Epidermal Growth Factor Receptor Tissue Expression Level as a Predictor for the Aggressiveness of HPV-associated Laryngeal Carcinoma

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ABSTRACT

This study was designed to evaluate the frequency of human papilloma virus (HPV) infection in patients with laryngeal squamous cell carcinoma (LSCC) through identification of the viral DNA using PCR analysis and to determine the tissue levels of epidermal growth factor receptor (EGFR) as a trial to find a relation between HPV infection, EGFR expression and clinicopathological findings in patients with LSCC. The study comprised 32 patients with suspected LSCC; 25 males and 7 females; with mean age 53.3±12.2; range: 25-72 years. All females and 5 males were non-smokers; while 20 males were smokers. Patients were subjected to full history taking and clinical examination. Direct laryngoscopy was performed under light general anesthesia in the operating room for evaluation of the larynx and the entire upper aerodigestive tract for accurate clinical staging according to TNM classification, to determine the full extent of the local spread of the tumor and to obtain tissue biopsy. Fresh tumor tissue specimens were divided into two parts, the first was studied and graded pathologically according to World Health Organization (WHO) classification and the second was stored at -70°C until processed and examined by PCR technique for the presence of HPV-DNA and analyzed for EGFR expression expressed as femtomol/mg (fM/mg) protein. Squamous cell carcinoma was detected in 29 cases (90.6%) and 3 cases were excluded off the study; 21 patients (72.4%) had lesions clinically staged as stage I, while 3 (10.3%) and 5 (17.3%) had lesions of stages II and III, respectively. Patients had Stage I lesions were significantly (p<0.05) younger than patients with stage II and III lesions and 9 lesions were detected in non-smokers. Laryngoscopy defined 23 (79.3%) glottic lesions, 2 (6.9%) supraglottic and 3 (10.3%) subglottic lesions and one case (3.4%) had an extensive squamous cell carcinoma of the larynx involving the subglottic region, the glottis and the supraglottic areas. There were 21 (72.4%) polypoid lesions and 8 (27.6%) ulcerative lesions. According to WHO classification, 14 specimens were type 1, 9 specimens type 2 and 6 specimens were type 3. PCR could detect HPV-DNA in 16 (55.2%) specimens (viral specimens) and could not be detected in the other 13 specimens (non-viral cases). Four specimens of WHO type 1, 6 specimens of WHO type 2 and 6 specimens of WHO type 3 were viral specimens. Mean tissue expression level of EGFR was 37.7±32.2 fM/mg protein and was significantly higher in viral (54.7±27.8 fM/mg protein) compared to
non-viral cases (16.8±24.6 fM/mg protein) and in specimens of WHO type 2 and 3 compared to those of type 1. Moreover, there was a positive significant correlation between the pathological WHO types and presence of viral infection, \( r=0.568, p=0.001 \) and the tissue expression levels of EGFR, \( r=0.720, p<0.001 \) and a positive significant correlation between tissue expression of EGFR and the presence of viral infection, \( r=0.595, P=0.001 \). Using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) to determine the specificity of the presence of HPV infection and tissue expression of EGFR as a predictor of cancer aggressiveness manifested as WHO pathological stage revealed that tissue expression of EGFR is more specific (AUC=0.731) than the presence of viral infection (AUC=0.583). It could be concluded that laryngeal infection with HPV may predispose to carcinogenesis through activation of certain growth factors as EGF and both were found significantly correlated with the aggressiveness of LSCC with the level of tissue expression of EGFR being a specific determinant of tumor aggressiveness manifested as pathologic stage.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer worldwide. In 2005, 400,000 cases of HNSCC were diagnosed worldwide. Laryngeal carcinoma is the second most common type of head and neck cancer after skin cancer and accounts for 3% of total cancer risk\(^{(1)}\).

