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original article

Prevalence genotypes and distribution of human papillomavirus infection in women with abnormal cervical cytology in Catania, Italy

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SUMMARY: **Prevalence genotypes and distribution of human papillomavirus infection in women with abnormal cervical cytology in Catania, Italy.**

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Objective. *HPV infection has a strong regionalism, and HPV infection rates and distribution in different countries or regions are different; therefore, studying the distribution of HPV in a particular region provides important guidance for developing and applying vaccines. This study aimed to assess the distribution of different HPV types in women, with atypical cervical cytology, living in Catania.*

Material and Methods. *We analyzed data from 359 patients that had cytologically abnormal cervical samples during routine cervical cancer screening. The patients were from the outpatient services of the Department of Gynecology & Obstetrics of the University Hospital "Policlinico Vittorio Emanuele" of Catania.*

Results. *171 (47,6%) was HR-HPV, 36 (10%) was LR-HPV and the remaining 152 (42,4%) was multiple infection caused by different HPV, variously combined. In the HR-HPV samples, the most prevalent genotypes were HPV 16 (24,5%), HPV 31 (5,3%), HPV 18 (3,4%), HPV 56 (2,2%), HPV 58 (2,2%), HPV 39 (1,7%) HPV 45 (1,4%) HPV 33 (1,4%) HPV 66 (1,4%), HPV 51(0,8%), HPV 59 (0,8%) HPV 35 (0,8%), HPV 52 (0,3%), HPV 68 (0,3%), HPV 82 (0,3%). We calculated the odds ratio (OR) of having a viral infection caused by HPV16, HPV18, HPV31, HPV33, HPV45.*

Conclusions. *The most common HPV genotypes detected in most previous European studies were HPV 16 and 31 and in the USA were HPV 16, 45 and 51, whereas the present study showed the most prevalent genotypes in our patients to be HPV 16, 31 and 18.*

KEY WORDS: Epidemiology - High Risk-HPV - Human papillomavirus (HPV) - Prevalence - Catania - Sicily.

Introduction

Epidemiology and basic research have confirmed that human papillomavirus (HPV) infection is a major cause of cervical intraepithelial neoplasia and cervical cancer (1).

High-risk genotype of HPV (HR-HPV) is more likely to lead to the development of cervical cancer and the forth most common cause of death from cancer in women worldwide (2).

It is estimated that each year approximately 493,000 new cases are diagnosed and 274,000 women die from cervical cancer worldwide (3).

Furthermore, many vaginal, vulvar, anal, and penile cancers, as well as head and neck cancers are attributable to HR-HPV genotypes. Low-risk genotype of HPV (LR-HPV) has an etiological role in genital warts and respiratory recurrent papillomatosis (4).

The cofactors may be genetic, immunological as well as sociodemographic, e.g. lower age of conception, high parity, use of oral contraceptives, diet, smoking, etc. It was also evidenced that women co-infected with multiple HPV-type infections comprising of one or more high-risk types were prone to persistent HPV infection (5).

General improvement in socioeconomic status and educational level of the population tends to have a good effect on the risk of cervical cancer by altering some of the known risk factors such as age at marriage, parity and health-care seeking behavior. Other strategies such as low-intensity cytology screening (e.g., 1 Pap smear every 10 years after age 35) and visual inspection need to be better evaluated in randomized controlled trials to determine their cost-effectiveness (6).

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To date, more than 200 HPV genotypes have been identified, and ~40 HPV genotypes have been detected in the female genital tract. HPV16 and HPV18 are well known as oncogenic genotypes; additionally, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV69, and HPV 82 are also closely associated with cervical cancer. Therefore, all of these genotypes are classified as "high-risk" HPV. Meanwhile, "low-risk" genotypes, including HPV6, HPV11, HPV42, HPV43, and HPV44 are the causative agents for benign or low-grade changes in cervical cells, such as genital warts.

The current HR-HPV detection is supposed to serve as an additional approach for the early diagnosis of cervical cancer, and was implemented to complement less sensitive and non-objective cytology-based methods (7). The high negative predictive value of HR-HPV testing is applicable for the indication of a low-risk population, in which the cervical cancer screening interval can be safely extended (7).

Based on the results of clinical trials, several European countries will implement HR-HPV testing as the primary screening modality (8).

