant difference of HPV prevalence among women across a broad age range representative of the population (Figure 2): from 36.45% (95% CI, 28.44-45.28) in the 26-35 years group to 14.63% (95% CI, 6.78-28.76) in those aged 46 years or more (p = 0.037) (Table 2). There was also a statistically significant difference of HPV prevalence related to marital status: 42.76% (95% CI, 35.68-50.14) in the single group that increased to 61.54% (95% CI, 39.87-79.43) in the widowed/separated/divorced group vs 14.18% (95% CI, 9.38-20.89) in the married/living with partner group ($p < 0.001$). No statistically significant difference was found in relation to geographic zone and education. There was a statistically significant difference of HPV prevalence related to low BMI (< 18.7: HPV prevalence 48.15% (95% CI, 32.39-64.28) ($p < 0.05$)), a high number of lifetime sex partners (≥ 3: HPV prevalence 84.21% (95% CI, 73.28-91.21) ($p < 0.006$), and to cigarette smoke (smokers: HPV prevalence 46.15% (95% CI, 37.29-955.27) ($p < 0.001$). Particularly, HR-HPV infections were associated with tobacco users. Indeed, compared with non-smokers, current smokers were at increased risk of HR-HPV infection: in fact the HR-HPV infection was 39.56% (95% CI, 30.79-49.05) in smokers vs 14.90% (95% CI, 10.72-20.35) in no smokers. Also a high prevalence of multiple infections were associated with tobacco users: 29.67% (95% CI, 21.54-39.33) in smokers compared to 11.06% (95% CI, 7.48-16.05) in non-smokers. No statistically significant difference was found in relation to menopausal state, pregnancy, cancer familiarity, utilization of oral contraceptives, history of previous HPV-related pathologies (previous LSIL/HSIL), and age of first sexual intercourse (Table 2). Among the 299 women screened, 271 (90.64% (95% CI, 89.14-91.94)) had normal cytology, ten (3.34% (95% CI, 1.81-6.10)) showed ASC (atypical squamous cells), thirteen (4.35% (95% CI, 2.54-7.34)) showed a LSIL, and five (1.67% (95% CI, 0.70-3.95)) a HSIL. Eight women with ASC resulted HPV-negative and only two ASC resulted HPV-positive; both HPV-positive cases developed LSIL during follow-up. Each case of LSIL and HSIL resulted HPV-positive and the cytological diagnoses were confirmed by colposcopic examination and cervical biopsy (Table 3). Six LSIL (46.15% (95% CI, 24.57-69.28)) showed a CIN-1 at histology and seven LSIL (53.85% (95% CI, 31.76-74.52)) showed normal histology. Three HSIL resulted as CIN-2 (60.0% (95% CI, 26.39-86.26)) and two HSIL (40.0% (95% CI, 11.45-77.46)) showed as CIN-3.

LSIL were treated with diathermocoagulation (DTC) or laser vaporization (5/13) or repeated cervical cytology and colposcopy at six and twelve months, allowing time for the abnormality to be resolved (8/13). HSIL were treated with a surgical conization of the cervix (5/5) (Table 3). No cancer was detected.

HPV-16 was detected in 4/5 cases (80.0% (95% CI, 51.79-93.71)) of HSIL and in 1/13 cases (7.69% (95% CI, 1.08-38.91)) of LSIL. HPV-6 was detected only in one case (20.0% (95% CI, 2.84-68.15)) of HSIL (associated to HPV-45 and-53) and no cases of LSIL or ASC. HPV-11 and HPV-18 were not detected in any patient with cytological abnormality. However, because there was a low number of samples, the difference did not reach statistical significance. The 84.44% (95% CI, 80.0-88.05) (76/90) of HPV-positive patients cleared the infection in 24 months.