Alcohol/tobacco consumption is the most important risk factor for that neoplasia; nevertheless, since 1983 it has been suggested that human papilloma viruses have a role in HNSCC, mainly in the oropharynx. In the last years, some authors showed the implication of the HPV in the development of pre-carcinogenic lesions and of squamous cell carcinoma\(^{(2)}\). The infection by HPV has been associated to hyperplastic epithelial lesions, papilloma and warty carcinoma in skin and in different types of mucosa, including the anogenital, cervical, urethral, tracheobronchial, nasal, laryngeal and oral mucosa tracts. The viral high-risk geno-types (oncogenic) such as 16, 18, 31, 33 and 35 are frequently associated to leukoplakia and squamous carcinoma\(^{(3)}\).

Human papilloma virus is a small double-stranded DNA virus that is capable of infecting cutaneous and mucosal epithelium, resulting in a variety of pathological lesions\(^{(4)}\). Among the more than 100 different types of HPV identified, HPV16 is the most common high-risk virus. Its contribution to neoplastic progression is predominantly through the action of the viral oncoproteins E6 and E7. Expression of HPV16 E6 and E7 proteins is sufficient for the immortalization of primary human epithelial cells and induces histological abnormalities reminiscent of premalignant HPV-associated squamous intraepithelial lesions\(^{(5)}\).

According to the modern theory of carcinogenesis, a complex and multistep process is likely in the development of LSCC. Cancer is caused by uncontrolled proliferation of cells, which is itself induced by abnormalities of cell cycle regulatory
mechanisms or activation of growth factors involved in cell proliferation and differentiation\(^{(6)}\). The p53 tumor-suppressor gene regulates cell cycle progression through induction of apoptosis at the G1/S checkpoint. It has been well documented that E6 and E7 oncoproteins alter normal cell growth control mechanisms by inactivating two well-characterized tumor suppressor proteins, p53 and retinoblastoma protein, respectively\(^{(7)}\).

There is evidence that inherited differences in cell cycle control systems, DNA repair systems, and carcinogen-metabolizing enzymes or altered dominant and recessive oncogenes, genetic instability, and growth factor-linked signal transmission pathways could increase the risk for laryngeal cancer\(^{(8)}\). The transforming activity of high-risk HPV seems to depend primarily on deregulated expression of E6 and E7 HPV oncoproteins. The E5 HPV oncoprotein, also, seems to play a role in early growth stimulation of HPV-infected cells by an interaction with some cellular proteins, and in particular with growth factors receptors, and to alter the cell response to signals for growth and differentiation\(^{(9)}\).

Epidermal growth factor receptor is composed of extracellular domains, including a ligand-binding domain, a hydrophobic transmembrane region and a tyrosine kinase-containing cytoplasmic region. Stimulation of the EGFR by endogenous ligands, EGF or transforming growth factor-\(\alpha\) (TGF-\(\alpha\)), results in a conformational change in the receptor, permitting it to enter into dimers and other oligomers. Dimerization results in activation of intracellular tyrosine kinase, protein phosphorylation and stimulation of various cell signaling pathways that mediate gene transcription and cell cycle progression\(^{(10)}\). The EGFR is expressed on normal human cells but higher levels of expression of the receptor have, also, been shown to be correlated with malignancy in a variety of cancers. The activation of the EGFR participates in oncogenesis by inducing cell proliferation, cell mobility and angiogenesis, and inhibiting apoptosis. This activation might be due to numerous abnormalities, including increased expression of its ligand\(^{(11)}\).

The current study was designed to evaluate the frequency of HPV infection in patients with LSCC through identification of the viral DNA using PCR analysis and to determine the tissue levels of EGFR as a trial to find a relation between HPV infection, EGFR expression and clinicopathological findings in patients with LSCC.

