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The Variability of Cervical Human Papillomavirus is not Clinically Important

KEYWORDS: Human papillomavirus, Cervical infections, HPV variability, Brazilian study

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ABSTRACT *Human genital tract harbors approximately 40 papillomavirus types that vary in prevalence and pathogenicity. The goal of the present study was to trace the spectrum of uncommon HPV types in Rio de Janeiro State according to cytological status. A meta-analysis study included 891 women recorded in five Brazilian studies. The overall HPV prevalence was 32.8%. Thirty-three HPV genotypes have been identified. HPV 16 was the prevalent type, found in 27.7% of infected people. The types 26, 35, 39, 51, s82 (high risk), 40, 42, 44, 54, 70, LX100 (low risk), and 32, 62, 69, 83, and 84 (undetermined), were found in a low frequency and, except HPV 39, were not associated with abnormal cytology. Their predominance in young women, the fact that they have been more easily found in co-infections and the unlikely link with risk for cervical cancer allow labeling them as opportunistic infections of little clinical importance.*

INTRODUCTION

Human genital tract harbors approximately 40 types of papillomavirus, included in the Alphapapillomaviridae genera (Bernard et al., 2010). Some types are considered oncogenic due to their association with high grade cervical lesions, while others types are found in benign lesions. Recently some types were redefined as established high risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), probably high risk (26, 53, 66, 68, 73, 82) and undetermined risk (30, 32, 34, 62, 67, 69, 71, 74, 83, 84, cand 85, 86, 87, 90, 91) due to poor epidemiological confirmation (Munõz et al., 2006). The most common oncogenic types are included in A9 and A7 species, while HPV related to rare or undefined carcinogenic outcome are distributed in the remaining genders (Schiffman et al., 2009). The oncogenic HPV 16 is the most prevalent type regardless the cytology result (Weller and Stamberry, 2007).

The aim of this study was to demonstrate that HPV circulating in a low prevalence in Rio de Janeiro state act as opportunistic infections not associated with high grade cervical lesions but they are important not only to record the diversity of HPV types in this area, but also identified them from the pathogenic types not covered by the anti-HPV vaccines.

MATERIAL AND METHODS

Patients

A cross-sectional study population included 891 women whose cervical smears have been taken from 2000 to 2010 to investigate HPV infection. Women included in this work had at least one sexual partner in their lifetimes. Pregnancy and hysterectomy were excluding factors. No women were HPV vaccinated.

Cervical samples

Cervical samples were collected after clinical examination. Two cervical specimens were taken from each participant using a cervical brush. The first sample was spread onto a glass slide to be processed for Papanicolaou staining. The remaining specimen were placed in Tris EDTA buffer and stored at -20°C. According to the Bethesda nomenclature, cervical smears were classified as normal/inflammatory, atypical squamous cells of undetermined significance/ atypical glandular cells of undetermined significance (ASCUS/AGUS), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), and cervical cancer (CA) (Solomon et al., 2002).

HPV detection

DNA was extracted from the cervical samples with phenol-chloroform-isoamyl alcohol. HPV DNA was detected by polymerase chain reaction (PCR) amplification using MY09/11 consensus primers. HPV typing was performed by restriction fragment length polymorphism analysis (RFLP). The resultant 450 base pair PCR products were digested by six restriction enzymes (BamHI, DdeI, HaeIII, HinfI, PstI, RsaI; Invitrogen, São Paulo, Brazil). The RFLP pattern of each sample was compared with the RFLP patterns for mucosal HPV (Bernard et al., 1994). HPV types were clustered according to biological and phylogenetic criteria (de Villiers et al., 2004). Specimens were classified as high risk if they had at least one high risk HPV type, and low risk if they had only low risk HPV types.

Statistical analysis

A data bank was generated using the Epi Info package. Statistical analyses were performed for laboratory diagnosis and HPV infection.

