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Cyclooxygenase-2 expression in squamous cell carcinoma of larynx: association with clinico-pathological factors and treatment outcomes

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ABSTRACT

Background: Squamous cell carcinoma (SCC) of larynx is widely prevalent in India and is one of the leading cancers in males. Tumor Cyclooxygenase-2 (Cox-2) expression can be used as a prognostic and predictive marker in laryngeal SCC. The primary objective of the present study was to determine the association between tumor Cox-2 expression and clinico-pathological characteristics and treatment outcome in patients with LSCC. Secondly, to evaluate the clinical utility of Cox-2 expression as a tool, to decide treatment strategy for LSCC.
Methods: Seventy two patients with stage III and IV LSCC who underwent upfront surgery were included in this study. Tumor Cox-2 expression was analysed by immunohistochemistry using standard Streptavidin biotin method.
Results: Thirty seven patients had pathological node involvement, ipsilateral in 35 and bilateral in two. Cox-2 was intensely expressed in patients with advanced (N2/N3) nodal disease and perineural invasion. There was no significant difference in 5 year disease free survival and overall survival when Cox-2 was correlated with perineural invasion, extra capsular spread and the T and N stage of the disease.
Conclusions: Preoperative Cox-2 analysis can be used to individualize need for routine neck dissection in cases of locally advanced laryngeal carcinoma.

Keywords: Cyclooxygenase 2, Larynx, Predictive marker, Reverse transcriptase polymerase chain reaction, Squamous cell carcinoma

INTRODUCTION

According to hospital based cancer registry data (2010) from the Regional Cancer Center (RCC), Trivandrum, India, laryngeal cancer accounts for 5.76% of all cancers in males and 0.18% of all cancers in females.[1] The male/female ratio for the incidence of laryngeal cancer is much higher than in other sub-sites of head and neck region.[2] Most of these tumors originate in the glottis (more than 60%) and supraglottis; the subglottis is an extremely rare site of origin (less than 5%).[3]

Early-stage laryngeal squamous cell carcinoma (LSCC) can be adequately treated with single-modality therapy, either surgery or irradiation, with a 5-year local control of 85–98%.[4-6] In locally advanced cancers, the treatment

approach is multidisciplinary which includes surgery, radiation and chemotherapy.[3] However, local regional failure and distant metastasis continue to remain a serious issue despite all advances in surgical techniques, modern radiotherapy delivery and use of chemotherapeutic drugs. Since similar patients, affected by tumours with similar clinico-pathological features and undergoing the same treatment differ widely in prognosis, the prognostic stratification of LSCC patients is inadequate. This may be due to the extreme biological heterogeneity of LSCC, which contributes to the lack of consistency in treatment planning. In this context, tumor markers have a promising role. There is an on-going need for development of molecular markers which can predict the response to therapy and can avoid overtreatment or under treatment of selected cases.

Cyclooxygenase (Cox-2) enzyme catalyses the formation of prostaglandins, which can affect cell proliferation and alter the response of the immune system to malignant cells. The Cox-2 is the inducible form of Cox-2 enzyme, which is found to have significance in carcinogenesis. Numerous studies have emphasized the importance of Cox-2 activity during tumorogenesis of the colon, oesophagus, head and neck, and breast. Recent work indicates that Cox-2 inhibitors also may be useful in the prevention and treatment of common epithelial malignancies. Non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit Cox-2, reduce the incidence of these tumors.[7,8]

A change in clinical approach is required to reduce laryngeal cancer-related mortality. The integration of the most promising biological markers in the phases of diagnosis, prognostic assessment and drug design would definitely reduce the laryngeal cancer-related mortality. Therefore, in this study the expression pattern of an inflammatory marker Cox-2, in locally advanced LSCC was studied. The correlation of intensity of Cox-2 expression with survival rate and with some of the known adverse prognostic factors was also identified. The present study also planned to identify the role of expression of Cox-2 to predict poor prognostic groups which need additional treatment approaches.

