Human papillomavirus, tobacco, and poor oral hygiene can act synergetically, modulate the expression of the nuclear factor kappa B signaling pathway for the development and progression of head and neck cancer in the Pakistani population

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Abstract

Background: Head and neck cancers (HNCs) are a heterogeneous group of tumors that progress owing to varied enviromental and genetic risk factors. Viral infections are threatening and adept at altering the expression of cellular transcription factors such as nuclear factor kappa B (NF-κB) and deregulation of other cellular proteins like NF kappa B inhibitor alpha (IκBα). The present study was conducted to detect high-risk genotypes of human papillomavirus (HPV) and protein expression of NF-κB signaling pathway in HNC patients with HPV infection.

Methods: For HPV detection, genomic DNA from 152 HNC tumors was extracted formalin-fixed paraffin-embedded tissue DNA kit. For genotyping, polymerase chain reaction (PCR) using a general primer, HPV type-specific primers and agarose gel electrophoresis were performed. Immunohistochemistry (IHC) was also performed on 4-μm thick tissue sections using HPV E6 monoclonal antibody. Protein expression analysis of NF-κB signaling pathway including p50, p65, and IκBα was performed using IHC.

Results: PCR analysis showed that 24.3% (37/152) of HNC cases were HPV positive. Among HPV positive, 86.5% (32/37) were tobacco users, while among HPV negative, 66.9% (77/115) were tobacco users. A significant association of HPV positivity and tobacco user was observed by univariate analysis [$P < 0.01$; odds ratio (OR): 0.310, 95% confidence interval (CI): 0.110 to 0.870]. More HPV positive patients were with poor oral hygiene (78.3%) when compared with patients with good oral hygiene (21.6%) [$P < 0.03$, OR: 2.440, 95% CI: 1.650 to 3.600]. The results of the logistic regression analysis showed that age, tobacco use and oral hygiene are significant predictors ($P < 0.02$). PCR and IHC staining results confirmed that HPV16 was predominant among HNC cases (64.8%) when compared with HPV18 (35.2%). Expression of NF-κB proteins (p50, p65, and IκBα inhibitor) were also observed in HPV and non-HPV infected HNC tissues. IHC expression of p50, and p65 showed nuclear staining, while IκBα inhibitor showed cytoplasmic staining. Protein expression in HPV cases was higher as compared to HPV naive cases ($P < 0.05$).

Conclusions: From the study, it can be established that the use of tobacco, oral hygiene, and HPV infection may be synergistically involved in modulating the expression of NF-κB signaling pathway for the development and progression of HNC in the Pakistani population.

Keywords: Human papillomavirus; NF-kappa B; p50; p65; IκBα inhibitor; Polymerase chain reaction; Immunohistochemistry; Head and neck cancer; Pakistani population

Introduction

Head and neck cancer (HNC) is a subset of malignancies at different anatomic sites in the upper aerodigestive tract including oral cavity, lips, oropharynx, hypopharynx, nasopharynx, and larynx.[1] It is a frequent type of cancer worldwide and accounts for > 650,000 cases and 330,000 deaths annually. Global Cancer Statistics 2020, compiled by the International Agency for Research on Cancer, estimates that oral cancer recorded for almost 2.0% of all cancer cases and 1.8% of all cancer deaths globally.[2,3] Oropharyngeal and tongue cancer are the principal forms of HNC in Western countries,[4] while oral cancer is widespread in South Asian countries.[5,6] More than 90% of cancers prevail in squamous cells of the oral cavity, oropharynx, and larynx,[7] and a steady increase has been witnessed in the prevalence of tonsil and oropharyngeal cancer worldwide.[8] Head and neck carcinomas stem from environmental and genetic factors; because it is

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particularly reported in people with extended exposure to the risk factors such as smoking, abnormal use of alcohol, and prolonged exposure to ultraviolet (UV) radiations, asbestos, and viruses.

Nuclear factor kappa B (NF-κB) is a pro-inflammatory transcription factor executing a key role in cell signaling pathways. NF-κB family includes five distinct members RelA [p65], RelB, c-Rel, NF-κB1[p50/p105], and NF-κB2 [p52/p100], which share an N-terminal Rel homology domain; a region responsible for DNA-binding and dimerization (homo-and heterodimerization) with nucleus localization sequence. NF-κB typically remains in a latent structure in the cytoplasm by restricting IκBs (inhibitory proteins), most remarkably by inhibitory protein (IκBα). Upon activation by cytokines, chemotherapeutic agents, developmental factors, lipopolysaccharide, bacterial or viral infection, and stress signals, it enters the nucleus and causes expression of over 100 downstream genes recruited for an array of cell functions including apoptosis, cell relocation, cell multiplication, and angiogenesis.[8]

Dysregulation of NF-κB protein expression and its instigation are frequently seen in malignancies, such as lymphoma, leukemia, myeloma, colon, pancreatic, breast, and cervical cancers.[8] Human papillomavirus (HPV) is viewed as a sexually transmitted infection that can be passed on by skin, mouth, and genital contact and is identified as an important etiological factor in the subset of HNC, especially oropharyngeal carcinoma.[2,9] HPV-associated oral cancers are extending in incidence worldwide and have distinct molecular, clinical, pathological, and epidemiological features.[10] Additionally, high-risk (HR) -HPV genotypes 16/18 produce early proteins E6 and E7 and deregulate cell cycle control.[11] HR-HPV16 has been remarked as a regulator for NF-κB initiation and articulation in various tumors including oral squamous cell carcinomas.[8]

