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RESEARCH

Human Papillomavirus Types Distribution in Eastern Sicilian Females with cervical lesions. A Correlation with Colposcopic and Histological Findings

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Abstract To determine human papillomavirus (HPV) types distribution in cervical lesions in a Southern Italian female population in Messina and their relationship between HPV type and grade of colposcopic and histopathological abnormality, a total of 253 women aged 17–68 years, with previous cytological abnormalities, were included in this study. HPV-DNA testing, colposcopy and biopsy were performed. For each sample, cervical cells were collected by centrifugation and DNA was extracted, followed by a PCR-based HPV-DNA assay and reverse dot blot genotyping. HPV-16 was found the most common type (46.6 %) followed by HPV-31 (26.9 %), −6 (18.6 %), −58 (8.8 %), −18 (6.7 %), −66 (5.7 %), −52 and −53 (4.7 %). Out of 62 women with abnormal transformation zone (ATZ) area compatible with squamous intraepithelial lesion (SIL) or cervical cancer (CC), 64.5 % was found high risk (HR) HPV-positive. Moreover the severity of the colposcopic diagnosis was positively correlated with the higher HPV oncogenicity risk (HPV-16 P=0.023; and HPV-53 P=0.047). The HPV-16 was found the most prevalent type within each histological category: 66.7 %, 31.2 %, 44 % and 37.2 % of CC, high grade (H)SIL, low grade (L)SIL and chronic cervicitis respectively; followed by HPV-31 present in 25 %, 8 %, and 13.3 % of HSIL, LSIL and chronic cervicitis respectively. A higher HPV incidence than the rest of Italy was found, in agreement with that detected by other authors for the South of the country. These data provide further information about the types prevalence in women with cervical lesions living in Eastern Sicily, suggesting the introduction of new targeted vaccines against a wider spectrum of HPV.

Keywords Human papillomavirus · Genotypes · Distribution · Colposcopy · Cervical lesion

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Introduction

Because of development of human papillomavirus (HPV)-type-specific prophylactic vaccines, many studies have assessed the prevalence of HPV infection and its subtypes in different populations to provide more information regarding variations in the HPV type spectrum according to geographical region, and the association to cervical lesions, in order to apply the correct diagnostic, prophylactic and therapeutic measures in each area.

In Italy, despite the incidence of cervical cancer (CC) together with the Pap-test coverage is slightly lower in the South than in the Centre and the North, the high risk (HR)-HPV prevalence detected for the South is higher than the rest of the country [1].

The different overall HPV prevalence found, is depending on selection criteria of study population, asymptomatic recruited into screening program [2–4], or based on the presence of abnormal cytology or cervical lesions [5–12], and on different HPV DNA detection methods [13–16].

The objective of this study was to determine the prevalence of HPV infection and its types distribution in a selected Southern Italian female population in Messina, who referred to the Outpatient Clinic of cervical cancer prevention and colposcopy of our Department, because of cytological abnormalities at the Pap test.

Moreover, another objective was to establish a relationship between HPV types and colposcopic and histopathological findings.

Methods

The patients included in this study came from a health area of the province of Messina (Eastern Sicily region, Southern Italy), and referred to Outpatient Clinic of cervical cancer prevention and colposcopy of our Department for routine cervical screening between January and December 2009. Based on the presence of cytological abnormalities, after verbal informed consent was obtained, a cervical smear for HPV DNA testing, was performed. From 4,528 women evaluated, 253 women aged between 15 and 69 years, who underwent for the first time HPV-DNA testing were enrolled and subdivided in four age groups: ≤24 years; 25–34 years; 35–44 years; ≥45 years.

Out of 253 patients, 182 underwent colposcopy and cervical biopsy . The main inclusion criterion was the presence of cytological alterations in the Pap smears according to Bethesda classification. The collected data were the patient age, HPV types, colposcopy and biopsy diagnosis.

