RESEARCH ARTICLE

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Prevalence and Distribution of High- and Low- Risk HPV Genotypes in Women Living in the Metropolitan Area of Naples: A Recent Update

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Abstract

Introduction: Human papillomavirus (HPV) can infect both male and female genitals, skin, and mucous membranes, causing benign or malignant lesions. HPV is a common sexually transmitted infection and it is the main cause of cervical cancer. The present retrospective study updated the previously published data on HPV genotypes distribution among women living in Naples. **Materials and methods:** In this study, 502 cervical scrape specimens were collected from women with abnormal cytological indication and analyzed for HPV DNA identification by Linear Array HPV genotyping test. **Results:** The HPV infection rate was 24.1%. HPV-16 (14.6%) was the most representative HR-HPV genotypes, followed by HPV-31 (13.8%), -18 (9.2%), and HPV-51 (8.5%). In addition, HPV-42 (16.4%) was the most prevalent genotype among LR-HPV genotypes (low-risk human papillomavirus). It was also found that women at the age group of 23-29 years (42.5%) were at the highest risk of HPV infection. It was found that the HPV-16 frequency decreased, but HPV-31 and -18 frequency increased a little. The LR HPV-53 frequency decreased, leaving the first place for abundance to the LR HPV-42. HPV-6 frequency did not change. LR HPV -11 was no more present. Merging <23 and 23-29 age classes into one class followed the same result. **Conclusion:** HPV prevalence declined in comparison to the previous data. A frequency variation was recorded for several genotypes in this study. Data can be useful to implement the preventative strategies and to promote HPV vaccination.

Keywords: HPV- cervical cancer- vaccines- prevention- COVID-19

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Introduction

Identified in 1981 for the first time and defined as "human warts virus", the Human papillomavirus (HPV) contains over two hundred different species of viruses (Staquet et al., 1981). It belongs to *Papillomaviridae* family. HPVs are small double-stranded DNA viruses, non-enveloped, and epitheliotropic, which are capable of infecting cutaneous epithelia in humans and causing benign lesions, such as warts and/or papilloma of particular areas, namely hands, feet, face, as well as genital and oral tissues. On the other hand, they can cause anogenital, vagina, vulva, penis, anus, and oropharynx (the base of the tongue and tonsils), and cutaneous epithelial cancers (de Villiers et al., 2004). HPV infection is therefore a major risk factor for cervical cancer, which is known as one of the most common sexually transmitted infections (STI) (Schiffman, 1992). Based on the Global Cancer Statistics 2020 reported by the American Cancer Society (ACS) and the International Agency for Research on Cancer (IARC) collaboration, cervical cancer ranks fourth among the most common type of cancers in women, with an incidence of 6.5%. There were an estimated 604,000 new cases of cervical cancer and 342,000 related deaths worldwide in 2020 (Sung et al., 2021). The U.S. Cancer Statistics Data Briefs (N°. 18 September 2020) reported the number of new HPV-Associated cancer cases as about 45,300 in the United States each year, of which 25,405 diagnosed among females and 19,925 among males since 2013 to 2017 (Prevention, 2020). Italian Association of