Even though chemotherapy, radiation therapy, and surgical procedure remain the key therapeutic tools to treat and manage HNC at an initial stage, the overall survival rate is still < 65%.[10] This can be due to inadequate validity of treatment strategies, detection of tumor growth at later stages, and the environmental factors for HNC development, especially the involvement of pathogenic viruses. However, its mechanism of action in fostering cancer is unclear and data have not shown a confirmed association between HPV, NF-κB signaling pathway, and occurrence of HNC worldwide.[12] The aim of the present study is the detection of high-risk genotypes of HPV and the expression pattern of the proteins within the NF-kB signaling family and its inhibitor IκBα in different stages and different anatomic subsites of the HNC in the Pakistani population.

Methods

Ethical approval

This study was prior approved by Ethics Committees of Quaid-i-Azam University, Islamabad, Pakistan (Approval No. IRB-QAU-151), and collaborating hospitals and was conducted per the guidelines described in the *Declaration of Helsinki* (II) (18th, 1964). Data were collected using the specifically designed questionnaire via a personal interview after obtaining the written consent of each patient and their family.

Sample collection and patient's data

Tumor samples and clinical data of pathologically confirmed HNC patients were collected from different hospitals of Pakistan including Pakistan Institute of Medical Sciences (PIMS, Islamabad), Nuclear Medicine, Oncology and Radiotherapy Institute (Islamabad), Ayub medial complex (Abbottabad), Institute of Radiotherapy and Nuclear Medicine (Peshawar). Almost every patient all over Pakistan visits PIMS, Islamabad. The clinical history of selected patients was obtained from patient's files maintained at the hospitals. All records related to patients, for example, age, gender, name, ethnicity, occupation, smoking history, area of cancer, any infection, histopa-thology report, year of diagnosis, and treatment, were collected. Different anatomic subsites of the head and neck were classified following the International Classification of Diseases 10th Revision classification system and the 8th tumor–nodes–metastases (TNM) staging system was adopted.[13] Patients with a history of any other type of cancer and other diseases were excluded from the study. Formalin-fixed paraffin-embedded (FFPE) tissue specimens and slides of 152 tumors samples stained with hematoxylin and eosin were retrieved from hospitals. All slides were re-examined for confirmation of the diagnosis.

Genomic DNA isolation and polymerase chain reaction (PCR)

Deoxyribonucleic acid (DNA) was extracted from the patient's tumor using GeneAll® Exgene™ DNA kit (GeneAll Biotechnology, Seoul, Korea) from FFPE tissues. DNA of all samples was quantified by gel electrophoresis and UV transilluminator (Bio-Rad, Puchheim, Germany). Primers for HPV high-risk genotypes selected for the current study (HPV16, 18, 31, 33, and 45) were adopted from Kumar *et al*[14] and amplified through PCR.

HPV genotyping and gel electrophoresis

PCR products were run on 2% agarose gel stained with EtBr (Merck, Darmstadt, Germany). HR-HPV positive cervical DNA sample was used as positive control and UltraPure™ DNase/RNase free distilled water (Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, CA, USA) was used as a negative control in each run of PCR.

Confirmation of HPV positive cases via immunohistochemistry (IHC)

The expression of HPV E6 protein (anti-HPV16/E6 and anti-HPV18/E6 mouse monoclonal antibody; Abcam, Waltham, MA, USA) in the tissue biopsies of HNC were also evaluated by IHC. IHC was performed on thick tissue sections obtained from FFPE samples. Evaluation of the cytoplasmic immunoreactivity intensity was done semi-

quantitatively using a score of 0 to 3. IHC staining to detect HPV positivity was scored for the presence of strong nuclear and cytoplasmic staining in > 70% of cancer cells, while 0 was used for HPV negative staining. Scores of 1, 2, and 3 were used to represent weak, moderate, and strong staining, respectively.

Expression analysis of NF-κB proteins and IκBα using IHC

The expression of NF-κB pathway proteins p50, p65, and IκBα inhibitor (NF-κB/p50, NF-κB/p65, and IκBα) were analyzed in the samples selected for study along with positive and negative controls. Polyclonal antibodies (Host Rabbit) (cat# E-AB-32226, cat# E-AB-32232, and cat # E-AB-10086, Elabscience, Houston, TX, USA) were used to carry out all immunochemical reactions with a concentration of 1:100 (1 mg/mL), 1:100 (1 mg/mL), and 1:50 (0.1 mg/mL), respectively. Tumor cells were counted and observed under both $10 \times$ and $40 \times$ for measuring immunoreactivity, which was calculated as, Immunoreactive score (IRS) = proportion score (PS) × intensity score (IS).