In addition to HPV screening, HPV vaccination has been shown to be an effective strategy against HPV infection and has been recently implemented in most western countries. Although Cervarix (HPV16/18) and Gardasil (HPV6/11/16/18) protect against infection by HPV16 and HPV18, these vaccines provide no effect on some of the HR-HPV types found in at least 25% of cervical cancers (9). Furthermore, the role of non-vaccine HPV types in the development of lesions remains unknown, and it remains possible that non-vaccine HPV types could replace these vaccine types as the causative agents for cervical precancerous lesions and cancer in vaccinated cohorts without sufficiently broad cross-protection (9).

HPV infection has a strong regionalism, and HPV infection rates and distribution in different countries or regions are different (10); therefore, studying the distribution of HPV in a particular region provides important guidance for developing and applying vaccines.

Material and methods

Study Population

In this study the database consisted of 359 women from the outpatient services of the Department of Gynecology & Obstetrics of the University Hospital "Policlinico Vittorio Emanuele".

Patients were enrolled between September 2012 and October 2015. Participants'ages mainly ranged from 19 to 55 years, with a median age of 37 years. Inclusion criteria was a cytologically abnormal cervical samples during routine cervical cancer screening.

Exclusion criteria included current pregnancy, <3 months post-partum and a history of either hysterectomy or treatment for cervical cancer.

All patients have undergone pap smear, cytology exo-endocervical that were collected in ThinPrep solution to be subjected to extraction of total nucleic acids (DNA) for research and genotyping of viral DNA.

HPV-DNA Test

HPV-DNA from cervical samples was extracted and HPV genotyping was performed by polymerase chain reaction using Nested PCR.

Statistical analysis

The population for the statistical analysis was the histologically adequate HPV+ group. The HPV infection rate was calculated by dividing the number of each HPV (16, 18, 31, 33, 45) positive sample by the total number of samples that were positive for HPV. We calculated the odds ratio (OR) of having a viral infection caused by HPV16, HPV18, HPV31, HPV33, HPV45. Furthermore, a binomial 95% confidence interval (95% CI) was estimated for each calculation of the prevalence of HPV. All statistical analyses were conducted using Microsoft Office Excel 2013.

Results

In this study, HPV prevalence was espressed as percentage of HPV samples against all HPV tested cases. Furthermore, HPV samples were divided in high risk HPV (HR-HPV) and low risk HPV (LR-HPV).

In these 359 samples, from patients with dysplasia detected by routine pap smear, 171 (47,6%) were HR-HPV, 36 (10%) were LR-HPV and the remaining 152 (42,4%) were multiple infection caused by different HPV, variously combined.

In the HR-HPV samples, the most prevalent genotypes were HPV 16 (24,5%), HPV 31 (5,3%), HPV 18 (3,4%), HPV 56 (2,2%), HPV 58 (2,2%), HPV 39 (1,7%) HPV 45 (1,4%) HPV 33 (1,4%) HPV 66 (1,4%), HPV 51(0,8%), HPV 59 (0,8%) HPV 35 (0,8%), HPV 52 (0,3%), HPV 68 (0,3%), HPV 82 (0,3%).

In the LR-HPV samples, the most prevalent genotypes were HPV 6 (2,2%), HPV 54 (1,7%), HPV 42 (1,7%), HPV 53 (1,4%), HPV 73 (1,4%), HPV 81 (0,8%) HPV 11 (0,3%), HPV 61 (0,3%), HPV 70 (0,3%).

In the samples characterized by the combination of different HPV, the virus mostly present with other genotypes was HPV 16 (15%), HPV 31 (5,3%), HPV 18 (2,8%). Furthermore, in the multiple infections the HPV combinations mostly present was HPV 16 with HPV 31 (2,5%), HPV 16 with HPV 18 (2,3%) and HPV 18 with HPV 31 (0,5%) (Table 1).

TABLE 1 - HPV GENOTYPES.

HR-HPV	Overall	HPV 16	HPV 31	HPV 18	HPV 56	HPV 58	HPV 39	HPV 45	HPV 33
	171 *(47,4%)*	88 *(24,3%)*	19 *(5,3%)*	12 *(3,3%)*	8 *(2,2%)*	8 *(2,2%)*	6 *(1,7%)*	5 *(1,4%)*	5 *(1,4%)*
LR-HPV	36 *(10%)*	HPV 6 8 *(2,2%)*	HPV 54 6 *(1,7%)*	HPV 42 6 *(1,7%)*	HPV 53 5 *(1,4%)*	HPV 73 5 *(1,4%)*	HPV 81 3 *(0,8%)*	HPV 11 1 *(0,3%)*	HPV 61 1 *(0,3%)*
Multiple infections	152 *(42,1%)*	with HPV16 54 *(15%)*	with HPV 31 19 *(5,3%)*	with HPV 18 10 *(2,8%)*	with HPV 33 10 *(2,8%)*	with HPV 45 7 *(1,9%)*	with HPV 16+18 8 *(2,2%)*	with HPV 16+31 9 *(2,5%)*	with HPV 18+31 2 *(0,5%)*
Negative HPV 2 *(0,55%)*									
Overall cases	*361*								

