

**Research Report****The role of human papillomavirus in advanced laryngeal squamous cell carcinoma****Fauziah Fardizza, Bambang Hermani, Susyana Tamin**

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Faculty of Medicine Universitas Indonesia/ Dr. Cipto Mangunkusumo Hospital  
Jakarta**ABSTRACT**

**Background:** Many studies had been conducted regarding the association of human papillomavirus (HPV) with laryngeal cancer. HPV was assumed to be one of the etiology of squamous cell carcinoma (SCC) besides smoking and alcohol consumption. Neck lymph node metastasis which is found in advanced laryngeal cancer could decrease the 5-year survival rate up to 50%. **Purpose:** This study aimed to investigate the role of HPV infections in the oncogenesis of the advanced laryngeal SCC and to evaluate the role of HPV in neck metastasis. **Methods:** Cross-sectional, double blind study with planned data collection. Data were taken from Formalin Fixed Paraffin Embedded (FFPE) of laryngeal cancer specimen after laryngectomy. Samples were analyzed by nested Polymerase Chain Reaction (PCR) and continuous flow-through hybridization for genotyping. Expression of Epidermal Growth Factor Receptor (EGFR), and Vascular Endothelial Growth Factor (VEGF) as metastasis biomarker were evaluated by immunohistochemistry. **Results:** Overall HPV proportion in laryngeal cancer was 28.7%. A total of 9% laryngeal cancer patients were infected with high risk HPV type and HPV 16 was found in 5 out of 7 samples. Mantel-Haenszel multivariate analysis found that HPV infection did not play a role in neck metastasis even though there were positive evidences of metastasis biomarker. On the contrary, in the absent of HPV, high expression metastasis biomarkers increased the risk of neck nodes metastasis: in EGFR 3.38 and VEGF 5.14 fold. **Conclusion:** HPV was found to be an oncogenic factor of laryngeal SCC, and HPV 16 was the most frequently observed type of HPV. HPV had protective function towards lymph node metastasis.

**Keywords:** Human papillomavirus, advanced laryngeal squamous cell carcinoma, expression of epidermal growth factor receptor, vascular endothelial growth factor, formalin fixed paraffin embedded

**ABSTRAK**

**Latar belakang:** Berbagai penelitian telah dilakukan untuk melihat hubungan antara Human papillomavirus (HPV) dengan karsinoma sel skuamosa (KSS) laring. HPV dianggap sebagai etiologinya, selain dari merokok dan konsumsi alkohol. Metastasis kelenjar getah bening (KGB) leher merupakan salah satu faktor yang menurunkan angka kesintasan lima tahun sebanyak 50% dan sering kali terjadi pada KSS laring yang telah lanjut. **Tujuan:** Penelitian ini ingin mengetahui peran HPV sebagai faktor onkogenesis dan faktor risiko kejadian metastasis KGB leher pada KSS laring lanjut. **Metode:** Cross-sectional, double blind study dengan pengumpulan data sekunder dari rekam medis. Data diambil dari parafin blok pasien KSS laring yang telah dilaringektomi. Semua data dianalisis dengan nested Polymerase Chain Reaction (PCR), dilanjutkan dengan flow-through hybridization untuk identifikasi tipe virus HPV. Dilakukan juga pemeriksaan imunohistokimia terhadap biomarka penanda metastasis yaitu Expression of Epidermal Growth Factor Receptor (EGFR), dan Vascular Endothelial Growth Factor (VEGF). **Hasil:** Secara keseluruhan didapatkan proporsi HPV pada KSS laring sebanyak 28,7%. Terdapat infeksi HPV tipe risiko tinggi pada 9% KSS laring, dan HPV 16 ditemukan pada 5 dari 7 sampel. Analisis multivariat Mantel-Haenszel mendapatkan infeksi HPV tidak berperan terhadap kejadian metastasis leher, meskipun didapati adanya biomarka penanda metastasis yang tinggi. Sebaliknya, pada ketiadaan infeksi HPV, adanya ekspresi biomarka metastasis yang tinggi meningkatkan risiko terjadinya metastasis KGB lokoregional pada EGFR 3,38 kali dan VEGF 5,14 kali. **Kesimpulan:** HPV dianggap sebagai faktor