The intensity score was categorized by the following scheme: 0 for negative intensity, 1 for weak intensity, 2 for moderate intensity, and 3 for strong staining intensity. Whereas, proportion score was categorized as 0 for no positive cell, 1 for ≤10% of positive cells, 2 for 11% to 50% of positive cells, 3 for 51% to 80% of positive cells, and 4 for > 80% distribution of positive cells. Immunoreactivity (range 0–12) was categorized as low immunoreactivity (IS 0–4) and high immunoreactivity (IS >4).[8]

Statistical analysis

Software Graph Pad Prism v7.01 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Correlation of clinical parameters with HNC and HPV infection was calculated by univariate analysis; χ^2 test with Fisher exact test, crude odds ratios (ORs), and 95% confidence intervals (CIs) were calculated for the association between demographic and clinical variables and HNC risk. A multiple logistic regression model using Software SPSS version 26 (IBM SPSS Statistics, USA) was used to find the synergetic effect of covariates such as the use of tobacco, oral hygiene, HPV infection, and risk of HNC. Correlations of protein–protein expression, proteins expression with risk factors and clinicopathological parameters were evaluated by Spearman correlations. A P value of <0.05 was used for statistical significance.

Results

Demographic data analysis

Data analysis of 152 HNC samples was performed, among them, 62.5% (95/152) were male patients while 37.5% (57/152) were females. A total of 47.3% (72/152) of the patients were in the younger age group (age < 40 years). In the participants, 71.7% (109/152) were tobacco users while 28.2% (43/152) were non-tobacco users. Cancer of the oral cavity was common cancer reported (49/ 152, 32.2%) [Table 1].

HPV-genotyping and IHC

One hundred and fifty-two HNC tumor samples were screened for HR-HPV typing using PCR followed by gel electrophoresis and IHC as shown in Figure 1. Results after PCR and IHC analysis revealed that 24.3% (37/152) were HPV positive, while 75.7% (115/152) were HPV negative. Among all patients, 86.5% (32/37) of HPV positive patients and 66.9% (77/115) of HPV negative patients are tobacco users. A highly significant association of HR-HPV positivity and tobacco use was observed by univariate analysis [$P < 0.01$; OR: 0.31 (95% CI:0.11–0.87)]. A total of 78.3% (29/37) of HPV positive samples were with poor oral hygiene [$P = 0.03$, OR: 0.38 (95%CI: 0.16–0.92)]. Patients coming from the urban areas accounted for 66.4% ($n = 101$) while 33.5% ($n = 51$) were from rural areas. A total of 11 (29.7%) HPV positive cases were from an urban area and 26 (70.2%) HR-HPV positive cases from a rural area, a significant difference was observed in HPV positive and negative patients, [$P < 0.0001$, OR: 0.11 (95%CI: 0.05–0.27)]. Concerning the anatomic site of head and neck cancer, the highest number of HPV positive cases was observed in the oral cavity 48.6% ($n = 18$) followed by oropharynx 21.6% ($n = 8$), hypopharynx 8.1% ($n = 3$), larynx 13.5% ($n = 5$), and nasal cavity 8.1% ($n = 3$). For histological grading of tumors, HPV positivity with the highest number of cases was in G2 (24.3%) and G3 (48.6%) as shown in Table 1. The percentage of patients positive for HPV at stage I and II were 78.3% (n=29), while advance stage tumor III and IV were 21.6% (n=8). No significant difference was observed among primary tumor stage (pTstage) with OR: 1.52 (95%CI: 0.63–3.66, $P < 0.34$).

From the binary multiple logistic regression analysis, it was estimated that the coefficients for age, tobacco, and oral hygiene have $P < 0.05$, indicating that these coefficients are not 0 using an α-level of 0.05, which concludes that these three independent variables have an impact on HPV-infected HNC. The results of the logistic regression analysis show that all the three independent variables together were statistically significant at $P < 0.01$ as shown in Table 2.

NF-κB and IκBα proteins expression in head and neck cancer cases

IHC for protein expression analysis of NF-κB pathway, p50, p65, and IκBα was performed on tumor samples collected for the present study (152 tumor samples). It was observed that NF-κB, p50, and p65 proteins were present in the nucleus of tumor cells and showed high expression as shown in Figure 2G and 2H. While IκBα inhibitor protein showed cytoplasmic expression in all samples as shown in figure 2C, F and I. IHC staining for p50, 72 (47.3%) showed strong, 67 (44%) showed moderate staining, and 13 (8.5%) showed weak immunostaining. IHC for p65 protein in tumor samples, 74 (48.6%) having strong staining intensity, 68 (44.7%) showed moderate staining intensity, and only ten (6.5%) showed weak staining intensity. IHC for IκBα inhibitor, 70 (46%) showed weak immunostaining, showing low expression of the protein in HNC cases, 62 (40%) have moderate

Table 1: Demographic and clinicopathological characteristics of HPV positive and negative head and neck cancer cases.