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Cancer Registries (AIRTUM) reported that about 2,400 new cervical cancer diagnoses were expected by 2020, equal to 1.3% of all incident cancers in females, and that 5-year survival rate is 68% (AIOM, 2020). Italian HPV cancer epidemiology released in 2020 reported that every year there are about 2,400 new cases, 1.3 % of all cancers diagnosed in women. Moreover, ISTAT 2018 data reported that every year over 500 women die from cervical cancer (ISTAT, 2020). Most HPV infections are asymptomatic and revert spontaneously due to the immune system, but persistent infections can progress to precancerous or cancer lesions. Several studies have reported that the cancer risk is not broadly distributed with all the HPV genotypes, but it is strongly related only with some of them. Indeed, the HPV genotypes can be subdivided in two different categories of low-oncogenic risk types (LR-HPV) and high-oncogenic risk types (HR-HPV) (Bzhalava et al., 2015; de Sanjose et al., 2010; N. Muñoz et al., 2003). Indeed, among the HR-HPV types, HR-HPV-16 and -18 represent about 70% of all oncogenic HPV genotypes worldwide (Bernard et al., 2013; Munoz et al., 2006), while LR-HPV types are more often found in conjunction with HR-HPV types (Clifford et al., 2003). The HPV genotypes coinfection is considered a risk factor driving carcinogenesis. During the last 40 years. It was found that higher risk of severe diseases related to HPV infection was represented by the "super-infection" (Hampson et al., 2020). In this scenario, we have two important weapons in order to counter the HPV infection and preserve the wellness of the community, namely the epidemiologic study of this infection and use of several vaccines available against the most of HR-HPV. Among all the proposed vaccines, actually we have three of them available on the market, including a bivalent form (which targets HPV16 and HPV18), a quadrivalent form (which targets HPV6, HPV11, HPV16, and HPV18), and a nonavalent form (which targets HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV45, HPV52, and HPV58) (Chatterjee, 2014; ECDC, 2012; ESMO, 2014; Martora et al., 2019). Precise diagnostic analysis of HPV genotype infection and vaccine diffusion can decrease the incidence of people affected by HPV infection in the next future. The aim of the present study was to update the information previously published about the same group (Barra et al., 2019), to evaluate and track differences about the incidence of HPV infections and the related distribution of genotypes with respect to age group.

Materials and Methods

Participants and specimen collection

From January 2018 to December 2019, 502 cervical scrape specimens from women aged from 19 to 63 years in Naples area were collected. The specimens were analyzed by the references molecular biology laboratory, section of microbiology and virology, University Hospital of Luigi Vanvitelli. This hospital covers a high urban density area of Naples (historic center of Naples). The cervical scrape specimens were collected by clinicians using an HPV-testing brush and plugged into a vial containing transport medium (PreservCyt®Solution, Hologic Inc., Marlborough, MA). The samples obtained were disciminated by clinicians as atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (L-SIL), and high- grade squamous intraepithelial lesions (H-SIL) according to the Bethesda classification for abnormal cervical cytological pathology. This information was not included in the paper since we analyzed the samples by a microbiological side and this study was based on database analysis. Next, the sample was processed for HPV genotyping within 2 days and stored until further processing.

Ethics approval from the Human Research Ethics Committee was not required for this retrospective study, because it was based on laboratory management data collected from databases. Patients' identifier were excluded to keep with confidentiality.2. HPV DNA extraction, PCR amplification, and genotyping

Multi-asymmetrical polymerase chain reaction (PCR) was used to detect and classify common HPV types. Genomic DNA of human cervical epithelial cells was extracted by lysing cervical cell specimens under denaturing conditions at elevated temperatures and in presence of proteinase K. Then, isolation and purification of DNA were done over a column according to the manufacturer's instructions (Roche Molecular Diagnostics, Milan, Italy). Amplification of HPV target DNA was performed using PCR technique. of a 450 base pairs fragment from the L1 HPV region and the human β-globin gene. An additional amplicon was used to provide a control for cell adequacy, extraction, and amplification. HPV detection and genotyping were performed by LINEAR ARRAY HPV Genotyping Test (Roche Molecular Diagnostics, Milan, Italy). In sum, 37 HPV-13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 24 LR-HPV types (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, CP6108) were detected. The LINEAR ARRAY HPV genotyping test was used to detect the presence of high-risk HPV DNA in cervical specimens. The sensitivity of test was about 96% and its specificity was about 99%.

Data analysis

Statistical analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA) for Windows. Categorical data were analyzed by $\chi 2$ tests, and confidence intervals (CI 95%) were calculated using unconditional logistic regression. All p-values were two-sided, and statistical significance was defined as $p<0.05$.