METHODS

The present study included a total of 72 patients with stage III and IV laryngeal squamous cells carcinoma at three different sub-sites (glottis, supraglottis and subglottis) who underwent primary surgery as initial treatment between January 2006 to December 2010 at Head and Neck Surgical Oncology unit of Regional Cancer Centre at Trivandrum in India. The study was approved by Institutional Ethics Committee and Informed Consent was taken from all the patients. Clinical and pathological staging was done based on AJCC TNM classification 2011.[9] All patients received post-operative radiation or chemo radiation and were followed up for 2 years (till December 2012).

Gene expression for Cox-2 mRNA was done using reverse transcriptase polymerase chain reaction (RT-PCR) by co-amplifying Cox-2 with housekeeping gene GAPDH, which served as an internal control. Total RNA from tissue samples was extracted using TRIZOL reagent following manufacturer's instruction. The isolated RNA was reverse transcribed to cDNA in a 25juI reaction mixture, containing 200U of MMLV Reverse Transcriptase (RT), 2 pg of random hexamer, 6U of RNA guard and l00 pM dNTP mix at 37°C for 1 hour. The enzyme was inactivated at 95°C for 5 minutes and chilled quickly. This cDNA was used for amplification (50 ul) containing 2.5U Taq DNA polymerase, 1.5 mM $MgCl_2$, 150 uM dNTP mix and 2.5 pmol of sense and antisense primers. PCR products were separated on a 1.5% agarose gel.

The intensity of staining of the marker was scored as 0 (<10%), 1+ (10-25%), 2+ (25-75%), and 3+ (>75%) for no staining, mild, moderate and intense staining, respectively. To make the data more compact and homogenous, nil and mild intensity of staining were analysed as a single category (mild/insignificant) and moderate and severe intensity of staining were analysed together as another category (intense/significant).

Data was analysed using STATA IC/11.2 software package. Descriptive analysis was done to evaluate the relationship between various clinico-pathological variables and Cox-2 expression. Chi squared test was used to test the association with various clinico-pathological variables. Life table method was used to estimate the overall and disease free survival (%). Logistic regression analysis was used to estimate the odd ratio and 95% confidence interval. Survival analysis was done by Kaplan –Meir method. Log rank test was used to test the equality of survivor functions.

RESULTS

A total of 72 surgically treated patients of laryngeal carcinoma enrolled in this study over a time period of 5 years. The disease characteristics of all the patients are depicted Table 1. The mean age of the patients was 57.2 ± 9.8 years. The epicentre of the tumor was identified as glottis in 47 (65.3%) cases, supraglottis in 19 (26.4%) and subglottis in 6 (8.3%) cases. There were 18 trans-glottic tumors. Radiological evidence of extra laryngeal spread was seen in 20 cases (27.78%). All patients underwent total laryngectomy with ipsilateral or bilateral neck dissection based on the clinical and radiological evidence of significant nodal disease. Thyroid gland involvement was seen in 18 cases (25%). Median follow up for the whole cohort was 2.54 years (range: 5 months to 72 months).

Treatment failure (defined as local or regional recurrence/second primary/distant metastasis) was seen in 21 (29.2%) patients. Local recurrence was seen in 3 patients, nodal recurrence in 8, distant metastasis in 6 and

second primary was seen in the rest 4 patients. At the time of last follow up, 47 (65.3%) patients were alive without any evidence of disease, 10 (13.9%) patients were alive with disease and 15 (20.8%) patients were dead.

Table 1: Patient and disease characteristics all the patients of laryngeal carcinoma.

Parameters	Subjects (N=72) (%)
Age (mean±SD, years)	57.2±9.78
Age group, n (%)	
<60 years	43 (59.7)
>60 years	29 (40.3)
Sub-site, n (%)	
Glottis	47 (65.3)
Supra-glottis	19 (26.4)
Sub-glottis	6 (8.36)
Pathological stage, n (%)	
T3	29 (40.3)
T4	43 (59.7)
N stage, n (%)	
N0	35 (48.6)
N1	19 (26.4)
N2	17 (23.6)
N3	1 (1.4)
Ipsilateral node, n (%)	35 (48.6)
Contralateral Node, n (%)	8 (11.1)
Grade of tumor, n (%)	
Well differentiated	18 (25)
Moderately differentiated	51 (70.8)
Poorly differentiated	3 (4.2)
Extra-capsular spread, n (%)	13 (18)
Perineural invasion (PNI), n (%)	19 (26.4)
Treatment failure, n (%)	21 (29.2)
Death, n (%)	15 (20.8)

Table 2: Correlation of Cox-2 expression with clinico-pathological variables.