Colposcopic examination of the cervix was performed by experienced colposcopists according to routine practice at Italian Society of Colposcopy and Cervico-Vaginal Pathology (SICPCV). Lesions were assessed by applying a 5 % acetic acid and iodine solution under 8×12 magnification. If colposcopy proved unsatisfactory, further exploration of the endocervix was carried out under 20× magnification using a Koogan speculum. The colposcopic findings were classified as follows : normal transformation zone (NTZ), abnormal transformation zone of low grade (ATZG1) with minor changes (regular mosaic and acetowhite thin epithelium); abnormal transformation zone of high grade (ATZG2) with major changes (irregular mosaic and acetowhite thick epithelium).

From 182 cervigrams a targeted biopsy was obtained. Biopsy specimens were formalin fixed and further paraffin embedded by means of manufacture's recommendation. All biopsies were examined as part of the daily routine in the Human Pathology Department.

The histological diagnosis was classified in four categories as follows: chronic cervicitis (inflammatory/reactive lesions), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and invasive squamous (CC).

A total of 253 cervical samples were examined in the laboratory of molecular biology at the Human Pathology Department. Details of DNA isolation and HPV genotyping employed have been previously described [17].

Briefly from each sample the cervical cells were collected by centrifugation and DNA was extracted (QIAamp DNA mini kit (Qiagen GmbH, Germany)); subsequently a PCR-based HPV DNA assay and reverse dot blot genotyping (HPV-HS Bio plus HPV-strip or HPV-type, AB Analitica, Padova, Italy) were performed allowing the identification of 11 types (HPV-6, −11, −40, −42, −43, −44, −54, −61, −70, −72, −81) considered low-oncogenic risk (LR) HPV and 18 types (HPV-16, −18, −26, −31, −33, −35, −39, −45, −51, −52, −53, −56, −58, −59, −66, −68, −73, −82) considered definitive or probable high-oncogenic risk (HR) HPV types. In some of the cases yielding unidentifiable results by line blot genotyping, the corresponding HPV DNA was sequenced by BMR Genomics (Padova, http://www.bmr-genomics.it).

A descriptive analysis of the variables was firstly carried out; the percentages were calculated for the qualitative variables and means and standard deviations for the numerical variables. Later, in order to assess the association between categorical variables (such as colposcopic and histological findings, HPV types, age classes) we estimated the Pearson Chi Square test and the relative significance. If the observation number per cell was <5, the Fisher's exact test was applied. The non parametric Spearman correlation test was applied in order to assess the existence of any significant interdependence between numerical parameters. Statistical analyses were performed using SPSS 11.0 for Window package. $P<0.05$ was considered to be statistically significant.

Results

A total of 253 samples for HPV typing were included in this study. The mean age at enrollment was 34.03±10.27 years. HPV type distribution in cervical samples is shown in Table 1; 193 patients (76.2 %) were positive for HPV DNA and 55 (21.7 %) were negative. Out of positive cases (n=193), 105 (54.4 %) were infected with a single HPV type, and 88 (45.5 %) were infected with multiple types. The majority of

Table 1 HPV type distribution in cervical samples detected by PCR in 253 women in Messina, oriental Sicily, Italy

HPV detection	N°	% study women[a]	% infected women[a]
Positive	193		
Negative	55		
Unidentifiable	5	2	2.6
Single infections	105	41.5	54.4
Multiple infections	88	34.7	45.6
2 types	62	24.5	32.1
>2 types	26	10.53	13.5
HR type specific			
16	90	35.6	46.6
18	13	5.1	6.7
26	2	0.8	1
31	52	20.5	26.9
33	7	2.8	3.6
35	1	0.4	0.5
39	7	2.8	3.6
45	5	2	2.6
51	3	1.2	1.5
52	9	3.5	4.7
53	9	3.5	4.7
56	7	2.8	3.6
58	17	6.7	8.8
59	7	2.8	3.6
66	11	4.3	5.7
68	1	0.4	0.5
73	6	2.4	3.1
82	2	0.8	1
LR type specific			
6	36	14.2	18.6
11	7	2.8	3.6
40	3	1.2	1.5
42	5	2	2.6
43	5	2	2.6
44	1	0.4	0.5
54	5	2	2.6
61	1	0.4	0.5
62	1	0.4	0.5
70	2	0.8	1
72	2	0.8	1
81	7	2.8	3.6

