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Original Article

Combined use of COX-1 and VEGF immunohistochemistry refines the histopathologic prognosis of renal cell carcinoma

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Abstract: The course of RCC is asymptomatic, resulting in 25-30% of patients presenting with metastatic disease at time of diagnosis. The development of novel agents targeting angiogenesis and signal transduction pathways has improved patient outcomes. Role of cyclooxygenase in cancer development has been the subject of close scrutiny. COX-1 has been recognized to be involved in regulation of angiogenesis. To date, no immunohistochemical studies have been performed to assess the possible association between COX-1 and VEGF in RCC. This study is designed to evaluate the relationship between these two proteins in RCC. Also, the relationship between their combined immunohistochemical expression and different clinicopathological prognostic parameters in RCC is investigated. Immunohistochemical expression of COX-1 and VEGF was evaluated retrospectively on 64 cases of primary RCC including: 45 clear cell carcinoma, 12 papillary carcinoma and 7 of chromophope carcinoma. High COX-1 expression was detected in 62.5% of RCCs with a significant association with tumor grade (P=0.028), and highly significant relationship with tumor size and stage (P=0.001). There was a highly significant relationship between the VEGF score and tumor size (P=0.001), and stage (P=0.006). There was a positive correlation between COX-1 and VEGF expression score (P=0.001). Combined expression of both markers predicts high stage tumors (stage III/IV). Immunohistochemical expression of COX-1 and VEGF is associated with poor prognostic parameters in RCC. Their combined expression has a beneficial role in prediction of high stage tumors (III/IV).

Keywords: Renal cell carcinoma, COX-1, VEGF, immunohistochemistry

Introduction

Renal cell carcinoma (RCC) accounts for ~2% of all malignant diseases in adults .It is the seventh most common cancer in males, the ninth most common cancer in females, and approximately 90% of all kidney malignancies [1]. RCC continues to be a devastating cancer and the worldwide incidence and mortality rates are rising at a rate of 2-3% per decade [2]. Furthermore, clinical course of RCC is asymptomatic, resulting in 25-30% of patients presenting with metastatic disease at the time of diagnosis [2].

Nephrectomy is effective in removing early tumors. However, when metastasis occurs, RCC is very difficult to be treated and has poor prognosis [3] The extensive histological variability long noted in RCC was shown to reflect the existence of a large number of distinct entities having differing prognoses and often harboring unique cytogenetic abnormalities [4-6]. Although the pathologic stage has been considered as the most powerful prognostic marker in patients with RCC, many investigations have been performed to discover a new predictive marker for this tumor [7].

Currently no prognostic biomarkers are available to independently validate the therapeutic effect in individuals with RCC [8]. Molecular biomarkers are expected to have an important impact on future diagnosis, prognostication and selection of therapeutic targets for RCC [8]. Central role is attributed to the enzyme cyclooxygenase (COX), which is also known as prostaglandin-endoperoxide synthase (PTGS). It is the rate-limiting enzyme that catalyses the first

Table 1. Relationship between COX-1 expression and different clinico-pathologicalfeatures in RCCs (n=64)

Clinico-pathological features	(n) (%)	COX-1 in RCC				*P*-value	Sig
		Low<5		High > 5			
		n	%	n	%		
		24	(37.5%)	40	(62.5%)		
Tumor Type	Clear (45) (70.3%)	13	28.9%	32	71.1%	0.029*	S
	Chromophobe/papillary (19) (29.7%)	11	57.9%	8	42.1%		
Tumor size	≤ 7 cm (39) (60.94%)	23	59.0%	16	41.0%	0.001*	HS
	> 7 cm (25) (39.06%)	1	4.0%	24	96.0%		
Tumor grade	I (9) (14.06%)	6	66.7%	3	33.3%	0.028*	S
	II (32) (50%)	14	43.8%	18	56.2%		
	III (16) (25%)	4	25.0%	12	75.0%		
	IV (7) (10.94%)	0	0.0%	7	100.0%		
Tumor stage	I (35) (54.7%)	23	65.7%	12	34.3%	0.001*	HS
	II (10) (15.6%)	1	10.0%	9	90.0%		
	III (16) (25%)	0	0.0%	16	100.0%		
	IV (3) (4.7%)	0	0.0%	3	100.0%		
Peri-renal fat invasion	Positive (15) (23.44%)	0	0.0%	15	100.0%	0.001*	HS
	Negative (49) (76.56%)	24	49.0%	25	51.0%		
Renal vein invasion	Positive (7) (10.94%)	0	0.0%	7	100.0%	0.03*	S
	Negative (57) (89.06%)	24	42.1%	33	57.9%		
Renal sinus invasion	Positive (11) (17.19%)	0	0.0%	11	100.0%	0.005*	HS
	Negative (53) (82.81%)	24	45.3%	29	54.7%		

*Chi-Square Tests n: number of cases; S: significant HS: highly significant.

step in prostaglandin (PG) biosynthesis and acts both as a dioxygenase and as a peroxidase. Two isoforms of COX have been identified: the constitutive COX-1 and the inducible COX-2, which differ in the regulation of their expression and tissue distribution. COX-1 is constitutively expressed in a broad range of cells and tissues where it mediates the synthesis of PGs required for physiological functions. COX-1 is also thought to be involved in cell-cell signaling and in the maintenance of tissue homeostasis [9, 10].

