HUMAN PAPILLOMAVIRUS VARIABILITY IN THE FEDERAL DISTRICT - CENTRAL BRAZIL

ABSTRACT

Human papillomaviruses (HPVs) are a heterogeneous group of viruses that have been consistently associated with cervical lesions. We review here the HPV genetic variability, based on data from the Federal District, Central Brazil. To define the prevalence of HPV types and variants in our region, 129 cervicovaginal scrape specimens from women whose cytological tests showed either pre-neoplastic or neoplastic lesions were screened. The MY09/MY11 L1 region of these samples was amplified and HPV types were defined by RFLP. HPV DNA prevalence in the Federal District was about 62%. A high diversity of genotypes was observed, including HPVs -16 (49.2%), -58 (13.4%), -31 (11.9%), -53 (6.0%), -18 and -33 (4.5% each), and each of HPVs -35, -52, -66, -CP8304, -6, -11, and -CP8061 in frequencies £ 3.0%. Some isolates that could not be typed by RFLP were sequenced, leading to the identification of one new variant of HPV-58 (Bsb-02), two of HPV-53 (Bsb-63 and -61), and one of HPV-66 (Bsb-68). The distribution of HPV-16 variants was also analyzed by the sequence diversity of E6 and L1 regions from 34 isolates. The most prevalent HPV-16 variant in the Federal District was the European (50%), followed by Asian-American (41.2%), African-1 (5.9%), and African-2 (2.9%). European and non-European variants appeared in equal frequencies among the cytological types of lesions. In countries such as Brazil, where HPV prevalence is high and ethnicity, as well as socio-demographic characteristics, varies according to different regions, HPV variability must be wider and has not yet been clearly defined.

INTRODUCTION

Evidence from experimental and epidemiological studies has shown a consistent association between human papillomavirus (HPV) infection and both cervical cancer and pre-cancerous neoplasias (Lehtinen et al. 2001). The existence of more than 100 different HPV genotypes has been previously reported (Villa 2003). All of these genotypes are specifically associated with the development of benign and in some cases malignant lesions of epithelial cells from the skin and mucosa. Mucosal HPVs, consisting of about 30 genotypes, are divided into low-risk (HPVs -6, -11, -40, -42, -43, -44, -54, -
61, -70, -72, -81, and -CP6108) and high-risk (HPVs -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -68, -73, and -82), according to their oncogenic potential (García-Carrancá 2003, Villa 2003).

Developing countries have both higher rates of invasive cervical cancer (ICC) and poorer survival of ICC patients than developed countries. Over 80% of deaths from ICC occur in developing countries, most notably in Latin America, India, and Sub-Saharan Africa (Pisani et al. 1999). In Brazil, for 2003, the frequency of deaths and expected new cases of cervical cancer were 4,110 and 16,480 respectively. In the Federal District the mortality and incidence rates expected for 2003 were 4.43/100,000 women and 27.95/100,000 women, respectively (Brasil 2003).

**HPV genetic variability.**

The specie Human papillomavirus is classified in the *Papillomavirus* genus of the *Papillomaviridae* family (van Regenmortel et al. 2000). The distribution of HPVs in genotypes is based on the analysis of nucleotide sequences from L1, E6, and E7 open reading frames (ORFs). Each type must have a similarity lower than 90% to the closest related type. Within the same type, isolates differing by 2-10% are considered subtypes. Moreover, a viral DNA having more than 98% nucleotide similarity to any other genotype in those ORFs is defined as a papillomavirus variant (Stewart et al. 1996, Yamada et al. 1997).

The role of intratyp HPV variants in determining the risk for developing HPV-associated disease is becoming an active area of epidemiologic investigation. HPV protein sequence variation may affect virus assembly, carcinogenic potential and host immunologic response (Yamada et al. 1997). Sequence variation within HPVs may be related to host immune response, persistence, or risk for cervical lesions and may also be relevant to the generation of rational vaccine strategies (Wheeler et al. 1997). The possibility that these naturally occurring variants have different biological and biochemical properties and thus differ in pathogenicity has attracted the interest of the scientific community. This has lead to an intense research effort to improve understanding of this virus group diversity, so that diagnosis, treatment, and control of HPV infections may be optimized (Ong et al. 1994).

Reports on the prevalence of HPV genotypes indicate that HPV-16 is the most prevalent worldwide (Muñoz 2000). HPV-16 variants are clustered in 5 groups: African-1 (Af-1), African-2 (Af-2), Asian (As), Asian-American (AA), and European (E) (Stewart et al. 1996, Yamada et al. 1997). The frequency of other HPV genotypes and variants may vary according to geographic, demographic, and clinical-pathological factors, as well as to the methods used for characterization (Meyer et al. 1998, Lo et al. 2001, Camara et al. 2003).