Results

Incidence of positive samples, infection types distribution, and genotypes frequency

Among 502 women samples tested during the study period, 121 (24.1%) (95% CI 20.4-27.8) were positive for HPV DNA. Among 121 HPV-positive patients, 46 (38%) (95% CI 29.4-46.7) were infected with a single type (ST) of HPV and 75 (62%) (95% CI 53.3-70.6) with multiple infections (MT). In addition, 92 cases with ≥1 HR-HPV genotype (76.0%) (95% CI 68.4-83.6) were

Table 1. Prevalence of a) HR-HPV and b) LR-HPV genotypes among 121 women positive for HPV DNA detection. Salmon pink lane in the table represent the genotypes used for the nonavalent vaccine. * indicates important variations in order of genotypes frequency with respect to the previous data.

a

HR GENOTYPES	PREVIUS DATA 2011-2017	UPDATE 2018-2019
*16	23.76%	14.6%
*18	6.91%	9.2%
*31	12.06%	13.8%
33	4.79%	2.3%
35	1.77%	2.3%
39	5.32%	6.2%
45	5.14%	4.6%
51	7.45%	8.5%
52	5.14%	7.7%
56	4.79%	7.7%
58	7.45%	7.7%
59	7.62%	6.2%
66	7.98%	6.9%
68	2.48%	2.3%

b

LR GENOTYPES	PREVIUS DATA 2011-2017	UPDATE 2018-2019
6	8.69%	8.6%
*11	1.42%	0.0%
26	0.18%	0.8%
40	1.06%	0.8%
*42	9.40%	16.4%
*53	12.41%	10.2%
54	7.09%	5.5%
55	4.08%	4.7%
61	6.03%	7.0%
62	9.93%	9.4%
64	0.00%	0.0%
67	1.06%	2.3%
69	0.00%	0.0%
70	2.66%	1.6%
71	0.89%	0.0%
72	0.53%	0.0%
73	8.16%	9.4%
81	1.77%	4.7%
82	0.71%	0.8%
83	1.24%	3.9%
84	5.32%	4.7%
IS39	0.18%	0.0%
CP6108	9.22%	9.4%

Table 2. a) HR-HPV and b) LR-HPV genotypes distribution in single and multiple infections. Salmon pink represent the genotypes included in nonavalent vaccine.

a

a	PREVIOUS DATA 2011-2017		UPDATE 2018-2019	
HR GENOTYPES	MT %	ST%	MT %	ST%
16	56.7	43.3	84.21	15.79
18	87.2	12.8	75.00	25.00
31	82.4	17.6	88.89	11.11
33	78.0	22.0	66.67	33.33
35	70.0	30.0	66.67	33.33
39	70.0	30.0	100.00	0.00
45	80.5	20.5	83.33	16.67
51	81.0	19.0	81.82	18.18
52	72.0	28.0	80.00	20.00
56	92.6	7.4	90.00	10.00
58	48.6	52.4	80.00	20.0
59	81.4	18.6	75.00	25.00
66	80.0	20.0	77.78	22.22
68	100	0.0	66.67	33.33

b

	PREVIOUS DATA 2011-2017		UPDATE 2018-2019	
LR GENOTYPES	MT %	ST%	MT %	ST%
6	75.5	25.5	81.8	18.2
11	88	12	0.0	0.0
26	100	0	100.00	0.0
40	83	17	100.00	0.0
42	75.5	25.5	90.50	9.50
53	68.6	31.4	84.60	15.40
54	85	15	71.40	28.60
55	88	12	100.00	0.00
61	76	24	77.80	22.20
62	58.8	40.2	83.30	16.70
64	0	0	0.00	0.00
67	100	0	100.00	0.00
69	0	0	0.00	0.00
70	60	39	50.00	50.00
71	80	20	0.00	0.00
72	100	0	0.00	0.00
73	83	17	83.30	16.70
81	100	0	66.70	33.30
82	100	0	0.00	100.00
83	58	42	80.00	20.00
84	80.5	19.5	66.70	33.4
IS39	0	100	0.00	0.00
CP6108	78	22	83.30	16.70

identified. The most prevalent HR-HPV genotypes was HPV-16 (14.6%), followed by HPV-31 (13.8%), HPV-18 (9.2%), HPV-51 (8.5%), HPV-52-56-58 (7.7%).The

prevalence of other HR-genotypes was lower than 7%. Considering the LR-HPV genotypes, the most prevalent ones were HPV-42 (16.4%), HPV-53 (10.2%), HPV-62, -73, -CP6108 (9.4%), and HPV-6 (8.6%). The prevalence of the other LR-genotypes was lower than 7%. Results on genotypes distribution during the studied period are shown in Table 1.

