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Original article

Significance of TDO2 And FOXP3 Expression in Renal Cell Carcinoma (An immune-histochemical study)

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Abstract

Background: Renal cell carcinoma (RCC) is the most aggressive form of genitourinary malignancy. Tryptophan 2, 3-dioxygenase (TDO2) is the primary enzyme that catalyzes tryptophan to kynurenine. TDO2 is overexpressed in a variety of malignancies. Forkhead box protein 3 (FOXP3) serves as a master regulator in maturation of regulatory T cells (Treg), but recently its expression found in cancer cells. **Aim:** To elucidate value of FOXP3 and TDO2 expression in RCC. **Materials and Methods:** In this retrospective study, 66 renal cell carcinomas and 10 cases of non-neoplastic renal tissue- were examined. TDO2 and FOXP3 immunohistochemical staining was conducted, evaluated for each case and correlated with clinic-pathological findings. **Results:** Significant statistical correlations were observed between TDO2 expression and grade, size, renal sinus invasion, nodal metastasis, and stage (P= 0.03, 0.0001, 0.005, 0.018 and 0.0001 respectively). Tumor FOXP3 expression and grade, renal sinus invasion, renal venous thrombosis, lymph node metastasis, and stage- were significantly associated (P= 0.0001, 0.0001, 0.003, 0.001, and 0.0001, respectively). The relationship between FOXP3+ Treg and grade, size, capsular invasion, renal sinus invasion, and stage- was significant (P= 0.0001, 0.034, 0.023, 0.033, and 0.002, respectively). The statistical correlations between the expression of TDO2 and tumor FOXP3 and FOXP3+Treg, as well as among the expression of tumor FOXP3 and FOXP3+Treg- were highly significant (P value < 0.01). **Conclusion:** TDO2 and FOXP3 may have role in tumorigenesis and progression of RCC and could be potential markers for targeted therapy.

Key words: Renal cell carcinoma, TDO2, FOXP3.

Abbreviations: Tryptophan 2, 3-dioxygenase (TDO2); Forkhead box protein 3 (FOXP3); Renal cell carcinoma (RCC).

Introduction

Renal cell carcinoma (RCC)- the most aggressive genitourinary malignancy- is the fourteenth most prevalent malignancy worldwide and accounts for 2.2% of all neoplasms. RCC accounts for ninety percent of all renal malignancies **(1).**

It is diagnosed in 434,419 patients annually, leading to 155,702 deaths representing 1.6% of all cancer-related mortality worldwide**.** In Egypt, RCC represents 1.5% of all cancers, ranking the fourteenth most common cancer, based on GLOBOCAN 2022 estimates **(1).**

Although, many treatment options for RCC are available, but the outcome of patients still unfavorable, so, more therapeutic targets and potential biomarkers needed to be studied **(2).**

A tetrameric 167-kDa heme-containing enzyme known as Tryptophan-2,3-dioxygenase (TDO2) catabolizes tryptophan (Trp) into Kynurenine (Kyn). The TDO2 gene on chromosome 4q32., encodes it **(3)**.

The modulation of the immunosuppressive microenvironment in human malignancies and the tumorigenesis of numerous cancers are facilitated by tryptophan (Trp) and its catabolites of the Kyn pathway **(4).** Numerous cancer cells exhibit an overexpression of TDO2. Therefore, TDO2 could be an interesting therapeutic target **(5).**

Tumor-promoting and tumor-antagonistic cells, are the two categories into which tumor-associated immune cells can be divided. The primary cells that promote tumor growth are regulatory T cells (Treg) **(6).** The FOXP3 gene, situated on chromosome Xp11.23, encodes a transcription factor that serves as the Treg development primary regulator **(7)**. FOXP3 expression is also being noticed in tumor cells, which is becoming more apparent **(8).**

The objective of this work is to assess the significance of FOXP3 and TDO2 expression in RCC.

Material and methods

Study Groups:

This is a retrospective study performed on selected formalin-fixed paraffin-embedded biopsies taken from 66 RCC cases, and classified as; thirty cases of clear cell renal cell carcinoma (ccRCC), twenty cases of papillary renal cell carcinoma (pRCC) and sixteen cases of chromophobe renal cell carcinoma (ChRCC). The control group consisted of ten renal tissue specimens that were not neoplastic. Pathology department and Early Cancer Detection Unit, Faculty of Medicine, Benha University, Egypt, provided these cases between 2011 and 2023. All specimens were obtained by radical nephrectomy. The clinico-pathological data of the cases were taken from the patient files.