Socio-demographic characteristics	Total (*N* = 152)	HPV positive (*n*=37)	HPV negative (*n*=115)	OR (95% CI)	*P* value
Use of tobacco*					
No	43	5 (13.5)	38 (33.0)	Ref	–
Yes	109	32 (86.5)	77 (66.9)	0.310 (0.110, 0.870)	0.01
Oral hygiene					
Good	56	8 (21.6)	48 (41.7)	Ref	–
Poor	96	29 (78.3)	67 (58.2)	0.380 (0.162, 0.915)	0.03
Area of living					
Urban	101	11 (29.7)	90 (78.2)	Ref	–
Rural	51	26 (70.2)	25 (21.7)	0.110 (0.051, 0.270)	<0.001
Education					
Illiterate	93	23 (62.1)	70 (60.8)	Ref	–
Educated	59	14 (37.8)	45 (39.1)	1.050 (0.492, 2.264)	0.880
Anatomic site					
Oral cavity	49	18 (48.6)	31 (26.9)	Ref	–
Oropharynx	38	8 (21.6)	30 (26.0)	0.780 (0.322, 1.897)	0.290
Hypopharynx	29	3 (8.1)	26 (22.6)	0.300 (0.086, 1.063)	0.030
Larynx	22	5 (13.5)	17 (14.7)	0.900 (0.308, 2.637)	0.420
Nasal cavity	14	3 (8.1)	11 (9.5)	15.790 (4.110, 60.680)	<0.001
Tumor grading†					
G1	39	8 (21.6)	31 (26.9)	Ref	–
G2	45	9 (24.3)	36 (31.3)	0.700 (0.302, 1.647)	0.200
G3	55	18 (48.6)	27 (23.4)	1.990 (0.940, 4.245)	0.036
Unknown	13	2 (5.4)	11 (9.5)	–	
pT stage					
T0-T1	110	29 (78.3)	81 (70.5)	Ref	–
T2-T4	42	8 (21.6)	34 (29.5)	1.520 (0.630, 3.660)	0.340
N stage					
Positive	60	24 (64.8)	36 (31.3)	Ref	–
Negative	92	13 (35.1)	78 (68.6)	4.050 (1.854, 8.852)	<0.001

Data are shown as *n* (%). *P* value computed from two-tailed Student *t* test. *Use of tobacco, moist snuff, betel quid, and cigarettes. †Histological grade includes well-differentiated (G1), moderately differentiated (G2), and poor differentiated (G3) tumors. CI: Confidence interval; HPV: Human papillomavirus; N stage: Lymph node involvement; OR: Odds ratio; pT stage: Primary tumor.

immunostaining, and only 20 (13%) were with strong staining intensity.

Out of 37 HPV-positive cases, 30 (81%) were p50 positive with strong staining intensity, six (16%) with moderate, and only one (2.7%) with weak staining intensity, having no expression of p50. Twenty-five (67%) were p65 positive with strong staining intensity, 12 (32%) with moderate, and none of sample showed weak staining intensity. Among 115 HPV negative cases, p50 and p65 expression were also observed. Forty-two (35%) cases were with stronger intensity, 61 (53%) with moderate, and 12 (10%) showed weak immunostaining for p50. While in p65 protein expression, strong staining intensity was observed in 49 (42%), moderate in 56 (48%), and weak staining intensity in 10 (8.6%) samples.

The correlation of p50, p65, and IκBa inhibitor with risk factors and clinicopathological characteristics of head and neck cancer patients was calculated by Pearson correlation coefficient. A positive significant correlation was observed between p50 *vs.* p65 ($r = 0.249$, $P < 0.05$) and a negative significant correlation between p50 *vs.* IκBα ($r = -0.308$, $P < 0.0001$) in HNC cases. A negative correlation was also observed between p65 and IκBα ($r = -0.592$, $P < 0.001$). A positive significant correlation was observed between use of tobacco and oral hygiene ($r = 0.299$, $P < 0.05$), primary tumor (pT) stage and tumor grade ($r = 0.351$, $P < 0.0001$), and tumor grade and N stage ($r = 0.245$, $P < 0.005$). A negative correlation was observed between pT and N stage ($r = -0.092$) [Table 3].