The hypothesis of COX involvement in cancer progression has been strengthened by the effect of COX inhibitors that have been successfully used in the treatment of many cancers [11-15]. The mechanism responsible for these effects is still unclear [10]. Few studies have discovered that COX-1 may be a new site for molecular target therapy of RCC [16, 17]. However, further researches are needed to explore the exact molecular mechanism of COX-1 regarding the biology of renal cell carcinogenesis and progression.

COX-1, until lately, has been assumed to be of minor impact for carcinogenesis. However, reports on murine models of lung carcinogenesis [18] and colorectal carcinogenesis [19] as well as on some human tumor entities as ovarian cancer [20] cervical cancer [21], and breast cancer [22] suggest that COX-1 may be important for carcinogenesis. To the best of our knowledge, there was only one study concerning COX-1 immunohistochemical expression in human RCC [23].

Apart from being important for the regulation of apoptosis and immune surveillance, COX-1 has been recognized to be strongly involved into the regulation of angiogenesis [24]. Cell culture experiments using breast cancer [25], colon cancer [26], and ovarian cancer cell lines [20] indicate that one main mechanism is induction of proangiogenic growth factors of the vascular endothelial growth factor (VEGF) family [27]. There are several issues, which need to be further investigated, as whether cancer cells have receptors for VEGF and what the significance of

