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Correlation of High-Risk Human Papillomavirus (HPV 16/18) with p53 Immuno-Expression in Oral Carcinoma

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ABSTRACT

Background: *Oral cancer is a prevalent health concern in Sudan, with a rising incidence in recent years. This study aimed to assess the association between high-risk human papillomavirus (HR-HPV) subtypes 16 and 18 and oral cancer in Sudan.*

Methodology: *This was a retrospective, cross-sectional, descriptive study conducted on formalin-fixed paraffin-embedded (FFPE) tissue samples obtained from patients diagnosed with oral cancer between 2018 and 2022. We performed immunofluorescence (IF) to evaluate the p53 proteins' expression by using specific antibodies according to standard laboratory protocol. In addition, the presence of human papillomavirus (HPV) was determined by using a specific antibody against high-risk HPV subtypes 16 and 18.*

Results: *HPV positivity was observed in 17 cases out of 117 (14.5%), whereas HPV negativity was 100 out of 117 (85.5%). In contrast, p53 demonstrated a relatively high expression rate, positive in 79 cases out of 117 (67.5%), compared to 38 out of 117 (32.5%) negative cases.*

Conclusion: *This study found a strong connection between high-risk HPV (16/18) and oral cancer p53 expression, suggesting that HPV-mediated pathways may aid tumor formation by modulating tumor suppressor mechanisms. The difference in p53 expression between HPV-positive and HPV-negative cases suggests separate oral carcinogenesis molecular pathways. This shows that HPV status may alter tumor behavior and be a disease profiling biomarker. Understanding the HPV-p53 response may help us understand tumor biology and enhance prognosis and targeted therapies.*

Keywords
Oral cancer, Human papillomavirus, p53, Immunofluorescence, Sudan.

Introduction
Oral cancer is regarded as one of the most prevalent malignant tumors globally and represents a significant public health issue, particularly in developing nations. It is marked by elevated morbidity and death rates and is frequently detected at advanced stages, adversely affecting therapy outcomes and the mucosa [1,2]. Oral squamous cell carcinoma is the predominant histological subtype, frequently impacting the tongue and buccal mucosa [1]. Numerous risk factors are associated with oral cancer, including tobacco use, alcohol intake, and persistent inflammation [3]. Oral cancer in Sudan constitutes a considerable public health issue, intimately associated with the prevalent use of toombak, a type of smokeless tobacco with substantial carcinogenic potential [1,4]. Recently, human papillomavirus has been proposed as a potential factor in oral carcinogenesis, especially high-risk forms such as HPV 16 and 18 [5,6]. HPV-related cancers are linked to specific molecular pathways that include critical regulators of the cell cycle [5]. The oncoprotein disrupts tumor suppressor pathways, resulting in the aberration of normal cellular control. This leads to a modification in p53 activity [5,6].

Consequently, molecular markers like p53 have become increasingly significant in comprehending the tumor's behavior. If these indicators offer a more profound comprehension of the mechanisms driving carcinogenesis, they may enhance diagnostic and prognostic assessments [7,8], ultimately leading to improved treatment strategies and patient outcomes. This study seeks to assess the correlation between HPV infection and p53 expression in oral cancer patients.

Materials and Methods
This study was a retrospective, cross-sectional, descriptive analysis of formalin-fixed paraffin-embedded (FFPE) tissue samples from patients diagnosed with oral cancer between 2018 and 2022. The samples were collected from the National Public Health Laboratory in Sudan between 2022 and 2024. The study comprised 117 instances in all. We obtained all patients' clinical and demographic information from their records. We conducted immunofluorescence (IF) to assess the expression of p53 proteins utilizing particular antibodies in accordance with established laboratory protocols. The presence of human papillomavirus (HPV) was assessed using a particular antibody targeting high-risk HPV subtypes 16 and 18.

The staining was evaluated according to the proportion of positive tumor cells and classified as negative (0%), focal (1–10%), weak (11–50%), moderate (51–70%), and strong (>70%). Furthermore, staining intensity was classified as weak, moderate, or strong.