OR of HPV 16 infections was 17,45 (95% CI: 4,13-73,69; P <0,001); OR of HPV 18 infections was 1,98 (95% CI:0,46-8,61); OR of HPV 31 infections was 3,22 (95% CI: 0,75-13,81); OR of HPV 33 infections was 0,88 (95% CI: 0,19-3,99); OR of HPV 45 infections was 0,69 (95% CI: 0,15-3,23).

The OR of HPV infections 16, 18, 31, 33, 45 is graphically represented in Figure 1.

Conclusion

The prevalence of cervical infection with HPV varies greatly worldwide and is closely related to the corresponding risk of cervical cancer (10).

The objective of our study was to provide some cross-sectional figures on the local epidemiology of HPV infection in a population of women attending a colposcopy clinic in Catania, Eastern Sicily.

Regarding the other genotypes, a high variability is reported among studies, which could be due to geographical differences, different target populations, different methods for genotyping and random fluctuation for quite rare genotypes (11, 12).

Genotype HPV 16 was detected in 24,5% of positive samples, followed by 31, 18, 56 and 58. This is well

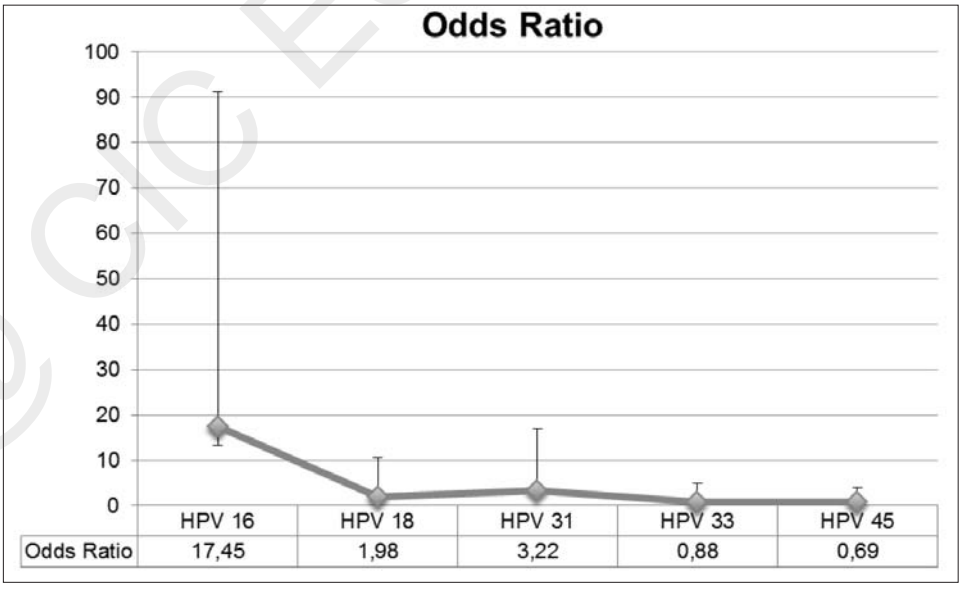

Figure 1 - The OR of HPV infections 16,18, 31, 33 , 45.