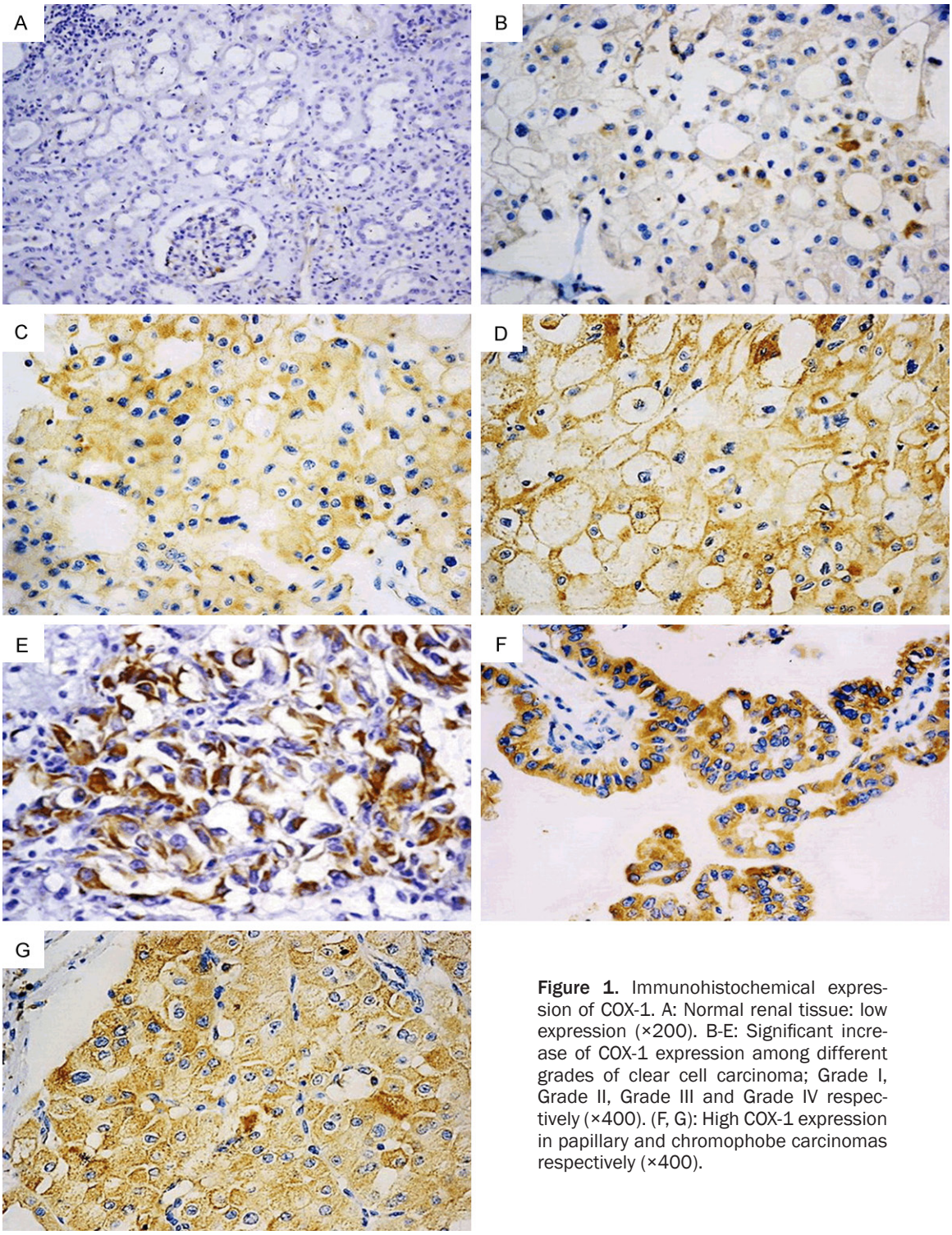

Figure 1. Immunohistochemical expression of COX-1. A: Normal renal tissue: low expression (×200). B-E: Significant increase of COX-1 expression among different grades of clear cell carcinoma; Grade I, Grade II, Grade III and Grade IV respectively (×400). (F, G): High COX-1 expression in papillary and chromophobe carcinomas respectively (×400).

this existence on the autocrine effect they might have. Despite evidence of vascular dilatation and increased permeability in RCC, the effects and regulation of VEGF are not clear in pathological angiogenesis [8].

To date, no immunohistochemical studies have been performed to assess the possible association between COX-1 and VEGF in RCC. This study is designed to evaluate the relationship between these two proteins in RCC. Also,

Table 2. Relationship between the VEGF expression score and different clinico-pathological parameters in RCC (n=64)

Clinicopathological parameters		VEGF expression score in tumour tissue		*P*-value	Sig
		Mean	± SD		
Tumor Type	Clear	2.51	1.14	0.057*	NS
	Chromophobe/papillary	1.95	85		
Tumor size	≤ 7 cm	1.87	89	0.001*	HS
	> 7 cm	3.08	95		
Tumor grade	I	1.89	1.17	0.034**	S[a]
	II	2.16	99		
	III	2.56	1.09		
	IV	3.29	95		
Tumor stage	I	1.83	95	0.006**	HS[b]
	II	2.80	92		
	III	3.06	93		
	IV	3.00	1.00		
Peri-renal fat invasion	Positive	3.07	96	0.003*	HS
	Negative	2.12	1.03		
Renal vein invasion	Positive	3.00	82	0.091*	NS
	Negative	2.26	1.09		
Renal sinus invasion	Positive	3.18	87	0.004*	HS
	Negative	2.17	1.05		

*Student t test n: number of cases; **ANOVA a 4 versus 1, 2 (S) b 1 versus 2, 3 (HS), 1 versus 4 (S); NS: non-significant S: significant HS: highly significant.

the relationship between their combined immunohistochemical expression and different clinicopathological prognostic parametres in RCC is investigated.

Material and methods

Tissue and patient data

This retrospective study was conducted on 64 cases of primary RCC that were retrieved from the archival files of the pathology labs of Ain Shams University Hospitals during the period from January 2011 until January 2013. The clinical data were obtained from the patients' medical records and included age, sex, tumor size, tumor grade and TNM stage. Hematoxylin and Eosin stained slides from all resected tumor specimens were reviewed by each author to re-evaluate and verify the histopathologic diagnosis (WHO, 2004) [4]. The study included 45 cases of clear type, 12 cases of papillary type and 7 cases of chromophope type.

Both tumor grading; using the established criteria of the Fuhrman nuclear grade [28], and TNM classification according to the Union for International Cancer Control [29] were revised. The availability of sufficient suitable material for the immunohistochemical studies was essential.

Ethics statement

The study was carried out with full local ethical approval from Research Ethical Committee at Faculty of Medicine, Ain Shams University.

Immunohistochemical staining

Four micrometer sections of formalin-fixed and paraffin embedded samples of 64 renal carcinoma cases were prepared. They included the tumor and the adjacent normal renal tissue. Immunohistochemical staining was performed using primary antibodies; COX-1 antibody (rabbit polyclonal antibody, Cat. #RB-10687-R7 (7.0 ml): Thermo Fisher Inc. Fremont, CA 94538, USA) (Ready-to-Use for Immunohistochemical Staining) and VEGF antibody (rabbit polyclonal antibody, Cat #RB-9031-R 7CA, USA dil (1:200): Thermo Fisher Inc. Fremont, CA 94538, USA) (Ready-to-Use for Immunohistochemical Staining). Avidin-Biotin immunoperoxidase complex technique was used according to Hsu et al. [30] by applying the super sensitive detection kit (Biogenex, CA, USA). The prepared tissue sections were fixed on poly-L-lysine coated slides overnight at 37°C. The paraffin embedded tissue sections were deparaffinized in xylene and rehydrated through absolute alcohol. Antigen retrieval in citrate buffer (pH9 Lab vision cat #AP9003) was used after the sections were treated in a microwave at 8° for 5-6 min, then at 3° for 10 min; the sections were then left to cool for 20 min. Peroxidase and protein block were done. Then, slides were incubated overnight with the primary antibodies at room tem-