Discussion

The prevalence of HPV infections was 24.3% in HNC cases. The research finding is consistent with the study conducted by Auguste *et al*.[15] It has been reported earlier that most of HPV positive oropharyngeal cancer patients were younger in age and mostly had no history of tobacco and alcohol,[16] and data analysis revealed that HPV positive HNC patients were higher in female and younger group (age < 40 years). Results of the current study are in support with previous studies, which may point toward its association with sexual behavior.[17-19] It is generally explained that HPV infection can be transmitted through skin contact and damages the epithelial cells in the oral mucosa, genital mucosa, and skin.[20] We have found the data stating that tobacco and betel quid users have a higher frequency of HPV infection, and other studies have
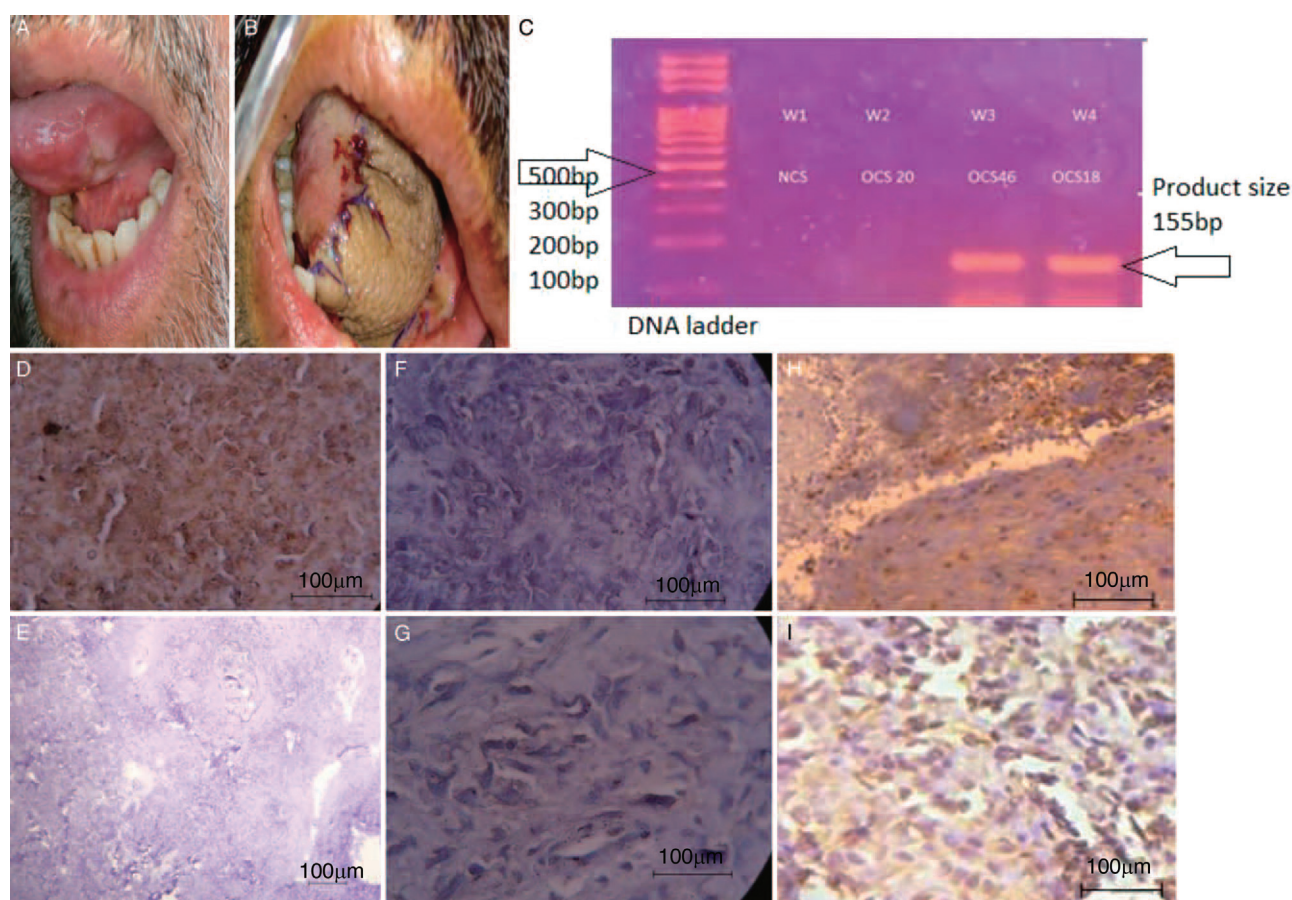
Figure 1: (A) A 56-year-old man with typical initial stage oral carcinoma T0 lesion involving the lift buccal mucosa and tongue. (B) A 41-year-old man with initial stage T1 lesion involving the lift buccal mucosa, tongue, and overlying skin, both using betel quid and tobacco frequently. (C) Electropherogram of amplified product of general primer for HPV detection. The figure also shows DNA ladder (100 bp) with the size of specific amplified PCR product was 155 bp general primer (GP), w1–w4 indicate wells on the agarose gel. (D-I) Immunohistochemical staining of HNC tissues with HPV16/18 E6 antibody staining in various tissues (Original magnification × 40). (D, E) Positive and negative control; (F, G) HPV negative; (H, I) strong staining of HPV positive. HNC: Head and neck cancer; HPV: Human papillomavirus; PCR: Polymerase chain reaction.

Table 2: Binary logistic regression analysis of risk factors predicting the likelihood of head and neck cancer patients.

Variables	B	SE	Wald value	df	P value	OR (95%CI)
Area of living	−0.576	0.423	1.861	1	0.173	0.562 (0.245,1.286)
Age	−2.118	0.823	6.620	1	0.010	0.120 (0.024,0.604)
Tobacco	−2.580	0.825	9.770	1	0.002	0.076 (0.015,0.382)
Oral hygiene	−1.748	0.878	3.965	1	0.046	0.174 (0.031,0.973)
Age_Oral hygiene_Tobacco	1.089	0.316	11.883	1	0.001	2.970 (1.599, 5.515)
Constant	5.986	2.634	5.165	1	0.023	397.867

CI: Confidence interval; df: degrees of freedom; OR: Odds ratio; SE: Standard error.

also shown an association of tobacco with HPV infection worldwide,[14,21] signifying the fact that tobacco-associated carcinogens may alter genetic pathways which may lead to molecular changes, making the individual susceptible to HR-HPV infection. When the concentration of nicotine and its exposure to the cells increases, it leads to altered antigen-mediated signaling pathways such as NFκB and Akt.[22,23] Results from the current study showed a significant correlation between HPV positivity and poor hygiene with an OR of 2.440 (P < 0.03, 95% CI:1.65–3.60), the same trend was observed in other studies worldwide.[24-26] This suggests that poor oral

hygiene plays an important role in the etiology and increases infection of HPV and the risk of cancer by chronic inflammation.[24]