Distribution of single type and multiple type infections

The distribution of single type (ST) and multiple type (MT) infections for all the HPV genotypes is depicted in Table. 2 . Among HR-HPV genotypes, HPV-33, -35 and -68 displayed the highest rate for ST-HR (33.3%) (95% CI 0-86.68). However, HPV-39 was presented only in MT infections (100%). HPV-56, -31, and -16 was detected in 90.0%, 88.9%, and 84.2% of MT infections, respectively (95% CI 71.41-100; 75.37-100; 67.81-100). HPV -18 was detected in 75% of MT infections (95% CI 50.50-99.50). Among the LR genotypes, HPV-26, -40, -55, -67 were noticed only in association with other genotypes (100% in MT infections). HPV-42 was fond in 905% of MT infections (95% CI 77.92-100). HPV-70 was observed in 50% of MT infections and appeared as a single genotype in 50% of the patients. In ST infections, HPV-82 had the highest prevalence (100%), while HPV-81 and -84 were detected in 33.3% of ST infections (95% CI 0-71.05). LR-genotypes were also detected in MT infections(more frequently associated with other genotypes).

Distribution of HPV positivity with respect to age groups

The analysis of total HPV infections distribution with respect to age group showed that the highest rate of infection was detected in women aged 23–29 years (42,53%), followed by those aged <23 years (41.18%), with a significant decrease ($p < 0.001$). In addition, it was found that both HR and LR genotypes had the highest distribution in the age group of 23–29 years (52.9% for HR-HPV; 51.7% for LR-HPV) compared to age group <23 years (47.1% for HR-HPV; 41.2% for LR-HPV, and age group ≥ 30 years 19.1% for HR-HPV). This difference was found to be statistically significant ($p < 0.001$) (Table 3 a).

In Table 3 b, the age groups <23 and 23-29 were combined then were compared (for positivity rate of total HPV, HR and LR HPV) to the 2011-2018 results.

Discussion

The mode of viral transmission is mainly sexual, so HPV infection affects not only the female population but also the males. HPV is one of the major causes of death among women nowadays. The weapons to prevent the spread of infections are several, of which prevention by vaccines is fundamental. However, vaccines are not 100% efficient because they cannot protect humans against pre-existing HPV infections or other HPV genotypes (Nubia Muñoz et al., 2010). Therefore, it seems essential to control behaviors that increase the chances of contracting the infection, such as having unprotected sex or a large number of sexual partners.

The World Health Organization (WHO) on 17 November 2020 suggested a global strategy to increase the elimination of cervical cancer as a public health problem. Among the many objectives of this global strategy are vaccination of 90% of girls with HPV vaccine by age 15 years, screening 70% of women with a high-performance test by 35-45 years of age, and providing treatment to 90% of women with cervical disease (90% of women with precancerous lesions treated, and 90% of women with invasive cancer managed) (Organization, 2020). In Italy, the vaccination plan is managed by regions according to a national prevention plan. Our aim is to monitor the HPV incidence and the relative distribution of genotypes in our region, also in relation to age groups, to provide epidemiological data that are always updated. The purpose is to provide useful information to evaluate the effects of vaccinations on the population during the years, and to try to raise awareness of screening actions in the Campania region. Certainly, variations were considered in this update about the positivity incidence (24.1%

Table 3. a) Distribution of HR- and LR- HPV positivity by age group. b) Comparison of the update data with the previous data reported and combining the age groups <23 and 23-29 into less than 30.

a

Age group/ patients	Positive/ Total (n.)	Positive/ Total (%)	Positive HR-HPV/Total (n.)	Positive HR-HPV/Total (%)	Positive LR-HPV/Total (n.)	Positive LR-HPV/Total (n.)
<23	7-17	41.18%	Aug-17	47.10%	Jul-17	41.20%
23-29	37/87	42.53%	46/87	52.90%	45/87	51.70%
>30	77/398	19.35%	76/398	19.10%	75/398	18.80%