**MATERIALS & METHODS**

This prospective selective study was conducted at Departments of Otorhinolaryngology and Clinical Pathology, University hospital in conjunction with Medical Biochemistry and Pathology Departments, Faculty of Medicine, Benha and Minoufiya Universities. The study comprised 32 patients with suspected LSCC. They were 25 males (78.1%) and 7 females (21.9%) with mean age 53.3±12.2; range: 25-72 years. All females and 5 males were non-smokers; while the other 20 males were smokers.
Patients were subjected to full history taking, clinical examination with respect to laryngeal region and underwent CT imaging. Direct laryngoscopy was performed under light general anesthesia in the operating room for evaluation of the larynx and the entire upper aerodigestive tract for accurate clinical staging, to determine the full extent of the local spread of the tumor and to obtain tissue biopsy. Cases were categorized clinically according to TNM classification and only cases with SCC were included in the study.

Sample Preparation
Fresh tumor tissue specimens were divided into two parts, the first was studied and graded pathologically according to the World Health Organization (WHO) classification, and the second was kept frozen at -70°C until processed and examined by PCR technique for the presence of HPV-DNA and analyzed for EGFR expression.

A- Preparation of Cytosolic and Membrane Fractions
Tumor specimens were finely minced and homogenized in 5 volumes of ice-cold buffer consisting of 25 mM Tris, 1.5 mM EDTA, 5 mM NaN3, 0.1% monothioglycerol, and 20% glycerol using Plotter-Elvehjem homogenizer. The crude homogenate was centrifuged at 3000 revolve/min (rpm) for 20 min at 0°C, and the supernatant was further centrifuged at 100 rpm for 75 min at 0°C to obtain a cytosolic fraction for a membrane fraction for EGFR assay.

EGFR Measurements
The membrane pellet was resuspended in 25 mM Tris, 1.5 mM EDTA, 5 mM NaN3, 20% glycerol, and 10 mM MgCl2. Aliquots of the suspension (100 µl containing 300–500 µg of protein) were incubated with 125I-EGF (2.6 nM; 800,000 Ci/mmol; NEN, DuPont, Wilmington, DE) for 12–16 h at room temperature in a final volume of 400 µl. Binding was blocked by the addition of 3 ml of ice-cold 25 mM Tris, 20% glycerol, 5 mM NaN3, and 0.1% BSA. After centrifugation at 2000 rpm for 20 min at 0°C, the supernatant was carefully aspirated, and pellets were counted on Berthold Gamma Counter LB 2104 (Wallac, Inc., Gaithersburg, MD). Results were expressed as femtomol/mg (fM/mg) protein. EGFR status was defined using the arbitrary cutoff value corresponding to the median value of EGFR levels.

B- HPV DNA Extraction and Detection
a. DNA extraction using proteinase K in presence of 1% sodium dodecyl sulfate (SDS), phenol-chloroform and ethanol precipitation.

b. PCR amplification:
   Primer used: The primer pair MY11-MY09, supplied at concentration of 100 pmol/ml (Biometra, Germany); which were selected from a highly conserved region (L1) within the HPV genome. Amplification was carried out in a programmable Thermal Cycler 480 (Perkin Elmer, Norwalk, CY). An initial denaturation for 5 min at 94°C (1 cycle) followed by 45 sec at 95°C, 45 sec at 60°C, 60 sec at 72°C (35 cycles); then 10 min at 72°C (1 cycle).

c. Electrophoretic separation and identification: An aliquot of
amplified DNA was analyzed by 1.5% agarose gel electrophoresis, at constant current 100 volt for 45 min and ethidium bromide (0.5 mg/ml) staining to visualize fragments of the expected size (450 bp). Bands were visualized under ultraviolet illumination and photographed. The experiment was performed in parallel with a negative control in which PCR reaction was performed without a template DNA. To assess the integrity of the genomic DNA of HPV-negative samples, a region of the human ß-globin gene was amplified as described previously. Negative controls included reaction mixtures lacking any DNA template and reaction mixtures containing human DNA without HPV target sequences. Procedures to prevent specimen contamination and PCR carryover were vigorously observed at every step in this analysis.

**Statistical analysis**

Data were analyzed using t-test and Chi-square test. One-way ANOVA test was used to study variance of levels expression of EGFR. Possible relationships were investigated using Pearson linear regression. Specificity of the presence of HPV infection and tissue expression of EGFR as a predictor of cancer aggressiveness manifested as WHO pathological stage were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC). Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. P value <0.05 was considered statistically significant.

