A Phase II Study of the Role of Selective Cyclo oxygen ase-2 (COX-2) Inhibitor and Concurrent Chemoradiation as Adjuvant Postoperative Treatment in Patients with Locally Advanced Cancer Larynx

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Abstract

Objectives: The primary study objective is to determine the tolerability, acute toxicity profile and loco-regional recurrence (LRR) rate, 2 year relapse free survival (RFS) and overall survival (OS) with the use of selective COX-2 inhibitor, Celecoxib, concurrently with chemoradiotherapy (CRT) as adjuvant treatment for locally advanced squamous cell carcinoma (LASCC) of the larynx. Secondary end point is to study COX-2 expression and assess its prognostic significance with the pathological features and treatment outcomes.

Patients and Methods: This study included 36 patients with histologically confirmed diagnosis of LASCC of the larynx after curative surgery stage III-IVA. All patients received Cisplatin 100mg/m^2 days 1,22 and 43 concurrently with external beam radiotherapy and celecoxib in a dose of 300mg twice daily during the whole course of radiotherapy. EBRT was delivered with conventional fractionation in a dose of 1.8-2 Gy/f to 50-54 + boost to high risk areas to 60-66 Gy in 30-33 fractions over 6-7 weeks using (6MV) with isocentric techniques. Tissue samples or paraffin blocks were obtained from eligible patients for detection of COX-2 expression by immunohistochemical method.

Results: More than 75% of the patient had > T2 tumors, 78% had positive lymph nodes and 80% had GI & II tumors. Concurrent CRT and celecoxib were tolerable thus, 32 (89%) patients could proceed to receive a full course of Celecoxib, chemotherapy and radiotherapy. Overall, G3,4 acute toxicities were observed in 18/36 patients (50%) mainly chemotherapy related, but were well tolerated. Most G3 & 4 neuropenia which reported in 22% were transient and not complicated with toxic death on the protocol therapy. The worst nonhematologic toxicities were mucositis (44%), GIT (36) and dysphagia (30%). With a median follow-up period of 27.6 months locoregional recurrence was observed in 17% of the patients. Kaplan-Meier method revealed two-year RFS of 67% and the two-year overall survival of 72%. COX-2 was expressed in 61% of the patients and was significantly increased in Cox-2 positive patients (73% Vs 27%) which was statistically significant (p<0.05). Additionally COX-2 positivity was associated with decreased 2 years RFS and 2-year overall survival but was not statistically significant (p>0.05).

Conclusion: COX-2 may have prognostic value in predicting disease recurrence. Addition of Cox-2 inhibitors concurrently with (CRT) was safe and tolerable, however its advantage over the standard CRT regimen couldn't be proved. Confirmatory randomized phase III trial on larger number of patients and longer follow-up is encouraged.

Key Words: COX-2 inhibitor – Cancer larynx – Postoperative.

Introduction

CANCER larynx is the most common cancer of the head and neck [1]. At NCI Cairo, Egypt it ranks number 10 among the most common important tumors. It contributed 27.84% of malignant tumors of the respiratory organs and 1.77% of total malignancies [2].

Although surgery alone may be adequate treatment for early-stage disease, additional therapy is required to prevent disease recurrence, even after an apparently complete resection of locally advanced (LA) disease. At least 50% of patients will recur, within the first 2 years so, patients with locally advanced disease have a poor prognosis with conventional treatment that consists of total laryngectomy or a combination of laryngectomy and postoperative radiotherapy [3].

A number of pathologic poor risk factors have been associated with higher recurrence rates after surgery, including positive margins of resection, extracapsular extension of disease from a lymph
node, involvement of 2 or more lymph nodes, perineural, and perivascular disease and vascular tumor emboli [4,5,6].