The inclusion criteria: Patients whose clinicopathological data available regarding the age, sex, tumor size, grade, and lymph node status of the cases, capsular invasion, renal sinus invasion, renal venous thrombosis, and stage.

The exclusion Criteria: Patients with preoperative chemotherapy, partial nephrectomy specimens and cases with unavailable clinical data.

The Faculty of Medicine Ethical Committee at Benha University authorized the study code (MD 1-8-2022).

Histopathological studies:

Hematoxylin and eosin slides of cases were revised for diagnosis, histologic typing, and nuclear grading. The WHO/ISUP grading system was used to grade ccRCC and pRCC cases, but current WHO recommendation is to not grade ChRCC **(9).** The cases were staged according AJCC 8th edition 2018 **(10).**

Immunohistochemical method:

According to instructions of manufacture, Four-micron tissue sections were taken on positive-charged slides. Following xylene deparaffinization, the sections were rehydrated in descending grades of alcohol followed by distilled water. A 10 mmol/L citrate monohydrate buffer (pH 6.0) was employed to conduct the antigen retrieval procedure. The TDO2 antibody **(Rabbit polyclonal antibody, 100µl concentration, Chongqing Biospes company, Cat. ♯ YPA2534, conc. China)** and FOXP3 antibody **(100µl concentration, Chongqing Biospes company, Cat. ♯ YPA 2193, conc. China)-** were incubated with the slides at a dilution of 1:50. The standard labeled streptavidin-biotin system was employed to conduct immunodetection ***(Dako Cytomation, Denmark, A/S)*.** Fresh prepared chromogen diaminobenzene **(DAB, Envision TM Flex /HRP-Dako, REF K 8000)-** was used. "The counter satin was Mayer's hematoxylin." Sections of normal liver tissue and tonsil were taken as positive controls for the TDO2 and FOXP3 antibodies, respectively, **(11) (12).** The primary antibody omitted during the procedure for the negative control.

Interpretation of TDO2 expression:

The extent and intensity of cytoplasmic brown coloration of stained cells used to evaluate immunoreactivity. Positive cells percentage was calculated as: 0 for no positive cells, 1 for 1–25% positive cells, 2 for 26–50% positive cells, 3 for 51–75% positive cells, and 4 for 76–100% positive cells. The reaction's intensity was rated as follows; there is no color reaction when the value is 0, a faint reaction when the value is 1, a moderate reaction when the value is 2, and an intense reaction when the value is 3. Score index, obtained when the intensity of staining score (0-3) and the percentage of positive cells score (0-4) are multiplied, used to evaluate immunoreactivity: **Negative**; score 0-3; **Weak expression**; score 4-6; **Strong expression**; score 8-12 **(13).**

Interpretation of FOXP3 expression:

a) FOXP3 expression in tumor cells:

The extent and intensity of cytoplasmic brown coloration of stained cells are used to evaluate immunoreactivity. FOXP3 expression was determined to be positive in a minimum of 25% of the

tumor cells, and intensity of staining was categorized as either weak or strong **(14).**

b) FOXP3 expression in Treg cells:

Expression of FOXP3 in Treg detected as nuclear brown color. FOXP3+Treg cells counted in 10 high-power fields (x400) manually. The extent of FOXP3+ Treg cells was evaluated using the following scores: 0 indicates no positive cells, while 1+ indicates 1–25% positive cells, 2+ indicates 26–50% positive cells, and 3+ indicates 51–100% positive cells. Scores of 2+ and 3+ considered positive and indicative of high infiltration, while scores of 0 and 1+ were negative and indicative of low or absent infiltration **(15).**

Statistical analysis:

SPSS version 22 was employed to conduct the statistical analysis **(SPSS Inc, Chicago, IL, USA).** Descriptive statistics such as mean, standard deviation (± SD), median, standard error (± SE), and range were used to characterize numerical data. Frequency and percentage were employed to analyze non-numerical data. The test of Chi-Square was employed to examine the correlation among two qualitative variables. In situations where the anticipated count is less than 5 in more than 20% of the cells, Fisher-Exact/Monte-Carlo test implemented to examine the correlation between two qualitative variables. Significant P values defined as those that are less than 0.05, while highly significant P values are ones that are ≤ 0.01.