	Total N (%)	Mild N (%)	Intense N (%)	P value
Subjects age group	72 (100)	37 (51.4)	35 (48.6)	
<60 years	43 (59.7)	21 (48.8)	22 (51.2)	0.598
>60 years	29 (40.3)	16 (55.2)	13 (44.8)	
Sub-site				
Supraglottis	19 (51.4)	6 (31.6)	13 (68.4)	0.044
Glottis	47 (65.3)	28 (59.6)	19 (40.4)	0.057
Subglottis	6 (15.3)	3 (50)	3 (50)	1.00
T stage				
T3	29 (40.3)	10 (34.5)	6 (48.3)	
T4	43 (59.7)	10 (23.3)	11 (25.6)	0.746
N stage				
Node negative	35 (48.6)	35 (100)	0	0.001
Node positive	37 (26.4)	2 (10.5)	35 (94.6)	
Grade				
Well differentiated	18 (25)	13 (72.2)	5 (27.8)	
Moderately differentiated	51 (70.8)	22 (43.2)	29 (56.8)	0.358
Poorly differentiated	3 (4.2)	2 (66.7)	1 (33.3)	
Extra capsular spread				
Absent	59 (81.9)	28 (47.5)	31 (52.5)	0.155
Present	13 (18)	9 (69.2)	4 (30)	

Continued.

	Total N (%)	Mild N (%)	Intense N (%)	P value
Peri-neural invasion				
Absent	53 (73.6)	25 (47.2)	28 (52.8)	0.232
Present	19 (26.4)	12 (63.2)	7 (36.8)	
Treatment failure				
Absent	51 (70.8)	26 (50.9)	25 (59)	0.914
Present	21 (29.2)	11 (52.4)	10 (47.6)	
Death				
Alive	57 (79.2)	29 (50.8)	28 (49)	0.866
Dead	15 (20.8)	8 (53.3)	7 (46.6)	

Table 3: Cox-2 expression with clinic-pathological factors in disease free and overall survival.

Factors			OS			DFS		
			2 yr (%)	5 yr (%)	P value	2 yr (%)	5 yr (%)	P value
1) Age	< 60	Mild	95.2	80.6	1	100	100	0.23
		Intense	89.5	89.5		94.6	78.8	
	> 60	Mild	85.7	38.1	1	79.3	14.5	0.46
		Intense	83.9	41.1		76.3	38.5	
2) T stage	T3	Mild	93.5	55.1	0.	100	75.7	1
		Intense	76.9	38.5		100	71.4	
	T4	Mild	77.1	77.1	1	85.4	48.9	0.76
		Intense	85.3	73.1		80.7	46.7	
3) N stage	N0	Mild	90.7	62	0	90	60.3	0.4
		Intense	0	0		0	0	
	N+	Mild	100	100	0.73	33.3	33.3	0.5
		Intense	87.3	56.3		87.4	55.1	
4) ECS	No	Mild	92.3	72.4	0.3	91.6	76.4	0.3
		Intense	89.3	57.4		89.3	56	
	Yes	Mild	88.9	36.9	0.3	50.8	16.9	0.2
		Intense	75	75		75	75	
5) PNI	No	Mild	87	50	1	67.3	56.9	1
		Intense	84.3	52.6		84.3	58.3	
	Yes	Mild	88.9	75.2	0.5	72	60.9	1
		Intense	100	100		77.8	38.9	

OS**:** overall survival, DFS: disease free survival, ECS: extra capsular spread, and PNI: perineural invasion.

