original article

High COX-2 immunostaining in papillary thyroid carcinoma is associated with adverse survival outcomes

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BACKGROUND: Thyroid carcinoma is one of the most common malignancies worldwide. More than 70%-80% are papillary thyroid carcinoma (PTC). Many factors influence the PTC pathway of development such as genetic mutations, growth factors, and radiation. More biological understanding of the genetic and molecular pathways is needed in PTC to determine tumor behavior, and initial clinical assessment.

OBJECTIVES: Investigate the relation of COX-2 immunostaining in thyroid carcinoma with clinicopathological parameters to assess whether immunostaining results have prognostic significance.

DESIGN: Retrospective study

SETTING: Pathology department, tertiary care center

METHODS: Records of PTC were retrieved and tissue microarrays were constructed. Tissue sections were stained using anti-human COX-2 monoclonal antibody. Immunostaining results were recorded and analysed.

MAIN OUTCOME MEASURES: Relationship of COX-2 immunostaining in thyroid carcinoma with clinicopathological parameters.

SAMPLE SIZE: 139 tissue samples from 139 patients

RESULTS: High versus low COX-2 immunostaining showed no significant differences for most clinicopathological parameters. However, high COX-2 immunostaining showed borderline association with tumor multifocality (P=.05), lower overall (log-rank=8.739 and P=.003), and disease-free survival (log-rank=7.033, P=.008).

CONCLUSION: The study showed a positive association of high COX-2 immunostaining with lower survival outcomes in PTC. COX-2 immunostaining could be a potential prognostic factor for survival in PTC. Additional molecular and clinical investigations are needed for further understanding the molecular pathways of COX-2 in PTC and the feasibility of using inhibitors of COX-2 as adjuvant therapy along with current chemotherapy.

LIMITATIONS: Relatively low number of PTC variants, and no testing of other thyroid carcinomas.

CONFLICT OF INTEREST: None.

Papillary thyroid carcinoma (PTC) is considered the most common thyroid cancer, originating from follicular thyroid cells. It constitutes up to 80% of all histological subtypes. PTC is observed frequently in females with an estimated prevalence of two to four times greater than male population.[1] Moreover, PTC is considered among the top 10 most frequent carcinoma seen in women. Although it is less common in males, men are predisposed to a higher mortality rate, probably due to late diagnosis, old age of presentation, and advanced tumor stage. PTC are commonly diagnosed at the ages of 30–40 years. The estimated 10-year life survival expectancy is 90%. Radiation exposure is a well documented risk factor for PTC development.[1] Tumor pathogenesis is related to patient age and gender, maximum tumor size, extrathyroid extension, lymph nodes statues and extranodal spread, distant metastasis, and pathologic tumor stage. However, more useful objective biological prognostic factors are required for better understanding of PTC tumorigenesis, good clinical assessment and better outcomes. Cyclooxygenases activate prostaglandin production from arachidonic acid. Cyclooxygenase-1 (COX-1), is a housekeeping gene, which is constantly expressed in most normal epithelial tissues. Cyclooxygenase-2 (COX-2), also known as prostaglandin endoperoxide H synthase-2, is found on chromosome 1 and encodes a 70-kDa protein. Unlike COX-1, COX-2 is expressed at baseline levels and is not commonly detected in normal tissues.[2] COX-2 overexpression may encourage tumor carcinogenesis by apoptosis inhibition and promoting tumor angiogenesis, cellular proliferation, and enhancing cell invasion.[3,4] The association between COX-2 overexpression and thyroid carcinogenesis needs more study to assess the exact COX-2 tumorigenesis pathway and any relationship to survival rate, which may affect clinical behavior and disease outcome. The aim of the current study was to investigate the relation of COX-2 immunostaining in thyroid carcinoma with clinicopathological parameters to assess whether immunostaining results have prognostic significance.

