TITLE: MOLECULAR DIAGNOSIS OF HPV: MY9/MY11 AND PGMY09/PGMY11 COMPARATIVE ANALYSIS IN WOMEN CERVICAL SAMPLES.

AUTHORS: FERRARI, I.C.; SANTOS, C. M.; FERRO, L. M. T.; SCHWAMBACH, A. F.; BRABES, K.C.S.; SOUZA, G.H.A.; RADAI, J.A.S.; SIMIONATTO, S.; NEGRÃO, F. J.

INSTITUTION: UNIVERSIDADE FEDERAL DA GRANDE DOURADOS, DOURADOS, MS (Rodovia Dourados/Itahum, Km 12 - Unidade II | Caixa Postal: 364 | CEP: 79.804-970)

The Human Papillomavirus (HPV) is a small virus double circular DNA genome belonging to the family Papillomaviridae. HPV transmission occurs mainly through sexual contact and more than 95% of infections are asymptomatic or develops benign lesions, however HPV is related to the development of 99% of cervical cancers and 50% of cancers of the penis. The HPV are commonly detected from clinical samples by consensus PCR methods. The study aim was compare the MY09-MY11 primers to the set of five PGMY11 primers in simultaneous use with the set of 13 primers PGMY09 (PGMY09-PGMY11) for the detection and genotyping of HPV infection cervical samples of women between 18 and 65 years were diagnosed with cervical cytological abnormalities, DNA extraction was done using the Wizard® Genomic DNA Purification Kit (Promega) and each sample was amplified with the two different sets MY09 / MY11 (at the 20 pmol concentration of each primer) and PGMY09-PGMY11 (at 20 pmol concentration of all primers), and as beaglobin GH20 and PC04 primers (5 pmol of each primer), the reaction conditions were 1x PCR buffer, 3 mM MgCl 2, 200 mM (each) dATP, dCTP, dGTP and dTTP and 5 U of Platinum Tag DNA Polymerase DNA polymerase (Invitrogen). Amplifications were performed in thermocycler under the following conditions 95øC for 3 min and 40 cycles of 95øC for 1 min, 55øC for 1 min and 72øC for 1 minute, followed by a final extension at 72øC for 5 min the fragments were observed on GelRed® stained 1.5% agar (pv) stained in ultraviolet light. Fifty - six samples of the uterus were collected from women during cytopathological examination (Papanicolau). The PGMY09 / PGMY11 system appeared to be significantly more sensitive than the MY09/MY11 system, detecting 21 HPV positive specimens and 15 with the MY09/MY11 system. The two multiple infections were only detected with the PGMY09 / PGMY11 system. The set of PGMY09 (five primers) and PGMY11 (thirteen primers) primers, although presenting a higher initial cost, provides an increase in amplification sensitivity when compared to the MY09/MY1 primer system, proving to be more useful in HPV typing analysis and assessment of the natural history of HPV infections.

Keywords: Molecular diagnosis, HPV, cervical samples

Acknowledgments: Universidade Federal da Grande Dourados. Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul - FUNDECT