The correlation of Cox-2 expression with clinico-pathological variables is displayed in Table 2. Out of the 18 cases of intense expression, 14 (77.7%) were primarily supraglottic malignancy and 4 were of other sub-sites (p=0.04). For glottic malignancy 19/47 (40.4%) were of intense expression. The Cox-2 was intensely expressed in 25.6% of patients with T4 stage and in 48.0% with T3 stage (p=0.74). Among 37 node positive cases, intense expression was seen in all cases of N2 and N3 (18/18) and 17 of 19 N1 cases (94.6% of all node positive cases) as compared to none in 35 of N0 cases (p=0.001).

Of the 13 patients with extra-capsular invasion, 4 showed intense Cox-2 expression (30%) compared to 31/59 (52.5%) without extra-capsular invasion (p=0.155). Of the 21 patients who recurred after treatment, 6 showed no Cox-2 expression in primary surgical specimen as against 5 each with mild, moderate and intense expression. Of the 10 significant expression cases who failed, 3 were local failures, 2 were second primary and 5 were nodal failures. Of the 15 patients who died; 7 (20.5%) had intense expression of Cox-2 and 8 (11.5%) had only minimal expression compared to 28/57 (49%) with intense expression and 29/57 (50.8%) with mild expression in those who were alive (p=0.866).

The 2 and 5 year disease free survival of the study population was 71.6 and 51.8%. The overall survival of the study population at 2 and 5 years were 81.3 and 56.9%, respectively. Cox-2 was intensely expressed in 18 (25%) patients, moderately in 17 (23.6%) and mildly in 17 (23.6%). There was no expression of Cox-2 in 20 (27.8%) patients. The overall survival at 5 years for mild and intense expression Cox-2 was 64.7% and 22.9%, respectively (p=0.88). The disease free survival at 5 years for the mild and intense expression cases were 58.5% and 55.1%, respectively (p=0.88).

DISCUSSION

The main role of cyclooxygenases (COX) is to catalyse the transformation of arachidonic acid into the intermediate prostaglandin H2, which is the precursor of a variety of prostanoids with diverse and potent biological actions. Cox-1 is a housekeeping gene that is expressed constitutively in most tissues and in contrast, Cox-2 is an immediate-early response gene that is induced by a variety of stimuli. Multiple studies have confirmed that high expression levels of Cox-2 were closely related to the development and prognosis of many tumors.

In the present study, Cox-2 was intensely expressed in 35 (48.6%) patients and insignificant in 37 (51.4%) patients with LSCC. In the study by Chen et al, 47.5% expression of Cox-2 was found in 80 cases of laryngeal carcinoma treated by primary surgery in a 2 year.[10] In a retrospective analysis of 123 patients with early stage laryngeal carcinoma, high levels of Cox-2 expression were observed in 66.6% and 6.7% of laryngeal squamous cell carcinoma and epithelial dysplasia, respectively, but no Cox-2 expression was detected in the normal epithelium.[11] Ranelletti et al, reported Cox-2 over-expression in 31% (19 of 61) of tumours that comprised 18 cases of stage I or II laryngeal cancers and 43 cases of stage III or IV laryngeal cancers.[12]

The potential of Cox-2 as a prognostic factor and as a target for therapeutic intervention was evaluated in many studies on head and neck cancers. But studies specifically in advanced laryngeal cancers are infrequent. In this study, out of the 35 cases of intense expression of Cox-2, 22 (62.9%) were in T4 stage, showing an increasing trend of its expression as the primary stage advances.

Current study also showed that, among 37 node positive cases, intense expression of Cox-2 was seen 35 cases (89.4%) compared to insignificant expression in all (35/35) node negative cases which was statistically significant (p=0.001). So, the presence of significant expression of Cox-2 was highly predictive of nodal involvement. This was unique feature demonstrated in this study which was not found in other studies till date.

Among the 35 cases of ipsilateral nodal disease, significant expression was seen in 31 cases (88.5%). There was significant expression of Cox-2 in all cases with contralateral node involvement. This proves the positive correlation of Cox-2 expression with the nodal disease and the advanced nodal stage. It can be hypothesised that patients having significant Cox-2 expression warrants prophylactic neck treatment (surgery/radiation) in N0 neck.

