Section: Dental surgery

Original Research Article

THE PREVALENCE OF HPV16 AND 18 GENOTYPES IN ORAL LEUKOPLAKIA: A PILOT STUDY FROM MACHILIPATNAM, ANDHRA PRADESH

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Abstract

Background: The global incidence of oral cancer is highest in India, with potential malignant disorders such as leukoplakia often preceding the development of oral cancers. Tobacco use is a well-known risk factor for oral cancer, while the role of viruses in oral carcinogenesis, particularly human papillomavirus (HPV). The primary objective of this study is to ascertain the frequency of high-risk genotypes HPV 16 and 18 in oral mucosa of leukoplakia and potentially malignant oral lesions individuals with the intention of evaluating its potential as a biomarker for disease severity assessment in patients. **Materials and Methods:** The study includes 85 clinicopathologically confirmed oral leukoplakia cases were included in the study and the samples were collected from Government General Hospital Dental OPD, Machilipatnam, Andhra Pradesh. We used polymerase chain reaction (PCR) assay capable of detecting HPV 16/18 genotypes. **Results:** HPV DNA was detected in 93% of the oral samples, with no significant variation in HPV positivity. HPV 16 was present in 91% of the specimens, HPV 18 in 7.6% among all age (20-64 yrs) groups of study population. **Conclusions:** This study explores the epidemiological data of the prevalence and genotype distribution of HPV in patients with oral leukoplakia in, Machilipatnam population, Andhra Pradesh.

INTRODUCTION

The global incidence of oral cancer is highest in India, with potential malignant disorders such as leukoplakia often preceding the development of oral cancers.[1] The primary risk factor of oral leukoplakia is tobacco use, while the role of viruses in oral carcinogenesis, particularly human papillomavirus (HPV) and often in conjunction with other contributing factors. This condition may regress unpredictably, remain unchanged, or progress to carcinoma. Oral squamous cell carcinoma (OSCC) is recognized as the predominant malignant neoplasm affecting the oral cavity, with a fatal outcome and a concerning rise in global incidence.[2] In Indian subcontinent, oral cancer represents 23% of all cancer related fatalities, with an age related adjusted rate of approximately 20 cases per 100000 individuals in India.[3] In a significant number of instances, potentially malignant conditions including oral lichen planus, leukoplakia, and oral submucous fibrosis have been implicated in the development of oral cancer.[4,5] While tobacco, alcohol, and betel nut consumption have been extensively studied as the primary etiological agents, viruses like human papillomavirus are also under investigation. The precise involvement of human papillomavirus (HPV) in the pathogenesis of vitiligo and oral squamous cell carcinoma (OSCC) is a topic of ongoing debate within the medical community. While the role of HPV in carcinogenesis is well-established, research has highlighted its association with OSCC and oral leucoplakia.[5]

The participants included in this research were individuals diagnosed with leukoplakia, who had undergone HPV testing on their oral mucosa and oral tissue at the Outpatient Department of Government General Hospital in Machilipatnam, Andhra Pradesh, between October 2023 and January 2024. Subsequently, the HPV genotypes were determined, and the prevalence of different HPV types among various age groups was assessed and examined.

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MATERIALS AND METHODS

The study was carried out at the Government General Hospital Dental OPD in Machilipatnam, Andhra Pradesh, India. Approval was granted by the ethics committee and written informed consent was acquired from all participants. White spots or plaques that cannot be definitively classified as any other known diseases, and are linked to a physical or chemical ailment aside from smoking, do not contribute to a definitive diagnosis. A total of 85 oral mucosa samples were gathered from patients.

The study utilized the PCR (Polymerase chain Reaction) technique to detect specific high-risk human papillomavirus (HPV) strains responsible for oral cancer and OSCC.[6] The quality of the DNA samples was evaluated by PCR amplification of the β-globin housekeeping gene as an internal control. HPV positive samples were confirmed by PCR using the consensus primers GP5 and GP6. The PCR reaction was set up in a 20µL volume, with the following final concentrations of reagents: primers (GP5 primer- 5'- TTT'GTT ACT GTG GAT ACT AC-3', GP6 primer – 5'- GAA AAA TAA ACT GTA AAT CAT ATT C-3', 50mM KCl, 10mM Tris, 200µM dNTP's mix, 1.5mM MgCl2, and 2U of Taq DNA polymerase using a CFX96 (Bio-Rad). The PCR cycling parameters were: initial denaturation at 94°C for 4 minutes, followed by 45 cycles for 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, with final extension at 72°C for 10 minutes.

Statistical Analysis

The mean age of our study population is 39.5yrs. The Chi-square test was used to compare the differences in HPV genotype distribution among the histopathologically different lesions and all age (20-64 yrs) groups.

RESULTS

The study group consisted of 85 clinicopathologically confirmed cases of leukoplakia, with 53 cases in males and 22 in females (Fig-1). In this study, the prevalence of oral leukoplakia was found to be higher on the buccal mucosa, with 52 cases, followed by the labial mucosa with 5 cases, the dorsal surface with 12 cases, and the lateral border of the tongue with 2 cases. HPV positivity was found in 79 cases using GP5 and GP6 primers in PCR analysis. Samples that tested negative (n=6) for HPV by PCR also tested negative in other investigations.