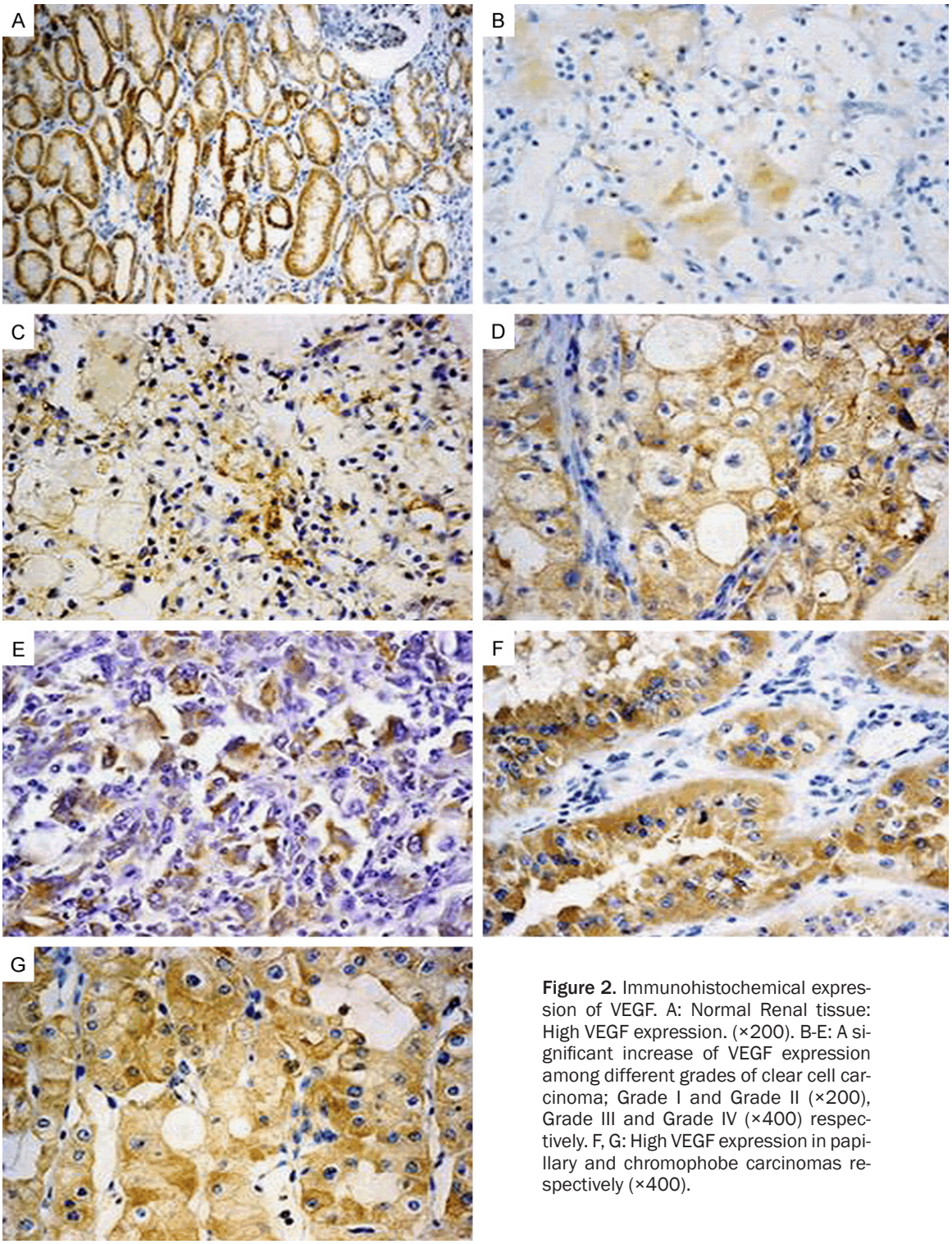

Figure 2. Immunohistochemical expression of VEGF. A: Normal Renal tissue: High VEGF expression. (×200). B-E: A significant increase of VEGF expression among different grades of clear cell carcinoma; Grade I and Grade II (×200), Grade III and Grade IV (×400) respectively. F, G: High VEGF expression in papillary and chromophobe carcinomas respectively (×400).

perature, followed by rinsing in PBS (pH 7.6). This was followed by the secondary biotin conjugated antibody for 1 hour and finally the peroxidase conjugated streptavidin for another hour. Diaminbenzidine tetrachloride (DAB) (freshly prepared) was added for 25 min, then counterstained in Harris Hematoxylin, followed by dehydration, clearing and mounting. Positive control for COX-1 antibody was esophageal tissue, while positive control for VEGF was angio-