Despite recent advances in radiotherapy the survival of patients with HNSCC has not improved significantly [7]. Concurrent chemoradiotherapy (CCRT) used in locally advanced HNSCC including Cancer larynx has shown a statistical significant improvement in overall survival (OS) (absolute improvement 8%) with the use of platinum based chemotherapy either alone or combined with 5 FU as adjuvant treatment concurrent with radiotherapy in several studies [3-68].

A large body of evidence from a variety of experimental system by many authors [9,10] reported that Prostaglandins (PGS) regulated by cyclooxygenase-2 (Cox-2) are upregulated in transformed cells malignant tissues and believed to be important in carcinogenesis due to their special effects on apoptosis, angiogenesis, cell proliferation and immune surveillance [11,12,13]. COXs catalyze the synthesis of PGS from arachidonic acid. There are two isoforms of COX: One is constitutively expressed (COX-1) and the other is inducible (Cox-2) [14]. The COX-2 gene is an immediate, early response gene that is induced by cytokines, growth factors, oncogenes, carcinogenes and tumor-promoting pherbol esters. Opposing to the constitutive isoform, COX-1, is essentially unaffected by these factors and expressed at a constant level throughout the cell cycle by almost all tissues [15,16].

Chan and colleagues [18] stated that levels of COX-2 are increased in HNSCC and selective inhibitors of Cox-2 may be useful in chemoprevention and/or treatment of this disease. Later, Jaeckel et al. [17] observed that the levels of COX-2 mRNA were approximately 2 to 5 fold higher than that of the normal control. More recently, Nix et al. [18] studied COX-2 in a total of 122 patients with early laryngeal carcinoma (61 patients were considered radiosensitive and 61 were radiosensitive). COX-2 expression was detected in 67% (41/61) of patients with radiosensitive carcinoma and 41% (25/61) of patients with radiosensitive tumors. In this study overexpression was significantly associated with more radiosensitive tumors (p=0.004).

Selective COX-2 Inhibitors include the common pain relievers celecoxib (Celebrex), inhibit an inflammation-promoting enzyme COX-2, which also plays a role in blood vessel growth (angiogenesis) that feeds tumor cells. Preclinical studies have suggested that COX-2 inhibitors have promoted chemo sensitivity and radiosensitivity and have shown that COX-2 inhibition will improve the response to radiotherapy without marked affection of normal tissue radiation response [19,20]. Early studies indicated that they may enhance the anti-tumor effects of standard chemotherapy and radiation and may cause head and neck neoplasm to regress and reduce the recurrence [19,20,21].

Later, Raju and colleagues [22] observed that Celecoxib is a powerful enhancer of in vitro radiosensitivity of head and neck human carcinoma cells. At a clinical dose of 2Gy, celecoxib was effective and reduced the survival fraction from the radiation-only value of 0.67 to 0.48 (enhancement factor of 1.4). Furthermore, treatment with celecoxib changed the shape of the radiation-dose survival curve by reducing the "shoulder" region of the curve, suggesting that celecoxib may have blocked the repair of sublethal DNA damage which is a major mechanism responsible for its radiosensitizing action.

Taken together, these observations provide a strong rationale to perform a trial to evaluate its possible role of COX-2 selective inhibitors with concurrent chemoradiation as adjuvant treatment in patients with LA laryngeal cancer with high risk of treatment failure.

Objectives:

The primary study objective is to determine the tolerability, acute toxicity profile and loco-regional recurrence (LRR) rate, 2 year relapse free survival (RFS) and overall survival (OS) with the use of selective COX-2 inhibitor, Celecoxib, concurrently with chemoradiotherapy (CRT) as adjuvant post-operative treatment for locally advanced squamous cell carcinoma (LASCC) of the larynx. Secondary end point is to study COX-2 expression and assess its prognostic significance with the pathological features and treatment outcomes in LASCC of the larynx.

Patients and Methods

This study was carried out at Ain Shams Clinical Oncology Department (ASCOD) in the period from October 2005-November 2008. This study included 36 patients with histologically confirmed diagnosis of locally advanced Squamous Cell Carcinoma (LASCC) of the larynx after having curative surgery.

