ISSN: 03875547
Volume 44, Issue 03, June, 2021

Human Papilloma virus-16 and tumor stage; is there a correlation?

Mohammed A. Gomaa[1*], Usama Abdel Nabi[1], Ahmed Abdel Rahman[1], Rasha A. Abdel Moniem[1], Ahmed A. Sadek[1]

From the Department of ORL-Head and Neck Surgery, Minia University, Egypt[1]

Corresponding Authors: 1*

Keywords:
Laryngeal squamous cell carcinoma, HPV-16, Tumor stage.

ABSTRACT

Many patients with Laryngeal squamous cell carcinoma (LSCC) do not have any of the traditional risk factors associated with head and neck squamous cell cancers. Epidemiological and molecular studies have identified human papillomavirus (HPV) as a causative agent. The aim of the study is to find the relation between HPV-16 infected LSCC and T and N stage of the tumor. Prospective, cross section Tertiary university hospital. The current study was conducted on 47 cases suffered from LSCC, all patients subjected to clinical, radiological and endoscopic assessment of the tumor. Biopsy was taken from each patient and stained by H&E to confirm the clinical diagnosis and also Immunohistchemical staining was done to evaluate infected tumors by HPV-16. SPSS program version 16 was used to assess the correlation between HPV-16 affected tumors and T and N stage of the LSCC. The study sample pointed out that the majority of patients were in T2 and N0 stage. The current study showed that most of the patients infected with HPV-16 presented in T2 stage (six out of nine patients 66.7%), while the majority HPV-16 negative patients also presented in the same stage (36 patients 94.7%). There is no difference between HPV-16 positive and negative patients in N stage as the majority of both groups presented in N0, 66.7% in positive cases compared to 76.3% in negative cases. Our results reveal that 22.2% of HPV positive cases were in stage T3 and T4 compared to 0% in the HPV negative cases in the same stages. The second common presenting N stage of HPV-16 positive cases was N2, while in HPV negative patients was stage N1. Our results showed that there were no correlation between T nor N stage of HPV-16 infected LSCC, but indeed not statistically significant we found that HPV positive cases tend to be presented more in advanced T and N stage compared to HPV negative cases.

1. INTRODUCTION

Epidemiologic and molecular studies have identified human papillomavirus (HPV) as a causative agent in the head and neck cancer and viral infected tumors presenting a better survival and being important risk

factors together with the long-established predisposing factors (e.g. tobacco and alcohol consumption) [1]. Cancer of the larynx accounts for more than 3% of all cancers, making it the sixth most common cancer worldwide. The potential oncogenic role of HPV infection in the development of laryngeal cancer has been well recognized in recent decades [2]. Papillomaviruses exhibit a high degree of specific cellular tropism for squamous epithelial cells and have been associated with various clinical manifestations ranging from benign hyperplastic epithelial proliferative innocuous lesions (warts, papillomas) to invasive cancer [3]. HPV infection, especially infection due to the high-risk type HPV-16, was found to be significantly associated with the risk of laryngeal squamous cell carcinoma [2]. The aim of the study is to assess the correlation between tumor and Lymph node stages of the laryngeal cancer and infectivity by HPV-16.

2. Patients and Methods

The current study is cross sectional prospective study that was done on 47 patients with laryngeal carcinoma recruited from outpatient clinic of Minia University Hospital, from March 2016 to July 2018. The study was approved by The Research Ethics Committee of the Faculty of Medicine, Minia University and informed singed consent was obtained from every patient that enrolled in the study. The age of the patients ranged from 45 to 70 years with 46 male and one woman. We enrolled in the study patients with Squamous cell carcinoma (SCC) of the larynx that proved by histopathology, we excluded from the study, patients with benign laryngeal lesions and patients prove to be cancer larynx but not SCC. Patients were subjected to an assessment protocol that included a detailed medical and sexual history. Patients underwent a comprehensive general and otorhinolaryngological examination that included an indirect endoscopic laryngeal examination, Radiological evaluations by computerized tomography (CT) and both clinical and endoscopic examinations were used to stage the tumor and assess its extent according to the TNM staging system. Biopsy specimens were obtained from each patient at operating room under general antiasthma and the biopsy divided into two sections: the first was subjected to H&E staining, and the other was used for immunohistochemical (IHC) staining for HPV-16. The IHC staining level was assessed and scored by two independent pathologists. The expression of HPV-16 was defined as positive if distinct nuclear immunoreactivity was detected in laryngeal tumor cells, cytoplasmic and nuclear staining was present, staining was moderate to strong and should be diffuse, and staining was present in at least 50% of tumor cells.

2.1 Statistical analysis

Statistical analysis was performed using SPSS program version 16. Data are presented as means ± standard deviation (SD). Student's t-test, analysis of variance (ANOVA) and the Bonferroni post-hoc test were used for comparisons. The level of statistical significance was $P \leq 0.05$.