Table 3. Correlation between COX-1 expression and individual and mean VEGF Score (n=64)

		cox-1 in tumor tissue					
		Low		High		*P*-value	Significance
		n	%	n	%		
		24	37.5%	40	62.5%		
VEGF expression score	1	18	75.0%	1	2.5%	0.001	HS*
	2	5	20.8%	10	25.0%		
	3	1	4.2%	18	45.0%		
	4	0	0.0%	11	27.5%		
		cox-1 in tumor tissue					
		Low		High		*P*-value	Significance
		Mean	± SD	Mean	± SD		
VEGF expression score		1.29	.55	2.98	.8.0	0.001	HS**

*Chi-square test; **Student t test; HS: highly significant; n: number of cases.

sarcoma. Negative controls were done by excluding the primary antibody and its replacement with a non-immune antibody.

Interpretation of immunohistochemical staining

Immunohistochemical analysis of COX-1 and VEGF was blindly performed by the two pa thologists (the authors) without any prior knowledge of the clinicopathological data.

COX-1 and VEGF staining was evaluated for positive cytoplasmic and or cytomembranous staining of each sample. COX-1 Intensity of staining was graded as: 0, no staining; 1, weakly stained; 2, moderately stained; 3, highly stained [31, 32]. Percentage of cells showing positivity was graded: 1, 0-5%; 2, 6-25%; 3, 26-50%; 4, 51-75% and 5, > 75% [33]. All of these tissue sections were given final scores based on the multiplications of intensity scores and percentage scores. The optimal cut-off value was calculated and final score of more than 5 was considered as high expression of COX-1 and < 5 as low expression [23].

VEGF was evaluated semi-quantitatively according to the percentage of positive cells in at least five areas at a magnification of 400×, and assigned to one of the four following categories: 1 < 25%; 2, 26-50%; 3, 51-75% and 4, 76-100% [34].

Statistical analysis

Continuous variables are expressed as mean and Standard Deviation. Categorical variables are expressed as frequencies and percents. Student t test was used to assess the statistical significance of the difference between two study group mean. ANOVA and post hoc test were used to assess the statistical significance of the difference between more than two study group mean. Chi square and Fisher's exact test were used to examine the relationship between Categorical variables. Logistic Regression Model was used to combine information of COX-1 and VEGF for prediction of stage III/IV tumour. A significance level of $P < 0.05$ was used in all tests. All statistical procedures were carried out using SPSS version 15 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Clinicopathological results

Our retrospective study included 45 cases of clear renal cell carcinomas (70.3%), twelve cases of papillary carcinomas (18.8%) and seven cases of chromophobe carcinomas (10.9%). Fifty patients were males (78.1%) and 14 were females (21.9%). The mean age was 56.63 ± 9.32 (16 cases (25%) with age ≤ 50 and 48 cases (75%) with age > 50). Thirty five cases (54.7%) were stage (I), 10 cases (15.6%) were stage (II), 16 cases (25%) were stage (III) and 3 cases (4.7%) were stage (IV). Data not tabulated.

Immunohistochemical results of COX-1

COX-1 immunoreactivity was almost exclusively cytoplasmic and/or cyto-membraneous in all the examined tissue specimens. It was observed in the tumor cells where high COX-1 expression was detected in 40 cases (62.5%) and low expression in 24 cases (37.5%). However, the adjacent non-neoplastic renal tubular epithelial cells showed negative expression in 45 cases (70.3%) and low expression in 19 cases (29.7%).

A significant association was found between COX-1 immunostaining and tumor grade (*P*=

Table 4. ROC curve for COX-1 in RCCs, VEGF score and both markers combined in prediction of tumor stage III/IV

Variable	AUC	Std. Error	95% Confidence Interval	*P*-value
COX-1	0.740	0.0726	0.616 to 0.842	0.003
VEGF	0.759	0.0709	0.636 to 0.857	0.001
Combined	0.775	0.0693	0.653 to 0.870	

0.028), as well as renal vein invasion (*P*=0.03). Moreover, a highly significant relationship was detected between COX-1 score and tumor size (*P*=0.001), peri-renal fat invasion (*P*=0.001), renal sinus invasion (*P*=0.005) and consequently tumor stage (*P*=0.001). On further comparison among several histopathologic tumor types of renal cell carcinoma; Chi-square test revealed a significant high COX-1 expression in 71.1% of clear type of renal cell carcinoma versus only 42.1% cases of other types of RCC (*P*=0.029).

No significant association was observed between COX-1 expression and patients' age and sex (*P*=0.074 and *P*=0.435 respectively). The relationship between COX-1 expression and different clinicopathological features is presented in **Table 1**.

Figure 1 shows representative areas for COX-1 expression in RCC tissue and adjacent normal renal tissue.

Immunohistochemical results of VEGF

Cytoplasmic and/or perimembranous VEGF expression was relatively strong in non-neoplastic renal epithelium.