The study by Nix et al did not show significant association of Cox-2 overexpression with T stage, degree of tumour differentiation, or laryngeal sub-site in a study of Cox-2 on radio resistant laryngeal cancer.[13] The positive expression of Cox-2 was related to the clinical stage in another study, Takatori et al studied on 228 cases of esophageal carcinoma and found that overexpression of Cox-2 was associated with tumor invasion, advanced clinical staging, poor histological differentiation and poor prognoses than those with low or no expression of Cox-2.[14,15]

The high expression of the two proteins namely Cox-2 and VEGF-c was observed in the study by Kyzas et al which was correlated with the presence of lymph node metastasis at the time of diagnosis.[16] Chen et al also showed that Cox-2 expression levels correlate with invasion and lymph node metastasis in LSCC.[10] They showed that high levels of Cox-2 was present in the patients with lymph node metastasis, advanced stage and poorly differentiated tumors than in those with no lymph node metastases, early staging and well or moderately differentiated tumors. Sun et al found that the patients with pathological grade III and IV disease showed a higher level of Cox-2 expression than those with grade I and II disease.[17] The rate of Cox-2 overexpression was higher in the cervical lymph node metastasis group than in the non-metastasis group.

Literature also showed that Cox-2 plays an important role in the process of development, growth and invasion of laryngeal squamous carcinoma; and its inhibitors may have an effect on the development of laryngeal carcinoma.[14]

The Cox-2 expression didn't show any significant difference in the survival, either overall or disease free survival. Chen et al showed that Cox-2 overexpression was an independent high-risk factor for prognosis, and the average survival time in Cox-2 positive group was lower than that in Cox-2 negative group.[10] He showed that Cox-2 was closely correlated with unfavourable survival (p=0.05). Ranelletti et al found that disease-free survival at 5 years was 84% in patients with Cox-2 positive tumors compared with 30% in those with Cox-2 negative tumors (p=0.0012).[12] Atula et al reported that Cox-2 expression does not have any prognostic significance in advanced oral and pharyngeal squamous cell carcinoma.[18] The disease free survival was significantly higher with stronger Cox-2 expression in a retrospective analysis of 97 cases of laryngeal cancer, although they had proved a higher grade of tumor with low Cox-2 expression.[19]

There is paucity of literature on the role of Cox-2 in predicting treatment failure after primary surgery, followed by chemo radiation. The overexpression of Cox-2 was found to be a predictor of increased risk of local recurrence after radiotherapy. Cho et al in their study on 123 patients with early stage LSCC treated by primary radiation therapy showed that elevated Cox-2 predicts local relapse.[20] Their study did not show an overall

survival benefit due to the fact that very few early-stage cancer patients died of their disease.

In patients with extra-capsular spread, those having significant expression of Cox-2 had worse outcome while those having insignificant expression had better outcome. Similarly, death rate among the patients with extra-capsular spread and significant Cox-2 expression was more compared to those with extra-capsular spread and mild Cox-2 expression. So, the marker expression along with the extra-capsular spread had statistically significant effect on both the overall and disease free survival. There was no correlation when combined with grade, nodal status or perineural invasion.

The inflammatory marker Cox-2 showed significant expression in supraglottic tumor samples and in the tumors with advanced nodal disease. It also had significant expression in cases with perineural invasion. The disease free survival in patients with and intense Cox-2 expression was significantly worse compared to those with extra-capsular spread and insignificant Cox-2 expression.

CONCLUSION

This study of Cox-2 expression in locally advanced LSCC showed significant relationship of Cox-2 expression with lymph node positivity. Since the presence of intense Cox-2 expression suggests nodal involvement, preoperative Cox-2 analysis will be useful to assess the need for routine neck dissection in carcinoma larynx cases undergoing surgical management in clinically N0 stage.

