Multiple HPV infection as possible marker of neoplastic progression

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Abstract

Background : Some studies in the literature suggest a possible role of multiple HPV infections as a prognostic factor in the development and progression of cervical neoplasia. we studied the cases of single and multiple HPV infection and analyzed the correlation with negative cases, and preneoplastic and neoplastic lesions of the uterine cervix with the aim of making a contribution to the prognostic factor under discussion

Methods: 921 women with clinical HPV manifestations were enrolled. Inclusion criteria were: positive at the cytology for HPV lesions, presence of preneoplastic and neoplastic lesions of the uterine cervix diagnosed by the histology examination All the patients underwent colposcopy and cervical biopsy with viral genotyping. The search for viral DNA was carried out using polymerase chain reaction. Genotype 16 is correlated with the majority of CIN2+; we divided the multiple HPV16 infections into “infections with 16 as the first genotype” (16mHPV) (e.g. HPV 16 , 31, 52) and into “ infections containing 16” (m16HPV) (e.g. HPV 31, 16 , 52), we then divided them based on the number of genotypes present: infections with 2 strains, 3 strains, 4 strains, and > 4 strains.

Results: We analyzed the differences between single and multiple infections with HPV16, the patients with single infections had a higher incidence of CIN2+ (83.3%) with respect to those with multiple infections (71.4%). The 16mHPV infection was significative for CIN2/CIN3. When the prevalence of the combinations between the genotypes was studied, we found that in 16mHPV infection HPV16, 18 and HPV 16, 31 were the most common combinations of mHPV infection (50%) and the most frequent in CIN2/CIN3 The 16mHPV infection with 2 genotypes, with respect to the infections with 3 or more genotypes, was significative with an OR= 7.94 (IC% 2.55-24.73).

Conclusions: Our results suggest that single HPV infections give a higher risk of SCC development with respect to multiple HPV infections. Among multiple infections, only 16mHPV infection with 2 genotypes is associated with CIN2/CIN3 in a significative way and it presents an 8 times greater risk of developing a high grade lesion.

Background

Human papillomavirus (HPV) infection of the cervix is a sexually transmitted disease and a significant risk factor for the development of Cervical Intraepithelial Neoplasia (CIN)[1]. However, only a small percentage of women with the infection develop CIN2+ (CIN2, CIN3, SCC). There are various risk factors that lead to cervical carcinoma: the viral genotype, age, viral persistence, and the woman’s immune state. All these factors have been well demonstrated by various authors, however, there has recently been a dispute concerning another element: multiple papillomavirus type infections (mHPV Infections) that have been recognized as a risk factor for cervical carcinoma[2].

Multiple papillomavirus types infections (mHPV) have been described most often in young women, in patients with cytological anomalies and in HIV positive patients [3]. The prevalence of the various viral

genotypes shows considerable differences in the various continents [4] and the increase in international migratory flows will inevitably lead to a continuous and constant increase in the diversification of the viral genotypes present in our country.

The clinical, virological and epidemiological significance of mHPV is still unclear: some studies in the literature suggest a possible role in the development and progression of cervical neoplasia, while other studies [5] show how the risk of development of precancerous lesions and invasive tumors in women with more than one type of HPV is no higher than that of women with only a single type infection. However, because of the difficulty in attributing the lesion to a particular HPV genotype, it has not yet been demonstrated if the association is given by the simple added risk or by a synergic interaction between the various types of HPV [6]. The studies of Liaw [7] are important as they associate HPV16 infection with an increased risk of acquiring multiple infections, while the study of Chaturvedi [8] reports that the A9 group made up of genotypes 16-31-33-35-52 and 58 is significantly less involved in multiple infections with respect to the genotypes belonging to other groups; it appears that the HPV types involved in multiple infections do not follow a particular logic of combination.

In the light of these considerations, we studied the cases of single and multiple HPV infection and analyzed the correlation with negative cases, and preneoplastic and neoplastic lesions of the uterine cervix with the aim of making a contribution to the prognostic factor under discussion.