RESULTS

Socio-demographic aspects of the studied population revealed that the patients aged 14-79 years old. While 75.5% of women presented normal cytology, the remaining of them showed ASCUS/AGUS (3.1%), LSIL (10.9%), HSIL (6.7%), CA (3.3%) and inconclusive cytological diagnosis (0.4%). The overall HPV prevalence was 32.8%. The HPV frequency among each cytological category was 19% (normal), 35.7% (ASCUS) , 80.4% (LSIL), 78.3% (HSIL), and 96.6% (CA).

A wide spectrum of HPV genotypes was found in the samples, totalizing 33 different HPV types. Sixteen types were found in prevalence rates lower than 1.5%: 26, 32, 35, 39, 40, 42, 44, 51, 54, 62, 69, 70, subtype 82, 83, 84, and LX100 among the 329 infections (Table 1 and 2).

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Table 1. HPV distribution of 16 high and probably risk genotypes in the Rio de Janeiro state

High risk				Probably high risk			
Species	Types	Na	%	Species	Types	Na	%
A5				A5	26	1	0.3
	51	1	0.3		S82	2	0.6
					53	17	5.1
A6	56	6	1.8	A6	66	11	3.3
A7	18	21	6.3				
	39	3	0.9				
	45	14	4.2				
	59	5	1.5				
A9	16	81	24.3				
	31	18	5.4				
	33	12	3.6				
	35	2	0.6				
	52	13	3.9				
	58	13	3.9				
Total		189	56.7			31	9.3

Na: Number of infections, in single or multiple infections.

Table 2. HPV distribution of 17 low and undeterminable risk genotypes in the Rio de Janeiro State. Na: Number of infections, in single or multiple infections. bUnique low risk type into A7 group.

Low risk				Undetermined			
Species	Types	Na	%	Species	Types	Na	%
A1	42	3	0.9	A1	32	2	0.6
A3	61	9	2.7	A3	62	3	0.9
	81	5	1.5		83	4	1.2
A7	70b	2	0.6		84	4	1.2
A8	40	2	0.6	A5	69	2	0.6
A10	6	30	9.0	A9	67	8	2.4
	11	14	4.2	A15	71	13	3.9
	44	1	0.3				
A13	54	4	1.2				
A15	LXV100	2	0.6				
		73	21.9			36	10.8

HPV 16 was the most prevalent type (24.3%) and highly associated with premalignant and malignant lesions. Out of 15 cases of HPV 18 single infections, 11 were present in severe lesions. None of these types, except the oncogenic HPV 39, was found in pre-malignant or malignant infection. In single infections, high-risk and probable high-risk HPV 51 and 26 as well as low-risk HPV 40, 83 and 84 were not associated to abnormal cytology. The distribution of HPV types according to cytological results is given at Figure 1.

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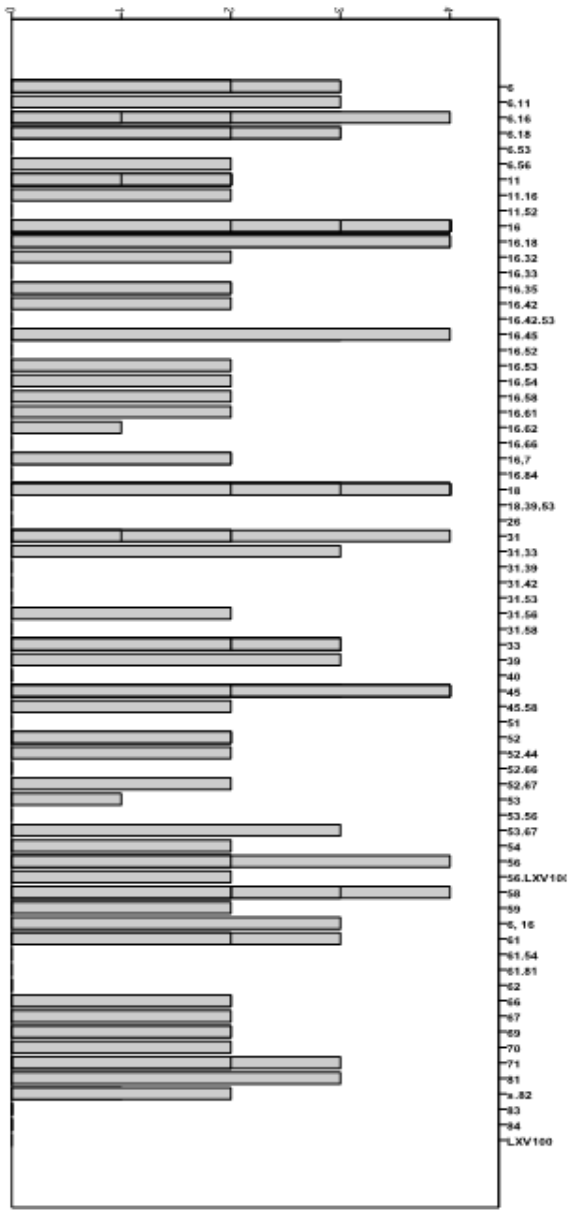

