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Abstract Title:- HPV DNA Genotyping by Real time PCR and its Surrogate marker p16 analysis through Immunohistochemistry in cervical cancer: A tertiary care experience

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Aims:-Human Papilloma virus (HPV) are the causative agents of cervical carcinoma (CC), but a small fraction, especially adenocarcinomas (ACs), are HPV-negative, constituting a more aggressive subgroup. WHO classifies cervical squamous cell carcinoma (SCC) and ACs into HPV-associated and HPV-independent tumors. However, studies on prevalence of HPV-negative CCs are limited. Immunohistochemical assessment of p16 expression is a reliable surrogate marker for HPV infections. P16 negative tumors tend to exhibit more aggressive behavior, emphasizing the clinical relevance of p16 in cancer management. The aim was to study p16 negative CCs, HPV independent CCs and their clinicopathological implications. Furthermore, we aimed to elucidate the relationship between HPV status and p16 immunoreactivity in CCs, contributing to an improved understanding of CC diagnostics and treatment strategies.

Methods:- In this study, histopathologically diagnosed cases of CCs, attending the Radiation Oncology OPD over a period of 1 year were analysed. 14 High-risk HPV DNA genotypes were assessed in cervical swabs using Real-time PCR and p16 expression was evaluated in cervical biopsies through Immunohistochemistry.

Results:- A total of 49 cases (mean age: 45years) of CC were analysed. SCC was the major histological type (76%). HPV was detected in 44 cases, with HPV 16 and 18 being the most common genotypes. Additional HPV genotypes detected were HPV 31, 33, 45, 52, 58, 56, 66, and 68. Five cases tested negative for HPV, and they were all ACs. P16 expression was positive in 90% of cases. Five cases exhibited negative p16 expression, comprising 2 SCCs and 3 ACs. No significant associations were found between p16 negativity and age, stage or grading. In most cases of SCC, both HPV positivity and p16 overexpression were observed. However, there were two cases where HPV was positive but p16 was negative. Within the AC group (n=12), 58% (n=7) were HPV positive with five displaying p16 immunoreactivity and the rest being negative. Among the five HPV-negative cases, one showed concurrent p16 negativity, while the others were p16 positive.

Conclusions:- The study emphasizes most CCs are HPV-associated, particularly SCC, but ACs can be HPV-negative. P16 is a reliable surrogate marker for HPV associated SCCs but a small subset can test negative for P16. Therefore, HPV testing is recommended in p16 negative patients for better prognosis and management. Additionally, some HPV-negative cervical AC can be exhibit p16 positivity, hence emphasizing the need for HPV testing in all cases of adenocarcinoma. Further studies are required for a comprehensive understanding of clinicopathological features in p16 negative and HPV independent CCs.

Keywords:- Cervical carcinoma, Human Papillomavirus, p16 expression, Real time PCR, Immunohistochemistry