**High risk HPV positive cervical squamous cell carcinomas: correlation of tumor grades with virus load and expression of p16, p53 and Ki67**

Karina Biserova1\*, Daira Krišāne1, Svetlana Gebrila1, Juris Jansons2,3, Dmitrijs Perminovs4, Beatrise Orlova5, Marta Petrovska5, Simons Svirskis2, Jurijs Nazarovs1, Maria Isaguliants2, Liba Sokolovska1,2

1 Pathology Institute, Pauls Stradins Clinical University Hospital, Riga, Latvia;

2 Institute of Microbiology and Virology, Riga Stradins University, Riga, Latvia

3 Biomedical Research and Study Centers, Riga, Latvia

4 E. Gulbis Laboratory Ltd, Riga, Latvia;

5 Centrala Laboratorija Ltd, Riga, Latvia;

\*Presenting author

**Introduction** Cervical cancer is the fourth most common malignancy in females. The majority of cervical squamous cell carcinomas (CSCC) are associated with high-risk HPVs (hrHPV). Here, we investigated the associations between CC grades and genotypes of infecting hrHPVs, multiplicity of infection, virus load, and expression of cell cycle and immune check-points and proliferation markers p16, p53, and Ki-67.

**Methods.** Study group consisted of formalin-fixed paraffin embedded (FFPE) cervical tissues of women (n=76), median age 60 years, with primary CSCCs collected based on the ethical permit of RSU Ethical Committee N2-PĒK-4/415/2022 from 26/09/2022. FFPE were sectioned, DNA extracted from sections (Qiagen kits), subjected to quantitative or semiquantitative hrHPV specific PCR detecting 14 hrHPV genotypes (Anyplex HPV14 and Allplex HPV14, Seegene, South Korea). Tumor grades were determined histochemically by two pathologists. Expression of p16, p53, and Ki-67 was assessed immunohistochemically (ICH) using Flex kits, Autostainer Link-instrument (Dako) equipped with Eclipse 55i camera (Nikon). Slides were scanned, and intensity of signals in IHC was quantified by ImageJ.

**Results** All 76 cancer cases 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 presence of HPV16 and virus load of HPV39, and negatively, with the presence of HPV45 and presence and virus load of HPV33 (Fig. 1A, B). All tumors tested p16(+) with varying levels of p16 expression, overall increasing with increasing tumor grade. Median % of Ki67-expressing cells was 43.5% (IQR: 25.3 – 70.0%). Percent of Ki67-expressing cells tended to increase with increasing cancer grade (median: 52 vs 56 vs 65, p>0.05), positively correlated with HPV39 (R=0,2406, p=0,0362) and negatively with HPV18 loads (R=-0,3079, p=0,0067), while intensity of Ki-67 signal increased with increasing grade. Aberrant p53 expression, detected in six CC cases (7.9%), was most often in Grade 3 (5/6) in patients older than 60 (4/6), positively correlated with cancer grade (R=0,2936, p=0,0100) and HPV18 virus load (R=0,2783, p=0,0149).

**Conclusions** High grade CSCC were characterized by high loads of HPV16 and HPV39, and high level of p16 expression, all three showing positive correlation with tumor grade. High grade CSCC were also characterized with high levels of Ki-67 expression. Aberrant patterns of p53 expression, although a rare event, were typical to hrHPV(+) CSCC of high grades. Combination of three parameters “high p16/high Ki67/aberrant p53” in IHC jointly confirmed hrHPV infection, substituting for hrHPV DNA tests.

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**Key words:** cervical squamous cell carcinoma,tumor grade,high risk human papilloma virus (HPV), genotyping, immunohistochemistry, p16, Ki-67%, p53 markers

Fig. 1 Prevalence (A) and viral load (B) of high risk HPV genotypes (HR-HPV) detected in cervical carcinomas in cohort of women (n=76) diagnosed in Paul Stradins University Hospital, Riga, Latvia in the period 2016-2023.

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