Methods

From January 2014 to November 2017, at the out-patients clinic of Colposcopy and Cervical-Vaginal Pathology at the University of Catania, 921 women with correlated clinical HPV manifestations were enrolled. The data had been gathered in a database at the HPV Center and Colposcopy center of the University Hospital of Catania, Italy

The study protocol was approved by the Institutional Review Board of the Department and was conducted in accordance with the 1975 Declaration of Helsinki.

Inclusion criteria were the following:

- Positive at the cytology for HPV-correlated lesions
- Presence of preneoplastic and neoplastic lesions of the uterine cervix diagnosed by the histology examination

Women who were pregnant, immuno-depressed or with infections caused by the human immunodeficiency virus (HIV-positive), were not enrolled.

Patients' age was in the range 16 - 59 years old, mean 32 ± 8.3 years.

All the patients underwent colposcopy and cervical biopsy with viral genotyping. Histologic evaluation was performed on specimens collected by colposcopy directed biopsy and/or cone specimens collected

by the loop excision procedure. The histology were diagnosed according to the WHO classification as CIN2+ for all the cases of CIN2, CIN3 and SCC lesions.

In agreement with the LAST terminology, we treated CIN as simple viral lesions and we considered only CIN2+ lesions as true preneoplastic lesions.

The search for viral DNA (HPV-test) was carried out using polymerase chain reaction (PCR) after the extraction of the cervical sample using Thin-prep. The automated DNA extraction was carried out on the Nucli Senseasy MAG system (bioMérieux SA, Marcy l'Etoile, France) following the manufacturer's HPV 1.1 protocol. Amplification of HPV DNA was accomplished by HPV-HS Bio (AB Analitica s.r.l, Padova, Italy) nested-PCR for the detection of HPV-DNA sequences within the L1 ORF, according to the manufacturer's recommendations. HPV typing was carried out with specific probes for the most frequent HPV-types (HPV-type, AB Analitica s.r.l., Padova, Italy). HPV-typing identifies 11 LR-genotypes (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81) and 18 HR-genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53,56, 58, 59, 66, 68, 73, 82). Samples that were positive by nested-PCR but negative in reverse line blot for any of these types were considered as undetermined HPV. The cervical swab for the HPV test was taken from the endocervical canal and the transformation zone. In multiple infections, the order in which the various genotypes that are isolated are expressed depends on the power with which they manifest: the first genotype has a "strong" expression and is followed by the other genotypes with weaker expression. Genotype 16 is correlated with the majority of CIN2+; we therefore divided the multiple HPV16 infections into "infections with 16 as the first genotype" (16mHPV) (e.g. HPV 16, 31, 52) and into " infections containing 16 HPV" (m16HPV) (e.g. HPV 31, 16, 52), we then divided them based on the number of genotypes present: infections with 2 strains, 3 strains, 4 strains, and > 4 strains.

Statistical Analysis

The statistical analysis of the data was made with the software SPSS 15.0 package (SPSS Inc.; Chicago, IL, USA). The analysis of the data was made using the $\chi 2$ test and, when appropriate, Fisher's exact test was used to calculate the significance (p value) of the difference between the groups. Contingency tables were made to evaluate the correlation between single, multiple or viral genotype infections. Values with $p < 0.05$ were considered statistically significative.

Results

Viral genotyping gave a positive result for infections by a single HPV genotype for 363 patients (48 %), 394 patients (52 %) were, instead, positive for multiple infections, 127 women (13.7 %) were negative for the viral genotypes present in the kit that was used (tab.1). Thirty-six samples analyzed, from all the samples (3.9 %), were inadequate; these patients and negative women were excluded from the study group.

At the histologic examination, carried out after the biopsy, 475/758 patients (62.6 %) presented one low-grade precancerous lesion (CIN 1); 63 women (8.3 %) had one moderate-grade lesion (CIN 2), 100 women

(13.2%) one sever-grade lesion (CIN 3); 5 women (0.6%) squamous carcinomas (SCC). Finally, 113 (14,9%) patients had a negative histological diagnosis (Table 1).