The expression of p53 was evaluated based on the proportion of positive cells and the intensity of staining. HPV expression was assessed using immunofluorescence labeling of tumor cells and classified as positive or negative based on the presence or absence of staining.

Data Analysis
The data were evaluated statistically utilizing the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL). The correlation between HPV status and p53 expression, together with clinicopathological factors, was evaluated using the Chi-square test. A p-value of less than 0.05 was deemed statistically significant.

Results
The study comprised 117 individuals aged 7–85 years, with a mean age of 57.7 ± 16.9 years. Males outnumbered females, 74/117 (63.2%) to 43/117 (36.8%). The majority of patients were between the ages of 56-65, with ≥71 and between the ages of 41-55 years accounting for 32/117 (27.4%), 27/117 (23.1%), and 23/117 (19.7%), respectively. (Table 1 and Figure 1). demonstrate that most HPV positives were aged ≤ 40 years, followed by those aged 41–55, which accounted for 36.0% and 17.0%, respectively. Female patients are more likely to be HPV positive than male patients (23.0% versus 9.0%). HPV positivity was strongly linked with age (p=0.031) and gender (p=0.041).

Table 1: Distribution of HPV by patient age and sex.

Category	HPV Positive	HPV Negative	Total
Age range			
≤ 40 years	8	14	22
41 - 55	4	19	23
56 - 65	3	29	32
66 - 70	0	13	13
≥71	2	25	27
Total	17	100	117
Sex			
Males	7	67	74
Females	10	33	43
Total	17	100	117

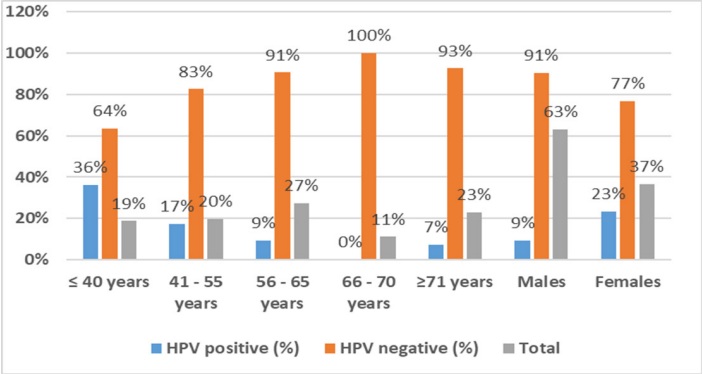

Figure 1: Description of HPV by patient age and sex.

Most patients had lesions in the 25 (21.4%) buccal and palatal regions, followed by the 16 (13.7%) tongue and 14 (12.0%) lips. Table 1 and Figure 1 show that the majority of HPV-positive cases occurred in the tongue and oropharynx, accounting for 7 out of 16 (44.0%) and 4 out of 9 (44.0%), respectively. followed by buccal and palate with 5 out of 25 cases (20.0%) and lip with 1 out of 14 cases (7.0%). The remaining specimen types were HPV negative, and an association was found ($p > 0.001$), as illustrated in (Table 2 and Figure 2).

Table 2: Distribution of HPV by specimen site.

Specimen site	HPV Positive	HPV Negative	Total
Tongue	7	9	16
Cheek	0	9	9
Mandible	0	12	12
Parotid	0	2	2
Buccal and Palate	5	20	25
Lip	1	13	14
Gingival	0	5	5
Maxillary	0	7	7
Retromolar	0	4	4
Submandibular	0	8	8
Oropharynx	4	5	9
Other	0	6	6
Total	17	100	117

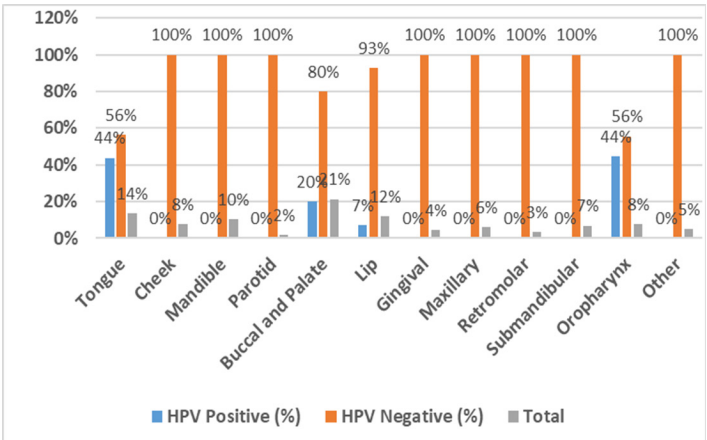

Figure 2: Description of HPV by specimen site.