METHODS

Archival formalin fixed paraffin embedded blocks representing tumor tissue from patients with PTC for the period from 1996-2014 were retrieved from the archive of the Department of Pathology, King Abdulaziz University, Jeddah, Saudi Arabia. The study was approved by the research committee of the biomedical ethics unit of the research institute (Reference No: 1127-13). The patients gave informed written consent for using the material in research.

Tissue microarray

Tissue microarrays were constructed using the retrieved archival blocks. Malignant tissues were selected and many representative areas were selected and marked on haematoxylin and eosin-stained slides. Two tissue cores (a diameter of 1.5 mm) were biopsied from selected tumor areas in the donor block and inserted into new recipient paraffin blocks by using a tissue microarrayer instrument (TMA Master 1.14 SP3, 3DHistech Ltd. Budapest, Hungary). Orientation of blocks was guided by the use of placenta tissue.[5]

Immunohistochemistry

Sections (4 µm thick) were cut from tissue microarray blocks, and mounted to positive-charged slides (Leica Microsystems Plus Slides). Immunostaining was performed using automated immunostainer (BenchMark XT, Ventana Medical systems Inc., Tucson, AZ, USA), followed by clearance and rehydration. Pre-treatment was performed (60 minutes) by a pre-diluted cell conditioning solution. The primary antibody (mouse anti-human COX-2 monoclonal antibody (Dako Cytomation Norden A/S, Glostrup, Denmark) was used in a 1:50 dilution and incubated at 37oC for 20 minutes before applying a Ventana I-view DAB detection kit. Slides were washed, counterstained using Mayer's haematoxylin and mounted using positive and negative control slides.

COX-2 immunostaining interpretation

A semi-quantitative assessment was used to report COX-2 immunostaining. The COX-2 positive tumor was calculated and expressed as a percentage. A cut-off point of 10% was chosen as the threshold. Results were divided as low COX-2 immunostaining when 10% or less of the examined tumor cells showed COX-2 immunostaining or as high COX-2 immunostaining when more than 10% of the examined tumor showed COX-2 immunostaining.[6-8]

Statistical analysis

Statistical tests were used in IBM SPSS program (version 16) and the statistical significance was determined when *P* value is ≤.05 and 2-sided. The chi-square of association was used for comparisons of demographic and clinicopathological data with COX-2 immunostaining, which were all categorical. The survival analyses were tested by using the Kaplan-Meier procedure. The end-point for patients was survival in months (alive was defined as having appeared for a follow-up visit).

RESULTS

In 139 formalin-fixed blocks, immunostaining for COX-2 revealed cytoplasmic localization in malignant cells in 101/139 tumors (72.7%) (**Figure 1**). Patient and tumor characteristics are shown in **Tables 1 and 2**. Low COX-2 immunostaining was seen in 76 tumors (54.7%) while high COX-2 immunostaining was detected in 63 tumors (45.3%) (*P*=.270). There was no statistically significant difference in COX-2 immunostaining among different variants of PTC (*P*=.761) (**Table 3**). There were no statistically significant differences between low COX-2 immunostaining and high COX-2 immunostaining for most of the clinicopathological parameters with the exception of tumor multifocality where high COX-2 immunostaining showed borderline significance with more cases where multifocality was present (*P*=.05) (**Table 4**).

COX-2 immunostaining and survival outcomes

The survival analyses showed that there were statistically significant lower overall survival rates in patients with high COX-2 immunostaining than in patients with low COX-2 immunostaining (log rank test=8.739 and *P*=.003). Disease-free survival was statistically significantly lower in tumors with high COX-2 immunostaining (log rank=7.033, *P*=.008). Survival curves are shown in **Figures 2 and 3**.