*onkogenik KSS Laring, dan HPV 16 merupakan tipe yang paling sering ditemukan. HPV mempunyai fungsi protektif terhadap kejadian metastasis KGB lokoregional.*

**Kata kunci:** *Human papillomavirus, advanced laryngeal squamous cell carcinoma, expression of epidermal growth factor receptor, vascular endothelial growth factor, formalin fixed paraffin embedded*

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## INTRODUCTION

Head and neck malignancy accounts for 5% of all malignancies and 3% of those results in death. Laryngeal carcinoma is placed as the third most common malignancy after nasopharyngeal and paranasal sinus carcinoma.<sup>1</sup> World Health Organization (WHO) reported, cited by Hermani,<sup>2</sup> that 1.5 of 100,000 population died because of malignant tumor. In Indonesia, the incidence of laryngeal carcinoma is estimated to reach 1% of all malignancies in the otolaryngology head and neck area. The American Cancer Society reported in 2006, that America had 12,000 new cases with 4,740 mortality cases caused by laryngeal carcinoma.

Siddiqui<sup>3</sup> reported that Spain is also one of the countries with a high incidence of laryngeal carcinoma, with number of 20 out of 100,000 population. Countries with a high incidence of laryngeal carcinoma are Italy, France, and Poland. In India, data from the Pathology Anatomy Department, Patna Medical College Hospital, during 2002–2006, reported 5,479 tumor cases: 768 cases in the head and neck region, 455 cases were diagnosed as benign tumors, and 214 cases of malignant tumors with 18.7% of those cases were diagnosed with squamous cell carcinoma (SCC) of the larynx, placing above of nasopharyngeal cancer. From the data of the Pathology Anatomy Department, Faculty of Medicine Universitas of Indonesia/Cipto Mangunkusumo Hospital (FMUI-CMH), in the period of 2000–2005, there were 3,344 cases of malignant tumors of the head and neck region with nasopharyngeal carcinoma as the most common case with 948 cases

(28.3%), while laryngeal carcinoma with 225 cases (6.73%). Histologically, SCC comprises 95–98% of all laryngeal carcinoma cases.<sup>4</sup> Otolaryngology Head and Neck Department FMUI-CMH reported 310 laryngeal carcinoma cases in 2006–2014.<sup>5</sup>

Human papillomavirus (HPV) is the most common virus researched which is correlated with laryngeal carcinoma. Human papillomavirus is one of the factors that have the role as an oncovirus in developing countries. The prevalence of this virus involvement varies between 0–85%. Because of the wide variation of prevalence, it is important to know the prevalence of this oncovirus in Indonesia. Tumor with HPV involvement has a better prognosis and better response to therapy compared to tumor without HPV infection.<sup>6</sup>

Commonly, human papillomavirus infection is detected in malignancy cases, at high risk type, and among the high risk type, HPV 16 is the most common type of HPV found. In a molecular level, head and neck carcinoma with HPV has a different characteristic with malignancies without HPV. Malignancies with a positive HPV has a low expression of cyclin D, Retinoblastoma protein (pRb) and p53 wild type, also an increase of p16 that is not found in malignancies with negative HPV. This condition causes a difference in the molecular behavior that affects prognosis.<sup>7</sup>

The process of metastasis initially begins with a change in tumor tissue and its environment, starting with migration of cell tumors caused by the decrease of the cell

tumors adhesion, damage of the protecting barriers that confine the tumor, new growth of blood vessels, lymphatic vessels, and others. These changes can be detected by using biomarkers. Accurate biomolecular analysis development is expected to portray the status of a patient's tumor with or without metastasis. This biomarker finding can be used to determine the potential of metastasis to locoregional lymph nodes at the same time during the biopsy of the primary tumor. This finding is expected to be used to determine the potential of micrometastasis so that an integrated management such as neck dissection could be executed coinciding with the definitive surgery. Molecular targeted therapy could also be done in patients with late stadium SCC or those with metastasis.<sup>8-10</sup>

