**Design and expression of HPV16 oncoproteins E6 and E7 for the dynamic analysis of the levels of specific serum antibodies in women with and without cervical lesions**

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**Background**

The humoral immune response against high risk HPVs (hrHPVs) has been suggested as a source of biomarkers for the early detection of cervical dysplasia and cancer. We aimed to characterize the dynamics of antibody response against oncoproteins E6 and E7 of HPV16 in women without and with cervical lesions, to determine its usefulness as early disease biomarker.

**Materials & Methods**

Amino acid sequences of E6 and E7 of HPV16 from Latvian patients were retrieved from GenBank (PQ215484-PQ215514). Consensus amino acid (aa) sequences were built (CLUSTAL MUSCLE algorithm). Expression optimized genes were synthesized and cloned into pET-based vector as fusion with His6-SUMO tag. Proteins were expressed in E coli, purified by Ni-column chromatography, Hus6-SUMO tag was cleaved by SUMO-specific protease Ulp1, Ulp1 was removed by affinity chromatography, and E6 and E7 were dialyzed to remove cleavage products. Women (n=82) aged 42.2±10.1 years, recruited into prospective study (Ethical Committee of Riga Stradins University (RSU) N2-PĒK-4/415/2022 dated 26/09/ 2022), visited gynecologist during up-to 2 years with 3-12 month intervals. At each, cervical smear was collected, and subjected to liquid cytology and PCR-testing for hrHPV DNA (Allplex, Seegene), both at Centrala Laboratorija (Riga, Latvia). Sera were collected at study entry and exit, and stored at -20o. Two to three repeated visits were completed by 48 healthy and 38 women with lesions and/or hrHPV infection. Study will be completed by 31/01/2025. Sera were subjected to indirect ELISA for anti-E6 and anti-E7 IgG and IgA on 96-ELISA plates (Maxisorb, Nunc) using anti-human IgA and IgG conjugate (DakoPatts) and TMB as substrate. Sera of healthy women negative for hrHPVs at entry and exit served as negative controls. Control antigens were E6 and E7 (Abcam) (positive) and BSA (negative).

**Results**

Follow-up is completed by 75 participants. Of these, 22 (25,6%) were found hrHPV(+), majority (13; 59%) with hrHPVs of alpha-9 family, HPV16 (8/22, 36%) and HPV33 (5/22, 27%). The latter two are highly homologous in E6 and E7, motivating antibody screening using HPV16 E6 and E7. Consensus HPV16 E6 and E7 aa sequences were designed based on HPV16 isolates sequenced in the current project (n=32) and from the East-European and Baltic regions (n=185). Consensuses were found to be identical to the reference HPV16 strain (NCBI: NC\_001526.4). Expression-optimized genes encoding E6 and E7 were expressed in E coli as SUMO fusions, SUMO-tag cleave with repeated affinity chromatography yielded 95% pure proteins. A trial panel of sera of women without (Controls, C) and with cervical lesions and/or hrHPV-infection was selected (Study Group, SG) and subjected to indirect ELISA on E6- and E7-coated plates. Test ELISA runs demonstrated similar performance of in-house to commercial E6 and E7. Analysis using in-house E6 and E7 revealed differences in E6 and E7 antibody responses. Significantly higher OD values in E6 IgG ELISA were observed in SG compared to C, up-to serum dilution 1:5000, in HPV16(+) and). HPV33(+) patients (alpha-9 hrHPV-positive) compared to controls (p<0.05). Furthermore, SG were significantly more likely to be E6 Ab-positive (titer >7000) than C groups. Significantly higher OD values in E7 IgG ELISA were also observed in alpha-9 hrHPV-positive SG compared to C, but only at serum dilution 1:500 (p<0.05). Alpha-9 hrHPV-positive patients had significantly higher end-point anti-E6, but not anti-E7, serum titers than controls (Fig. 2). SG and C sere behaved similarly in ELISA using BSA. Reactivity of SG and C sera in IgA-based E6 and E7 ELISA did not differ. Associations between positivity and end-point titers of anti-E6 and anti-E7 with dynamics in positivity for hrHPV DNA and development/regression of cervical lesions will be presented.

**Conclusions**

E6 and E7 of HPV16 were efficiently expressed in E. coli from synthetic genes, purified and successfully approbated for detection of the levels of anti-E6 and anti-E7 of HPV16. Sera of women without and with varying degrees of cervical lesions differed in anti-E6 IgG, but not IgA. Positivity for anti-E6 and levels of anti-E6 IgG were significantly higher in women with lesions infected with hrHPVs of alpha-9, but not other hrHPV families. Increase in the levels of anti-E6 IgG can be used to predict development of cervical lesions associated with infections with hrHPVs of alpha-9 family.

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**Figure 1.** Comparison of the end-point titers of sera of control (Cont) and hrHPV(+) study groups (Pt), positive or negative for hrHPV of alpha-9 (a9+ or a9-, respectively) in ELISA for IgG antibodies against HPV16 oncoproteins E6 and E7 (Mann-Whitney test, \* p<0,05).

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