Inclusion criteria:

Patients were eligible for study entry with age > 18 years, resectable stage III-IVA with high risk of relapse, Eastern Cooperative Oncology Group
performance status (ECOG) 0-2, adequate organ function i.e normal CBC neutrophils > 1.5 x 10⁹/l, Platelets count > 100 x 10⁹/l, haemoglobin > 10g/dl, liver (total bilirubin < 1.5 x upper reference range ASAT and ALAT < 2.5 x upper reference range, Alkaline Phosphatases < 5 x upper reference range) and kidney profiles (serum creatinine < 1.5 times the upper limit of normal) and negative pregnancy test in women.

Exclusion criteria:

Patients were excluded from this study if they had ECOG Performance status >2, a prior history of SCCCHN, T4b or N3 disease, metastatic disease, recurrent or persistent tumor following surgery, uncontrolled hypertension, severe congestive heart failure, asthma caused by (COX-2) inhibitors or nonsteroidal anti-inflammatory drugs (NSAIDs), active peptic ulcer disease, gastritis/esophagitis, or inflammatory bowel disease.

Pretreatment evaluation: Pretreatment diagnostic procedures included history and physical examination, direct laryngoscopy under anesthesia, performed by an otolaryngologist, mirror or fiberoptic laryngoscopy and biopsy. Radiological assessment included chest X-ray, or Chest CT with contrast, (CT or MRI) of the larynx and neck was required in all patients and pretreatment dental evaluation and oral hygiene. All patients were staged according to the American Joint Committee on Cancer (AJCC) Tumor-Nodal-Metastasis Classification System 2002 [23].

Study design: All eligible patients were treated with primary curative surgery. Tissues samples or paraffin blocks from the primary tumors were collected for detection of COX-2 expression.

Treatment schedule: All patients treated with adjuvant post operative concurrent chemoradiotherapy by external beam radiotherapy (EBRT) with the intent to cure. Concurrent chemotherapy during EBRT was given in the form of Cisplatin 100mg per square meter of body-surface area/21 days (days 1,22 and 43 of radiotherapy) with the regular prophylactic hydration and antiemetic measures. All patients were given Celecoxib in a dose of 300mg twice daily starting from day 1 of EBRT and continuing daily through out the course of radiotherapy and the morning dose was given 3h before EBRT.

Dose modification: Cisplatin therapy was postponed if neutrophils count was 1000 per cubic millimeter or the platelet count below 75,000 per cubic millimeter. Celecoxib was permanently stopped for all other non-hematological (cardiovascular or gastrointestinal) grades 3 or 4 acute events that were considered to be drug related.

Radiation therapy: EBRT was delivered with conventional fractionation in a dose of 1.8-2Gy/f to 50-54Gy followed by boost to areas that were at high risk for malignant dissemination to 60-66Gy in 30-33 fractions over 6-7 weeks using linear accelerators (6MV) with the use of isocentric techniques. Radiotherapy was maintained whenever possible; any treatment interruptions resulting from adverse effects had to be kept as short as possible.

Patient evaluation during and after treatment:

During concomitant chemoradiotherapy (CCRT) treatment, close weekly follow-up was necessary to assess any treatment related toxicities. Labortaory studies were done before each cycle of chemotherapy. After end of treatment all patients were followed regularly for the possibility of tumor relapse. Patients were evaluated by H & P every 2 months for 1 year, every 3-4 month in the second year and every 6 months thereafter. Additionally, serum TSH was evaluated every 6-12 months. Evaluation of LRR was done using fiberoptic laryngoscopy every 12-16 weeks and CT scan or MRI every 3-4 months for 2 years. Chest imaging was done when clinically indicated.

Concurrent celecoxib and CRT acute treatment related toxicities (<90 days from CCRT start) were scored according to the Common Toxicity Criteria of the National Cancer Institute, (CTC version 2.0) [24]. Late RT toxicities (>90 days from RT start) were scored according to the Radiation Therapy Oncology Group/European Organisation for Research and Treatment of Cancer (RTOG/EORTC) Late Morbidity Scoring Schema criteria for radiotherapy [25].