**RESULTS**

Squamous cell carcinoma was detected in 29 cases (90.6%) and 3 cases (2 males and one female) were excluded off the study; 2 cases with lymphoma and one case with spindle cell carcinoma. Twenty-one patients (72.4%) had lesions clinically staged as stage I, while 3 (10.3%) and 5 (17.3%) had lesions of stages II and III, respectively. Patients had Stage I lesions were significantly (p<0.05) younger than patients with stage II and III lesions with a non-significant (p>0.05) difference between patients staged II and III. One female patient had lesion of stage III, but the other 5 female patients had lesions of stage I, all other patients were males. Nine lesions were detected in non-smokers; one stage II and another of stage III; whereas the other 7 had stage I lesions. Laryngoscopy detected 23 (79.3%) glottic lesions (18, 2 & 3, according to stage respectively), 2 (6.9%) supraglottic and 3 (10.3%) subglottic lesions. One case (3.4%) had an extensive squamous cell carcinoma of the larynx with the tumor involving the subglottic region, the glottis and the supraglottic areas. There were 21 (72.4%) polypoid lesions, (19, 1& 1 according to stage, respectively) and 8 (27.6%) ulcerative lesions; 2, 2 & 4 according to stage, respectively, (Table 1).
Table (1): Patients' distribution according to lesion character

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>29</td>
<td>21 (72.4%)</td>
<td>3 (10.3%)</td>
<td>5 (17.3%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.9±13.2 (25-72)</td>
<td>46.6±11.5 (25-63)</td>
<td>65.3±5.8* (62-72)</td>
<td>66.2±1.6* (65-68)</td>
</tr>
<tr>
<td>Sex; M:F</td>
<td>23:6</td>
<td>16:5</td>
<td>3:0</td>
<td>4:1</td>
</tr>
<tr>
<td>Smoker: Non-smokers</td>
<td>20:9</td>
<td>14:7</td>
<td>2:1</td>
<td>4:1</td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supraglottic</td>
<td>2 (6.9%)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Glottic</td>
<td>23 (79.3%)</td>
<td>18</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Subglottic</td>
<td>3 (10.3%)</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>All site</td>
<td>1 (3.5%)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Character</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypoid</td>
<td>21 (72.4%)</td>
<td>19</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ulcerative</td>
<td>8 (27.6%)</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, ratios & numbers; ranges & percentage in parenthesis
*: Significant difference versus stage I

According to WHO grading classification, histopathological examination of the 29 biopsies taken revealed the presence of 14 specimens WHO type 1, 9 specimens of WHO type 2 and 6 specimens WHO type 3, (Table 2). There was a non-significant difference between clinical staging and histopathological grading, (X^2=2.1, p>0.05).

Table (2): Clinical and histopathological categorization of cases of laryngeal carcinoma

<table>
<thead>
<tr>
<th></th>
<th>Clinical staging</th>
<th>Histopathological typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I (T1N0M0)</td>
<td>20 (71.9%)</td>
<td>WHO type 1 (squamous cell carcinoma)</td>
</tr>
<tr>
<td>Stage II (T2N0M0)</td>
<td>4 (12.5%)</td>
<td>WHO type 2 (non-keratinizing carcinoma)</td>
</tr>
<tr>
<td>Stage III (T3N0M0-T1, T1N1M0)</td>
<td>5 (15.6%)</td>
<td>WHO type 3 (undifferentiated carcinoma)</td>
</tr>
</tbody>
</table>