1. Single HPV infection

The single infections in our study group were 363(48%). The typology of lesion with the highest percentage of single infection was SCC with 100%, followed by CIN3 with 84%, CIN2 with 60,3% and finally negatives and CIN1 respectivelly with 49,5% and 37,8%.

The most frequent genotype of single infections was genotype 16, present in 153/363cases (42.1%), followed by genotype 31 with 29/363 cases (7.9%) then genotype 18 with 13 cases (3.6%), and finally genotypes 33 and 45, both with 7 cases (1.9%).

These genotypes, all at high risk, are responsible for 97.6% (82/84 cases) of CIN3, 89.4% (34/38 cases) of the cases of CIN2, 61.8% (73/118 cases) of the cases of CIN1 and we found 16/32 cases (50%) negative at histology (Table 2). Genotype 16 was responsible for 85.7 % of the cases of CIN3, genotype 31 for 6.56 %, genotypes 18 and 35 for 2.63%, and genotypes 45 and 81 for 1.3 % of the cases.

In the present study the patients with one single HPV 16 infection had a higher incidence (85, 3%) of CIN2+ with respect to those with other genotypes (17, 8%). In particular, the presence of the HPV16 genotype in our study was associated with a 10 times greater risk of developing a high grade lesion (CIN2 +) (OR=10.04; IC95%=5.66-17.83). Thus, also in our clinical study, genotype 16 was at the highest oncogenic risk.

2. Multiple HPV infections

There were 394 (52%) multiple infections in our study. The typology of the lesion with the highest percentage of multiple infections was CIN1 with a frequency of 62.1%, and negative cases were 50,5%. The percentage decreases decisively in CIN2 (39.7%) and progressively in CIN3 (16%), reaching zero in carcinoma exclusively characterized by single infections (Fig.1).

There were 17 high risk (HR) genotypes that were isolated 666 times (Table 3), and 19 at low risk (data not shown).

We calculated the incidence of each genotype for histologic lesion, the four most diffused HPV genotypes were HPV 16, 51, 59 and 31 (Table 4), also in multiple types infections the most common genotype was HPV 16 , which was found 150 times, and was significatively higher in patients with CIN2/CIN3, 71.4% (OR=4,85; IC 95%=2.39-9,78).

We analyzed the differences between single and multiple types infections with HPV16, the patients with single infections had a higher incidence of CIN2+ (83.3%) with respect to those with multiple infections (71.4%).

The ORs of the single infections of HPV 16 associated with CIN2+ were more than those of HPV16 multiple infections (Table 5).

We studied genotype 16 in particular and we divided the infections into “16mHPV infection”, the cases in which genotype 16 was the first of the strains present (e.g.: 16, 31, 52), and in “m16HPV infection”, the cases in which genotype 16 was among the strains that made up the infection (e.g: 52, 16, 31).

There were 100 women (66,6%) with 16mHPV infections, in 64 cases we had an infection with 2 genotypes, in 19 cases with 3 genotypes, in 13 cases with 4 genotypes and in 4 cases with 5 genotypes (Table 6).

In the 50 cases (33.3%) of m16HPV infection we had: 11 cases of infection with 2 genotypes, 32 cases with 3 genotypes, 4 cases with 4 genotypes and 5 cases with 5 genotypes.

We then correlated genotype 16 with the number of strains and histological diagnosis (Table 7).

In the 16mHPV group we had 12 cases of CIN3, 13 cases of CIN2, 65 cases of CIN1 and 10 negative cases.

In the m16HPV group we had 3 cases of CIN3, 2 cases of CIN2, 35 cases of CIN1, and 10 negative cases. Only in this group, 1 CIN3 was caused by an infection with 2 genotypes, the other lesions were caused by 3 or more genotypes.