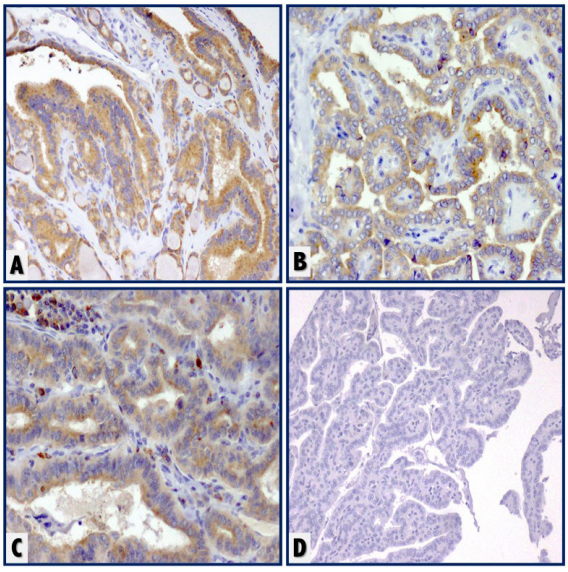

Figure 1. Immunostaining of COX-2 in papillary thyroid carcinoma using immunohistochemical labelling with anti-COX-2 antibodies with diaminobenzidine used as the chromogen and haematoxylin as counterstain. (A, B, and C) Positive COX-2 cytoplasmic immunostaining in different papillary thyroid carcinomas (200×). (D) Negative COX-2 immunostaining papillary thyroid carcinoma (200×).

Table 1. Patient demographic and clinicopathological characteristics (n=139).

Gender	
Female	107 (77)
Male	32 (23)
Age (median 39) (range 9-93)	
<45 years	87 (62.6)
≥45 years	52 (37.4)
Extrathyroid extension	
Absent	121 (87)
Present	18 (13)
Multifocality	
Absent	83 (59.7)
Present	56 (40.3)
Lymphovascular invasion	
Absent	123 (88.5)
Present	16 (11.5)
Capsular invasion	
Absent	122 (87.8)
Present	17 (12.2)
Primary tumor	
T1	61 (43.9)
T2	44 (31.7)
T3	17 (12.2)
T4	17 (12.2)
Nodal metastasis (n=55)	
Absent	21 (38.2)
Present	34 (61.8)
Margin status	
Free	103 (74.1)
Involved	36 (25.9)
Recurrence	
Negative	124 (89.2)
Positive	15 (10.8)

Data are n (%). T1: tumor size ≤2 cm in greatest dimension and s limited to the thyroid.; T2: tumor size >2 cm but ≤4 cm, limited to the thyroid; T3: tumor size >4 cm, limited to the thyroid or any tumour with minimal extrathyroidal extension.; T4: Advanced disease; tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve, prevertebral fascia or encased carotid artery or mediastinal vessel.

DISCUSSION

When expression of COX-2 is investigated in different types of carcinomas, most show over-expression of COX-2 when compared to normal counterparts.[6,9,10] COX-2 may play a considerable role in the invasiveness of tumor cells in different human neoplasms.[11] The exact role of COX-2 pathogenesis and progression to carcinoma is not clearly understood. The role of tissue inflammation, expression of COX-2 and cellular defence reactions, which may lead to thyroid carcinoma, should be considered. Chronic inflammatory cells in the thyroid gland may enhance tumor carcinogenesis and elevate genetic instability in mutational pathways.[12,13] Overexpression have been documented in various tumor carcinogenesis through enhancing tumor angiogenesis, apoptosis inhibition, increase cellular invasion, and enhancement of malignant cells proliferation.[14,15]

In the current study, COX-2 immunostaining was demonstrated in 72.7% of tumors. On further classification high COX-2 immunostaining was reported in 45.3% of PTC examined. These findings are similar to previous observations.[15-23] In the current study, COX-2 immunostaining was not associated with most clinicopathological variables which has not been reported previously.[15] In contrast, some previous studies reported COX-2 association with age.[1,15,16,25-27] Also, studies reported associations with large extrathyroidal extension, tumor size, advanced stage of disease, nodal stage, and invasive front of PTC[1,16,20,22,27,28] Others reported that COX-2 is associated with adverse prognosis through either invasion or metastasis.[26] In the current study, high COX-2 immunostaining with associated with tumor multifocality. This finding is consistent with a previous study.[22] The staining of COX-2 was also examined among different variants of PTC; however, our study showed no statistically significant difference. Some previous studies reported a higher COX-2 expression in papillary microcarcinomas than other PTC variants. Subsequently they suggested that COX-2 expression may play a role in early stages.[15] Another study found this correlation with solid, and trabecular variants.[16] The inconsistency in the results reported from different studies may be related to the number of tumors examined and the scoring methods of COX-2 immunostaining.