T1: Tumor limited to normally mobile vocal cords (involving anterior and/or posterior commissure)
T2: Tumor extending to the supraglottis or subglottis with impaired vocal cord mobility
T3: Tumor confined to the larynx with vocal cord fixation
T4: Tumor invading through thyroid cartilage and/or with direct extralaryngeal spread
N0: No lymph nodes (LN)
N1: Metastases to 1 ipsilateral cervical LN≤3 cm in greatest dimension
N2: Metastases to a single ipsilateral cervical LN>3cm but <6cm in greatest dimension
N3: Metastasis in lymph node>6cm
M0: no distant metastases. M1: distant metastasis
PCR detected HPV-DNA, (Fig. 1) in 16 (55.2%) specimens (viral specimens) and were not detected in the other 13 specimens (non-viral cases). Four specimens of WHO type 1, 6 specimens of WHO type 2 and 6 specimens of WHO type 3 were viral specimens, (Table 3). Mean tissue expression levels of EGFR was 37.7±32.2; range: 3-98 fM/mg protein. The mean level of EGFR in viral cases was 54.7±27.8; range: 10-98 fM/mg protein with a significant (P<0.05) increase in comparison to non-viral cases that had a mean level of 16.8±24.6; range: 3-78 fM/mg protein. There was increased tissue expression levels of EGFR in viral cases of all grades but showed a significant (P<0.05) increase in specimens of WHO type 2 and 3 compared to those of type 1 with a non-significant increase in specimens of WHO type 3 compared to those of type 2, (Table 3, Fig. 2).

Fig. (1): Agarose gel electrophoresis of the PCR product a DNA-positive LSCC specimen. M: molecular weight marker VI (Boehringer Mannheim), C+ and C−, positive and negative PCR controls, respectively. Lanes 1, 4, 9, 12, and 18 are negative specimens; Lanes 2, 3, 5–8, 10, 11, 13–17, 19, and 20 are positive specimens

Fig. (2): Mean tissue expression of EGFR in specimens categorized according to WHO type and presence of HPV
Table (3): Patients distribution according to HPV DNA detection and level of tissue expression of EGFR level in relation to WHO pathological types

<table>
<thead>
<tr>
<th>Data</th>
<th>Total</th>
<th>WHO type 1</th>
<th>WHO type 2</th>
<th>WHO type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>29</td>
<td>14</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Viral: Non-viral</td>
<td>16:13</td>
<td>4:10</td>
<td>6:3</td>
<td>6:0</td>
</tr>
<tr>
<td>Tissue EGFR expression level (fM/mg protein)</td>
<td>Viral</td>
<td>54.7±27.8‡</td>
<td>37±2.8‡</td>
<td>52.3±35.9†</td>
</tr>
<tr>
<td></td>
<td>Non-viral</td>
<td>16.8±24.6</td>
<td>6.3±1.9</td>
<td>47.3±32.7</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, ratios & numbers.
†: Significant difference versus WHO type 1
‡: significant versus non-viral

Moreover, there was a positive significant correlation between the pathological WHO types and presence of viral infection, ($r=0.568$, $p=0.001$) and the tissue expression levels of EGFR, ($r=0.720$, $p<0.001$) and a positive significant correlation between tissue expression of EGFR and the presence of viral infection, ($r=0.595$, $P=0.001$), (Table 4, Fig. 3a-c). Using ROC curve analysis to determine the specificity of both the presence of HPV infection and tissue expression of EGFR as determinant of the WHO pathological stage revealed that tissue expression of EGFR is more specific with AUC=0.731 while just the presence of viral infection had an AUC=0.583, (Fig. 4).

Table (4): Correlation coefficient "r" between WHO types, presence of HPV infection and EGFR expression level

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EGFR expression level</th>
<th>Presence of HPV infection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>&quot;r&quot;</td>
<td>p</td>
</tr>
<tr>
<td>WHO types</td>
<td>0.720</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of HPV infection</td>
<td>0.595</td>
<td>=0.001</td>
</tr>
</tbody>
</table>
Fig. (3): Correlation between WHO pathological types, EGFR expression levels and presence of HPV infection

Fig. (4): ROC curve analysis for specificity for WHO stage
DISCUSSION

Laryngeal carcinoma is the second most common type of head and neck cancer after skin cancer and accounts for 3% of total cancer risk\(^{(18)}\). The optimal primary treatment for laryngeal cancer is still a matter of international debate. The treatment proposed is often based on the extent of the disease, the clinician’s experience and training, and geographic practice preferences\(^{(19)}\).