We calculated the OR of the 16mHPV infection with respect to m16HPV for CIN2/CIN3, 16mHPV infection was significative ($p<0.05$) with an OR=3 (IC% 1.07-8.39). (Table 8)

When the prevalence of the combinations between the genotypes was studied, we found that in 16mHPV infection HPV16, 18 and HPV 16, 31 were the most common combinations of multiple types infection (50%) and the most frequent in CIN2/CIN3. Genotype 16 was present in 94% of the cases of CIN3 (15/16) of multiple types infections and they were all lesions with two genotypes.

From the analysis of the OR, 16mHPV infection with 2 genotypes, with respect to the infections with 3 or more genotypes, was significative with an OR= 7.94 (IC% 2.55-24.73) (Table 8).

Discussion

To date, only a few studies have reported a relationship between mHPV infections and SCC, and the results have not been conclusive. Some studies have suggested a possible role of mHPV in the development of SCC [8, 9, 10], while other studies have reported that the risk of SCC in women infected by mHPV was not greater than that with single type infections [11], [12].

Fife KH [10] found an average of three different types of HPV in cervical dysplasia, with respect to a single type of HPV in samples with normal cytology, supporting a possible role of multiple HPV types infections in the development of cervical dysplasia; Becker [13] studied the presence of multiple

papillomavirus infections in immune-competent women, demonstrating that these infections are associated with a greater risk of dysplasia, second only to positivity for HPV16. It has also been hypothesized that various types of HPV act co-operatively in neoplastic transformations. Rousseau [14] on the other hand, suggested that the persistence of papillomavirus infection can be independent from the presence of more viral genotypes.

Rolòn [15] did not find a significatively higher risk of carcinoma in women with multiple HPV types infections with respect to those affected by a single viral genotype.

The patients affected by the HIV virus often present multiple infections, with percentages that are quite different from those reported for the general population (up to 80%) [16], suggesting a possible role of systemic immunodeficiency as a determining factor for the development of multiple papilloma virus types infections, which would facilitate acquisition.

In immune-competent women the prevalence of mHPV varies from 24.8% to 52.6% among all the HPV positive women [17].

In our study, the prevalence of multiple infections was 52%, in particular, 62.1% in patients with CIN 1, up to 18% in those with CIN 3; the greatest prevalence of multiple infections was in the group of patients with a negative histologic exam (62%).

These data seem to confirm that multiple infections are not correlated with the grade of dysplasia. In fact, coinfections are relevant only in the early stages of the lesion (CIN 1 and CIN2), while they are absent in carcinoma where there are only single lesions.

From the pathogenetic point of view, the data seem to suggest the hypothesis [18] that different cells are infected with different viruses, rather than that in the same cell there co-exist various viral genotypes. In HSIL and invasive tumors, a monoclonal line develops from one infected cell with a single virus at high risk, with the consequence of finding few multiple infections in high-grade lesions and only single infections in carcinoma [19]. Therefore, a persistent infection from a high-risk genotype is surrounded by cells with transitory HPV infection.

Conclusion

Our results suggest that single HPV type infections give a higher risk of SCC development with respect to multiple HPV types infections. Multiple HPV types infections are relevant only in the early stages of the lesion (CIN1- CIN2), while they are absent in carcinoma, where the lesions are only a single type. Also in our study the genotype 16 is the one with the highest oncogenic risk, therefore genotyping is important, to study HPV 16 positive women [20].

In particular, among multiple infections, 16mHPV infection with 2 genotypes is associated with CIN2/CIN3 (21/30) in a significative way and it presents an 8 times greater risk of developing a high grade lesion, OR= 7.94 (IC% 2.55-24.73).

Thus, it is probable that only specific combinations of HPV (HPV16,18 - HPV 16,31) can be associated with a significative clinical impact, while other combinations can simply be correlated because of the route of common infection or the diagnostic method used. Thus, 16mHPV infection with two high-risk genotypes is at greater risk of CIN2/CIN3.