Angiogenesis role that is stated by the high expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) at the time of diagnosis show a risk of metastasis to neck lymph nodes. Detection of these biomarker can predict metastasis to the neck lymph nodes, enabling determination of the right choice of therapy.<sup>11</sup>

According to this background, the author is interested in conducting a research regarding the analysis of laryngeal SCC, how the biomarkers expression of EGFR, MMP-9, and VEGF in laryngeal carcinoma with or without HPV infection, and also to observe the role of the biomarker in determining the potential of metastasis by correlating it with locoregional lymph node involvement.

## METHODS

This research used a cross sectional design, double blinded with a diagnostic research approach with expression of biomarkers EGFR, MMP-9, TIMP-1, E-cadherin and collagen type IV, also the infection of Human papillomavirus and its subtype in laryngeal SCC to determine the

diagnosis of locoregional lymph node that has potential for metastasis.

This research was conducted in Laryngopharyngeal Division, Department Otolaryngology HNS FMUI-CMH. HPV examination used the GenoFlow Human Papillomavirus Array Test-Kit, DiagCor with a technology of flow through hybridization. The assessment of the protein expressions EGFR, MMP-9, and VEGF was done by immunohistochemistry. All examinations for HPV and protein expressions were performed in the Department of Pathology Anatomy FMUI-CMH.

The research population were laryngeal SCC patients that have undergone laryngectomy with or without neck dissection in the Department of Otolaryngology HNS FMUI-CMH with a paraffin block of the surgical tissue kept in the Department of Pathology Anatomy FMUI-CMH that were matched the inclusion criteria of no history of previous chemotherapy and/or radiation before surgery and no chronic illnesses such as TBC or hepatitis. The exclusion criteria was when the data of the laryngeal SCC patient's medical record were incomplete and/or the paraffin block of the patient could not be used for laboratory analysis. Research subjects were included by random sampling from the CMH medical record of 2006–2014. The number of subjects were counted from the minimal research subject equation to answer the purpose of this research as large as 87 cases.

This research had been initially begun by identifying patients with the diagnosis of laryngeal SCC in the Department of Otolaryngology HNS FMUI-CMH through tumor medical records in the Laryngopharyngeal Division (from 2006–2014). Classification of the tumor stadium had been done according to UICC 2002. Then, the data recorded into a special research status. Human papillomavirus subtype examination was conducted using GenoFlow Human

Papillomavirus Array Test-Kit, DiagCor, (GenoFlow; DiagCor Bioscience, Hong Kong) with a technology of flow through hybridization. DNA extraction was conducted with DNeasy and Steinau et al<sup>12</sup> modification from the paraffin block of laryngeal SCC. The HPV genotyping results were read from the cellulose membrane in the form of dot blots in a probe with a specific marker.

Immunohistochemistry (IHC) tissue had been processed from the paraffin block that has been re-evaluated using Hematoxylin Eosin (HE) staining by the Department of Pathology Anatomy FMUI-CMH. The HE staining and IHC for all expressions of EGFR, TIMP-1, MMP-9, VEGF, E-cadherin and collagen type IV of laryngeal SCC tissues were conducted in the Department of Pathology Anatomy FMUI-CMH. Immunohistochemistry staining used specific antibodies for each protein. The examination results were evaluated using the binocular light microscope Leica, with a 400x enlargement. Image captured was taken from each corner and evaluated using ImageJ software (Wayne Rasband, Bethesda, Maryland). The final score for each research subject were the mean value of five examinations.

