Summary

Infection of High Risk Human Papillomavirus (HR HPV) is the necessary etiological cause for the development of cancer in Uterine Cervix (CaCx). Globally, the prevalence of cervical HPV infection is about 10%, although wide variations in its prevalence and type distributions can be seen. About 80% of such initial asymptomatic infections are cleared off immunologically, inefficiency of which makes nearly 20% women susceptible to cervical carcinogenesis due to persistent HR HPV infection. In India, such large scale population based studies are only a few, however; in the global CaCx burden India ranks first. So, understanding of HR HPV prevalence in women population of India and detection of susceptible immunogenetic alleles would be imperative. During development of CaCx, the HR HPV16 oncoprotein E6 and E7 play important roles. Their expression, in turn, depends on the genetic (physical status, viral copy number, sequence variation of the HPV16 genome) and epigenetic (methylation of the viral enhancer and the early-late promoters) profile of HPV16 genome. However, the change of the profiles, if any, during development of CaCx from asymptomatic infection is not well understood. Similarly, how these profiles of HPV16 change due to therapeutic interventions has also not been evaluated in details for better prognosis of the disease.

In the Indian women population studied (N=4500), the prevalence of HPV infection in cervix was 7.6% and prevalence of HR HPV16 and HR HPV18 were 2.7% and 1%, respectively. With the development of cervical abnormalities, significant gradual increase in HPV viral load was also seen. The younger women (25-34 years; OR=1.22 (0.84, 1.76) who were married at early age (<20 years; OR=6.81 (3.80, 12.19) and having parity >3 (OR=4.48 (2.59, 7.76) were found to be at more risk of cervical HPV infection. Moreover, the IL-1β -511T-allele was associated with development of pre-neoplastic cervical lesions [OR=3.68 (0.97-16.35), p=0.03; OR=3.59 (0.92-16.38), p=0.03] and CaCx [OR=2.03 (1.03-5.2), p=0.02; OR=2.25 (0.96-5.31), p=0.04], whereas, the HLA-DQB1*03 A-allele was associated [OR=2.56 (1.42-4.62) and 2.07 (1.12-3.87), p=0.01] with development of CaCx, in presence of HR HPV16/18. More importantly, the women containing both the HLA-DQB1*03 A-allele and IL-1β -511T-alleles were more susceptible to CaCx due to the persistence of HRHPV16/18 infection in cervix.
During development of CaCx, the episomal form of HPV16 was prevalent in asymptomatic (N=93) and LSIL/HSIL (N=28) samples followed by significant increase (p=0.01) in integrated form in CaCx samples (N=98). Comparable frequencies of methylation in HPV16 enhancer (29-34%) were evident in asymptomatic, LSIL/HSIL and CaCx samples, despite the change in physical form. In asymptomatic infection, comparatively higher early-promoter methylation (52%) and lower late-promoter methylation (41%) was seen in episomal form, in contrast to the lower early-promoter methylation (40%) and higher late-promoter methylation (50%) in the integrated form. In LSIL/HSIL samples comparable frequencies of early-promoter and late-promoter methylation was seen (44%) in the episomal form, whereas, in the integrated forms the methylation profile was similar to the asymptomatic samples with integrated HPV16. In CaCx, higher methylation was seen in late-promoter (53-66%) than early-promoter (37-47%), irrespective of HPV16 physical status. Thus, increased HPV16 integration and late promoter methylation along with lower early promoter methylation seems to be important for malignant transformation of cervical epithelium.

Prevalence of HPV16 in pre/post-therapy plasma was significantly associated (p=0.03) with high viral load in the corresponding primary tumor site of cervix. In pre-therapy plasma (N=29), prevalence of integrated/mixed form of HPV16 was comparatively higher with significantly increased hypomethylation of early promoter (14/29, Z=4.47, p<0.01) and hypermethylation of late promoter (20/29, Z=3.74, p<0.01), than the corresponding CaCx. Integrated form of HPV16 was more persistent in cervical swab (22/29), after therapy, with hypomethylation of the early enhancer (6/29, Z=2.0, p<0.05) and late-promoter (18/29, Z=4.4, p<0.01). In post-therapy plasma, the physical status of HPV16 was similar (19/24) to its corresponding cervical swab, along with hypomethylation of early promoter and hypermethylation of late promoter (8/24, Z=2.6, p<0.01). Interestingly, the patients with differential methylation of HPV16 promoters in plasma and higher viral-load in primary tumor site showed poor prognosis with metastasis in distant organs.

Thus, our data indicated that the persistent HR HPV16 infection in the asymptomatic women could change its genetic and epigenetic profile during development of CaCx. This suggests that changes in its profile are important for malignant transformation of infected cervical epithelium. The presence of HR HPV16 in plasma and its profile in pre and post therapeutic CaCx patients has prognostic importance.