COX-2 detection:

Patient samples: Tissue samples or paraffin blocks were obtained from eligible patients with primary laryngeal squamous cell carcinoma who underwent curative radical surgery. The anatomical locations of the tumors were variable as shown in Table (1). The surgical specimens were fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned and stained with H&E, these specimens were subjected to detailed pathological examination, for pathological tumor staging.

Immunohistochemical staining: Paraffin embedded blocks were sectioned at about 4-mm thickness, deparaffinized and rehydrated. After microwave pretreatment in citrate buffer (pH 6.0) for
antigen retrieval, slides were immersed in 0.3% hydrogen peroxide for 20 min to block the endogenous peroxidase activity. After washing, slides were incubated overnight at 4 °C with the polyclonal antibody against COX-2 (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) in a dilution of 1:50. After a second incubation with a biotinylated anti-goat antibody, slides were incubated with peroxidase-conjugated streptavidin (DAKO LSAB + kit; Dako Corp, Carpinteria, CA). Reaction products were visualized by immersing slides in diaminobenzidine chromogen and finally counterstained with Mayer’s hematoxylin. We performed control immunostaining using preabsorption of anti-COX-2 antibody with human synthetic COX-2 peptide (Santa Cruz Biotechnology) to determine the specificity of primary antibody.

Positive staining of smooth muscle cells within the muscle coat provided an internal positive control. The percentage of positively stained tumor cells was graded semi-quantitatively and each sample was assigned to one of the following categories: A simple scoring system analyzing only tumor cells was used to interpret the staining patterns according to Mighell et al. and Nix et al. [18,26].

Sections were regarded as positive if >5% of the tumor cells stained, sections with ≤5% of the tumor staining were considered negative. In order to reduce sampling error the whole biopsy section for each tumor was analyzed.

Descriptive statistics and statistical consideration:

Locoregional recurrence (LRR) was defined as recurrence of disease at site within the upper aerodigestive tract anatomically contiguous with the primary tumors or within the cervical lymphatic system. Distant metastasis (DM) was considered if disease recurred in a site that didn’t meet the definition of LRR and wasn’t considered to represent a second primary tumor based on its histological or clinical manifestations.

Relapse-free survival (RFS) was measured from the first day of starting the treatment to the time of discovery of the first recurrence event after treatment of any tumor (local-regional or metastatic) or death from any cause. Overall survival (OS) was measured from the first day of starting treatment to the date of death from any cause.

Statistical analysis of the correlation between COX-2 expression in the tumors and clinicopathological parameters calculated with the Chi-square test and Spearman correlation matrix test. \( p<0.05 \) was selected as the statistically significant value. RFS and OS were examined with the Kaplan-Meier method.

Results

Patient’s characteristics:

A total of 36 patients were accrued onto the study and all were included in the analysis of the patient characteristics. The TNM stage distributions and the baseline disease characteristics are shown in Table (1). More than 75% of the patients had >T2 tumors, 80% had GI&II tumors and 78% had nodal involvement. Most of the patients (64%) had stage III disease and 36% had stage IVA disease. Perineural involvement was observed in 28% and high risk factors were found in a total of 58%. The median follow-up for all patients for this analysis was 27.6 Months (ranged from 21 to 35 months).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (36)</th>
<th>%</th>
<th>Variable</th>
<th>Number (36)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>33/3</td>
<td>92/8</td>
<td>Lymph nodes status</td>
<td>N0</td>
<td>8</td>
</tr>
<tr>
<td>Median age (range)-yr</td>
<td>59.4 (41-73)</td>
<td>28</td>
<td>N1</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>ECOG Performance Status</td>
<td>10</td>
<td>53</td>
<td>N2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>28</td>
<td>Grade</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>53</td>
<td>Well differentiated G I</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>19</td>
<td>Moderately differentiated GII</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td>Poorly differentiated</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Supraglottic</td>
<td>23</td>
<td>64</td>
<td>COX-2 status</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Glottic</td>
<td>13</td>
<td>36</td>
<td>COX-2 negative</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>8</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>18</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>10</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Treatment results:

Tolerance and compliance:

Concurrent chemoradiotherapy and celecoxib were delivered per protocol. Treatment protocol was tolerable thus, 32 (89%) patients could proceed to receive a full course of Celecoxib, chemotherapy and radiotherapy. Celecoxib compliance was acceptable, however it decreased with the end of chemoradiation. 4 patients (11%) stopped celecoxib 1-2 weeks before the end of radiotherapy 2 for G4 nausea and vomiting and 2 for G4 mucosal reaction. The specified radiotherapy was delivered in 34/36 (94%) of patients. Eight patients (22%) required treatment interruption for the following reasons: neutropenia 5, anemia 1, thrombocytopenia 1 and skin reaction 1.

Treatment-related acute toxicities:

Overall, G3,4 acute toxicities were observed in 18/36 patients (50%) mainly chemotherapy related, but were well tolerated. Three patients required cisplatin dose modification, two for renal toxicities and one for leucopenia. Most G3 & 4 toxicities were observed in (33%) patients. The most common form of late radiotherapy toxicity was mucositis (44%), GIT (36), dysphagia (30%) and xerostomia (27%). Table (2) lists the major G3 & 4 acute toxicities of the protocol therapy according to the CTC v. 2.0 criteria.

Treatment-related late toxicities:

Late radiotherapy toxicity, was scored according to the RTOG/EORTC Late Morbidity Scoring Scheme. With a relatively short median follow-up of 27.6 months, the incidence of G3&4 late toxicities were observed in (33%) patients. The most common form of late radiotherapy toxicity was xerostomia (30%) followed by dysphagia (25%).

Table (2): Common high grade acute toxicity.

<table>
<thead>
<tr>
<th>Toxic effect*</th>
<th>G3 No. (%)</th>
<th>G4 No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All hematologic</td>
<td>13 36 3 8 16 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>3 8 1 3 4 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>7 19 1 3 8 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>3 8 1 3 4 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>1 3 0 0 1 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis</td>
<td>14 39 2 5 16 44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xerostomia</td>
<td>8 22 2 5 1 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin reaction (within the RT field)</td>
<td>13 36 1 3 14 39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIT (Nausea and vomiting)</td>
<td>11 30 2 5 13 36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysphagia</td>
<td>10 27 1 3 11 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal or genitourinary</td>
<td>2 5 0 0 2 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Patient may develop >1 toxic effect.

Loco-regional control and pattern of relapse:

So far, at 2 years with median follow-up of 27.6 months, locoregional recurrence was observed in 6/36 (17%) of the patients, relapse at distant sites (DM) was observed in a total of 5 (14%) patients (1/5 with LRR developed DM). Lung metastasis was the commonest site which represented 60% of all distant metastasis.

Survival outcomes:

At the time of analysis, with a median follow-up period of 27.6 months twenty-six patients were alive, 9 patients died with disease progression and 1 patient died with no evidence of disease after 16 months from unrelated cause. Twenty-four patients were alive with no evidence of disease, Kaplan-Meier method revealed two-year RFS of 67% and the two-year overall survival of 72%. Table (3) summarizes the treatment outcome end points.

Table (3): Treatment outcomes end points.