Radiotherapy with concurrent cisplatin is the standard alternative to total laryngectomy for patients with locally advanced laryngeal cancer and patients with complete response to induction chemotherapy in laryngeal carcinoma have a high probability of cure after hyperfractionation radiotherapy. However, hyperfractionation radiotherapy induces a high degree of toxicity that reduces the laryngeal function preservation rate and may jeopardize overall survival. Thus, a prerequisite of fundamental importance in dealing with the dilemma of treatment selection in patients with laryngeal cancer is the identification of unfavorable prognostic determinants that can serve as guidelines in addressing the treatment to the individual patient\(^{(20)}\).

Several lines of evidence support the EGFR as a molecular target for therapy of various SCC. First, overexpression of EGFR is one of the most common molecular alterations in SCC and the level of EGFR expression on HNSCC is elevated relative to expression on normal adjacent squamous mucosa in 83-100% of cases. Second, increased receptor content is often associated with increased production of ligands, such as transforming growth factor-\(\alpha\), by the HNSCC\(^{(21)}\). Furthermore, treatment with EGFR-targeted therapy such as the chimeric monoclonal antibody cetuximab (C225) that binds competitively and with high affinity to the EGFR, such binding prevents stimulation of the receptor by endogenous ligands and results in inhibition of cell proliferation, enhanced apoptosis, and reduced angiogenesis, invasiveness and metastasis. Binding of cetuximab to the receptor also results in internalization of the antibody-receptor complex which leads to an overall downregulation of EGFR expression. The EGFR is a prime target for new anticancer therapy that inhibits EGFR signaling and potentiates the effects of chemotherapy or radiation\(^{(22)}\).

Moreover, Pivot et al.\(^{(23)}\) reported that EGFR determination appears to be a powerful prognostic parameter for patients with laryngeal and hypopharyngeal cancer treated by induction chemotherapy followed by exclusive radiotherapy and that laryngectomy seems to erase the prognostic impact of EGFR expression. They concluded that the obtained results prefer the use of EGFR targeting therapy for this category of patients.

Thus, the current study was designed to evaluate the frequency of HPV infection in patients with LSCC through identification of the viral DNA using PCR analysis and to determine the tissue levels of EGFR as a trial to find a relation between HPV infection, EGFR expression and...
The present study included 29 patients with LSCC with evident sex predilection to occur in males (M:F ratio = 23:6) and with higher percentage of smokers (69%); thus illustrating the relationship between smoking and laryngeal carcinogenesis. These data go in hand with that reported by Alagić-Smailbegović et al. who reported a frequency of smokers of 93%, however, such high frequency could be attributed to exclusion of female patients in their study and inclusion of only males in whom the frequency of both the disease and smoking is high.

PCR detected HPV-DNA in 16 (55.2%) of specimens (viral specimens); 4 specimens of WHO type 1 and 12 specimens of WHO type 2 and 3 with a positive significant correlation between the presence of viral infection and the severity and aggressiveness of the cancer manifested as pathological stage. This result signified that a well-defined subset of LSCC may be etiologically linked to HPV infection and in such a subset of LSCCs, HPV infection would be an early event in a multistep process of laryngeal carcinogenesis and could address tumoral progression. This result agreed with those of Zhao et al. and Wang et al. using PCR, they reported a rate of positive cases with HPV infection of 45.6% and 68% of their patients with laryngeal carcinoma, respectively. Also, Makowska et al. reported that 10 out of 23 studied cases (43.5%) of laryngeal carcinoma revealed presence of HPV DNA. Almadori et al. reported a significant correlation between HPV infection and cyclin D1 gene amplification and suggested the involvement of HPV infection in laryngeal carcinogenesis and that HPV positive laryngeal cancers may constitute a different subset of tumors with a peculiar molecular pattern and thus with a different clinical behavior.