Discussion

The authors have studied the prevalence of the HPV types in the Molise region, one of smaller Italian regions (321,000 inhabitants of which nearly 97,500 women aged between 17-64 years). HPV is a common infection among females in Molise [10,11]. The present data indicated that the burden of prevalent HPV infection among females was greater then previous estimates in Italy 30.1% vs 7%-16% (official data of Superior Health Institute) [12], however the prevalence of HPV vaccine types was relatively low for HPV-6 1.34% (95% CI, 0.50-3.51), HPV-11 1.00% (95%CI,0.32-3.06), and HPV-18 1.00% (95% CI, 0.32-3.06). Only HPV-16 (6.69% (95% CI, 4.36-10.13)) was representative in Molise but it followed a strict geographic distribution: 65% (95% CI, 48.02-78.87) of them were detected in high Molise (Isernia) and only 35% (95% CI, 18.55-56.01) in low Molise (Campobasso and Termoli). Perhaps, this is possibly due to the micro-economy in Molise which is primarily a rural and mountain economy, therefore the social and cultural exchanges are not favorable [13]. These data, also observed in other isolated regions [14], are in contrast to the study of Agarossi *et al*. [15] where no differences were observed in the prevalence rates of HPV infection among various Italian geographic areas; yet they are justified if we consider that different geographical areas have social, cultural, economic, and historical differences [16]. Other most common HR-HPV types detected in Molise were: HPV-52, HPV-53, HPV-66, HPV-59, HPV-58, HPV-73, HPV-45, and HPV-39. Some of them (HPV-16, 45, 52, and 58) are included in the eight most common HPV types involved frequently in pathogenesis of invasive cervical cancer (HPV-16, 18, 31, 33, 35, 45, 52, 58) and cervical adenocarcinoma (HPV-16, 18, 45) [17]. Multiple HPV infections are common and were observed in 16.2% of the study samples (55.64% of all HPV positive samples) with a mean of three viruses per woman (range: 2-8). The prevalence reported in the literature ranged from 1% to 20%, particularly in relation to HPV detection methods used [18]. Currently, there is a lack of consensus within

Table 2. — *Prevalence of HPV infection by demographic physical and behavioral characteristics*.

Demographics characteristics	Sample Size	Prevalence % (95% Confidence interval)		*p* value
Overall (Aged 17-64 years)	299	30.10	(25.39-35.28)	
Age (years)				
17 – 25	68	35.29	(25.51-46.49)	
26 – 35	107	36.45	(28.44-45.28)	0.037
36 – 45	83	25.30	(17.34-35.35)	
46 – 64	41	14.63	(6.78-28.76)	
Geographic zone				
Campobasso	123	27.64	(20.72-35.84)	0.74
Isernia	122	31.97	(24.68-40.26)	
Termoli	54	31.48	(21.04-44.21)	
Marital status				
Single (unmarried)	145	42.76	(35.68-50.14)	< 0.001
Married/living with partner	141	14.18	(9.38-20.89)	
Widowed/separated/ divorced	13	61.54	(39.87-79.43)	
Education				
Graduated (degree)	45	26.67	(16.13-40.74)	
Senior high school	136	36.03	(28.89-43.84)	0.2
Primary or junior high school	118	24.58	(17.83-32.85)	
B.M.I.				
< 18.7	27	48.15	(32.39-64.28)	0.05
18.7 – 23.8	188	30.32	(24.44-36.92)	
> 23.8	84	23.81	(16.10-33.73)	
Menopausal state				
Yes	17	11.76	(2.99-36.59)	0.09
No	282	48.35	(42.06-54.70)	
Previous pregnancies				
Yes	117	22.22	(15.74-30.41)	0.20
No	182	35.16	(28.97-41.90)	
Cancer familiarity				
Yes	70	30.00	(20.83-41.10)	0.98
No	229	30.13	(24.78-36.08)	
Utilizzation of oral contraceptive				
Yes	66	36.36	(26.37-47.69)	0.17
No	233	28.33	(23.12-34.19)	
Previous pathologies HPV related				
Yes	59	37.29	(26.71-49.24)	0.23
No	240	28.33	(23.2034.10)	
Age at first sexual intercourse. y				
< 16	105	32.38	(24.54-41.35)	0.61
≥ 16	194	28.87	(23.16-35.33)	
No. of lifetime sex partners *				
1	15	33.33	(15.38-57.91)	
2	13	38.46	(18.22-63.689	0.006
3-5	19	84.21	(73.28-91.21)	
> 6	4	75.00	(40.17-93.06)	
Smoke				
Yes	91	46.15	(37.29-55.27)	< 0.001
No	208	23.08	(17.98-29.11)	
Smoke and HR-HPV infections				
Yes	91	39.56	(30.79-49.05)	0.008
No	208	14.90	(10.72-20.35)	
Smoke and multiple HPV infections				
Yes	91	29.67	(21.54-39.33)	< 0.001
No	208	11.06	(7.48-16.05)	

* Date available only for 51 of 299 patients.

Table 3. — *Management of patients with cytological abnormality*.