HPV human papilloma virus, *HR* high risk, *LR* low risk

[a] Due to multiple HPV infection, the overall percentage of HPV types exceeds 100 %

multiple infection was caused by 2 types (32.1 %); three or more types was only obtained in 26 (13.5 %) of total positive cases. Considering also the multiple infection cases, HPV-16 was the most common type (90/193, 46.6 %) followed by HPV-31 (52/193, 26.9 %), −6 (36/193, 18.6 %), −58 (8.8 %), −18 (6.7 %), −66 (5.7 %), −52 and −53 (4.7 %). The other genotypes identified (HPV-11, −40, −42, −43, −44, −54, −61, −62, −70, −72, −81, −26, −33, −35, −39, −45, −56, −59, −68, −73, −82) were below 4 %. Only 5 cases (2.6 %) were caused by an unclassified HPV type.

HPV prevalence (any type) was 20.7 % at age ≤24 years, 32.1 % at 25–34 years, 22.8 % at 35–44 years and 14.5 % at age ≥45 years. With respect to relationship between the patients'age and the HPV presence or not, or the HPV type, statistical significant differences in the age ranges were not observed (Pearson chi square=8.686, p=0.069). Association between HPV-DNA detection and the younger age was not encountered.

Out of 182 cervigrams, a NTZ was present in 20 patients (11 %), an ATZG1 was diagnosed in 147 women (80.8 %), an ATZG2 was shown in 15 cases (8.2 %). The histological findings obtained were: 113 chronic cervicitis (62 %), 50 LSIL (27.5 %), 16 HSIL (8.8 %) and 3 CC (1.6 %). There was a positive correlation between colposcopic and histological finding that was statistically significant in the more severe diagnosis (P<0.001).

HPV genotyping results were initially grouped according to HPV risk group and diagnostic category. We found 162 ATZ areas compatible with HPV-negativity in 44 cases (27.2 %) and with oncogenic HPV-positivity in 118 cases (72.8 %). The rate of HR HPV infection was highest in ATZG2 cases (46.6 %); a significant correlation was found between ATZG2 and HR HPV-16 (P=0.023) and −53 (P=0.047); only one case of ATZG2 was negative for HPV infection.

ATZG1 correlated positively with the presence of LR HPV- 42 (P=0.036) and −70 (P=0.041); NTZ with LR HPV-70 (P=0.04).

The mean age of patients with NTZ, ATZG1, ATZG2 was 35.4±11.2 (range 17–54), 33.7±10.7 (range 17–68), 40.1±11.1 (range19–63) years, respectively.

Distribution of 182 cases according to HPV risk group and histological diagnosis is shown in Table 2; HPV infection was present in the 76.1 %, 72 %, 68.7 %, and 88.8 % of chronic cervicitis, LSIL, HSIL and CC cases respectively. Two or more HPV types (up to six types in a single case) were identified in 61/182 (33.5 %) cases studied, with the highest percentage in chronic cervicitis category (38.9 %).

Considering multiple infections, in total 218 individual HPV types were identified in the 182 biopted cases. The HR HPV types were the dominant group in both HSIL (68.7 %) and CC (66.7 %) cases when all individual HPV types were included (considering the single highest risk HPV type in case of multiple infection), as shown in Table 3. HR HPV types were more frequent in LSIL cases as well (66 %). Out of three CC cases, two were positive for HPV-16 and in one of them there was a coinfection with HPV-53 with a significant correlation (P=0.007).