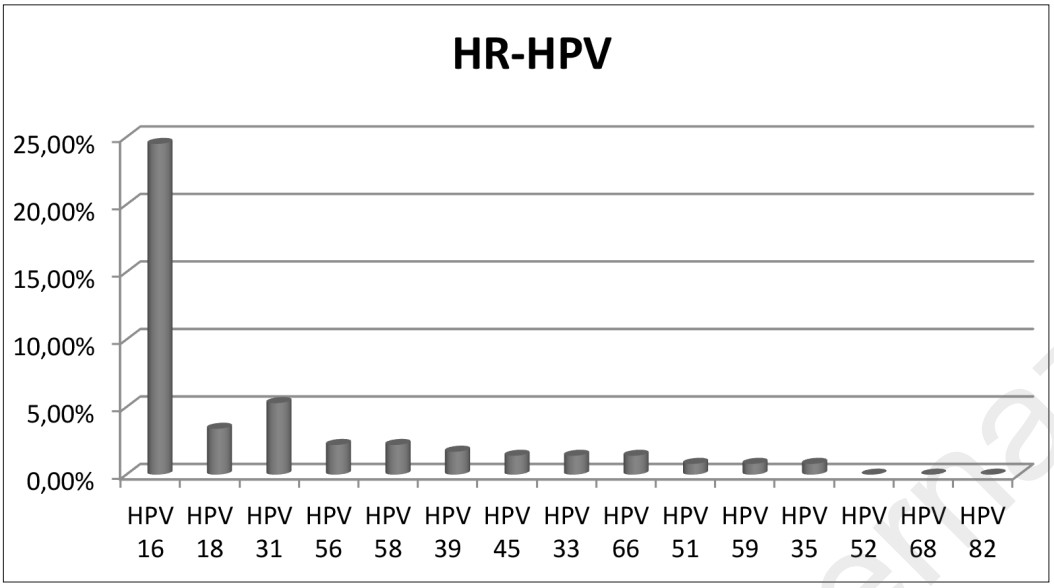

Figure 2 - Prevalence of HR-HPV.

consistent with the results of a comprehensive meta-analysis on HPV positive women with normal cytology conducted by de Sanjosè et al. (13), which reported HPV 16, 31 and 18 among the five most common types worldwide. In Italy, HPV 16 resulted to be the genotype most frequently detected in all studies and HPV 31 was frequently reported as the second most common genotype (14-17).

Unlike the rest of Europe, our study found that specimens exhibited higher rates of HPV 16 and HPV 31 infections than HPV 16 and HPV 18 infections in this area.

The prevalence of HR- HPV genotype is depicted in Figure 2.

The relative risk (RR) of developing CIN2+ lesion among HPV16-positive women compared to women positive for other HR-HPV types has been shown to be elevated (RR= 4.5) (18).

Detection of HPV16 is an useful stratifier of risk (19) for deciding the clinical management of CIN2 lesion diagnoses: HPV16-negative CIN2 diagnosed in young women with ASCUS or LSIL cytology could be managed less aggressively through increased surveillance while HPV16-positive CIN2, the most strongly linked with CIN3 must be treated. In 2012 the last terminology recommends treatment of CIN 2 if the protein p16 is positive (20).

The most common HPV genotypes detected in most previous European studies were HPV 16 and 31 and in the USA were HPV 16, 45 and 51, whereas the present study showed the most prevalent genotypes in our patients to be HPV 16, 31 and 18.

Multiple genotypes were detected in 42,4% of infected patients, the highest figure among those reported so far in Turin, 8%; in Vicenza, 3.6%; in Apulia, 26.7%; and in Palermo, 30.9% (21-24).

The finding that all multiple HPV infections contained at least 1 high-risk type, with only one exception has been already reported. Such an observation could be explained by clearing of low-risk HPV types and by persistence of high-risk HPV types. The epidemiologic and clinical relevance of multiple HPV infections still warrant further studies (25, 26).

Furthermore in our study also males partners were tested. They are often associated with the presence of HR-HPV, indicating that male sexual partners of women with cervical intraepithelial neoplasia might constitute a reservoir for HR- HPV.

Several variables have been found to be significant associated with HPV infection both positively (previous sexually transmitted diseases, number of sexual partners, number of sexual partners in the last three months) and negatively (age, onset of sexual activity), confirming the validity of the immunization strategy, mainly aimed at girls aged 12 years, before starting of sexual activity.

References

1. Pierce Campbell CM, Curado MP, Harlow SD, Soliman AS. Regional variation in histopathology-specific incidence of invasive cervical cancer among Peruvian women. Int J Gynaecol Obstet. 2012 Jan;116(1):47-51.
2. World Health Organization. World Cancer report 2014. International Agency for Research on Cancer.

http://apps.who.int/bookorders/anglais/detart1.jps?codch=275&codcol=80&codlan=1. Publihsed 2014. Accessed October 12, 2014.9