<table>
<thead>
<tr>
<th>End points</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local-regional recurrence</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Grade 3-4 acute toxicity</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Grade 3 &amp;4 late toxicity</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>2 Years Relapse-free survival</td>
<td>24</td>
<td>67</td>
</tr>
<tr>
<td>2 years Overall survival</td>
<td>26</td>
<td>72</td>
</tr>
</tbody>
</table>

COX-2 expression and its prognostic significance:

We investigated the expression of the COX-2 protein immunohistochemically in 36 patients with LASCC of the larynx. Normal laryngeal mucosa did not stain for COX-2, however it was observed in smooth muscle cells, fibroblasts and inflammatory mononuclear cells of the desmoplastic stroma. Additionally, epithelial cells showing dysplasia adjacent to tumor tissue were also strongly immunoreactive to COX-2 protein.

Most laryngeal carcinoma cases showed positive cytoplasmic staining with anti-COX-2 antibody, 22 out of 36 (61%) exhibited positive immunoreactivity of COX-2 protein (Fig. 1a,b), while (14 out of 36, 39%) cases exhibited negative immunoreactivity (Fig. 1c).

We evaluated the relationship between expression of COX-2 protein and various clinicopathological parameters of laryngeal cancer patients. The COX-2 expression showed no significant correlation neither with age nor sex. Although COX-2 expression observed more with advanced pathological T stage (T3&4), however this difference was not statistically significant (p>0.05). On
the contrary, COX-2 expression in laryngeal carcinoma was significantly related to tumor grade I&II, supraglottic site and positive lymph nodes, \((p<0.05)\) (Table 4).

Table (4): Cox-2 correlation with disease characteristics.

<table>
<thead>
<tr>
<th>Tumor characteristics</th>
<th>COX-2 +ve</th>
<th>COX-2 -ve</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>No.=22</td>
<td>No.=14</td>
<td></td>
</tr>
<tr>
<td>Supraglottic</td>
<td>23</td>
<td>6</td>
<td>0.05</td>
</tr>
<tr>
<td>Glottic</td>
<td>13</td>
<td>8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Grade:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI&amp;II</td>
<td>29</td>
<td>9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GIII</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>T stage:</td>
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<td></td>
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<tr>
<td>T2</td>
<td>8</td>
<td>4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>T3</td>
<td>18</td>
<td>6</td>
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<tr>
<td>T4</td>
<td>10</td>
<td>4</td>
<td></td>
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<tr>
<td>LN status:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LN +ve</td>
<td>28</td>
<td>8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LN -ve</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Prognostic significance of cox-2 expression on treatment outcomes:

The correlation between COX-2 expression in the tumors and treatment outcomes calculated with the Chi-square test and Spearman correlation test. Over all relapse was significantly increased in cox-2 positive patients. Out of the 11 patients who developed relapse, 8 (73%) patients were Cox-2 positive Vs 27% with COX-2 -ve which was statistically significant \((p<0.05)\) but with no significant difference regarding relapse site.

Results from the Kaplan-Meier log-rank tests for associations between COX-2 and 2-year locoregional control rate (LRC) did not show difference between the 2 groups, however, it demonstrated that COX-2 positivity was associated with decreased 2 years RFS and 2-year overall survival but was not statistically significant \((p>0.05)\) Table (5).

Table (5): Cox-2 correlation with treatment outcome.

<table>
<thead>
<tr>
<th>Treatment outcome</th>
<th>Total relapse (n=11)</th>
<th>2 years LRC</th>
<th>2 years RFS</th>
<th>2 years OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cox-2 +ve</td>
<td>8 (73%)</td>
<td>81%</td>
<td>63%</td>
<td>64%</td>
</tr>
<tr>
<td>Cox-2 –ve</td>
<td>3 (27%)</td>
<td>86%</td>
<td>71%</td>
<td>86%</td>
</tr>
<tr>
<td>(p) value</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Discussion

For patients with laryngeal cancer who are at a high risk of cancer recurrence, evidence from four randomized trials [4,5,27,28] support that the addition of chemotherapy to postoperative radiotherapy results in statistically and clinically significant improvements in locoregional control, progression or disease free survival and overall
survival. Pooled data across the trials, with and without sensitivity analysis, confirm the significant improvement in locoregional control and overall survival with radiochemotherapy when compared with radiotherapy alone. Differences in median survival were also consistently in favour of radiochemotherapy but were non-significant in one trial [5].