The limits of our study are linked to the sample study, the women selected came from second level center.

Further clinical studies are necessary to determine the mechanism of these mHPV infections and the clinical results correlated with a particular combination of HPV types.

Abbreviations

HPV= Human Papillomavirus;

CIN2+= all the cases of CIN3, SCC lesion;

hrHPV= high riskHPV;

SCC= Squamous Cervical Carcinoma

mHPV= multiple HPV infection

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on

request.

Authors' contributions

MTB designed the study; CN and SB collected the data; MTB and SB drafted the manuscript; SG compiled the statistical data. All authors were involved in editing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the Department and was conducted in accordance with the 1975 Declaration of Helsinki. We do not have informed consent because we conducted a retrospective study. We designed the protocol for the analysis of information according to the guidelines of the Declaration of Helsinki ensuring respect for all human beings and protect their health, their individual rights and confidentiality of personal information. This analysis protocol information was submitted for review and was approved.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

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Figures

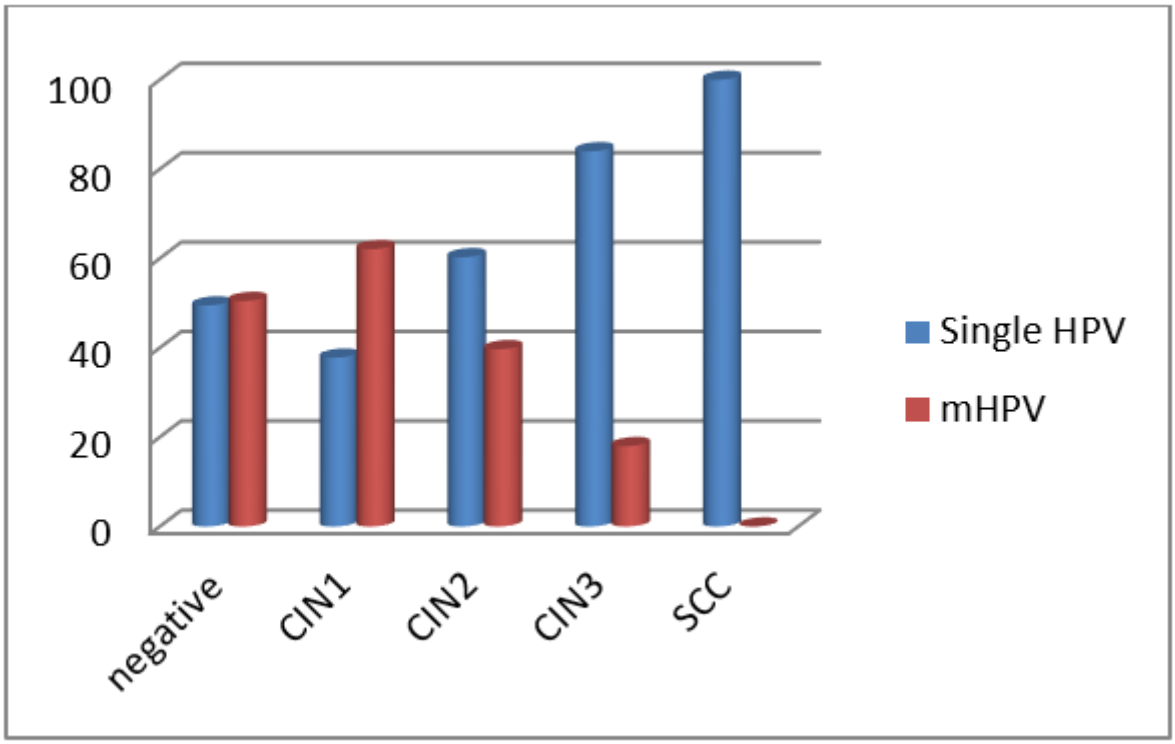

Figure 1

Percentage of lesion grades for multiple and single infection

Supplementary Files

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