It was also expressed in all neoplastic tissues, with a heterogeneous immunostaining pattern detected in 35 cases (54.7%) and a diffuse pattern was in 29 cases (45.9%). ANOVA test; revealed a significant correlation between VEGF score among high grade tumors (grade IV) versus VEGF score in low tumor grades (I and II) (*P*=0.034). Moreover, there was a highly significant correlation between VEGF score and tumor size (*P*=0.001), peri renal fat invasion (*P*=0.003) and renal sinus invasion (*P*=0.004). Consequently with further ANOVA test; a high VEGF expression was positively correlated with tumor stages (stage I versus II, III (highly significant), and stage I versus stage IV (significant) (*P*=0.006) (**Table 2**).

No significant relationship was detected between VEGF expression and patients' age and sex (*P*=0.694 and 0.379 respectively). Also, there was no significant difference between histopathologic types of RCC regarding VEGF score (*P*=0.057) (**Table 2**). **Figure 2** shows representative areas for VEGF expression in RCC and adjacent normal renal tissue.

Correlation and combined expression of COX-1 and VEGF

Chi-square test revealed a positive correlation between COX-1 and VEGF expression score and also Student t test revealed a positive correlation between COX-1 expression and the mean VEGF score (*P*=0.001 each) (**Table 3**). The ROC curve for COX-1 immunostaining in tumor tissue revealed "Area under Curve" (AUC) =0.740, while in VEGF expression AUC was =0.759. AUC for combined markers expression was =0.775. Combined expression of both markers predicts high stage tumors (stage III/IV) (**Table 4**; **Figure 3**).

Discussion

Renal cell carcinoma (RCC) remains the most frequently occurring cancer in the kidney. Epidemiologic studies suggest a continued rise in the incidence and mortality of renal cell carcinomas worldwide over the last 30 years [35, 36]. Moreover, about 30-50% of RCC patients with curative surgery may be expected to develop a recurrence with distant metastases. The prognosis of RCC patients with metastatic or recurrent diseases is poor, with a 5-year survival of less than 20% [37, 38].

RCC is comprised of several histological cell types; each type has differences in origin, genetics, morphology and behavior [39, 40].

A better understanding of tumor molecular pathways that lead to tumor appearance and growth may help in the development of new strategies for the early detection and treatment of RCC. In recent years, the development of novel agents targeting angiogenesis and signal transduction pathways has markedly improved patient outcomes [40]. However, few utility of these molecular markers for RCC exist till now, probably because of lack of knowledge at the molecular level regarding the biology of renal cell carcinogenesis and progression [41, 42].

The role of cyclooxygenase (COX) in cancer development has been the subject of close