b

		Total HPV			HR-HPV			LR-HPV		
		Absolute frequence	Relative Frequence	P.value	Absolute frequence	Relative Frequence	P.value	Absolute frequence	Relative Frequence	P.value
Age	<30	44/104	42.31%	<0.001	579/1265	51.92%	<0.001	52/54	50.00%	<0.001
	>30	77/398	19.35%		130/502	19.10%		75/298	18.84%	
Studies	2018-2019	563/1265	44.51	<0.001	579/1265	45.77%	<0.001	519/1265	41.03%	<0.001
	2011-2017	121/502	24.10		130/502	25.90%		127/502	25.30%	

versus 44.5%) and the distribution of the most abundant genotypes. In fact, a redistribution of the genotype frequency on the total of positive patients emerged from the small population were analyzed and compared to the published data in this study (2011-2017). Although the variations in terms of frequency percentages were different in most of the genotypes, we highlighted merely those related to the most representative genotypes. Similar to previous findings, the most frequent genotype among HR-HPV genotypes was HPV-16, albeit its frequency was slightly decreased (from 23.8% recorded in the previous study to 14.6%). The second most frequent genotype was HPV-31, which decreased from 12.1% to 13.8%. HPV-18 frequency increased from 6.9 to 9.2, becoming the third most frequent genotype. Among LR genotypes, the redistribution was more remarkable. HPV-53 genotype was no longer the most frequent one, decreasing in percentage from12.4% to 10.2% and occupying the second place in terms of frequency. HPV-42 increased in frequency percentage (9.4% versus 16.4%), ranking the first in terms of frequency. HPV-11, which had low frequency in previous data, was not found in any of the positive samples in this study. The frequency of HPV-6 remained constant. Rahmat et al., in 2021 reported that the prevalence of HPV infection in Malaysian women aged 20-74 was 14.0% (107/764) with high-risk type at 10.7% (82/764) and low-risk type at 3.27% (25/764). The most common HR HPV types was HPV-52, followed by 66, 33, 39, and 58. The most frequent LR HPV types were HPV-6, 40, and 81. The Malaysian data is worrying as genotype 52 is not included in vaccination coverage and cervical cancer ranks third as the cause of death in Malaysian women. The most abundant genotypes in our study, which were 16 (14.6%), 31 (13.8%), 18 (9.2%) fell within the vaccine coverage, hoping that their share will decrease more and more (Rahmat et al., 2021). Similarly, in Japanese female, HPV-52 was the most recurrent genotype (8.6%) and HPV genotypes prevalence was 28.1% and 30.5% for HR and LR, respectively (Kitamura et al., 2021). However, one study conducted on Iranian women aged 17- 78 revealed that HPV16 was the predominate genotype (30.5%), followed by HPV53 (17.3%) and HPV39 (13.3 %). Among LR genotypes, HPV6 (60.6%) was the most frequent, followed by HPV11 (17.9%) and HPV81 (8.6%) (Chalabiani et al., 2017) in aforementioned study. This difference in findings highlights the diversity of HPV genotype distribution in various geographic contexts.

It was also found that, among HR genotypes, HPV-56 and -31 maintained a high percentage of association with other genotypes (90% and 88.9% in MT infections, respectively). The tendency of genotype HPV-18 to be associated with other genotypes was maintained (75% compared to previous 87.2%). HPV-16 percentage increased from 70% to 84.2% in MT infections. The frequency of HPV-68 changed from 100% to 667% in MT infections and 333% in ST infections. HPV-39 frequency increased from 70% to 100% in MT infections. With respect to LR genotypes, HPV-26 and -67, their presence exclusively in association (100% in MT infections) was confirmed. About the genotypes HPV-40 and -55 are found in association in 100% of the time. Genotype -81 frequency decreased from 100% to 76.7% in MT infections while its frequency increased in ST infections. Radical inversion was found for genotype -82, which had 100% frequency in MT infections in previous data but it had 100% frequency in ST infections in our data. Genotype HPV-11 was not present in our data, whereas previously it was found at 88% of MT infections. HPV-6 maintained a similar trend, that is it was detected in 81.1% of MT infections in our study (95% CI 59.03-100) while it was present in 75.5% of MT infections previously. HPV-53 frequency in MT infections switched from 68.6 to 84.6% (95% CI 65.00-100). Therefore, by focusing on the genotypes that make up the tetravalent vaccination, we found that -16 and -11 decreased in MT infections in terms of frequency and percentage , -18 increased in frequency but its presence in MT infections slightly decreased. These fluctuations may be influenced by the administration of vaccines, although a real estimate of the vaccinated is complex to draw up due to the wide variability between Regions / PP.AA. for all cohort studies. According to "Ministero della Salute" report on December 31, 2018, emerged that in Italy is confirmed the negative trend of the surveys for the last active call cohort (the coverage data relating to the 2006 cohort settled at 61.7% for the first dose and 40.3% for the complete cycle). On the other hand, the report on December 31, 2019, about 2006 cohort revealed a slight improvement compared to the previous year, but in any case, lower than the historical data (41,60% in 2019 versus 40.34% in 2018, 49,9% in 2017, 53,1% in 2016, 56.2% in 2015). This slight increase from 2018 to 2019 could be reflected in the decrease in the 16 genotype's frequency and the total absence of genotype 11 present in both tetravalent and nonavalent vaccinations. Currently, the worrying finding is that in no Italian region ≥95% of the vaccinated population has been reached as foreseen by the PNPV 2017-2019. Furthermore, the disparity of data between the various autonomous regions does not allow an accurate estimate and suggests the need to adopt increasingly targeted approaches.