3. Results

The current study was conducted on 47 patients with laryngeal SCC. The age of the patients ranged from 45 -70 years, with a mean age of 58 years, including 46 (97.9%) male patients and one (2.1%) female patient.

3.1 Correlation between HPV-infectivity and Tumor stage

The present study revealed that the association of HPV-16 in laryngeal SCC was 19.1% (9 cases), table1. The study sample pointed out that 42 patients (89.4%) were in T2 stage (Table 2). While most patients (35 patients, 74.5%) were presented in N0 stage (Table 3) The current results indicate that most of the patients infected with HPV-16 presented in T2 stage; 6 out of 9 patients (66.7%), while in HPV-16 negative patients 36 patients (94.7%) were also in T2 stage. Table 4 When we compared other T stages: the results showed that 22.2% of HPV-positive cases were in stage T3 and T4 compared to 0% in the HPV- negative cases were in the same stages.

3.2 Correlation between HPV-infectivity and lymph node stage

Table 5 reveals that there was no significant difference between HPV-16 positive and HPV- negative patients (66.7% vs. 76.3% in HPV-positive and HPV-negative respectively) in No stage, however; patients with HPV- positive specimens had higher N2 stage (2 patients (22.2%) compared to 3 patients (7.9%) in HPV-negative patients. So, the second common presenting N stage of HPV-16 positive cases was N2, while in HPV-negative patients was stage N1. Our results pointed out that, there was no correlation between HPV-16 positivity and T nor N stages of SCC of laryngeal cancer P = 0.075 and 0.340 respectively, this was presented in table 6. HPV-positive patients tend to have an advanced T and N stages, even if it wasn't significant but was apparent from frequency. We only find correlation between T and N stage of the tumor.

4. Discussion

The current study tried to find any relation between HPV-16 and tumor stage in laryngeal SCC. Our results showed that there was no correlation between T and N stage of the patients with HPV- 16 infected laryngeal SCC, however; we found that HPV-positive patients tend to be presented in advanced T and N stages compared to HPV negative cases. Our data is supported by our previous results that showed a significant correlation between tumor grad and HPV-positivity in laryngeal SCC [4]. As the grade of the tumor goes up towards undifferentiating, the tumor spreads more locally (T stage) and regionally (N stage) and the response to chemoradiation modality of treatment becomes better. The recent articles about prognosis of HPV positive cases in laryngeal SCC showed that assumption. Patients whose tumors test positive for HPV have at least half the risk of death from HNSCC and respond better to treatment than those who test negative [5], [6] This includes selection of patients for organ preservation therapy, which may be more successful in patients with HPV- positive HNSCC [7]. These results support our assumption. Stephen et al, reported that there was no correlation between HPV status and the tumor stage, however; regarding the prognosis according to HPV status, HPV-positive LSCC patients showed better survival outcomes when compared to HPV−negative after chemo-radiation. These findings support our results [8]. Similar to our results, Morshd and Jiang et al, indicated that no significant correlation was found between the incidence of HPV and the epidemiological, histological grade and clinical stage of tumors. In their results, HPV is also detected more frequently in T2-T4 stage and in patients with nodal relapse [9- 11] found that in oropharyngeal SCC, HPV positive cases are frequent in early T stage and advanced N stage and reported also that HPV patients had histopathology of tumors more frequently in poorly differentiated grade. Our findings agree with Urban study in the N stage, this raise the issue that HPV infected tumors is biologically different from HPV negative cases. [12] stated that the overall 5-year survival rate in head and neck SCC was 62% in HPV-16 positive cases compared to 26% in negative cases treated with radiotherapy. These findings support our assumption of biological difference between HPV positive and negative cases.

Anwar et al, suggested that multiple factors are involved in laryngeal SCC and that the simultaneous over-expression of p53 and the presence of ras mutation may be related to the advanced stage of laryngeal carcinoma not HPV- 16 positivity. [13] Kava et al. [14] and Hernandez et al. [15] found no correlation between HPV positivity and tumor stage in laryngeal SCC., These findings are matching our results. The use of p16- IHC staining as a marker of infection with HPV in OPC is widely used in clinical trials based on the high concordance between this method and various other HPV detection methods, including type specific HPV-DNA detection by situ hybridization (ISH) [16]. So our results are comparable to others as we used IHC. Although our study is limited with a small number with HPV-positivity, however; it reported clearly that HPV-positive patients were more presented in advanced T and N stages compared to HPV-negative patients. These findings warrant further evaluation in multi-centers with larger number of patients.