Paraffin blocks from laryngeal SCC that had undergone laryngectomy from the year 2006–2014 with or without neck dissection were included in the study until the minimal number of subjects was reached. Anonymosity was executed in the collection of the data without attaching the name when transferring data from the medical record. The collected data was included the demographic characteristic, gender, age, cigarette smoking consumption, alcohol consumption, and clinical characteristics such as clinical symptoms of hoarseness, difficulty in swallowing, size of the tumor, tracheotomy before surgery, total laryngectomy, pathological anatomy differentiation, therapy modality, the time distance to radiation postsurgery, and the existence of locoregional

lymph node enlargement according to UICC criteria. The existence and type of HPV examination with GenoFlow Human Papillomavirus Array Test-Kit, DiagCor was examined by using formalinized laryngeal SCC tissue in the form of paraffin block. Identification of HPV subtype was conducted using the technology of flow through hybridization in the Molecular Pathology Division and the protein expressions of EGFR, MMP-9, and VEGF were assessed using the immunohistochemistry examination in the Immunology Division, Department of Pathology Anatomy FMUI-CMH.

All numerical data were tested for normality values with Shapiro Wilk test. Quantitative data analysis that have an abnormal distribution was presented in median and range. Statistical correlation testing between 2 qualitative variables was done using Chi Square or Fisher Exact test if it does not comply with the parametric conditions. Multivariate analysis was done by using binary logistic analysis. Variables that have a correlation with the locoregional lymph node status ( $p < 0.25$ ) from the bivariate analysis were included in the binary logistic regression. This was performed to investigate which independent variable had the strongest correlation with the dependent variable and then included in a multivariate logistic regression model.

Mantel Haenszel multivariate analysis was done for biomarker expression EGFR, MMP-9, VEGF and also HPV against locoregional lymph node metastasis subject.

## RESULTS

The characteristic of subjects found that male were more dominant than female with a ratio 16:1, with the biggest age group was the adult (93%), smokers (86.2%) with smoking period of more than 20 years (44%), and more than 20 cigarettes a day (44%) (Table 1).

**Table 1. Demographic profile of patients**

Variables	(n)	Percentages (%)
<b>Sex</b>		
Male	82	94.3
Female	5	5.7
<b>Age</b>		
Elderly (>60)	37	42.5
Adult (45-60)	45	51.7
Young adult (18-44)	5	5.8
<b>Smoking</b>		
Yes	75	86.2
Cigarettes		
>20 cigarettes	42	56.0
≤20 cigarettes	33	44.0
Smoking periods		
>20 years	70	93.3
≤20 years	5	6.7
Not smoking	12	13.8

Ninety seven point eight percent of subjects were advanced laryngeal carcinoma. Lymph node spread were found in 51.7%, there was no distant metastasis. Keratin tumor was 88.5%, well–moderate differentiated tumor G1-G2 was 86.4%. Chemoradiation was 56.3%, and recurrence was 25%.

DNA HPV were found in 25 cases of Laryngeal Carcinoma with *nested PCR* using MY09/MY11 and GP5+/GP6+ (Figure 3 and 4). Positive nested PCR continue with flow-through hybridization (DiagCor) for genotyping. Sample result from DiagCor genotyping seen in Figure 5.

We found 7 cases of high-risk HPV and 8 cases of the low-risk type, as well as 3 cases of universal (unknown genotype) (Table 3).

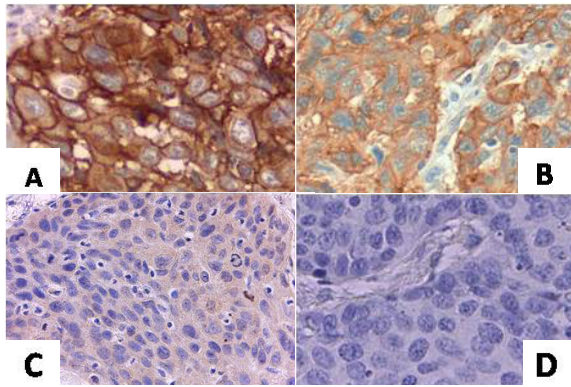
The Mantel-Haenszel test listed three kinds of positive biomarkers expression towards HPV infections that were able to be used to determine the potential of locoregional lymph node metastasis, such as EGFR, MMP-9, and VEGF high expressions with p value <0.05. This study had proven, when there was