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REFERENCES

1. Annual Report. Regional Cancer Center T, India; 2010-2011.
2. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer statistics, 2005. CA: a Cancer J Clin. 2005;55(1):10-30.
3. Shah JP, Karnell LH, Hoffman HT, Ariyan S, Brown GS, Fee WE, et al. Patterns of care for cancer of the larynx in the United States. Archives Otolaryngol Head Neck Surg. 1997;123(5):475-83.
4. Franchin G, Minatel E, Gobitti C, Talamini R, Vaccher E, Sartor G, et al. Radiotherapy for patients with early-stage glottic carcinoma. Cancer. 2003;98(4):765-72.
5. Reddy SP, Mohideen N, Marra S, Marks JE. Effect of tumor bulk on local control and survival of patients with T1 glottic cancer. Radiotherap Oncol. 1998;47(2):161-6.
6. Rudoltz MS, Benammar A, Mohiuddin M. Prognostic factors for local control and survival in T1 squamous cell carcinoma of the glottis. Int J Radiation Oncol Biol Physics. 1993;26(5):767-72.
7. Soslow RA, Dannenberg AJ, Rush D, Woerner B, Khan KN, Masferrer J, et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer. 2000;89(12):2637-45.
8. Liu B, Qu L, Yan S. Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. Cancer Cell Int. 2015;15(1):106.
9. Sobin LH, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours: John Wiley & Sons; 2011.
10. Chen YF, Luo RZ, Li Y, Cui BK, Song M, Yang AK, et al. High expression levels of COX-2 and P300 are associated with unfavorable survival in laryngeal squamous cell carcinoma. European Arch Otorhinolaryngol. 2013;270(3):1009-17.
11. Cho NP, Han HS, Soh Y, Lee KY, Son HJ. Cytoplasmic HuR over-expression is associated with increased cyclooxygenase-2 expression in laryngeal squamous cell carcinomas. Pathology. 2007;39(6):545-50.
12. Ranelletti FO, Almadori G, Rocca B, Ferrandina G, Ciabattoni G, Habib A, et al. Prognostic significance of cyclooxygenase-2 in laryngeal squamous cell carcinoma. Int J Cancer. 2001;95(6):343-49.
13. Nix P, Cawkwell L, Patmore H, Greenman J, Stafford N. Bcl-2 expression predicts radiotherapy failure in laryngeal cancer. Br J Cancer. 2005;92(12):2185-9.
14. Chang BW, Kim DH, Kowalski DP, Burleson JA, Son YH, Wilson LD, et al. Prognostic significance of cyclooxygenase-2 in oropharyngeal squamous cell carcinoma. Clin Cancer Res. 2004;10(5):1678-84.
15. Takatori H, Natsugoe S, Okumura H, Matsumoto M, Ishigami S, Owaki T, et al. Predictive value of COX-2 for the effect of chemoradiotherapy on esophageal squamous cell carcinoma. Oncology Reports. 2005;13(4):697-701.
16. Kyzas PA, Stefanou D, Agnantis NJ. COX-2 expression correlates with VEGF-C and lymph node metastases in patients with head and neck squamous cell carcinoma. Modern Pathol. 2005;18(1):153-60.
17. Sun X, Ge R, Dong Q, Zheng C. The expression of cyclooxygenase-2 in laryngeal squamous cell carcinoma and its relationship with MVD. J Clin Otorhinolaryngol. 2005;19(21):967-70.
18. Atula T, Hedström J, Ristimäki A, Finne P, Leivo I, Markkanen-Leppänen M, et al. Cyclooxygenase-2 expression in squamous cell carcinoma of the oral cavity and pharynx: association to p53 and clinical outcome. Oncol Reports. 2006;16(3):485-90.
19. Kourelis K, Vandoros G, Kourelis T, Papadas T, Goumas P, Sotiropoulou-Bonikou G. Low COX2 in tumor and upregulation in stroma mark laryngeal

squamous cell carcinoma progression. Laryngoscope. 2009;119(9):1723-9.
20. Cho EI, Kowalski DP, Sasaki CT, Haffty BG. Tissue Microarray Analysis Reveals Prognostic Significance of COX-2 Expression for Local Relapse in T1–2N0 Larynx Cancer Treated with Primary Radiation Therapy. Laryngoscope. 2004;114(11):2001-8.

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