Furthermore, stratification by age group showed the highest incidence in the 23-29 age group, in contrast to the data obtained in the previous study. In detail, a higher incidence was observed in age group of 23-29 years (42.53%) compared to age groups <23 years (41.18%) and > 30 years (19.35%). Conversely, Martora et al., reported a higher prevalence in the age group <23 years (58.5%), compared to age groups of 23-29 years (54%) and> 30 years (38.2%). Based on our study, there was an association between HPV positivity and the age group. In particular, based on our findings, the age groups <23 and 23-29 were at the highest risk , while the age group >30 was at the least risk than the others. The highest risk in the age group of 23-29 is not surprising due to sexual activity, partner changes, and less exposure to the vaccine. This decrease in the age group < 23 is heartening, because it falls within the studied cohort concerned, probably due to greater vaccination adhesion. Based on the data obtained in our study, two genotypes of HR-HPV and LR-HPV had higher frequency in 23-29 age group (HR-HPV 52.9%; LR-HPV 51.7%) compared to the data reported in the reference study. On the other hand, we performed

age groups analysis ex novo based on the actual data, merging <23 and 23-29 age groups and confirmed that <29 was at high susceptibility of HPV infection, also for the HR and LR genotypes. This different susceptibility was statistically significant for the total, HR, and LR analysis performed ($p < 0.001$). According to our findings, HPV Total, HR-HPV, and LR-HPV positivity prevalence decreased statistically significant. This decreasing of prevalence in respect the previous data results could be confused by the different distribution of the age classes.

The data were collected prior to the outbreak of COVID-19 pandemic. There are few papers studying how the global pandemic has affected vaccination planning. In fact, the management of the emergency may have delayed the administration of the planned vaccines and affect the development of epidemics of other infectious diseases. Therefore, it will be even more important to monitor the epidemiological data of vaccine preventable diseases (VPD). On the other hand, vaccination for COVID-19 may increase community awareness abot vaccines, so it could be useful to reprogram and strengthen vaccination campaigns riding the wave.

Since the recent update of the Guidelines on anti-HPV vaccination of the Advisory Committee on Immunization Practices (ACIP), the American Cancer Society (ACS) has recommended early vaccination from the age of 9, catch-up HPV vaccination for all people up to 26 years of age, and lack of HPV vaccination for adults> 26 years of age, which is a shared clinical decision in all inadequately vaccinated age group of 26-45 years old (Saslow et al., 2020).

In this study, some variations about the frequency of single genotypes and their presence in association with other genotypes with respect to age groups were highlighted and compared to the previous reported data.

Author Contribution Statement

All authors contributed equally in this study.

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Study limitations

The limitations of this retrospective study were the lack of detailed patient training, such as whether they have been vaccinated for HPV or not. Moreover, the population taken into consideration was limited to the age range of 19 to 63 years. Another limitation was non-involvement of males.

Conflicts of Interest

The authors declare no conflict of interest.

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