Table 2 Distribution of HPV infection in 182 women with known histological diagnosis, according to HPV risk group

HPV category	Histological category				
	Chronic cervitis N[a] (%)	LSIL N(%)	HSIL N(%)	CC N(%)	Total HPV N(%)
HR	32 (28.3)	20 (40)	7 (43.7)	1 (33.3)	60 (33)
LR	8 (7.1)	2 (4)	1 (6.2)	0	11 (6)
Unidentifiable	2 (1.8)	1 (2)	0	0	3 (1.6)
Multiple	44 (38.9)	13 (26)	3 (18.7)	1 (33.3)	(33.5)
Positive	86 (76.1 %)	36 (72)	11 (68.7)	2 (88.8)	135 (74.2)
Negative	27(23.9)	14 (28)	5 (31.2)	1 (33.3)	47 (25.8)
Total cases	113 (100)[b]	50 (100)	16 (100)	3 (100)	182 (100)

HPV human papilloma virus, *HR* high risk, *LR* low risk, *LSIL* low grade squamous intraepithelial lesion, *HSIL* high grade squamous intraepithelial lesion, *CC* cervical cancer

[a] *N* number of cases in each histology diagnostic category according to the risk HPV group identified. The percentage is calculated down the row with the total number of cases used as the denominator

[b] Percent is calculated on the total number of cases in the column

The distribution patterns of HPV types in the intraepithelial lesions was highly varied. HSIL cases showed HPV-16 and −31 as the most frequent HPV types present (31.2 and 25 % respectively) followed by HPV-53 and −73 (6.2 % each).

The most frequent HPV type found in LSIL cases were HPV-16 (44 %) and HPV-31 (8 %), followed by HPV-18 (6 %), HPV-35, −45, −58 and −66 (2 %). A significant correlation was found between LSIL diagnosis and HR HPV-18 (P=0.03). Chronic cervicitis cases harbored HPV-

Table 3 Distribution of HPV types in 182 women with known histological diagnosis, according to the single highest risk HPV type identified

HPV	Chronic cervitis N[a] (%)	LSIL N(%)	HSIL N(%)	CC N(%)	Total HPV N(%)
HR-types	75(66.4)	33(66)	11(68.7)	2(66.7)	121(66.5)
16	42 (37.2)	22 (44)	5 (31.2)	2 (66.7)	71 (39)
18	3 (2.6)	3 (6)	0	0	6 (3.3)
31	15 (13.3)	4 (8)	4 (25)	0	23 (12.6)
33	1 (0.9)	0	0	0	1(0.5)
35	0	1 (2)	0	0	1 (0.5)
39	4 (3.5)	0	0	0	4 (2.2)
45	0	1(2)	0	0	1(0.5)
51	1 (0.9)	0	0	0	1 (0.5)
52	3 (2.6)	0	0	0	3 (1.6)
53	1 (0.9)	0	1 (6.2)	0	2(1.1)
56	3 (2.6)	0	0	0	3 (1.6)
58	1 (0.9)	1(2)	0	0	2(1.1)
66	1 (0.9)	1(2)	0	0	2(1.1)
73	0	0	1(6.2)	0	1(0.5)
LR-types	9 (8)	2(4)	0	0	11(6)
6	4 (3.5)	1(2)	0	0	5 (2.7)
42	2 (1.8)	0	0	0	2 (1.1)
43	0	1 (2)	0	0	1 (0.5)
54	2 (1.8)	0	0	0	2 (1.1)
81	1(0.9)	0	0	0	1 (0.5)
Unidentifiable	2 (1.8)	1 (2)	0	0	3 (1.6)
Negative	27 (23.9)	14 (28)	5(31.2)	1(33.3)	47 (25.8)
Total cases	113 (100)[b]	50 (100)	16 (100)	3 (100)	182 (100)

HPV human papilloma virus, *HR* high risk, *LR* low risk, *LSIL* low grade squamous intraepithelial lesion, *HSIL* high grade squamous intraepithelial lesion, *CC* cervical cancer

[a] *N* number of cases in each diagnostic category sorted according to the single highest risk HPV type identified. The percentage is calculated down the row with the total number of cases used as the denominator

[b] Percent is calculated on the total number of cases in the column

16 as the most frequent type as well (37.2 %), followed by HPV-31 (13.3 %) and −6 (3.5 %).