Figure 1. Distribution of HPV types in single and multiple infections according to citology from 2000 to 2010 in Rio de Janeiro State. Cytology:0 –normal/inflamatory, 1 – ASCUS/ARGUS, 2- LSIL/HPV, 3 - HSIL, 4- CA

Virus belonging to A7 and A9 species was responsible for the majority of infections, even when not considering the most prevalent type, HPV 16 (101 infections). Multiple infections were found in 19.2% of HPV positive cases. In spite of being the most frequent genotype, HPV 16 presence was greatly reduced when comparing single and multiple infections (52 versus 18), notwithstanding to maintain a high prevalence in the latter group.

DISCUSSION

Knowing the HPV types circulating in a population is important for developing prophylactic strategies. A large survey held by the Brazilian Minister of Health (2013) showed that among the large variety of HPV types in Brazil, the most common are 16, 31, 18, 6, 35, 58, 33, 11, 54 and 68 in normal cytology samples; 16, 51, 31, 18, 58, 33, 35, 39, 56 and 59 in low grade lesions; and 16,11, 58, 18, 31, 6, 33, 45, 66 and 35 in high grade lesions. With minor changes, our results are in agreement with these official data.

With regard the variability of infections, the types 26, 35, 39, 51, s82 (high risk), 40, 42, 44, 54, 70, LX100 (low risk), and 32, 62, 69, 83, and 84 (undetermined) were found in a low frequency, usually not associated with severe dysplasia, regardless the recent categorizing of types according to their oncogenic potential (Munõz et al., 2006). The fact that most of the uncommon types were found in women under 24 years old strengthened the idea that these variants are involved in transient infections. It is important to emphasize that the highest frequency of HPV 16 was observed in women between 45-54 years old, suggesting a persistent infection.

Akcaly et al. (2013) reported 35, 40, 42, 54, 62, 82, and 84 types in frequencies up to 0.5%. A study with Brazilian HIV infected people reinforced our findings: high risk 26, 35, 39, 51and 82 were no found or they were present in a low prevalency in cervix, vagina or anal site (Gonçalves et al., 2008). If we compare the frequency and the pathogenic potential between HPV 16 and these infrequent types we can see relevant differences such as the lack of association with severe cervical lesions, their predominance in young women, and the fact that they have been more easily found in co-infections. So, we can believe that these types are not only responsible by very few infections, but they have clinically minor importance. We speculate if they are circulating in a short time period, if they have less ability for transmission or cell adaptation or if they depend on host immunogenic factors.

CONCLUSION

This paper provides evidences for linking HPV variability, uncommon genotypes and their improbable risk for cervical cancer development. We have in mind that such cross sectional study is limited, with the results reflecting only a current status of the samples; however some types tend to be more persistent than others, particularly the oncogenic types. The establishment of these infections, regardless to be classified in low or high risk, may be useful to focus HPV types that effectively can cause damage to cervical tract in a particular geographic area. We hope this issue should be helpful to vaccination strategies.

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