**Table 2. Size of tumor, lymph node spreading, metastasis, characteristic and differentiated of tumor**

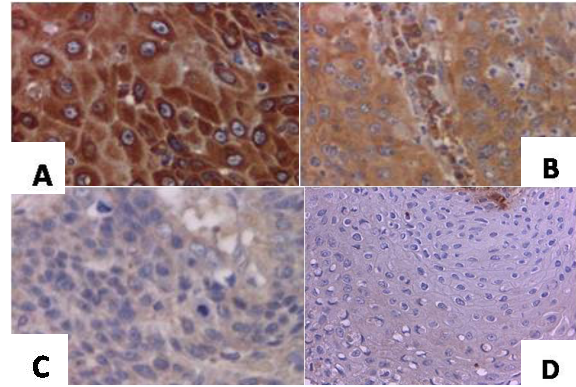
Variables	(n)	Percentages (%)
<b>Tumor size</b>		
T3-T4	85	97.8
T1-T2	2	2.2
<b>Lymph node spread (N)</b>		
N <sub>1-3</sub>	45	51.7
N <sub>0</sub>	42	48.3
Metastatic		
M <sub>0</sub>	87	100.0
<b>Tumor characteristics</b>		
Without keratin	10	11.5
Keratin	77	88.5
<b>Tumor differentiated</b>		
Undifferentiated (G3)	11	12.6
Well-moderate differentiated (G2-G2)	76	86.4
<b>Radiotherapy</b>		
Chemoradiation	49	56.3
Without chemoradioation	38	43.7
<b>Recurrence</b>		
Yes	22	25.3
No	65	74.7

no HPV infection, high expression of EGFR may pose the risk of N 3.814 times higher than low expression of EGFR. High MMP-9 expression was found to be 4.063 times likely than low expression of MMP-9, as well as the expression of high VEGF increased the risk to 4.473 times (Table 4).

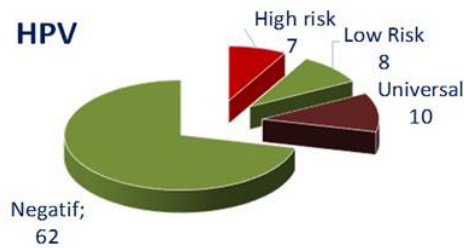
Multivariate statistics indicated the HPV infection in SCC laryngeal cancer, despite positive metastasis biomarkers (EGFR ≥3, MMP-9 and VEGF ≥2.9), patients were no longer at risk of locoregional lymph node metastasis. This study showed that HPV was statistically protective to locoregional lymph node.



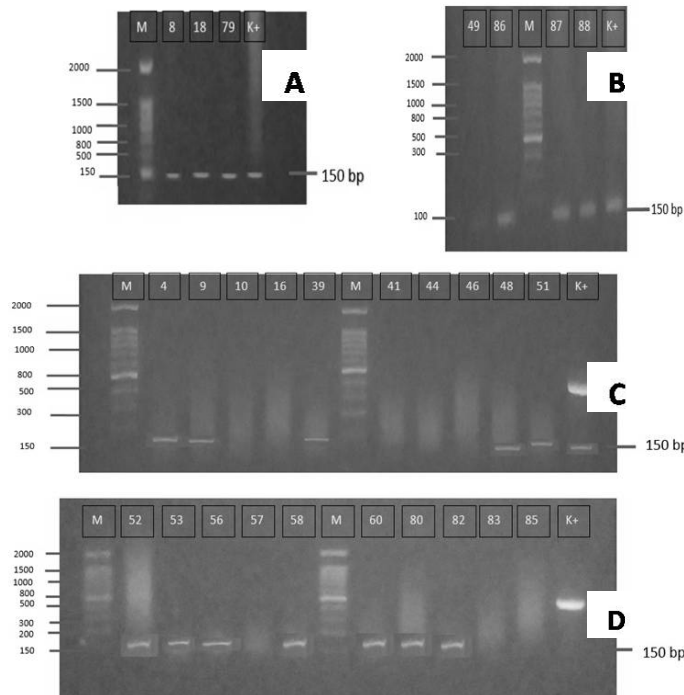
**Figure 1. Immunohistochemistry EGFR expression staining of laryngeal carcinoma**  
 Immunostaining positive on cell membrane and cytoplasm; (A) EGFR high positive expression, (B) EGFR positive expression, (C) EGFR low positive expression, (D) Negative control of EGFR expression (400x enlargement).



