

Multiple Types of Human Papillomavirus in Cervical Samples in Women in Campo Grande, MS, Brazil

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We analyzed 87 cervical samples from Campo Grande, Mato Grosso do Sul, with a PGMY/GP⁺ nested PCR system. Positive samples were typed using E7 type-specific primer pairs for HPV 6/11, 16, 18, 45 and 66. Eighteen samples (22%) were infected with HPV6/11, 18 samples (22%) with HPV66, 13 samples (15.9%) with HPV45, 8 samples (9.8%) with HPV18 and 7 samples (8.5%) with HPV16. Seventeen samples (20.7%) were infected by two HPV types, and five samples (6.1%) by three HPV types. We conclude that infection with multiple types is present at a high frequency in our population and that there is a relation between some types and cytological finds.

Key Words: Human papillomavirus, multiple types, nested PCR, TS-PCR.

Several molecular techniques have been used to detect HPV in cervical samples, including Hybrid Capture II (HCII) assay and the Polymerase Chain Reaction (PCR). The most common primers used in PCR to detect genital HPV are PGMY09/11 and GP5⁺/6⁺ [1-3]. Fuessel Haws et al. found that PGMY/GP⁺ nested PCR detected more HPV types than did MY/GP⁺ nested PCR [4].

The determination of the HPV types may be done by several methods including Type-Specific PCR (TS-PCR) and Restriction Fragment Length Polymorphisms (RFLP). The latter relies on distinct patterns of amplicon digestion with seven restriction enzymes. However, these patterns may be difficult to discriminate, especially when clinical specimens contain two or more HPV types. Alternatively, PCR with type-specific primers targeting E6 and/or E7 non-conserved regions is a highly sensitive method that is easy to interpret and allows characterization of infections by multiple types [5-7]. Using E7-type specific-primer pairs for HPV 6/11, 16, 18, 45 and 66, we studied samples tested by a PGMY/GP⁺ nested-PCR system to precisely characterize HPV types and to identify multiply-infected patients.

Eighty-seven samples were collected from women, attended at the Centro de Prevenção ao Câncer, Campo Grande, Mato Grosso do Sul, Brazil. All patients had clinical suspicion on HPV infection and came from public health service or medical clinics. Cytological diagnoses were reported using

the 2001 Bethesda System [8]. The positive products from the PGMY/GP⁺ nested PCR system were amplified using the techniques described by Walboomers et al. [9]; type-specific HPV primers were used, which amplify approximately 100bp in the E7 open reading frame of HPV 6/11, 16, 18, 45 and 66. Statistical analyses were performed using the Einfo software, version 3.2, 2004 (CDC, Atlanta, Georgia).

Forty-two samples were typed by TS-PCR, and the distribution of HPV types was: 18 samples (22%) were infected with HPV6/11, 18 samples (22%) with HPV66, 13 samples (15.9%) with HPV45, 8 samples (9.8%) with HPV18 and 7 samples (8.5%) with HPV16. Seventeen samples (20.7%) were infected by two HPV types, and five samples (6.1%) by three HPV types. In samples with atypical squamous cells of undetermined significance (ASCUS) cytology, a predominance of HPV 45 (71%) was observed, and in samples with low grade squamous intraepithelial lesion (LSIL) cytology there was a predominance of HPV6/11 (50%). High frequencies of HPV 16 (31.3%) and HPV 18 (25%) were observed in patients with LSIL cytology. Samples that were negative to intraepithelial lesion and malignancy (NILM) showed a high frequency of HPV6/11 (38.9%) and HPV 66 (38.8%). The sample that had high grade squamous intraepithelial lesion (HSIL) cytology was positive for HPV6/11 and HPV 66. The distribution of HPV 66 was similar among the ASCUS (43%), LSIL (44%) and NILM (39%) cytological groups.

Among the patients, 26.8% were infected with multiple HPV types, similar to previous reports. Huang et al. studied patients with cervical carcinoma in China; they found 28.9% of the tumors to be infected with multiple HPV types [10]. Munoz et al. examined cases from several countries, and 13.9% of them were infected with multiple HPV types [11]. Eluf-Neto et al. found 5.1% infection with multiple types in Brazil [12] and Levi et al. reported that 45% of the patients were infected with more than two HPV types, in a HIV-infected population from São Paulo, SP, Brazil [13]. Based on the wide range of

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these results, we suggest that geographical differences have an influence on the frequency of multiple HPV type infection.

Tawheed et al. found HPV66 in 2.5% of cervical lesions that had been diagnosed as high-grade cervical intraepithelial neoplasias, demonstrating a possible oncogenic potential of this HPV [14]. However, it has been classified as probably carcinogenic, an HPV that induces cervical intraepithelial neoplasia (CIN) I and II but rarely III [11,15]. Recently, the International Agency for Research on Cancer reassessed the carcinogenicity of HPV and classified HPV66 as carcinogenic for humans [16].

Munoz et al. reported 0.5% of the HPV66 among patients with invasive and *in situ* cervical cancer [11]; Poljak et al. reported 1.3% of this type in cervical specimens from women recognized as HPV-positive who were using an HCII high-risk cocktail [17]; Van Den Brule reported 2.7% HPV66 in specimens derived from women participating in cervical cancer screening [18]; Speich et al. reported 4.0% of this type in cervical samples of West German women [19]. There have been reports of a high frequency of this type in an HIV-infected population (9.4% and 20%) [13, 20]. However, Fuessel Haws et al. detected a higher frequency of HPV66 in samples positive for PGMY/GP⁺ nested PCR than in samples positive for MY/GP⁺ nested PCR (8.1% and 2.7% respectively) [4]. As we only typed samples that amplified for PGMY/GP⁺ nested PCR, there could have been preferential selection for this HPV type. Improvement of the PGMY system compared to MY for HPV 66 amplification could explain our results.

Van den Brule et al. reported an association of HPV16 and HPV18 with CIN I/II and III [18]. They observed that low-risk (LR-HPV) oncogenic types were predominant in samples with LSIL/NILM cytology and that HPV66 was equally present in all cytological groups [18]. We also observed a high prevalence of HPV 16 and HPV18 in patients with LSIL cytology and a predominance of LR-HPV types in patients with NILM cytology.

We conclude that infection with multiple HPV types is frequent in our population and that there is an association of some HPV types with cytological results.

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