Consistent with other reports, the study established a strong association between HR-HPV infection and oral cavity cancer patients,[27] and 48% of oral cancer patients had detectable HPV. These findings directed an anatomical preference of HR-HPV16 infection and are accordant with the literature.[28,29] Previous study has similarly described that TNM status might not be associated with HPV infection.[30] Overall 64% of HNC cases in the study tested
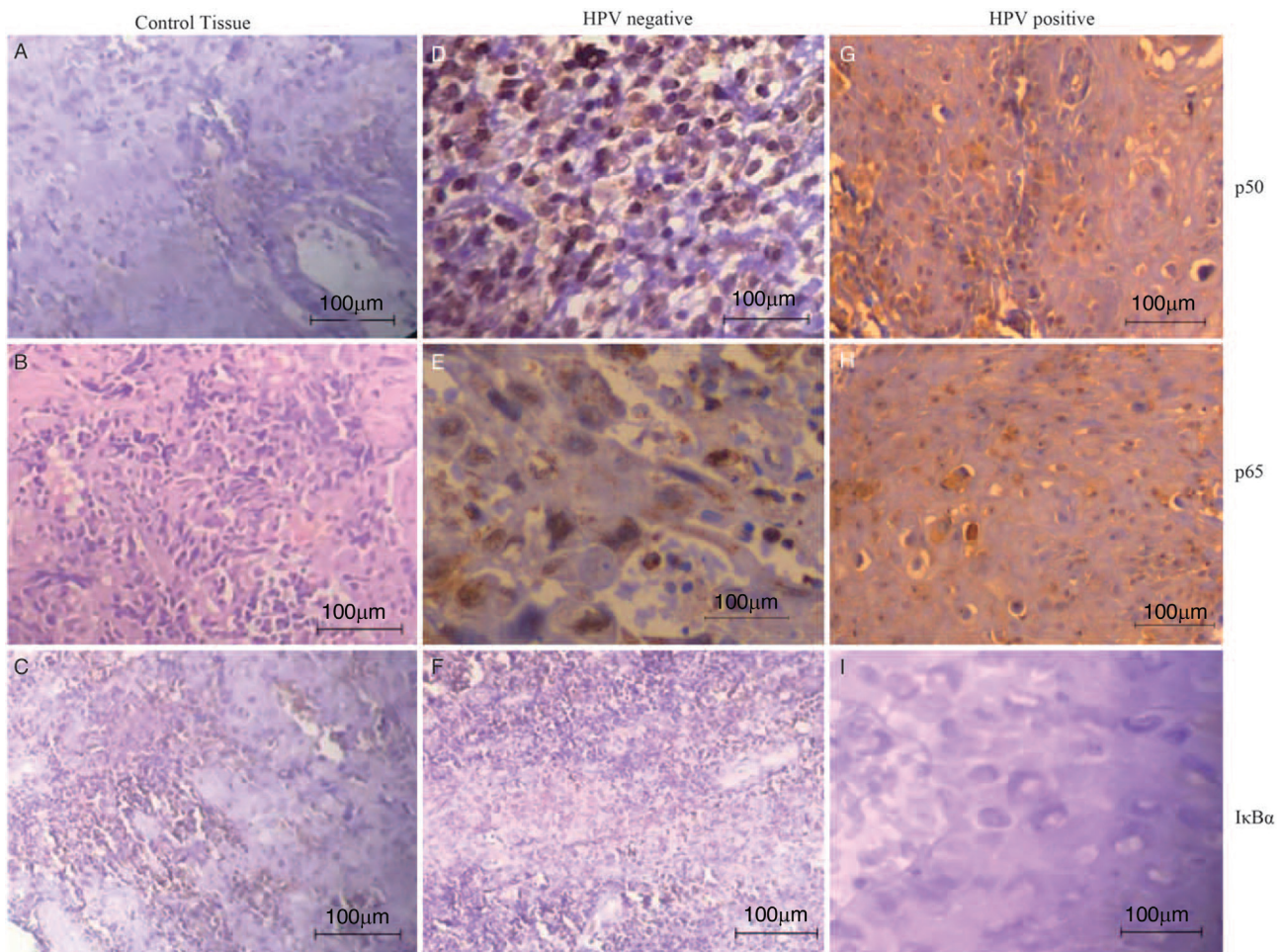
Figure 2: immunohiistochemistry of HNC tissues with NF-κB, p50, p65, and IκBα antibodies in various tissues. (A-C) Staining in control samples, showing no staining and protein expression of p50, p65, and IκBα, respectively. (D-F) Staining in HPV negative tissues, showing protein expression of p50, p65, and IκBα, respectively. (G-I) Staining in HPV positive tumor samples, of p50, p65, and IκBα respectively. Original magnification × 40. HNC: Head and neck cancer; HPV: Human papillomavirus; NF-κB: Nuclear factor kappa B.