Also, Du et al. reported activation and nuclear localization of factors with the ability to inhibit cell death and to maintain and promote the growth of cells as NF-κB and sequestration of p53, a cell cycle progression inhibiting factor may play a role in the development of human laryngeal squamous cell carcinoma infected with HPV and Gallegos-Hernández et al. found 50% of patients with laryngeal cancer patients were HPV positive.

Tissue expression levels of EGFR showed a significant increase in viral in comparison to non-viral cases irrespective of WHO pathological grade with significantly increased levels in specimens of WHO type 2 and 3 in comparison to specimens of WHO type 1. These results illustrated the relationship between viral infection and increased expression of EGFR, a mechanism that may underlay the role imposed by HPV infection in pathogenesis of LSCC. These results agreed with that of Bentzen et al. who reported that EGFR expression rate was significantly associated with histologic grade and microvessel density of HNSCC and concluded that EGFR might play a key role in determining the proliferative cellular response to fractionated radiotherapy in HNSCC and with Chung et al. who reported a significant correlation between HPV infection and cyclin D1 gene amplification and suggested the involvement of HPV infection in laryngeal carcinogenesis and that HPV positive laryngeal cancers may constitute a different subset of tumors with a peculiar molecular pattern and thus with a different clinical behavior.
who analyzed EGFR status in 86 tumor samples from HNSCC patients by fluorescent in situ hybridization (FISH) and found that high EGFR gene copy number by FISH is frequent in HNSCC, is a poor prognostic indicator associated with worse progression-free and overall survival.

In support of the obtained results, there was a positive significant correlation between the tissue expression levels of EGFR and both the pathological WHO types of LSCC and with the presence of HPV infection. Using ROC curve analysis identified the level of EGFR expression as a specific determinant of LSCC severity manifested as the pathological stage. These findings go in hand with those of Penault-Llorca et al. (10) who reported that the level of EGFR expression is a prognosis factor for several tumors and appears to be an indicator of poor prognosis which might influence treatments of HNSCC. Also, the obtained results coincided with the results of the experimental work of Knecht et al. (32) who investigated whether the addition of monoclonal antibodies against the EGFR could enhance the response rate of cisplatin, 5-FU and docetaxel and reported that the combination of cisplatin, 5-FU, docetaxel and the antibody resulted in highly significant complete tumor remissions, with no increased toxicity in the experimental animal. Also, Hitt et al. (21) reported that in patients with oral SCC, EGFR status and an oral cavity location of the tumor have independent prognostic value in patients with advanced head and neck carcinoma treated with induction chemotherapy.

Furthermore, Psyri et al. (11) found that patients with oropharyngeal SCC associated with high tumor EGFR expression levels had a local recurrence rate of 58% compared with 17% for patients with low EGFR tumor expression and that patients with high tumor EGFR levels had shorter disease-free survival compared with low expressors and concluded that in multivariate analysis adjusting for prognostic variables, high EGFR expression levels retained their prognostic significance. Pivot et al. (23) studied potential prognostic factors were age, gender, performance status, primary tumor localization, T status, N status, tumor volume and tumoral EGFR level in patients with laryngeal and hypopharyngeal SCC and reported that EGFR determination appears to be a powerful prognostic parameter for patients treated by induction chemotherapy followed by exclusive radiotherapy.

It could be concluded that laryngeal infection with HPV may predispose to carcinogenesis through activation of certain growth factors as EGF and both were found significantly correlated with the aggressiveness of LSCC with the level of tissue expression of EGFR being a specific determinant of tumor aggressiveness manifested as pathologic stage.