The mean age of each histological category chronic cervicitis, LSIL, HSIL and CC was: 34.6±9.9 (range 17–66), 32.6±11 (range 17–68), 35.4±9.2 (18–54), 51.6±14 (range 36–63) years respectively.

We found 62 ATZ compatible with SIL/CC in 19 HPV-negative women (30.6 %) and in 40 HR HPV-positive women (64.5 %) (see Table 4).

Discussion

The overall HPV prevalence found in women with cervical lesions living in Eastern Sicily, was 77.8 %, in agreement with the higher HPV incidence detected for the South Italy than the rest of the country by other authors [2–6, 12, 13, 18].

We found, as described in the medical international [14, 19, 20] and Italian [6–9, 11, 13, 19] literature, the HPV-16 as the most common type (46.6 %) followed by HPV-31 (26.9 %), −6 (18.6 %), −58 (8.8 %), −18 (6.7 %), −66 (5.7 %), −52 and −53 (4.7 %).

However, our data show that while the prevalence of HPV-18 was only of 6.7 %, HPV-31 represented the second common HR genotype, showing a prevalence of 26.9 %, similar to that described worldwide [19] and in a Northern Italian population [12], but higher than that encountered in the rest of Italy [5–7, 11, 12], because of the possible interference on detection procedures of high intra-type variation described for some genotypes as HPV-31 and the different pattern of geographical distribution of the virus [11, 20].

Table 4 Crosstabulations for HPV risk group and pathological colposcopic, histological findings

HPV	N[a]	Colposcopy		Histology
		ATZG1 N	ATZG2 N	
Negative	13	12	1	LSIL
LR	2	2	0	LSIL
HR	28	25	3	LSIL
Unidentifiable	1	1	0	LSIL
Negative	5	5	0	HSIL
HR	10	7	3	HSIL
Negative	1	1	0	CC
HR	2	0	2	CC
Total	62	53	9	

HPV human papilloma virus, *HR* high risk, *LR* low risk, *ATZG1* abnormal trasformation zone of grade 1, *ATZG2* abnormal trasformation zone of grade 2, *LSIL* low grade squamous intraepithelial lesion, *HSIL* high grade squamous intraepithelial lesion, *CC* cervical cancer

[a] *N* number of cases

No statistical significant differences were found between the patients'age classes and the HPV presence or not, or the HPV type; but the distribution of colposcopic and histological findings in relation to age has shown that the severity of the lesion is positively correlated with the age. As reported by other authors, the higher grade of colposcopic and histopathological abnormalities (ATZG2 corresponding to HSIL or CC) peaked in women aged ≥35 years, who otherwise were also the highest risk group, with the 40.1 % of HR HPV- positivity [7, 21]. Such differences may be ascribed to the sociodemographic and sexual behaviour typical of participants or to a reactivation of a latent HPV infection [6]. The positive correlation between colposcopy and histology, statistically significant in the more severe diagnosis, confirm that the identification of an acetowhite lesion is a highly sensitive indicator for the subsequent identification of HSIL, but because its low specificity, patient management cannot be based on colposcopic impression unless confirmed by biopsy [22–24]. Although the 3 CC cases were compatible with ATZ, none of them were identified at colposcopy, because not visible, which may explain in part the paucity of atypical cells detected on the Pap tests and the finding that the presenting cytology, although abnormal, was never diagnostic of cancer. On the other hand a low rate of women with NTZ or ATZG1 (5 % and 22.6 % respectively) was identified as HSIL at biopsy, suggesting that some lesions were missed or underestimated colposcopically [25].

In agreement with other authors, in our study as shown in Table 4, out of 62 acetowhite areas compatible with SIL/CC, 64.5 % were found in oncogenic HPV-positive women [1, 5–11, 18, 26].