Table 3: Correlation between expression of p50, p65, IκBα inhibitor, associated risk factors and clinical characteristics of patients with head and neck cancer.

Items	Use of tobacco	Oral hygiene	Anatomic site	Grade	pT stage	N stage	p50	p65	IκBα
Use of tobacco	1.000	0.299*	0.117	−0.115	−0.144	0.069	0.152	0.223*	−0.218*
Oral hygiene		1.000	0.069	0.057	0.031	0.117	0.155	0.004	−0.225*
Anatomic site			1.000	0.021	0.005	0.021	0.114	0.078	−0.032
Grade				1.000	0.351*	0.245*	−0.177	0.187	0.186
pT stage					1.000	−0.092	0.106	0.05	0.175
N stage						1.000	0.143	0.77	−0.121
p50							1.000	0.249*	−0.308*
p65								1.000	−0.592*
IκBα inhibitor									1.000

The expression levels of p50, p65, and IκBα inhibitor for HNC cases, based on the relative protein level. *$P < 0.05$. The P values were computed using one way analysis of variance and χ^2-test. N-stage: Lymph node involvement; pT: Primary tumor.

were positive for HR-HPV type 16, while 35% of cases were detected with HPV type 18. Previous studies have reported a similar detection rate among HNC samples with 90% of HPV type identified as HPV16.[22] HPV positivity in HNC cases can vary geographically and method used for detection, because PCR is highly sensitive.[31] Zhang et al[32] reported 28.6% positive cases of oral carcinomas with HR-HPV16 and HPV18 in the Chinese population, and in another study of 106 HNC patients in an Indian population, Kumar et al[14] reported HPV16 and HPV18 with a detection rate of 81.8% and 18.1%, respectively, our results correspond to both of these studies.

It was recognized that NF-κB signaling pathway proteins, p50, and p65 showed high expression in HPV-infected samples analyzed with IHC. The results of the current study were similar to the prior studies reported.[8,33] On that account, activation of the NF-κB pathway by viral oncogenes may be the mechanism employed in the head and neck cancer growth. The integration of HPV-DNA in the host cell DNA leads to the stimulation of several pathways involved in cancer development and progression. The HPV viral oncogenes E7 and E6 are the main sponsors to the expansion of HPV-induced cancers, probably due to the integration of the viral genes in the host cell genome. E7 and E6 protein-induced genetic instability results in the inactivation of tumor suppressor genes p53 and pRb, which is regular in the carcinogenesis of human cells. E6 and E7 oncogenes are key regulatory proteins available inside the host cells and are documented to be linked with the transcriptional activity of NF-κB proteins.[33]

Conclusions

This study highlights the detection of HPV infection in HNC patients and expression analysis of the NF-κB signaling pathway. From the study, it can be concluded that the use of tobacco, poor hygiene, and HPV infection are important environmental factors that can act synergetically in modulating the expression of NF-κB pathway proteins. Nevertheless, further research with a large sample size is requisite to elucidate the HPV infection-induced activation of the NF-κB signaling pathway leading to HNC carcinogenesis and to identify simple, reliable, and highly specific molecular biomarkers. Additionally, the disease can be managed by developing high standards of health in the local population, conserving hygiene conditions, and organizing awareness seminars concerning the disease that can reduce the risk of developing HNC.

Conflicts of interest

None.