In PTC, the 10-year survival rate has been reported to range from 80% to 90%.[29] Our survival analysis showed a lower survival outcome in patients with high COX-2 immunostaining both in overall survival and disease-free survival. This observation was only reported once very recently.[27] The result of survival analysis is in keeping with previous reports which correlated COX-2 immunostaining with advancing tumor stage, nodal metastasis and invasion. Interestingly, a previous report on survival found survival difference is related BRAF mutation status.[27] This finding is interesting and raises the importance of investigating COX-2 expression on the molecular level and its correlation with the molecular profile of PTC and other thyroid cancers, which may be valuable for stratification of patients amenable to anti-COX-2

Table 2. Histological subtyping of papillary thyroid carcinoma included in the study (n=139).

Variant	
Classic papillary thyroid carcinoma	71 (51)
Microcarcinoma variant	20 (14.4)
Follicular variant (PTC-FV)	40 (28.8)
Oncocytic variant	2 (1.4)
Hürthle cell variant	1 (0.7)
Insular variant	1 (0.7)
Columnar cell variant	1 (0.7)
Tall cell variant	3 (2.1)

Data are n (%).

Table 3. Distribution of COX-2 immunostaining in relation to papillary thyroid carcinoma variants (n=139).

Variant	Low COX-2 Immunostaining (n=76)	High COX-2 Immunostaining (n=63)	*P* value
Classic	42	29	.761
Follicular	20	20	
Microcarcinoma	10	10	
Others	4	4	

Data are n.

Table 4. Distribution of COX-2 immunostaining in relation to clinicopathological parameters of papillary thyroid carcinoma (n=139).

Parameter	Category	COX-2 Immunostaining		*P* value
		Low (n=76)	High (n=63)	
Gender	Female	59	48	.841
	Male	17	15	
Age (n=139, median 39) (range 9-93)	< 45 years	52	35	.12
	≥ 45 years	24	28	
Extrathyroid extension	Absent	66	55	.936
	Present	10	8	
Multifocality	Absent	51	32	**.051**
	Present	25	31	
Lymphovascular invasion	Absent	68	55	.691
	Present	8	8	
Capsular invasion	Absent	69	53	.234
	Present	7	10	
Primary tumor	T1	34	27	.384
	T2	26	18	
	T3	6	11	
	T4	10	7	
Nodal metastasis (n=55)	Absent	14	7	.231
	Present	17	17	
Margin status	Free	58	45	.514
	Involved	18	18	
Recurrence	Negative	67	57	.662
	Positive	9	6	

Data are number of cases. T1: tumor size ≤2 cm in greatest dimension and s limited to the thyroid.; T2: tumor size >2 cm but ≤4 cm, limited to the thyroid; T3: tumor size >4 cm, limited to the thyroid or any tumour with minimal extrathyroidal extension.; T4: Advanced disease; tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve, prevertebral fascia or encased carotid artery or mediastinal vessel. Statistical comparisons by chi-square of association.

therapies. Our data supports the use of COX-2 as prognostic marker for survival and subsequently may help in patient stratification for risk and therapy.

Limitations of this study included the relatively low number of PTC variants, and inability to test other thyroid carcinomas. The study showed a positive association of high COX-2 immunostaining with lower survival outcomes in PTC. The results support the use of COX-2 immunostaining as a prognostic factor for survival in PTC. Also, the results of our study provide another rationale for the usefulness of selective COX-2 inhibitors, which may protect against cancer progression. Further molecular studies are needed for greater understanding of the molecular pathway downstream of COX-2 in PTC. Also, these promising data need more clinical studies and trials on the feasbility of using inhibitors of COX-2 as adjuvant therapy in addition to current therapeutic approaches.