Moreover the severity of the colposcopic diagnosis was positively correlated with the HPV oncogenicity risk. The HPV-negative status found in 30.6 % of women with an acetowhite area compatible with SIL/CC may be due to the presence of focal lesions, not present in the material analysed [24, 27, 28], or to the presence of low copy of HPV DNA [29]. Another explanation could be that the HPV sample was likely taken far from the acetowhite epithelium; a false-negative enrollment could be also related to the location of the junction between the squamous epithelium of the ectocervix and the columnar epithelium of the endocervical canal [30, 31]; thus, the dynamic age-related changes in cervix influence HPV type–specific detection at the cervical os, where cervical specimens are routinely collected [30].

On the other hand, the presence of HPV infection in the 76.1 % of women without dysplastic lesions (chronic cervicitis), although in some cases it could be due to either sampling/interpretation errors or an occult lesion at the time of colposcopy [32], is another relevant aspect in order to avoid that healthy women without dysplastic atypical changes in the cervical epithelium could be considered ill, only based on positive HPV typing.

As previously reported by other authors, the HR HPV types continued to be the dominant group in both HSIL and CC [1, 6–12, 18–20, 33]. Other studies focused on the distribution of HPV types in women with cervical lesions; in the majority of them, HPV-16 and −18 cause 70–80 % of lesions, but the distribution patterns of HPV types in the intraepithelial lesions is highly varied [18]. In agreement with international [22] and Italian [2, 7–13] literature, HPV-16 was found to be the most prevalent type within each histological category: 66.7 % in CC, 31.2 % in HSIL, 44 % in LSIL, 37.2 % in chronic cervicitis; followed by HPV-31 present in 25 % of HSIL, in 8 % of LSIL and in 13.3 % of chronic cervicitis cases (see Table 3).

HR HPV types were more frequent in LSIL and cervicitis cases as well, when multiple infection was considered (66 and 66.4 % respectively), meaning that HPV could be present in cervical epithelial cells without produce atypical changes [34], although the risk for the development of HSIL and cancer appears to be related to HR HPV status [19].

Interestingly, HR HPV-53 was present in 6.2 % of HSIL cases and positively correlated with CC diagnosis (P= 0.007), whereas HPV-18, absent in CC and HSIL cases, was found in the 6 % of LSIL with a significant correlation (P=0.03) and in the 2.6 % of chronic cervicitis category.

One limitation of our study is the small size of the HSIL and CC cases examined.

Nevertheless, these data on the prevalence and distribution of HPV types in the Eastern Sicilian population and its association with cervical lesions may provide information regarding regional variations in the HPV type spectrum, at dawn of the introduction of HPV vaccination, that could prevent, in many countries, 70–80 % of HPV infections underlying HSIL or CC [15]. More epidemiological studies are necessary to know the distribution of certain HPV types in Sicily to take into account in the future production of vaccines targeting the HPV types which predominate in different geographical areas.

There are conflicting opinions about the predictive value of HPV testing in women with ASC-US; although HPV DNA detection as a triage test for ASC-US has been shown to predict CIN2 [35], according to Dane et al. its low positive predictive value, suggests that HPV-positivity could not be used for predicting the presence of CIN 2 to 3 [36]. The role of colposcopy in the prevention of cervical cancer continues to evolve. As the threshold for abnormal screening results has shifted now to detection of persistent oncogenic HPV in the presence of normal cytology, the task of identifying increasingly subtle preinvasive lesions has become more difficult [22]. In Italy, colposcopy is a more diffused diagnostic approach (like in Spain, Brazil, France and Swiitzerland) than in countries with Anglo-Saxon influence; it is a potential effective screening method, that adds to the risk stratification system based upon cytology and HPV status a further value as an independent risk stratifier [37].

Protocols have varied, considering what to do when the HPV test has been positive and the cytological result normal; HPV testing should be avoided, however, among women younger than 35 years, due to the high probability of positivity for HPV infection [38, 39]. Maybe guidelines for younger women entering into screening protocols need to be re-evaluated.

A risk-based approach should be adopted to guide clinical management, independent of current (e.g., cytology, carcinogenic HPV testing, and colposcopy) and future methods of measuring risk (e.g., HPV genotyping and p16 immunocytochemistry), to decrease costs and avoid to harm patients without benefit [40].

Conflict of Interest We declare that we have no conflict of interest.

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