Results

Clinico-pathological results: RCC cases had mean age of 61.38 years, and standard deviation of 8.063. The ages of patients ranged from 46 to 80 years. The ratio of males to females was 2.47:1. Complete clinico-pathological data illustrated in **Table (1).**

Immunohistochemical results:

TDO2 expression:

In the studied cases, TDO2 expression exhibited a statistically significant difference (P value < 0.01). Specifically, 83.3% of RCC cases were positive and 16.7% were negative, while all non-neoplastic cases (control group) were negative. In RCC cases, statistically significant correlation was observed between expression of TDO2 and grade (P value= 0.03), tumor size (P= 0.0001), nodal metastasis (P= 0.018), renal sinus invasion (P= 0.005), and stage group (P= 0.0001). Although TDO2 expression and RCC subtypes (P=0.940), renal vein thrombosis (P=0.129), and capsular invasion (P=0.160) exhibited an insignificant correlation, **(Table 2), (Figure 1).**

FOXP3 expression in tumor cells:

The cases studied exhibited a statistically significant difference in FOXP3 expression (P value < 0.01). In RCC cases, 74.3% exhibited positive cytoplasmic FOXP3 expression, while 25.7% were negative, but all non-neoplastic cases (control group) were negative. FOXP3 expression in tumor cells was statistically significantly influenced

by the grade of the tumor (P=0.0001), renal sinus invasion (P=0.0001), the metastasis of lymph node (P=0.001), renal vein thrombosis (P=0.003), and stage group (P=0.0001) in RCC cases. Furthermore, no significant correlation was observed between the expression of FOXP3 and RCC subtypes (P=0.102), tumor size (P=0.066), and capsular invasion (P=0.076), **(Table 3), (Figure 1).**

FOXP3 expression in Treg cells:

In RCC cases, 18 cases (27.3%) showed nuclear expression of FOXP3+Treg (high infiltration), while 48 cases (72.7%) were negative (absent or low infiltration). The presence of FOXP3+Treg was significantly associated with tumor grade (P=0.0001), renal sinus invasion (P=0.033), tumor size (P=0.034), capsular invasion (P=0.023), and stage group (P=0.002). The correlation with RCC subtypes (P = 0.568), renal vein thrombus (P=0.140), and metastasis of lymph node (P=0.06)- was insignificant, **(Table 4), (Figure 1).**

The statistical correlation among expression of FOXP3 in tumor cells and FOXP3+Treg, as well as TDO2 and expression of FOXP3 in tumor cells and FOXP3+Treg- was highly significant (P value < 0.01), **(Table 5).**

Table (1) Clinico-pathological data of studied 66 RCC cases

Clinicopathological data of studied 66 RCC cases	
Gender	
Male	47 (71.2%)
Female	19 (28.8%)
Age	
Mean	61.38, SD ± 8.063
Histologic Subtype	
ccRCC	30 (45.5%)
pRCC	20 (30.3%)
ChRCC	16 ((24.2%)
Tumor Grade	
Grade I	11 (22 %)
Grade II	24 (48 %)
Grade III	9 (18 %)
Grade IV	6 (12%)
Tumor Size	
≤ 4cm	14 (21.2%)
> 4 to ≤ 7cm	20 (30.3%)
> 7 to ≤ 10cm	22 (33.3%)
>10cm	10 (15.2%)
Capsular invasion	
Absent	60 (90.9%)
Present	6 (9.1%)
Renal sinus invasion	
Absent	46 (69.7%)
Present	20 (30.3%)
Renal vein thrombosis	
Absent	52 (78.8%)
Present	14 (21.2%)
Lymph node status	
N0	48 (72.7%)
N1	18 (27.3%)
Stage group	
Stage I	24 (36.4%)
Stage II	19 (28.8%)
Stage III	18 (27.3%)
Stage IV	5 (7.6%)

*****ccRCC: clear cell renal cell carcinoma, pRCC: papillary renal cell carcinoma, ChRCC: chromophobe renal cell carcinoma.