N=85 samples of oral mucosa were analyzed among them 79 samples showed positive for HPV infection. In n=72 samples gave positive for HPV genotype 16 i.e. around 91% and n=6 samples gave positive for HPV genotype 18 (7.6%) n=1 gave positive for both HPV genotypes 16 and 18 (Co-infection) (1.3%) of the leukoplakia patient are infected with HPV.

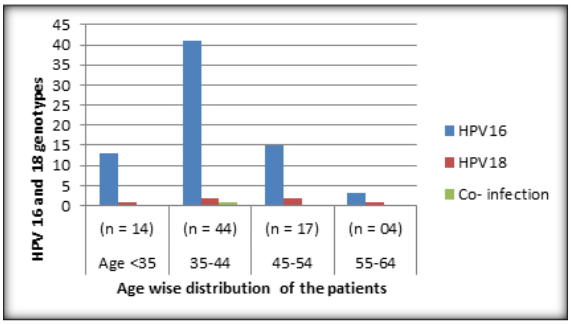

Figure 1: Distribution of HPV 16 and 18 genotype in patients with oral leukoplakia

Table 1: Genotype Distribution between Controls and Patients

	Age <35 (n = 14)	35-44 (n = 44)	45-54 (n = 17)	55-64 (n = 04)
HPV16	13	41	15	3
HPV18	1	2	2	1
Co- infection	-	1	-	-

DISCUSSION

Human Papillomavirus (HPV) is tropic to epithelial cells and can infect either the cutaneous or mucosal epithelium, depending on the specific genotype. The HPV genome is composed of three main regions: the early region (E), the late region (L), and the long region control. Within the early region, oncoproteins E5, E6, and E7 are present.[5] Structural proteins L1 and L2 are located on the viral capsid and are involved in the final stages of viral replication. Different genotypes of HPV, both low- and high-risk, are linked to benign proliferative growths, precancerous lesions, and cancerous epithelial abnormalities.[7,9,13-16]

Human Papillomavirus (HPV) has the potential to initiate epithelial proliferation or contribute to the initial stages of oral carcinogenesis through the action of E5, E6, and E7 oncoproteins. These oncoproteins are known to degrade tumor suppressor gene products such as p53 and Rb. Sufficient evidence supports the oncogenic capabilities of high-risk HPV genotypes in the development of cervical cancer. The correlation between HPV and Oral Squamous Cell Carcinoma (OSCC) is evident due to the similarities in morphology between oropharyngeal and genital epithelia.[8] Review of the literature confirms this association.[7]

The correlation between HPV and leukoplakia remains a subject of intense debate within the scientific community, with questions arising as to

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whether HPV should be classified as an etiological factor or a risk factor for malignancy in precancerous and cancerous oral lesions.[9] Various studies have detected high-risk HPV antigens and viral DNA in both potentially malignant and malignant oral disorders; however, notably high rates of HPV have also been observed in healthy oral mucosa. Robinson and colleagues have demonstrated the presence of HPV infection in freshly-collected samples.[10]

Several studies have demonstrated that HPV association with oral leucoplakia.[11,15,16] Cianfriglia et al. found the presence of HPV in oral leukoplakia and suggested that it may act as a cofactor in carcinogenesis.[11] In a study conducted by Cianfriglia, it was reported that the highest incidence of HPV (approximately 60%) was found in leukoplakia lesions, emphasizing HPV's specific risk factor in premalignant disorders.[11] Mathew et al. and Sugiyama et al. observed and discovered a high prevalence of HPV types 16 and 18 in leucoplakia.[6,12] A meta-analysis on HPV in oral carcinoma and potentially malignant disorders (OPMDs) identified a significant causal association between HPV and oral squamous cell carcinoma (OSCC), as well as in OPMDs such as oral leukoplakia, oral lichen planus, and epithelial dysplasias.[8]

In India, leukoplakia most commonly occurs in the buccal mucosa, followed by the labial mucosa, the lateral and dorsal surfaces of the tongue, and the floor of the mouth. Lesions found on the tongue and floor of the mouth have a higher likelihood of turning into malignant forms. Our study shows that leukoplakia is notably prevalent in the buccal mucosa, largely due to the widespread habit of gutkha chewing in this demographic. The presence of epithelial dysplasia in oral leukoplakia is a significant indicator of the potential for cancerous transformation, and it plays a crucial role in guiding clinical management.

CONCLUSION

The prevalence of HPV 16 and 18 was observed in cases of oral leukoplakia within the study population. Conducting a study with a larger sample size, excluding the influence of habits, and utilizing advanced molecular detection techniques would provide a more comprehensive understanding of the exclusive role of HPV in the process of carcinogenesis, starting from its initial stage. This, in turn, could enhance its potential use as a biomarker.

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Conflicts of interest: No conflicts of interest.

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