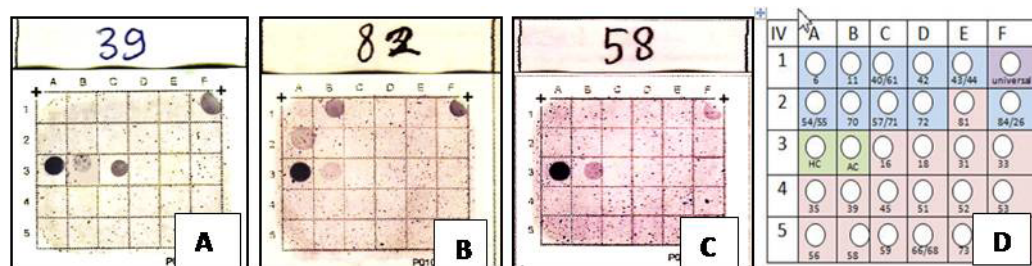
**Figure 2. Immunohistochemistry VEGF expression staining of laryngeal carcinoma**  
 Immunostaining positive on cell membrane and cytoplasm; (A) VEGF high positive expression, (B) VEGF positive expression, (C) VEGF low positive expression, (D) Negative control of VEGF Expression (400 x enlargement).



**Figure 3. DNA positivity and type of HPV in laryngeal carcinoma**



**Figure 4. Nested PCR with MY09/MY11 and Gp5+/Gp6+.**  
 Positive nested PCR seen as 150 pb band. DNA ladder/Marker 100 bp (Antagene, Inc. Santa Clara, CA 95054) used for marker (M). Positive DNA HPV found in sample with code no. 4, 8, 9, 18, 39, 48, 51, 52, 53, 56, 58, 60, 79, 80, 82, 86, 87,88.



**Figure 5. Genotyping DiagCor results**

(A) Sample 39, showed genotyping DNA HPV with high risk, type 16, with coordinate; (3;C);  
 (B) Sample no.82, showed genotyping DNA HPV with low risk, type 11 and 54/55, with coordinate (1;B) and type 54/55 (2;A);  
 (C) Sample 58, showed genotyping DNA HPV universal (1;F);  
 (D) Probe mapping for genotyping DNA HPV.

**Table 3. HPV type profile using *DiagCor***

No.	Code	Number histopathology	Result	Result			
				High-risk HPV	Low-risk HPV	HPV positive universal	Unclassified
1	8	0805834 IVD2	Positive	<b>16, 18, 51, 52</b>	57/71	No	No
2	9	0806426 IV	Positive	No	No	Yes	No
3	13	0907927 IIC1	Positive	<b>16</b>	11, 6	No	No
4	18	1005888 IB	Positive	<b>16</b>	No	No	No
5	21	1101117 ss IV F	Positive	No	No	No	Yes
6	23	1101859 IIE	Positive	No	No	Yes	No
7	25	1106459 VIC	Positive				Yes
8	30	1200874 IIE	Positive	<b>52, 16</b>	43/44, 11, 54/55, 70, 84/26,	No	No
9	36	1203984 VIII C	Positive	No	No	Yes	
10	38	1205448 VIIID	Positive	No	<b>11, 6</b>	No	No
11	42	1207099 IID	Positive	No	No	No	Yes
12	54	1303003 XC	Positive	No	No	No	Yes
13	60	1306106 IC	Positive	<b>18, 51, 52, 81, 16</b>	84/26, 57/71	No	No
14	68	1308236 IIB2	Positive	<b>51</b>	6	No	No
15	74	1309033 XIID	Positive	<b>51</b>	11, 6	No	No
16	76	0802448 IV	Positive	No	<b>11</b>	No	No
17	94	1100104 IIIB	Positive	No	No	No	Yes
18	95	1400638 IIE	Positive	No	<b>11, 43/44, 70</b>	No	No
19	96	1401005 IIB	Positive	No	<b>11</b>	No	No
20	98	1401979 IIE	Positive	No	<b>11, 6, 54/55</b>	No	No
21	99	1403882 IID	Positive	No	No	No	Yes
22	100	1405448 IIIC	Positive	No	<b>6</b>	No	No
23	102	1406398 IIB	Positive	No	<b>6</b>	No	No
24	103	1407036 IID	Positive	No	<b>6</b>	No	No
25	104	1404098 IIC	Positive	No	No	No	Yes