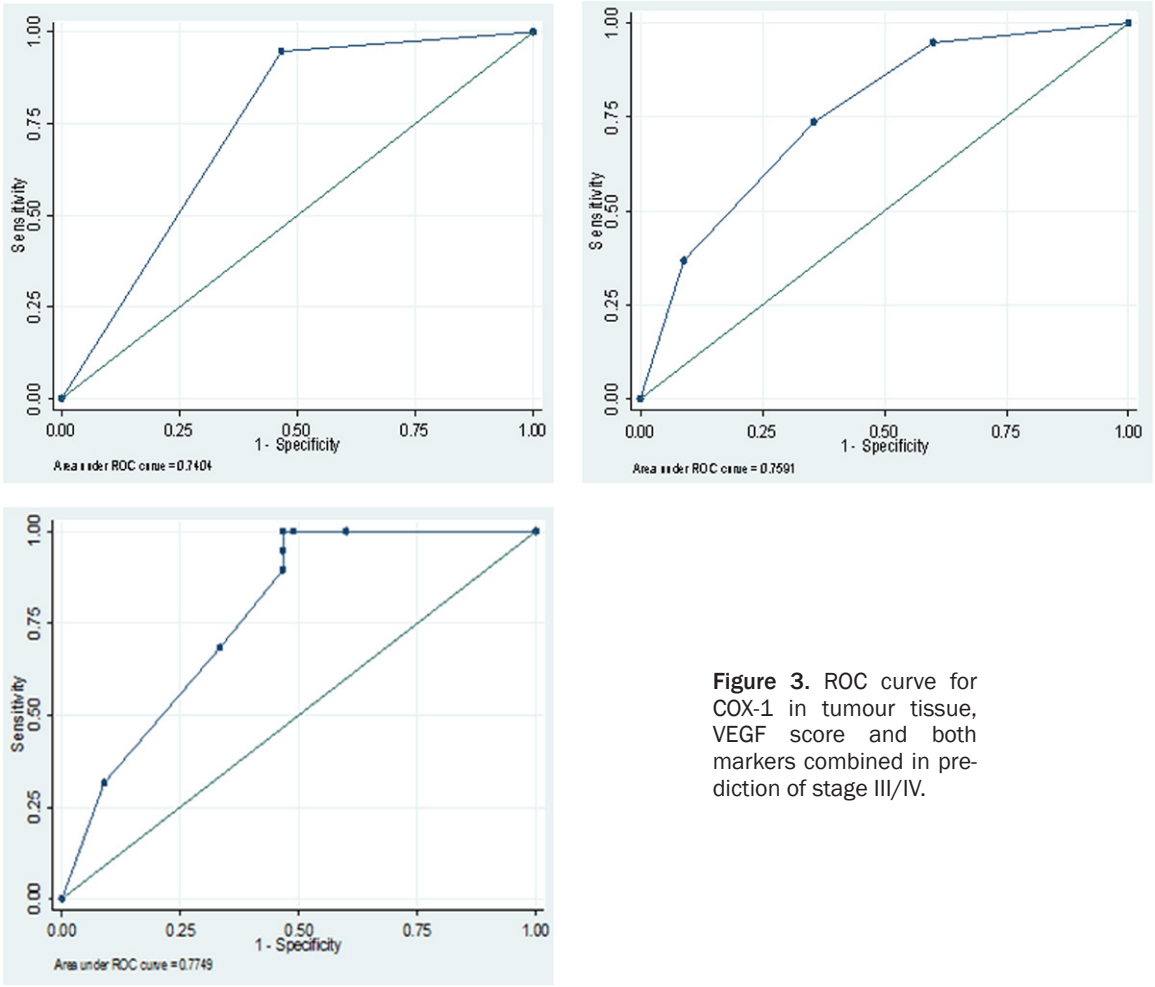

Figure 3. ROC curve for COX-1 in tumour tissue, VEGF score and both markers combined in prediction of stage III/IV.

scrutiny. COX-1 involvement in tumor growth has also been discussed in relation to several cancers [21, 43]. As for RCC, only one study has dealt with COX-1 immunohistochemical expression in human tissue [23]. Therefore, lack of data about COX-1 significance in the tumorigenesis of RCC has been the main impetus for the present study.

In this study, high COX-1 immunohistochemical expression was demonstrated in 62.5% of RCC tissues with low or negative immunostaining in non-neoplastic renal tubular epithelial cells. These figures were slightly lower than those of Yu et al [23], who detected COX-1 expression in about 69.3-75% of RCCs. Moreover, Okamoto, et al [44] reported up-regulation of COX-1 mRNA expression in 90% of experimental rat models of RCCs and cell lines.

Additionally, Mauro et al [10] revealed a progressive increase of COX-1 expression from normal oral mucosa towards hyperplasia, dysplasia and finally carcinoma. Similar observations were reported in many other solid tumors [20, 45]. Kino et al [45] reported significant increases of COX-1 by mRNA polymerase chain reaction in epithelial ovarian carcinoma (EOC) compared to normal ovarian tissues.

Sugimoto et al [46], Jang et al [47], Sales et al [48], demonstrated the autocrine-paracrine regulation of COX enzyme expression in epithelial and endothelial cells. Positive feedback circulation, constructed from prostaglandin (PG), c-AMP, inositol triphosphate (IP3), mitogen-associated protein kinase (MAPK), and phosphatidyl inositol 3 kinase-protein kinase B (PI3K/Akt), may promote COX-1 or COX-2 expression, and may thus result in tumor promotion.

RCC is a heterogeneous group of histological tumor subtypes of which clear cell carcinoma is the most frequent one making up more than 70% of all cases. However, papillary renal can-

cer makes up 10-15% and chromophobe carcinoma makes up to 5% [4-49].

In the current research, COX-1 over expression was observed in 71.1% of clear RCCs and in only 42.1% of other RCC subtypes. In contrast to Yu et al, [23], a statistical significant difference was detected in this study between RCC subtypes concerning COX-1 expression. This discrepancy may depend on difference in the number of the studied tumor samples. Also, it should be pointed out that this small number of cases analyzed may reduce the statistical power of the differences between the various tumor types.

Combined histological grade and clinical stage, which is considered to be the golden standard of prediction of patient's prognosis, cannot predict patient's prognosis accurately when used alone [50, 51]. By immunohistochemistry, we detected a progressive increase in COX-1 expression from normal renal tissue towards grade I-II, and grade III-IV carcinoma. Although Yu et al [23] didn't correlate COX-1 expression with tumor grade in their studied cases, yet our data are compatible with others who detected a correlation between COX-1 activation and tumor grades in their tumors [52].