COX-2 inhibitors have been combined with chemotherapy and/or RT in a number of settings including the treatment of lung, CNS, GI and head & neck malignancies and shown to be safe [29-31]. In a Phase I study performed at MD Anderson Cancer Center in unfavorable performance non small cell lung cancer patients treated to 66Gy in 33 fractions with concurrent celecoxib, the maximally tolerated dose was not reached and 800mg bid of celecoxib was observed to be safe [29]. Celecoxib related toxicity was observed in 3 of 47 patients in their study. One pilot study showed reasonable promise in the treatment of advanced pancreatic carcinoma cancer without increased toxicity [9], whereas other studies have shown increased toxicity without increased efficacy in the treatment of GI malignancies with chemotherapy [10,11].

The primary toxicity in our study was hematologic. Nevertheless, we experienced a significant rate of GI toxicity in this study. Although we observed acute Grade 3/4 rate of GI toxicity in 36% (13/36) of patients, the toxicities observed were principally nausea and vomiting, many of which were able to be controlled with medical intervention. We did not experience a significant number of adverse events attributable to upper GI toxicity which more readily could implicate the celecoxib therapy as gastritis (only 2 patients from 36; 5%) and bleeding. In some trials, coxibs showed no more upper GI toxicity than placebo while other studies showed no difference in GI bleeding rates compared with nonsteroidal anti-inflammatory agents [33-35].

In studies such as this, it is difficult to ascribe toxicities to a single agent when patients are receiving a complex regimen of external beam radiotherapy and chemotherapy. Although the acute toxicity in this trial was moderately high indicating the toxicity of the regimen, it was less than the overall rate of Grade 3 or greater toxicity seen in Radiation therapy oncology group 9501 (RTOG) with RT and cisplatin every 3 weeks for high-risk squamous cell carcinoma of the head and neck. The worst overall Grade 3 and 4 toxicity observed in this trial with daily celecoxib and cisplatin was 50%, compared with 72% observed in RTOG 9501 [5]. The European organization for research and treatment of cancer trial 22931 (EORTC) reported less toxicity than this study with 41% grade 3/4 toxicity using concomitant chemoradiotherapy for locally advanced head and neck cancer [4].

In this study, we were unable to demonstrate efficacy of celecoxib in addition to CRT. The LRC rate of 83% within the first 2 year of treatment is comparable to the experience with CRT alone, considering that most of the regimens in advanced laryngeal cancer achieve control rates of 70-75% [4,5,27,28]. Despite comparison between different trials is difficult, our result are very close to the 18% 2 years locoregional failure rate obtained in the RTOG in 42 patients and in the EORTC in 37 with cancer larynx and leaving 82% of the patient with LRC [36]. The survival rate in this study was also similar to that reported in previous studies of combined CRT [4,5,27,28].

One potential molecular marker in HNSCC that has drawn considerable interest is (COX-2). Using only pre-treatment tissue the present study demonstrated expression of COX2 in laryngeal carcinoma cases with positive expression in (61%) of the patients, meanwhile the normal mucosa did not express COX-2. We have found that Cox-2 overexpression has a greater association with tumor differentiation (grade 1 & 2), supraglottic site, and positive lymph nodes (p<0.05). This is in agreement with previous observations of Cox-2 protein expression in head and neck cancer. Chan et al. [15] reported that Cox-2 is expressed in 100% of tumors from a mixed group of 10 head and neck patients and Ranelletti et al. [37] who reported Cox-2 overexpression in 31% (19 of 61) of tumors that comprised 18 stage I or II laryngeal cancers and 43 stage III or IV laryngeal cancers. More recently, Nix and colleagues [18] found the same results and they reported that COX-2 is expressed in 67% of laryngeal squamous cell carcinoma cases.