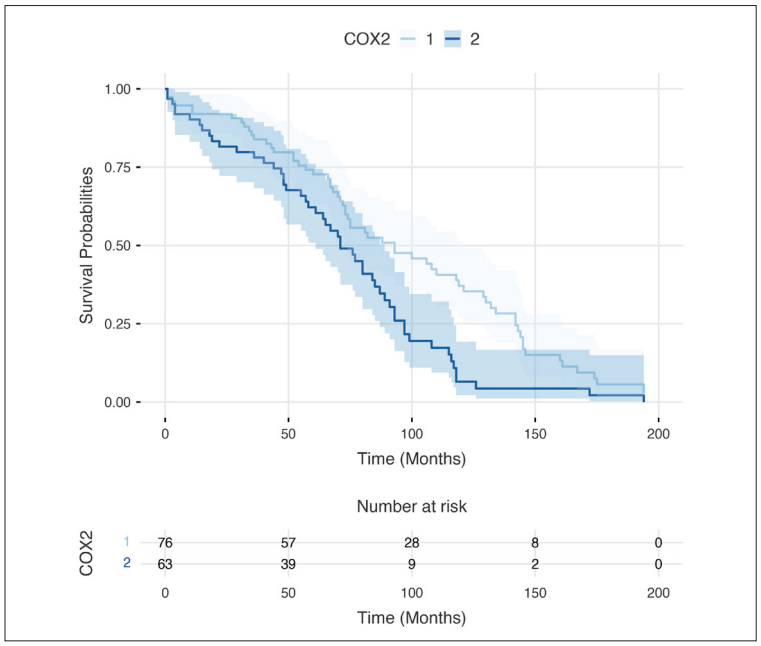

Figure 2. Overall survival curve (Kaplan-Meier) according to COX-2 immunostaining in papillary thyroid carcinoma (1: low COX-2 immunostaining; 2: High COX-2 immunostaining) (log-rank=8.739, *P*=.003).

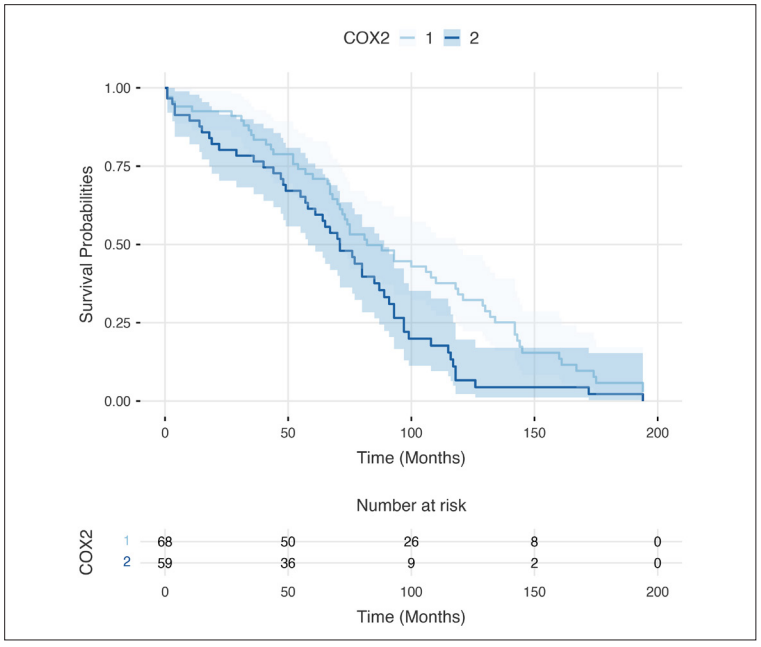

Figure 3. Disease-free survival curve (Kaplan Meier) according to COX-2 immunostaining in papillary thyroid carcinoma (1: low COX-2 immunostaining; 2: High COX-2 immunostaining) (log-rank=7.033, *P*=.008).

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