Table 3 shows the frequency distribution of HPV and p53 among the examined cases. HPV positivity was detected in 17 instances out of 117 (14.5%), while HPV negativity was found in 100 cases out of 117 (85.5%). In contrast, p53 had a reasonably high expression rate, being positive in 79 out of 117 (67.5%) instances and negative in 38 out of 117 (32.5%), as (shown in Table 3 and Figure 3).

Table 3: Distribution of immuno-expression of HPV by p53.

Marker	Positive	Negative	Total
HPV	17	100	117
P53	79	38	117

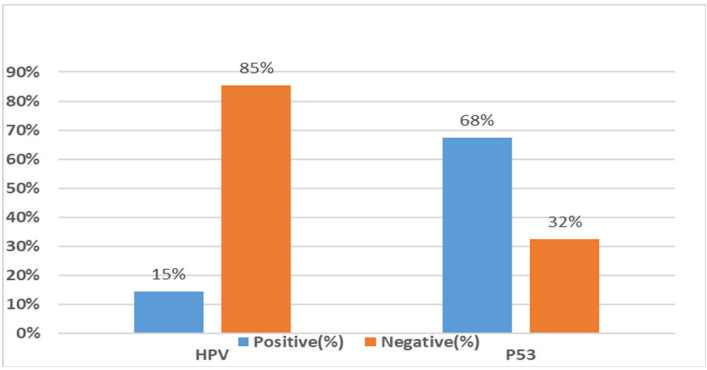

Figure 3: Proportions of immuno-expression of HPV by p53.

Altered p53 expressions were most common in HPV-positive cases, accounting for three out of seventeen (17.6%), whereas normal p53 expression was more common in HPV-negative patients. According to p53 scoring, negative and focal staining patterns were more common in HPV-positive cases, comprising 3 out of 17 (17.6%) and 11 out of 17 (64.7%), respectively, whereas moderate and strong staining were more frequently observed in HPV-negative cases, comprising 1 out of 17 (5.9%) and 0 out of 17 (0.0%), as shown in Table 4. p53 expression varied considerably by HPV status ($p<0.001$), with lower expression observed in HPV-positive patients.

Table 4: Association of HPV p53 expression by p53 scoring.

Variable	HPV Positive	HPV Negative	Total
P53			
Altered	3	76	79
Normal	14	24	38
Total	117	100	117
P53 Scoring			
Negative	3	4	7
Focal	11	20	31
Weak	2	31	33
Moderate	1	29	30
Strong	0	16	16
Total	17	100	117

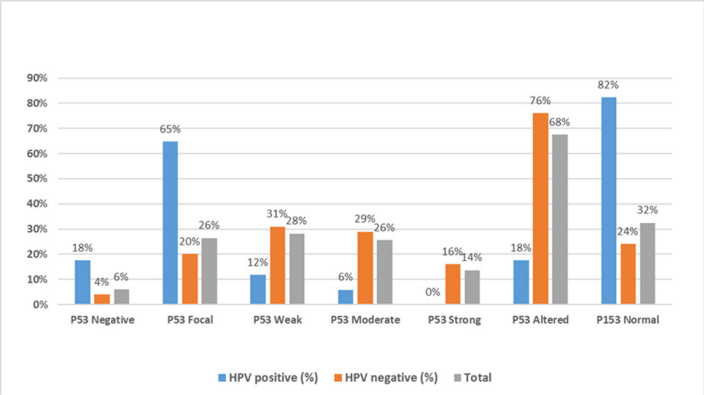

Figure 4: Proportions of HPV p53 expression by p53 scoring.