Cox-2 overexpression has been concerned in tumor response to radiotherapy. Tsujii and Dubois [38] demonstrated that over expression of Cox-2 in cells made them resistant to apoptosis, which is considered a critical pathway of cell death induced by radiation therapy. Inhibition of COX-2 has been found to diminish tumor growth in a countless ways including inhibiting vascular endothelial growth factor (VEGF), inhibiting new vessel growth, promoting apoptosis and sensitizing cells to radiation [39-43]. Pyo et al. [44] demonstrated that the Cox-2 inhibitor, enhances the effect of radiotherapy on human cells that overexpress
Cox-2 and this radiation-enhancing effects did not occur in cells deficient in Cox-2 expression. Additionally, more recently, Dittmann et al. [45] concluded that Celecoxib can make tumor cell radiosensitive by Inhibiting Radiation Induced Nuclear epidermal growth factor receptor (EGFR) Transport and DNA-Repair a signaling which is independent of COX-2 activity. This observation may have therapeutic implications, so COX-2 inhibitors may improve therapeutic efficacy of radiotherapy even in patients with radioresistant tumor that is not dependent on COX-2.

In our study the correlation between COX-2 expression in the tumors and treatment outcomes demonstrated that over all relapse was significantly increased in cox-2 positive patients (73% Vs 27% p<0.05) but with no significant difference regarding relapse site. Associations between COX-2 and 2-year locoregional control rate (LRC) did not show difference between the 2 groups. In a study by Cho et al. [46] reported on 32 patients T1-2 N0 stage with locally relapsed disease, for an actuarial 5-year local relapse-free rate of 70.4%. On multivariate analysis, the relative risk (RR) for local relapse after radiotherapy with COX-2 positivity was 2.57. This relationship may have potential therapeutic implications regarding the use of COX-2 inhibitors during radiation therapy for optimal outcome. In addition to the genetic evidence implicating COX-2 in tumorigenesis, selective inhibitors of COX-2 inhibit tumor formation in experimental animals [47].

Regarding survival analysis, our results demonstrated that COX-2 positivity was associated with decreased 2 years RFS and 2-year overall survival but was not statistically significant (p>0.05). These results are partially similar to Chang and colleagues results [48] on the prognostic significance of COX-2 in oropharyngeal SCC carcinoma which demonstrated that COX-2 positive tumors was associated with decreased 3-year overall survival, disease-free survival, and with any recurrence of disease (p=0.01, 0.05 & 0.05) respectively. When local recurrence, regional, distant, locoregional and any site recurrence were tested individually against COX-2 status, there was no significant association. This difference in significance may be attributed to the use of COX-2 inhibitors in our study, but in need to be confirmed.

Despite advances in chemoradiotherapy treatment for laryngeal cancer, patient survival has not improved significantly over the last two decades [8]. Patients whose tumors express high levels of COX-2 protein may be at risk for decreased 2 year OS and RFS if these studies are confirmed, COX-2 could become recognized as a basic factor in estimating prognosis and selecting treatment modalities for SCC of the larynx as a therapeutic target. Additionally, understanding the mechanism of tumor radioresistance will allow innovative therapies to be devised. Inhibitors of Cox-2 have the ability to enhance the effects of radiation. By using such inhibitors to radiosensitise tumors, enhanced radiotherapy cure rates may be attained. It is hoped that such strategies would improve the poor survival figures for laryngeal cancer. In light of these data, a clinical trial designed to assess the use of celecoxib in treatment of cancer larynx pre-operatively for possible increase in the rate of organ preservation seems justified. Consequently, confirmatory randomized phase III trial is encouraged.

Conclusion:

Cox-2 may have prognostic value in predicting disease recurrence. Cox-2 inhibitors as celebrex concurrently with chemoradiotherapy (CRT) was safe and tolerable, however its advantage over the standard CRT regimen couldn’t be proved. Confirmatory randomized phase III trial on larger number of patients and longer follow-up is encouraged.

References


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