**HPV genetic variability in the Federal District.**

It is still not known whether immunity to one HPV genotype can protect against infection with another. Identification of HPV genotypes may thus prove important to the rational design of diagnostic, therapeutic, and vaccine strategies (Stewart et al. 1996, Wheeler et al. 1997). However, one difficulty in developing such strategies is to determine which types to include, since geographical variations related to their prevalences are observed (Villa 2003). In this paper, we report the prevalence of the different HPV genotypes in women with pre-neoplastic and neoplastic lesions of the cervix from the Federal District (Camara et al. 2003) and the frequency of HPV-16 variants in these cervical specimens (Cruz et al. 2004). The presence of five isolates with uncharacterized HPVs allowed the further description of new HPV's 53, 58, and 66 variants (Cerqueira et al. 2003).

In order to study HPV genetic variability in the Federal District, epithelial cells were collected from the cervical surface of 129 outpatients from October 1998 to August 2001. These women, aged 17 to 73 (mean 37; median 35), reported first intercourses at the age of 12 to 30 years (mean 17.8; median 17.0) and periods of 2 to 59 years (mean 16.2; median 14) of sexually active life. Their number of sexual partners in the last five years ranged from 0 to 10 (mean 2.0; median 1.0). When considering information about sexually transmitted diseases (STDs), informed by 110 women, 70% answered negatively to any diagnosis of STD before the last Pap test and 30% answered positively. In the referred STD group, 54.5% had a previous diagnosis of HPV in cytological screening. Regarding smoking habits, 70.9% were nonsmokers and 29.1% reported that they have smoked 3 to 20 cigarettes a day. All patients studied had a cytological report of cervical intraepithelial neoplasia (CIN) grade 1, 2 or 3, squamous cell carcinoma (SCC), adenocarcinoma (ADENO), atypical squamous or
glandular cells of undetermined significance (ASCUS or AGCUS) or cytological alterations suggesting HPV infection (HPV). These cytologically diagnosed cervical lesions could be grouped according to severity as group 1 (G1), including CIN 2, CIN 3, SCC, and ADENO or group 2 (G2), including ASCUS, CIN 1, HPV, and AGCUS. In our sampling, G1 lesions were more frequent (73.6%) than G2 ones (26.4%) (Camara et al. 2003).

For the detection of HPV-DNA, the L1 MY09/MY11 consensus primers were used. Positive samples were typed by restriction fragment length polymorphism (RFLP) as previously described (Bernard et al. 1994).

The overall HPV DNA positivity in the Federal District was 62% (n = 80). This was lower than that previously reported by some authors: 76% (Rabelo-Santos et al. 2003), 78.5% (Lo et al. 2001), 86.2% (Lai et al. 1999), and 97% (Muñoz 2000), but higher than in González-Losa et al. (2004) (56.4%), Chan et al. (1999) (44.3%), and Riethmüller et al. (1999) (37.6%). Separately, we found an HPV prevalence of 66.3% in G1 and of 50% in G2 (OR = 1.97; 95% CI = 0.82-4.74; p = 0.09). In a study of Brazilian women with cervical lesions, Cavalcanti et al. (2000) have demonstrated HPV prevalences ranging from 85.6% in low-grade squamous intraepithelial lesions (LSIL) to 55.2% in SCC. In Germany, Meyer et al. (1998) reported HPV prevalence of 74% in LSIL patients and of 88% in those with high-grade squamous intraepithelial lesions (HSIL), indicating a prevalence rate of 1.2 (HSIL/LSIL). Although we could not consider as significant the association of DNA HPV positivity in G1 and G2 groups of lesions, our prevalence rate (1.3) was similar to that reported by Chan et al. (1999).

HPV-16 was the most prevalent (49.2%), followed by HPV58 (13.4%), -31 (11.9%), -53 (6%), -18 and -33 (4.5% each). Other genotypes (HPVs -35, -52, -66, -CP8304, -6, -11, and -CP8061) were less frequent (⩽ 3% each) (Camara et al. 2003). A study conducted in Goiânia, which is also located in Central Brazil, reported a high prevalence (57.1%) of HPV-16 among women with CIN 3 and invasive cervical cancer (Rabelo-Santos et al. 2003).