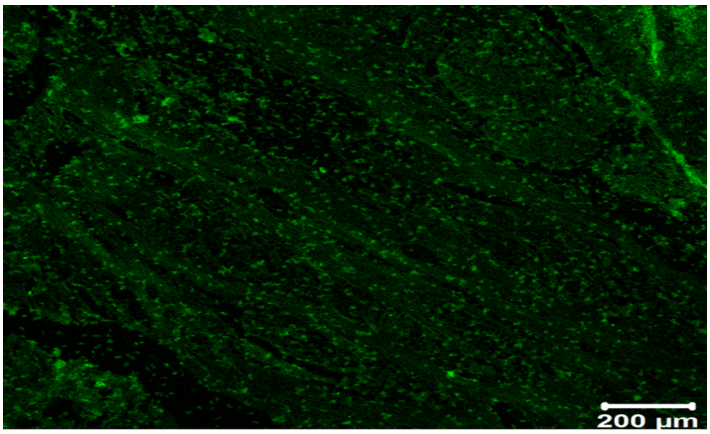

Image 1: Positive HPV expression in oral cancer tissue, showing green fluorescenc.

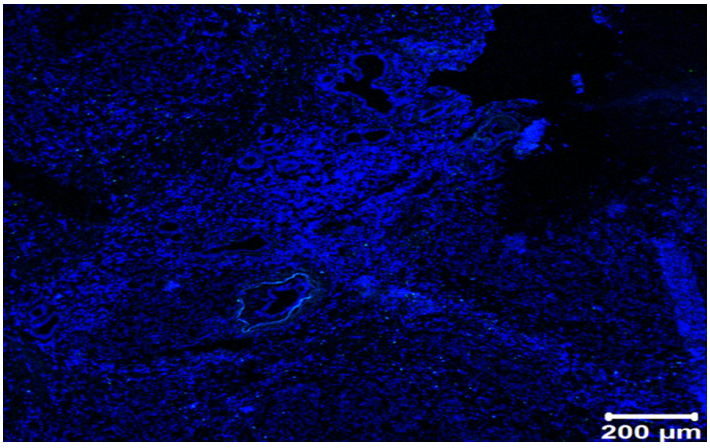

Image 2: Negative HPV expression in oral cancer tissue with only blue nuclear staining.

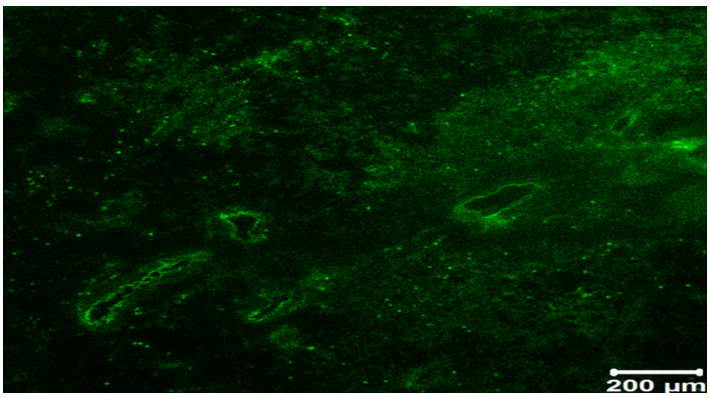

Image 3: Positive p53 expression showing green fluorescence in tumor cells.

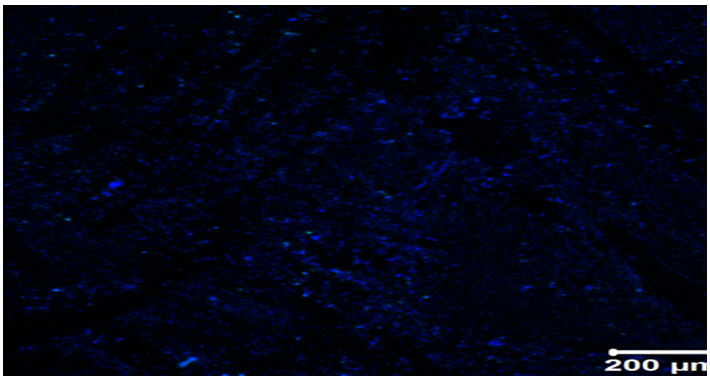

Image 4: Negative p53 expression showing absence of green fluorescence with blue nuclear staining.