REFERENCES


معدل استخراج الأنسجة لمستقبل عامل نمو البشرة كمكتب لتفاقم أورام الحنجرة المصاحبة للعدوى بفيروس البالبيوما البشري

ثناء حادث، سعد عودة، ابوعينة، ياسر، تادر راحظ، رابع الشاعري راس، جولة حداد بكر

أقسام الكيمياء الحيوية الطبية، البالبيوميا البيولوجية، الألقاب والآفات والمحتوى، البالبيوميا

صممت هذه الدراسة لتقييم معدل العدوى بفيروس البالبيوما البشري في مستشفى أورام الحنجرة من خلال
تحديد وجوب الحمض النووي للفيروس باستخدام أساليب البلمرة المتقدسة وتحديد معدل استخراج الأنسجة
مستقبل عامل نمو البشرة كمحاولة لإيجاد علاقة بينها ومتغيرات البالبيوميا البيولوجية في هذه الحالة، و
تمت بالدراسة 32 مريضا (25 ذكر و7 أنثى) بمتوسط عمر قدره 53.3 سنة، منهم 20 مدخنا، تم
فحص الجسم باستخدام منظور الحنجرة المباشر تحت تقدير خبير داخل هيئة العلاجات لتقييم التدرج الكليزيكي
и تحديد مدى انتشار السرير والعلماء وأخذ عينات من نسيج البرام وتقييماها لزائرين: الأول لل셀 بالبيولوجيا
لتقييم العدوى تبعا لتدريج منظمة الصحة العالمية وتم تقسيم الجزر الثاني للعوامل باستخدام أساليب الدرجة
المتقدسة وتحديد معدل استخراج الأنسجة لمستقبل عامل نمو البشرة.

تم استخدام 3 حالات وصنفت باقي الحالات الكليزيكي إلى 11 مريضا مصابة بورم من الدرجة الأولى،
ومن الدرجة الثانية و3 من الدرجة الثالثة وقد وجد أن المرضى المصابين بورم من الدرجة الأولى أصغر سنا من
الآخرين ووجد 9 أورام في غير المدخنين، الحمض الكليزيكي حدد 0.72 ووجد حيال المنطقة المزمار ووجد الزمرد 3 أورام تحت المزمار ووجد الزمرد 6 أورام تحت المزمار بالمناطق الثلاث في مريضا واحدا، وحول 21 ووجد في اثنين
الزوال العفوي و8 أورام على وزن جزء. تبعا لتقسيم منظمة الصحة العالمية كانت 14 عينة من النوع الأول,
9 عينات من النوع الثاني، 6 عينات من النوع الثالث.

وجد الحمض النووي للفيروس في 16 عينة (3 عينات في الأورام)؛ و4 من النوع الأول، 3 من النوع الثاني،
و6 من النوع الثالث، وكان متوسط معدل استخراج الأنسجة لمستقبل عامل نمو البشرة أعلى بدرجة ذات دالالة
الإحصائية في عينات الفيروسات الطبيعية والمصابة في عينات النوعين الثاني والثالث مقترنة بال النوع الأول.
وجده حضارة إيجابية ذات دالالة إحصائية بين وجود الحمض النووي للفيروس ونوع البرم لتقسيم منظمة
الصحة العالمية وتحديد معدل استخراج الأنسجة لمستقبل عامل نمو البشرة، وباستخدام تقدير منحنى الحمض
المكروبية تحت المذكور؛ تقييم الحمض النووي للفيروس وتحديد معدل استخراج الأنسجة
مستقبلي عامل نمو البشرة كمكتب لتفاقم أورام الحنجرة مثلة بوعي البرم، تبعا لتقسيم منظمة الصحة العالمية وجد
أن معدل استخراج الأنسجة لمستقبل عامل نمو البشرة أكثر خاصة في هذه الأورام البالبيوما.

يمكن استنتاج أن إصابة الحنجرة بالفيروس البالبيوما البشري لها تعدد أورام الحنجرة من خلال تنشيط
عوامل نمو معينة مثل كؤل عامل نمو البشرة ووجد أن جود الفيروس وتحديد معدل استخراج الأنسجة لمستقبل عامل
نمو البشرة له علاقة ذات دالة إحصائية بتفاقم أورام الحنجرة مثلة بوعي البرم، تبعا لتقسيم منظمة الصحة
العالمية واتباع معدل استخراج الأنسجة لمستقبل عامل نمو البشرة يعتبر مثبطاً ذو صحة عامة لتفاقم
أورام الحنجرة.