Although HPV-16 seems to be the most prevalent in all Brazilian regions, considerable variation can be observed when considering the other types. In the Federal District HPV-58 was the second most prevalent. In a study conducted by González-Losa et al. (2004) in Mexican women, HPV-58 was the most prevalent type and was found in 28.5% of all HPt positive women. In an evaluation of HPV infection at the United States-Mexico border, HPV-16 was the most common type. The second most prevalent were HPV-58, considering the Mexican population, and HPV-18 in the American one (Giuliano et al. 2001). Interestingly, a study in Spain found that 75% of women who were positive for type 58 had been born in Latin America (Touze et al. 2001). HPV-58 was also the second most common genotype in Japan (Sasagawa et al. 2001) and in China (Chan et al. 1999), with a significant trend to increase prevalence according to the increasing severity of lesions. In East Asia, Lai et al. (1999) questioned if HPV-58 may be partially responsible for cervical cancer in the older population and urged further investigation on the natural history of HPV-58-related cervical neoplasias. In Paraguay, HPV-58 was detected in 2.7% of cervical carcinomas (Rolón et al. 2000), showing that different areas in South America may have significant variations in the prevalence of HPV types. However, the studied population and the methods used for virus detection and typing may also vary among these studies.

The high prevalence of HPVs -58 and -31 showed by our data seems to be in accordance with what has been described in the North and Northeast regions of Brazil (Noronha et al. 1999, Lorenzato et al. 2000). Lorenzato et al. (2000) reported a HPV-58 prevalence of 8.2% among women with cervical lesions compatible with HPV infection in Recife. Noronha et al. (1999) observed that HPVs -31, -33, and -58 represented 21.2% of the types identified in individuals with CIN 2 or 3. The prevalence of HPVs -18 (4.5%) and -31 (11.9%) differed from that reported by Bosch et al. (1995) and Rolón et al. (2000) who cited prevalence of 14% and 5%, and 10% and 3.5%, respectively.

Following the HPV classification by phylogenetic tree (LANL 1997), the 11 genotypes isolated in the Federal District were classified in the super group A (mucosal/genital). A significant association between group A9, which includes HPVs -16, -31, -33, -52, and -58, and G1 was observed (OR = 6.39, 95% IC 1.15-37.45; p = 0.009).

**HPV variants in the Federal District.**
A total of 34 samples identified as HPV-16 were tested for variants classification, which were identified by automated sequencing of the MY09/
MY11 L1 region and of the entire E6 gene. The variant designation for the Federal District isolates followed the classification of Wheeler et al. (1997). Here, the most prevalent HPV-16 variant was the European (50%), followed by Asian-American (41.2%), African-1 (5.9%), and African-2 (2.9%). Among the European, only one E-350G variant was detected. HPV-16 non-European and European variants were present at similar frequencies among the cytological types of lesions, revealing a uniform distribution (Cruz et al. 2004).

HPV-16 European variants were the most prevalent in the Federal District (50%). Villa et al. (2000) detected European variants in 54% of the patients in São Paulo. However, in our study, the percentage of patients positive for Asian-American variants (41.2%) was higher than that reported by other authors. Villa et al. (2000) found Asian-American variants in 22% of the patients and Yamada et al. (1997), in an international study, detected Asian-American variants in 19.7% of the patients living in Central and South America.

Among the specimens analyzed by RFLP, five isolates, denominated Bsb-02, -08, -61, -63, and -68, showed atypical restriction fragment length profiles. They were further characterized by sequencing the MY09/MY11 region of the L1 ORF and classified as new HPV variants (Cerqueira et al. 2003).

The Bsb-02 and Bsb-08 isolates had 98% and 97% similarity to HPV-58 reference sequence, respectively. Isolates Bsb-61 and Bsb-63 showed 98% similarity to HPV-53 and the isolate Bsb-68 had 98% similarity to HPV-66. In these five isolates, intratype pairwise evolutionary distance varied from 2.05% to 1.26%, indicating that all might be variants of the respective HPV reference sequences. Although isolate Bsb-08 was 100% similar to HPV-58 IS417, to our knowledge there is no previous report on occurrence of this virus variant in Brazil, which was originally described in Mali. Isolate IS404, which was the closest in nucleotide sequence to Bsb-02, was also firstly described in Mali (Stewart et al. 1996).

Despite the predominance of HPV variants in certain continents, these are not restricted to a specific geographical area and the occurrence of close relationships among Brazilian HPV isolates and those from Mali can be explained by the extensive African immigration to Brazil during the colonization of the American Continent (Cerqueira et al. 2003).

Nucleotide sequences from these HPV variants identified in isolates from the Federal District have been submitted to GenBank and accession numbers have been assigned as follows: AY101597 to Bsb-68; AY101598 to Bsb-02; AY098920 to Bsb-08; AF516877 to Bsb-61; and AF16876 to Bsb-63.

In conclusion, we could mention that in Brazil HPV variability must be wider and not yet totally understood. More studies should be conducted in order to better define the prevalence of HPV genotypes in distinct geographical areas.

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