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Title: **HPV positive cervical squamous cell carcinoma: association of tumour grade and expression of p16 with the presence and load of the human papilloma viruses of high-risk genotypes**

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Abstract

Cervical cancer is one of the most common malignancies in females worldwide. Majority of cervical squamous cell carcinomas are associated with human papilloma viruses of high risk genotypes (hrHPVs). In this study we investigated the associations between cancer grades and genotypes of infecting hrHPVs, as well as virus loads. Our study panel consisted of formalin-fixed paraffin embedded (FFPE) cervical tissues of women (n=76), median age 60 years. Tumor grades were determined independently by two pathologists. Expression of p16 was assessed immunohistochemically (ICH). IHC expression of p16 was classified according to nuclear and cytoplasmic positivity, scored as positive when more than 10 continuous cells stained positive. DNA extracted from sections

of FFPE blocks was subjected to quantitative or semiquantitative PCR detecting 14 hrHPV genotypes. Grade 1 carcinomas were diagnosed in 5 (6,6%), Grade 2 in 45 (59,2%), Grade 3 in 26 patients (34,2%). Of 76, 70 (92,1%) were p16-positive. Of these 39% were Grade 3 and 61%, Grade 2 tumors. Grade 1 tumors were p16-negative. All 76 carcinomas were positive for at least one hrHPV, mainly HPV16 (73/76, 96.1%) followed by HPV33 (25/76, 32.9%). Other genotypes were significantly less prevalent ($p<0.05$). Increase in tumor grades correlated positively with the presence of HPV16 and virus load of HPV39, and negatively, with the presence of HPV45 and presence and virus load of HPV33. High grade CSCC were characterized by positivity for p16 and high loads of HPV16 and HPV39, both showing positive correlation with tumor grade. This data suggests that hrHPV detection and quantification can serve as a useful tool supporting differentiation of tumour grades in cervical cancer.