For further assessment of the prognostic value of COX-1 in RCC, the relationship between COX-1 expression and other clinicopathological variables was tested in this study. In agreement with Yu et al [23], a significant association was found in the current work between COX-1 expression and tumor size as well as tumor stage. Moreover Yoshimoto et al [53] reported a statistical significant correlation between COX-1 activation and progressive stages of carcinogenesis in other solid tumors. They declared that the mean immunostaining scores for COX-1, COX-2, mPGES, and TXS were significantly higher for non-small cell lung cancer (NSCLC) cases with more metastatic organs. This is crucial for COX-1 for being a target in the tumor chemoprevention; a previous study by Tanji et al [54] on prostatic adenocarcinoma stated that for those tumors for which aspirin or NSAIDs are known to be beneficial in reducing the risk of cancer development. The differential expression of COX-1 at various stages of tumor development could have profound implications for selecting the proper type of COX inhibitor to test as a potential chemopreventive agent.

The growing awareness of the central role of angiogenesis in the progression of tumors can be used in the development of antiangiogenic therapy, which specifically targets at suppressing tumor growth and metastasis [55]. Angiogenesis is controlled by angiogenic factors that provide the regulation of extracellular matrix remodeling, endothelial cell proliferation, capillary differentiation, and anastomosis necessary to establish blood supply. Angiogenic stimuli are released by tumor cells, stromal cells, and inflammatory cells recruited to the tumor site [56]. Among several identified peptides with angiogenic properties, the vascular endothelial growth factor (VEGF) is thought to play a major role in tumor angiogenesis [57].

Several studies have assessed the expression of VEGF and its receptors in RCC tumor cells but as RCC does not respond to any current treatment, there is need for further identification of tumor characteristics, and tumor angiogenesis [58-62].

In some immunohistochemical analyses, VEGF expression was not observed in normal kidney [63] whereas others detected VEGF in the cytosol of normal renal tubular cells [64]. In our study, positive immunohistochemical (IHC) staining of VEGF was observed in both RCC and adjacent renal cortex. The later usually showed a strong VEGF expression in the cytoplasm of tubular cells. This finding is in concordance with that previously demonstrated by other studies [64, 65].

In the present work, a higher VEGF score was found in RCCs with increased tumor size, high tumor grade and advanced tumor stage. These findings are in agreement with Parodis et al [66]; who reported positive correlation between VEGF expression and tumor size in conventional RCCs. This result supported the hypothesis that VEGF is associated with tumor growth and progression. Furthermore, they showed that cytoplasmic VEGF expression was an independent prognostic factor, suggesting that although most conventional RCCs express VEGF, only a high level of expression has prognostic significance. These cases were of a significantly higher grade and stage, and a worse prognosis, than those with no cytoplasmic VEGF immunostaining. Moreover, Baldewijns et al [67] and Yildiz et al [68] found a significant correlation between tumor grade and VEGF expression. In contrast, Kawai et al [69] found no significant

correlation between VEGF expression and tumor grade in their study. They suggested that the lack of this relationship may be attributed to VEGF gene polymorphisms which could play a critical role in altering VEGF expression and influence the progression of RCC and patient survival.

Concomitant with Yildiz et al [68] and Djordjevic et al [55], no significant difference was found in the present work between different RCC types concerning cytoplasmic VEGF expression.

Several studies have assessed VEGF expression in RCC [55, 67-69] However, to the best of our knowledge, this is the first study to analyze the relationship between COX-1 and VEGF and investigate their combined expression in relation to clinicopathological parameters in RCC.

In this study, a positive correlation was detected between COX-1 and VEGF expressions. The association between both molecules is not surprising as the same relationship was detected in other tumors [20, 27, 70]. COX-1 is a causal agent; likely due to its influence on PGE2 concentration [19, 71, 72]. PGE2 exerts its effects by engaging members of the G-coupled superfamily of receptors [73]. Upon ligation of the cognate receptors, signal transduction cascades are activated to modulate intracellular levels of cAMP and Ca++ that impact on various aspects of cell biology such as proliferation, adhesion, invasion, motility, cell morphology and survival of both tumor cells and surrounding tumor-associated stromal cells [74].

Moreover, a study by Li et al [70] has shown that COX-1 selective inhibitors inhibited the growth of tumor cells by inhibiting COX-1 activity, thereby reducing prostaglandin I^2 (PGI2) and PGE2 levels, inhibiting the production of angiogenic factors and ultimately impeding tumor angiogenesis [16, 75, 76].

In the present research, a combined expression of both markers predicted high stage tumors (stage III/IV).

In conclusion, immunohistochemical expression of COX-1 and VEGF is associated with poor prognostic parameters in RCC. Their combined expression has a beneficial role in prediction of high stage tumors (stage III/IV) and potentially progressive RCCs. Clustering of tumors based on the expression of both markers in RCC might provide means for determining tumors that will respond to anti-angiogenic therapies. Prospective studies are needed to confirm the value of combined prognostic role of COX-1 and VEGF expression, as well as their predictive value in patients treated with VEGF pathway targeting agents, preferably using quantitative methods of protein measurement. Additionally, identifying COX-1 and VEGF expression in RCC is warranted because of the availability and the lower cost of COX-1 inhibitor agent than the chemotherapeutic agents.

Disclosure of conflict of interest

None.

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