**Descriptions:**

High-risk HPV infections were caused by type 16, 18, 51, 52, 81. HPV-16 acquired from 5 out of 7 cases (71.4%) (sample code of 8,13,18,30,60). Low-risk HPV infections were caused by type 6, 11, 26, 43, 44, 54, 55, 57, 70, 71, 84. Multiple infections were found in 6 cases, sample code of 8, 13, 30, 60, 68,74. Single infection was found in one case of high-risk type (code 18) and in low-risk type were found 8 cases with sample code 38, 76,95,96,98,100,102,103. Three cases of Universal HPV were found (code 9,23,36) and seven cases of Unclassified HPV (sample code 21,25, 42,54,94, 99, 104).

**Table 4. HPV influence to the expression of the metastasis with locoregional lymph node metastasis biomarker**

Variables	p (cumulative)	Odds ratio (OR) cumulative
EGFR $\geq 3$	0.044	3.814
MMP-9 $\geq 2$	0.023	4.063
TIMP-1 $\geq 0.8$	0.537	2.755
VEGF $\geq 2.9$	0.030	4.473
E-cadherin $\leq 2.7$	0.163	0.398
Collagen type IV $\leq 0.6$	0.385	0.379

## DISCUSSION

This research found HPV DNA as the etiology of SCC in laryngeal cancer, besides smoking and alcohol consumption as risk factors. Literatures have established that high risk HPV had been proven to be the etiology of malignant tumors. This research found the proportion of HPV DNA infections in laryngeal SCC patients were up to 28.7%.

Fouret et al, cited by Torente,<sup>13</sup> found that the most common type of HPV infection in his research was HPV 16, reported 12 out of 19 (63.9%) in cervical carcinoma, all cases in laryngeal cancer, and 49 out of 55 (89%) cases in head and neck cancer. One research tried to compare between 93 laryngeal cancer and 49 cases normal laryngeal mucosa, 22 laryngeal nodules as control group. HPV DNA was detected by PCR, which they found in 33 (35.5%) patients, 4 out of 49 (8.2%) were normal mucosa. Gungor et al<sup>14</sup> used paraffin block in 99 patients and found 7.3 % was HPV-positive, which HPV type 6 and 11 were found to be the most common type, the only high risk type found was HPV 16. This research, utilized the same techniques, in 87 patients and acquired more than 28.7% which was higher than Gungor's, 28% were high risk HPV.

Baumann et al<sup>15</sup> detected HPV DNA in 6 out of 38 patients of early stage SCC laryngeal cancer ( $T_1$  and  $T_{is}$ ) and found subtypes 16, 26, 31, 39, and 52 HPV. HPV-positive SCC laryngeal cancer patients mostly found to be young adults and rarely had smoking habits. This research found 7 cases (100%) of HPV-positive in adults and elderly age group, which was on the contrary to the literature. HPV-positive were also found more in well and moderate differentiated tumors (G1/G2), these were 6 out of 7 cases, whilst the only 1 was found in the undifferentiated tumor (G3), otherwise, no HPV was found. This was true according to the study conducted by Morshed et al.<sup>16</sup> Morshed et al<sup>16</sup> studied 40 patients of SCC laryngeal carcinoma and 33 non-neoplastic laryngeal lesions as control group. Human papilloma virus was found in 6 cases of SCC laryngeal cancer; 5 out of 6 positive-HPV showed G2 tumor differentiation (*moderately differentiated*) and 1 (16,6%) cases of G3 (*poorly differentiated*), whereas no HPV infection tumor was considered as G1 (*well-differentiated*). Four out of 6 positive-HPV was supraglottic, 1 case of tumor located in the glottic, and 1 subglottic case. Five out of 6 (83.4%) cases of positive-HPV were  $T_3$ - $T_4$ , and one case was  $T_2$ . Three out of 6 HPV-positive tumors clinically showed no neck lymph node metastasis ( $N_0$ ), three of the positive-HPV