Discussion

A recent study found that female patients and people under the age of 40 were more likely to be positive for human papillomavirus. Traditional risk factors for oral and oropharyngeal malignancies have been thoroughly researched in elderly populations. However, recent research suggests that it is on the rise among young people. HPV has a high connection with OSCC and OPSCC in adults under 40 years old [9]. OSCC with HPV infection is more common in female patients [10]. This range is explained by variances in immune response, hormonal impacts, and behavioral exposure patterns, all of which might influence virus acquisition and persistence, particularly in the context of how these factors differ between genders and age groups, leading to varying rates of HPV infection and its associated cancers [11,12]. HPV-related carcinogenesis is particularly associated with long-term viral infection, rather than transient infection, which is more common in specific population groups [13]. Furthermore, the increased occurrence rate in younger patients lends credence to the idea that HPV-driven cancers are a separate biological entity that frequently appears before tumors induced by traditional carcinogens [14]. In contrast, an inverse connection was found between HPV status and p53 expression. This supports the well-established involvement of the HPV E6 oncoprotein in promoting p53 degradation via ubiquitin [15]. HPV-negative malignancies have more mutations

and functional losses in the p53 gene than HPV-positive cancers, which is due to the quick decay of non-mutant wild-type p53 under normal cellular conditions, making it difficult to detect. In contrast, mutant p53 has a prolonged half-life, causing it to accumulate within cells and increase IHC staining. The loss of p53 function hampers DNA damage response, apoptosis, and genomic stability, favoring tumor growth. This inverse pattern supports the molecular basis for HPV-associated carcinogenesis [16]. Overall, HPV-positive patients had a higher survival rate [17]. HPV-positive cancers and their early detection can serve as a valuable predictor of better prognosis and response to treatment, increasing interest in therapeutic de-escalation [18].

This study found no significant link between HPV status and tumor grade. These findings could relate to or imply that HPV-induced molecular alterations largely influence intracellular regulatory pathways rather than morphological differentiation. Tumor grading is based on histological characteristics, including cellular atypia and keratinization, which can indirectly indicate underlying molecular events. As a result, HPV-positive cancers may have similar histological grades to HPV-negative tumors despite having separate molecular pathways of progression [19]. The absence of a relationship between HPV and tumor grade suggests that traditional histological measures may not be sufficient to fully explain the tumor's biological activities. However, the availability of molecular markers like p16 provides functional insight into oncogenic pathways, particularly those involving cell cycle dysregulation, and may reveal additional values in tumor characterization that go beyond standard classification systems [18]. In this investigation, immunofluorescence gave increased sensitivity and better visibility of protein expression than standard immunohistochemistry, potentially improving marker detection accuracy. Overall, the findings of this study indicate that HPV-related oral cancer has a distinct molecular pattern that includes disruption of the key tumor suppressor pathways, particularly those involving p53. This points out the importance of combining genetic data with histological assessments to better understand tumor heterogeneity. These discoveries may have significant public health consequences, particularly in terms of HPV vaccination. The prevention of HPV infection with vaccination has the potential to minimize the incidence of HPV-associated mouth cancer. However, these findings also highlight the need for more research to better understand the predictive and therapeutic aspects of HPV-associated biomarkers. Additionally, further research is needed to better understand the efficacy of immunization in preventing oral HPV-related cancers. Improved understanding of these molecular pathways leads to better patient classification and the development of focused therapy options, which can ultimately enhance treatment outcomes for patients with HPV-associated cancers.

In conclusion, High-risk HPV (16/18) was highly connected to oral cancer associated with p53 expression, suggesting HPV-mediated pathways may alter tumor suppressor systems to promote tumor growth. The difference in p53 expression between HPV-positive and HPV-negative cases suggests different oral carcinogenesis pathways. This indicates that HPV status may influence tumor behavior and serve as a biomarker. Understanding the HPV-p53 response may enhance tumor biology, prognosis, and targeted therapy.

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