References

1. Sabatini ME, Chiocca S. Human papilloma virus as a driver of head and neck cancers. Br J Cancer 2020;122:306–314. doi: 10.1038/s41416-019-0602-7.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–249. doi: 10.3322/caac.21660.
3. Hanif M, Zaidi P, Kamal S, Hameed A. Institution-based cancer incidence in a local population in Pakistan: nine-year data analysis. Asian Pac J Cancer Prev 2009;10:227–230.
4. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011;29:4294–4301. doi: 10.1200/JCO.2011.36.4596.
5. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019;144:1941–1953. doi: 10.1002/ijc.31937.
6. Sabir M, Baig RM, Mahjabeen I, Kayani MA. Significance of cyclin D1 polymorphisms in patients with head and neck cancer. Int J Biol Markers 2013;28:49–55. doi: 10.5301/JBM.2012.9768.
7. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709–720. doi: 10.1093/jnci/92.9.709.
8. Senba M, Buziba N, Mori N, Fujita S, Morimoto K, Wada A, et al. Human papillomavirus infection induces NF-κB activation in cervical cancer: a comparison with penile cancer. Oncol Lett 2011;2:65–68. doi: 10.3892/ol.2010.207.
9. Braakhuis BJ, Snijders PJ, Keune WJH, Meijer CJLM, Ruijter-Schippers HJ, René Leemans C, et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. J Natl Cancer Inst 2004;96:998–1006. doi: 10.1093/jnci/djh183.
10. Haddad RI, Shin DM. Recent advances in head and neck cancer. N Engl J Med 2008;359:1143–1154. doi: 10.1056/NEJMra0707975.
11. Qatouseh LA, Sabri I, Alkhatib I, Atwa E, Arafat T. Detection of high-risk human papillomavirus genotypes 16 and 18 in head and neck squamous cell carcinomas in Jordan. Asian Pac J Cancer Prev 2017;18:1337–1341. doi: 10.22034/APJCP.2017.18.5.1337.
12. Cubie H, Seagar A, McGoogan E, Whitehead J, Brass A, Arends MJ, et al. Rapid real time PCR to distinguish between high risk human papillomavirus types 16 and 18. Mol Pathol 2001;54:24–29. doi: 10.1136/mp.54.1.24.
13. World Health Organization. (2015). International statistical classification of diseases and related health problems, 10th revision, Fifth edition, 2016. World Health Organization. https://apps.who.int/iris/handle/10665/246208.
14. Kumar R, Rai AK, Das D, Das R, Kumar RS, Sarma A, et al. Alcohol and tobacco increases risk of high risk HPV infection in head and neck cancer patients: study from North-East Region of India. PloS One 2015;10:e0140700. doi: 10.1371/journal.pone.0140700.
15. Auguste A, Gaëte S, Herrmann-Storck C, Michineau L, Joachim C, Deloumeaux J, et al. Prevalence of oral HPV infection among healthy individuals and head and neck cancer cases in the French West Indies. Cancer Causes Control 2017;28:1333–1340. doi: 10.1007/s10552-017-0966-z.
16. Chau NG, Rabinowits G, Haddad RI. Human papillomavirus-associated oropharynx cancer (HPV-OPC): treatment options. Curr Treat Options Oncol 2014;15:595–610. doi: 10.1007/s11864-014-0309-1.
17. Du J, Nordfors C, Ährlund-Richter A, Sobkowiak M, Romanitan M, Näsman A, et al. Prevalence of oral human papillomavirus infection among youth, Sweden. Emerg Infect Dis 2012;18:1468–1471. doi: 10.3201/eid1809.111731.
18. Dayyani F, Etzel CJ, Liu M, Ho CH, Lippman SM, Tsao AS. Metaanalysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). Head Neck Oncol 2010;2:15. doi: 10.1186/1758-3284-215.
19. Smith EM, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D, et al. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. Int J Cancer 2004;108:766–772. doi: 10.1002/ijc.11633.
20. Pereira N, Kucharczyk KM, Estes JL, Gerber RS, Lekovich JP, Elias RT, et al. Human papillomavirus infection, infertility, and assisted reproductive outcomes. J Pathol 2015;2015:578423. doi: 10.1155/2015/578423.

21. Jamaly S, Khanehkenari MR, Rao R, Patil G, Thakur S, Ramaswamy P, et al. Relationship between p53 overexpression, human papillomavirus infection, and lifestyle in Indian patients with head and neck cancers. Tumour Biol 2012;33:543–550. doi: 10.1007/s13277-011-0295-x.

22. Karin M, Greten FR. NF-kB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005;5:749–759. doi: 10.1038/nri1703.

23. Karin M. Nuclear factor-kB in cancer development and progression. Nature 2006;441:749–759. doi: 10.1038/nature04870.

24. Mazul AL, Taylor JM, Divaris K, Weissler MC, Brennan P, Anantharaman D, et al. Oral health and human papillomavirusassociated head and neck squamous cell carcinoma. Cancer 2017;123:71–80. doi: 10.1002/cncr.30312.

25. Hang D, Liu F, Liu M, He Z, Sun M, Liu Y, et al. Oral human papillomavirus infection and its risk factors among 5410 healthy adults in China, 2009-2011. Cancer Epidemiol Biomarkers Prev 2014;23:2101–2110. doi: 10.1158/1055-9965. EPI-14-0084.

26. Bui TC, Markham CM, Ross MW, Mullen PD. Examining the association between oral health and oral HPV infection. Cancer Prev Res (Phila) 2013;6:917–924. doi: 10.1158/1940-6207.CAPR-13-0081.

27. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24–35. doi: 10.1056/NEJMoa0912217.

28. Zhu C, Ling Y, Dong C, Zhou X, Wang F. The relationship between oral squamous cell carcinoma and human papillomavirus: a metaanalysis of a Chinese population (1994-2011). PloS One 2012;7:e36294. doi: 10.1371/journal.pone.0036294.

29. Kreimer AR. Prospects for prevention of HPV-driven oropharynx cancer. Oral Oncol 2014;50:555–559. doi: 10.1016/j.oraloncology.2013.06.007.

30. Ringström E, Peters E, Hasegawa M, Posner M, Liu M, Kelsey KT. Human papillomavirus type 16 and squamous cell carcinoma of the head and neck. Clin Cancer Res 2002;8:3187–3192.

31. Da Silva SD, Ferlito A, Takes RP, Brakenhoff RH, Valentin MD, Woolgar JA, et al. Advances and applications of oral cancer basic research. Oral Oncol 2011;47:783–791. doi: 10.1016/j.oraloncology.2011.07.004.

32. Zhang H, Zhang Y, Zhao H, Niyaz H, Liu P, Zhang L, et al. HPV infection and prognostic factors of tongue squamous cell carcinoma in different ethnic groups from geographically closed cohort in Xinjiang, China. Biochem Res Int 2016;2016:7498706. doi: 10.1155/2016/7498706.

33. Munger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, et al. Mechanisms of human papillomavirus-induced oncogenesis. J Virol 2004;78:11451–11460. doi: 10.1128/JVI.78.21.11451-11460.2004.