patients had N<sub>1</sub> or N<sub>2</sub> status. No HPV was found in control group. Morshed et al<sup>16</sup> mentioned 15% of HPV infection may suggest the etiology of SCC laryngeal carcinoma, even though they could not find the connection between the HPV incidence and the stage of infection.

Bivariate analysis was implemented to measure the correlation of HPV infection and locoregional lymph node metastasis. Three out of 7 positive-HPV (42.9%) were positive-N status and 4 (57.1%) cases were negative-N status. This indicates there was no statistical correlation ( $p=0.232$ , OR=0.36 and CI 95% 0.07–1.78).

The correlation of HPV infection and recurrency was found in 2 out of 7 cases of positive-HPV (28.6%), whereas the rest of the five (71.4%) cases were not recurrent. There were 17 recurrences in cases of negative-HPV (24.3%) and 53 (75.7%) cases were not recurrent, showing no statistically correlation.

Bozdayi et al<sup>17</sup> studied 89 subjects of *PCR assay* and found *HPV genome* in 65 cases due to inadequate tissue to amplify the G6PDH. Positive-HPV was found in 27 (41.5%) cases. Positive-HPV was also found to be correlated to neck lymph node metastasis. The odds ratio analysis indicated positive-HPV as an important factor in neck lymph node metastasis or HPV to be the predictor for neck lymph node metastasis.

Mendhelson et al<sup>18</sup> in his research studied the neck lymph node metastasis, HPV and p16. They stated that HPV and p16 may predict the poorly differentiated tumor. Tumor with positive-HPV and p16 increased the risk for neck lymph node metastasis ( $p=0.01$ , OR 23.9, CI 95% 2.2–265.1). Tumor with HPV and p16 positive showed *superior predictive value* towards lymph node metastasis compared to standardized histopathology examination using HE. A multidiscipline team managing the head and neck tumor with HPV and p16 must consider neck lymph node management carefully. This study stated a different result

than that of Bozdayi et al<sup>17</sup> and Mendhelson et al<sup>18</sup>. In this study, HPV infection also indicates a tendency to a different kind of SCC laryngeal carcinoma without HPV infection, having better nature, even though not yet proven statistically.

Mantel-Haenszel test analysis showed 3 kinds of positive biomarkers expression in the role of diagnosis of potential locoregional lymph node metastasis, which were EGFR, MMP-9, and VEGF. This analysis also stated that HPV infection statistically had a role in protecting locoregional lymph node metastasis. Further research is needed to prove this correlation. In SCC laryngeal carcinoma with HPV infection, with the presence of either high or low expression of the biomarkers (EGFR, MMP-9 and VEGF) statistically had no relationship with the N status having the same CI 95% of all 3 biomarkers, 0.14–1.17. On the contrary, without HPV infection, the locoregional lymph node metastasis was 3.814 times likely to occur in high expression of EGFR than the low expression. And so for MMP-9 expression, the risk is increased to 4.063 times in high expressed MMP-9, as well as high expression of VEGF, implying the risk 4.473 times higher. In cases of laryngeal SCC with or without HPV infection, there were differences in the profile expression of EGFR, MMP-9, TIMP-1, VEGF, E-cadherin and collagen type IV, between patients with positive and negative N status.

Human papillomavirus was found in 28.7 % of laryngeal SCC. HPV high-risk type was found in 9.1% cases and low-risk type in 10.4% cases. In laryngeal SCC without HPV infection, there was a difference in the expression of the biomarker profiles EGFR, MMP-9, and VEGF between the positive and negative N status, but in cases with HPV infection, the difference in the expression of the biomarker profile could not determine the N status.

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