P–40; **HUMAN PAPILLOMAVIRUS 16: EVALUATION OF VIRAL LOAD AND INTEGRATION**

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Background: Human Papillomavirus (HPV) natural history studies have revealed that human cancer is a rare consequence of an infec-

tion by some mucosa tropic high risk-HPV of this common sexually transmitted infection. HPV integration and persistent infection are critical events in progression to cervical carcinogenesis.

Objectives: Study of viral load and integration of HPV-16 in samples with different clinical/pathological status.

Materials and Methods: A total of 73 cervical samples infected with HPV-16 were retrospectively evaluated: 8 negative cytology, 4 ASC-H, 16 CIN1, 23 CIN2/3, 13 carcinoma and 9 treated carcinoma. DNA samples were extracted by a commercial kit (Qiagen). After spectrophotometrical quantification, the amplification was performed by *in house* PCR with set of primers: MY09/MY11 or PGMY09/PGMY11. β-globin was used as internal control. HPV types were determinated by RFLP, with RsaI and DdeI or by Microarrays (Papillocheck). Viral load was performed by real time PCR, with primers from E6 and E2 region. Caski cells were used as a positive control and albumin was used as an internal control. HPV-16 integration was determined by the quantitative ratio between E2 and E6 gene.

Results: Episomal, mixed and integrated form of HPV-16 was detected in 25%, 22% and 53% of all samples, respectively. The highest value of HPV-16 viral load was observed in carcinomas (both mixed and integrated form), CIN1 and CIN2/3 (episomal form). In treated carcinoma, the highest viral load was observed predominantly in the integrated form.

Conclusions: The carcinomas have a higher viral load than CIN1 and CIN2/3, in accordance with some authors. These data show that integration is a very early stage of the neoplastic progression to carcinoma. This methodology may not be the best approach to the determination of the integration status (Kalantari et al, 2001).