5. Acknowledgment

We acknowledge the work of Dr. Nisreen Abdeltwab, MD, and Dr. Rehab Mamdoh, MD, Assistant professor of Pathology, Faculty of Medicine, Minia University. The two pathologists evaluated tumor samples. We acknowledge also Mr. Mohammed Elmahdy. M.Sc statistics, for his statistical assessment of the results.

6. References

[1] Reka Fejer E1, Abram Z, Zs Egyed J, Voidazan S, Toth E, Szentirmay Z, Kasler M. Human papilloma virus status evaluation and survival description in selected oropharyngeal and laryngeal squamous cell carcinoma patients from Hungary. J BUON. 2016; 21(1):168-74.

[2] Li X, Gao L, Li H, Gao J, Yang Y, Zhou F, Gao C, Li M, Jin Q. Human papillomavirus infection and laryngeal cancer risk: a systematic review and meta-analysis. J Infect Dis. 2013; 207(3):479-488.

[3] Shirish S, Alok C, Sutapa M, Showket H, Rakesh K, Suresh H, Bhudev C. Infection of human papillomaviruses in cancers of different human organ sites. Indian J Med Res. 2009; 130:222-233.

[4] Gomaa M, El Gindy K, Nabi U, Mohammed H, Twab N, Rehab Mahmoud, Khalid M, Human Papillomavirus Subtype 16 and the Pathologic Characteristics of Laryngeal Cancer. OTO- Open. DOI:10.1177/2473974X17707925.

[5] Ang K, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010; 363:24–35.

[6] Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst. 2008; 100:261– 269.

[7] Fakhry C, Gillison ML. Clinical implications of human papillomavirus in head and neck cancers. J Clin Oncol. 2006; 24:2606–2611.

[8] Josena K, Stephen M, Kang Mei Chen et al. HPV outcomes in an access to care laryngeal cancer cohort. Otolaryngol Head Neck Surg. 2012; 146: 730–738.

[9] Morshed K. Association between human papillomavirus infection and laryngeal squamous cell carcinoma. J Med Virol. 2010; 82:1017–1023.

[10] Jiang H, Lin PF. Human papillomavirus infection a favorable prognostic factor in laryngeal squamous cell carcinoma is associated with the expression of proliferating cell nuclear antigen. Pak J Med Sci. 2013; 29: 1173–1177.

[11] Urban D, June C, Danny R. What is the best treatment for patients with human papillomavirus–positive and negative oropharyngeal cancer? Cancer, 2014; 15: 120:1462-1470.

[12] Lassen P1, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. J Clin Oncol. 2009 Apr 20; 27:1992-1998.

[13] Anwar K, Nakakuki K, Imai H, Naiki H, Inuzuka M. Over-expression of p53 protein in human laryngeal carcinoma. J Clin Oncol. 2009; 20; 27:1992-1998.

[14] Kaya H, Kotiloğlu E, Inanli S, Ekicioğlu G, Bozkurt SU, Tutkun A, Küllü S. Prevalence of human papillomavirus (HPV) DNA in larynx and lung carcinomas. Pathologica. 2001; 93: 531-534.

[15] Hernandez B, Goodman M, Lynch C, et al. Human papilloma virus prevalence in invasive laryngeal cancer in the United States. PLoS One. 2014; 29: 9(12).

[16] Jordan, R, Lingen, M, Perez-Ordonez B. Et al. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. Am J Surg Pathol. 2012; 36: 945-954.

(Table 1) HPV-16 in the studied sample

HPV	**Number**	**%**
+ve	9	19.1%
-ve	38	80.9%
Total	47	100%

(Table2) Frequency of T stage in the studied sample

T	**Number**	**%**
1	3	6.4%
2	42	89.4%
3	1	2.1%
4	1	2.1%
Total	47	100%

(Table 3) Frequency of N stage in the studied sample

N	**Number**	**%**
0	35	74.5%
1	7	14.9%
2	5	10.6%
Total	47	100%

(Table 4) comparison between HPV positive and negative cases in the studied sample, regarding T stage

T	HPV+ve (n=9)	HPV-ve (n=38)
1	1 (11.11%)	2 (5.26%)
2	6 (66.67%)	36 (94.74%)
3	1 (11.11%)	0 (0%)
4	1 (11.11%)	0 (0%)

(Table 5) comparison between HPV positive and negative cases in the studied sample, regarding N stage

N	HPV+ve (9)	HPV-ve (n=38)
0	6 (66.67%)	29 (76.32%)

1	1 (11.11%)	6 (15.79%)
2	2 (22.22%)	3 (7.89%)

(Table 6) Correlation between HPV and T, N stage of the studied sample, also correlation between T and N stage of the tumor.

		N	Correlation	Sig.
Pair 1	HPV & T	47	-.262	.075
Pair 2	HPV & N	47	-.142	.342
Pair 3	T & N	47	.465	.001

Paired Samples Correlations