3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55(2):74-108.
4. Baio G, Capone A, Marcellusi A, Mennini FS, Favato G. Economic burden of human papillomavirus related disease in Italy. PLoS One. 2012;7(11):e49699.
5. Srivastava S, Gupta S, Roy JK. High prevalence of oncogenic HPV-16 in cervical smears of asymptomatic women of eastern Uttar Pradesh, India: A population-based study. J Biosci. 2012;37(1):63-72.
6. Sankaranarayanan R, Bhatla N, Gravitt PE, Basu P, Esmy PO, Ashrafunnessa KS, et al. Human papillomavirus infection and cervical cancer prevention in India, Bangladesh, Sri Lanka and Nepal. Vaccine. 2008;26(Suppl 12):M43-52. doi: 10.1016/j.vaccine.2008.05.005.
7. Priebe AM. 2012 cervical cancer screening guidelines and the future role of HPV testing. Clinical obstetrics and gynecology. 2013;56(1):44-50.
8. Castle PE, de Sanjose S, Qiao YL, Belinson JL, Lazcano-Ponce E, Kinney W. Introduction of human papillomavirus DNA screening in the world: 15 years of experience. Vaccine. 2012;30 Suppl 5:F117-122.
9. Herrero R. Human papillomavirus (HPV) vaccines: limited cross-protection against additional HPV types. The Journal of infectious diseases. 2009;199(7):919-22.
10. Clifford GM, Gallus S, Herrero R, Muñoz N, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet. 2005;366:991-998.
11. Agodi A, Barchitta M, La Rosa N, Cipresso R, Guarnaccia M, Caruso M. Human papillomavirus infection. Low-risk and high risk genotypes in women in Catania, Sicily. Int J Gynecol Cancer. 2009;19:1094-1098.
12. Piana A, Sotgiu G, Castiglia P, Pischedda S, Cocuzza C, Capobianco G. Prevalence and type distribution of human papillomavirus infection in women from North Sardinia, Italy. BMC Publ Health. 2011;11:785.
13. De Sanjosè S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007;7:453-459.
14. Confortini M, Carozzi F, Zappa M, Ventura L, Iossa A, Cariaggi P. Human papillomavirus infection and risk factors in a cohort of Tuscan women aged 18–24: results at recruitment. BMC Infect Dis. 2010;10:157.
15. Agarossi A, Ferrazzi E, Parazzini F, Perno CF, Ghisoni L. Prevalence and type distribution of high-risk human papillomavirus infection in women undergoing voluntary cervical cancer screening in Italy.J Med Virol. 2009;81:529-535.
16. De Francesco MA, Gargiulo F, Schreiber C, Ciravolo G, Salinaro F, Manca N. Detection and genotyping of human papillomavirus in cervical samples from Italian patients. J Med Virol. 2005;75:588-592.
17. Mark H. Stoler, Thomas C. Wright, Jr, Abha Sharma, Raymond Apple, Karen Gutekunst, Teresa L. Wright, and the ATHENA (Addressing THE Need for Advanced HPV Diagnostics) HPV Study Group. High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. Am J Clin Pathol. 2011;135:468-475.
18. Castle PE, Sideri M, Jeronimo J, Solomon D, Schiffman M. Risk assessment to guide the prevention of cervical cancer. Am J Obstet Gynecol. 2007 Oct;197(4):356.
19. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC; Members of LAST Project Work Groups. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Arch Pathol Lab Med. 2012;136(10):1266-97.
20. Broccolo F, Chiari S, Piana A, Castiglia P, Dell'Anna T, Garcia-Parra R. Prevalence and viral load of oncogenic human papilloma virus types associated with cervical carcinoma in a population of North Italy. J Med Virol. 2009;83:278-287.
21. Del Prete R, Di Taranto AM, Lipsi MR, et al. Prevalence and genotypes identification of human papillomavirus infection in a population of South Italy. J Clin Virol. 2008;42:211-214.
22. Ronco G, Ghisetti V, Segnan N, et al. Prevalence of human papillomavirus infection in women in Turin, Italy. Eur J Cancer. 2005;41:297-305.
23. Capra G, Giovannelli L, Bellavia C, et al. HPV genotype prevalence in cytologically abnormal cervical samples from women living in south Italy. Virus Res. 2008;133:195-200.
24. Rassu M, Bertoloni G, Mengoli C, et al. HPV genotype prevalence in cervical specimens with abnormal cytology: a report from north-east Italy. Scand J Infect Dis. 2005;37:476-481.
25. Cuschieri KS, Cubie HA, Whitley MW, et al. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. J Clin Pathol. 2004;57:68-72.
26. Stevens MP, Tabrizi SN, Quinny MA, et al. Human papillomavirus genotype prevalence in cervical biopsies from women diagnosed with cervical intraepithelial neoplasia or cervical cancer in Melbourne, Australia. Int J Gynecol Cancer. 2006;16:1017-1024.