	Age	Cytology	HPV Types	Colposcopy	Hystology	Management
1	53	ASCUS	No HPV	—	—	—
2	44	ASCUS	No HPV	—	—	—
3	50	ASCUS	No HPV	—	—	—
4	45	ASCUS	No HPV	—	—	—
5	40	ASCUS	No HPV	—	—	—
6	31	ASCUS	No HPV	—	—	—
7	31	ASCUS	No HPV	—	—	—
8	36	ASCUS	No HPV	—	—	—
9*	27	ASCUS	62	Yes	Negative	Repeat cytology/six months
10*	26	ASCUS	52,58,59,62,66,7	Yes	Negative	Repeat cytology/six months
11	24	LSIL	42,59	Yes	Negative	Wait and see
12	22	LSIL	53,66	Yes	Negative	Wait and see
13	25	LSIL	16,55,62,66,CP6	Yes	CIN1	DTC
14	44	LSIL	39,42,45,53,54,C	Yes	CIN1	Laser vaporization
15	33	LSIL	62	Yes	Negative	Wait and see
16	27	LSIL	52,54,55,58	Yes	CIN1	DTC
17	24	LSIL	62,66	Yes	CIN1	Wait and see
18	32	LSIL	61	Yes	Negative	Wait and see
19	37	LSIL	16,51,56,59,61	Yes	CIN1	Laser vaporization
20	30	LSIL	53,59	Yes	Negative	Wait and see
21	42	LSIL	58,62	Yes	Negative	Wait and see
9*	27	LSIL	62	Yes	Negative	Wait and seet
10*	26	LSIL	52,58,59,62,66,7	Yes	CIN1	DTC
22	19	HSIL	16,33,45,53,73	Yes	CIN2/CIN3	Conization
23	28	HSIL	16	Yes	CIN2	Conization
24	32	HSIL	16,31,42,45	Yes	CIN3	Conization
25	26	HSIL	6,45,53	Yes	CIN2	Conization
26	22	HSIL	16,33,42,62	Yes	CIN2	Conization

* Patients 9 and 10 with ASCUS developed LSIL during the follow up.

the literature regarding the extent and implications of multiple HPV infections, but the present results are consistent with most recent international studies [19-22]. In the present research, multiple HPV infections were more frequently found in the youngest women; this observation is consistent with results of Mejlhede *et al*. [19] and suggests that a greater sexual activity of younger women may be associated with sexual transmission of multiple HPV types. According to the most epidemiological data [15], this study shows a high prevalence in young women aged 17 to 35 years and a continuous decline of prevalence rates of infection with increasing age; nevertheless the peak of prevalence in Molise is observed among females aged 26-35 years (36.45%). The result of multivariate analysis shows that a never-married and separated/-divorced status and total number of sexual partners in a lifetime are independent risk factors for HPV infections. These findings are in agreement with previous studies showing that having a never-married and separated/-divorced status and number of sexual partners are all factors associated with high prevalence of HPV infection [23]. Also a low BMI (< 18.7) is related to a high prevalence of HPV infection ($p < 0.05$). No association was found between the age at first sexual intercourse and the HPV infections or in relationship to menopausal state, pregnancy, cancer familiarity, a history of previous pathologies HPV-related (previous LSIL/HSIL), and utilization of oral contraceptives. Particularly, these last findings are in contrast with the study of Cotton *et al*. [24] that found an association between oral contraceptive use and HR-HPV infections. Previous research on the effect of oral contraceptive use on HPV infection showed inconclusive results [25]. Nonetheless, the findings in the present study do not support the hypothesis that oral contraceptive users may acquire HPV more often. Unlike in this study, HPV detection, especially those related to HR-HPV and multiple infections, was associated with tobacco consumption. It is possible that smoking could increase the likelihood of HPV infection through a local decrease of immune response in the cervix and through an indirect effect related to metabolism of female hormones [26]. During a follow up of 24 months, in agreement with the literature [27], the authors have detected 3.34% of ASC, 4.35% of LSIL, 1.67% of HSIL, and cancer cases. Only two cases of ASC were HPV-positive and developed LSIL during follow up. Every case of LSIL and HSIL resulted HPV-positive and the cytological diagnosis were confirmed by colposcopical examination and cervical biopsy. LSIL were treated with DTC or laser vaporization (5/13) or repeated cervical cytology and colposcopy at six and 12 months, allowing time for the abnormality to be resolved (8/13). Every HSIL was treated with a surgical conization of the cervix (5/5). However, HPV-16 was involved in 80% of HSIL but only in 15% of LSIL.

Conclusion

To the authors' knowledge, this study is one of the largest to assess HPV genotypes to date in Italy. It is clear that several HPV-types are involved in cervical lesions; in the authors' experience, the prevalence of HPV vaccine types (HPV-6, 11-, and 18) is relatively low in Molise and are not detected in any patient with cytological abnormality (excluded a coinfection with HPV-6 in a case of HSIL). Only HPV-16 is well-represented and is involved in 80% of HSIL, but follows a strict geographic distribution; therefore the vaccine in adolescents and younger women is profitable, but is limited by great number of genotypes implicated in the pathogenesis of cancer and by their dishomogeneous distribution [13]. In accordance with de Sanjose *et al*. [17], HPV types (16, 18, 31, 33, 35, 45, 52, 58, and others) should be given priority when the cross-protective effects of current vaccines are assessed, and for formulation of recommendations for the use of second-generation polyvalent HPV vaccines and should focus particularly on these HPV types. Currently, a good campaign of accurate and efficient screening is still necessary.

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