Table (2). Relation of TDO2 IHC with histo-pathological features of studied RCC cases:

		N	TDO2 Expression			P-value
			Negative	**Weak**	**Strong**	
RCC subtype	**ccRCC (%)**	**30**	**4 (13.3%)**	**12 (40%)**	**14 (46.7%)**	**0.940**
	pRCC (%)	**20**	**4 (20%)**	**6 (30%)**	**10 (50%)**	
	ChRCC (%)	**16**	**3 (18.8%)**	**6 (37.5%)**	**7 (43.7%)**	
Histologic grade	**Grade I (%)**	**11**	**5 (45.5%)**	**5 (45.5%)**	**1 (9%)**	**0.03***
	Grade II (%)	**24**	**3 (12.5%)**	**11 (45.8%)**	**10 (41.7%)**	
	Grade III (%)	**9**	**0 (0%)**	**1 (11.1%)**	**8 (88.9%)**	
	Grade IV (%)	**6**	**0 (0%)**	**1 (16.7%)**	**5 (83.3%)**	
Tumor Size	**≤ 4cm (%)**	**14**	**2 (14.3%)**	**10 (71.4%)**	**2 (14.3%)**	**0.0001****
	> 4 to ≤ 7cm (%)	**20**	**6 (30%)**	**9 (45%)**	**5 (25%)**	
	> 7 to ≤ 10cm (%)	**22**	**3 (13.6%)**	**2 (9.1%)**	**17 (77.3%)**	
	>10cm (%)	**10**	**0 (0%)**	**3 (30%)**	**7 (70%)**	
Capsular invasion	**Absent (%)**	**60**	**11 (18.3%)**	**23 (38.3%)**	**26 (43.3%)**	**0.160**
	Present (%)	**6**	**0 (0%)**	**1 (16.7%)**	**5 (83.3%)**	
Renal sinus invasion	**Absent (%)**	**46**	**11 (23.9%)**	**19 (41.3%)**	**16 (34.8%)**	**0.005****
	Present (%)	**20**	**0 (0%)**	**5 (25%)**	**15 (75%)**	
Renal vein thrombosis	**Absent (%)**	**52**	**11 (21.2%)**	**19 (36.5%)**	**22 (42.3%)**	**0.129**
	Present (%)	**14**	**0 (0%)**	**5 (35.7%)**	**9 (64.3%)**	
Lymph node status	**N0 (%)**	**48**	**11 (22.9 %)**	**19 (39.6%)**	**18 (37.5%)**	**0.018***
	N1 (%)	**18**	**0 (0%)**	**5 (27.8%)**	**13 (72.2%)**	
Stage group	**Stage I (%)**	**24**	**8 (33.3%)**	**15 (62.5%)**	**1 (4.2%)**	**0.0001****
	Stage II (%)	**19**	**3 (15.8%)**	**3 (15.8%)**	**13 (68.4%)**	
	Stage III (%)	**18**	**0 (0%)**	**6 (33.3%)**	**12 (66.7%)**	
	Stage IV (%)	**5**	**0 (0%)**	**0 (0%)**	**5 (100%)**	

N: Number, *: Significant, **: Highly significant, ccRCC: clear cell renal cell carcinoma, pRCC: papillary renal cell carcinoma, ChRCC: chromophobe renal cell carcinoma, TDO2: Tryptophan 2, 3-dioxygenase**.**

Table (3) Relation between FOXP3 IHC expression in tumor cells and histo-pathological features of studied RCC cases:

		N	FOXP3 Expression In Tumor Cells			P-value
			Negative	Weak	Strong	
RCC subtype	ccRCC (%)	30	4 (13.3%)	14 (46.7%)	12 (40%)	0.102
	pRCC (%)	20	6 (30%)	6 (30%)	8 (40%)	
	ChRCC (%)	16	7 (43.75%)	2 (12.5%)	7 (43.75%)	
Histologic grade	Grade I (%)	11	2 (18.2%)	9 (81.8%)	0 (0%)	0.0001**
	Grade II (%)	24	8 (33.3%)	10 (41.7%)	6 (25%)	
	Grade III (%)	9	0 (0%)	1 (11.1%)	8 (88.9%)	
	Grade IV (%)	6	0 (0%)	0 (0%)	6 (100%)	
Tumor Size	≤ 4cm (%)	14	6 (42.9%)	5 (35.7%)	3 (21.4%)	0.066
	> 4 to ≤ 7cm (%)	20	4 (20%)	10 (50%)	6 (30%)	
	> 7 to ≤ 10cm (%)	22	4 (18.2%)	7 (31.8%)	11 (50%)	
	>10cm (%)	10	3 (30%)	0 (0%)	7 (70%)	
Capsular invasion	Absent (%)	60	17 (28.3%)	21 (35%)	22 (36.7%)	0.076
	Present (%)	6	0 (0%)	1 (16.7%)	5 (83.3%)	
Renal sinus invasion	Absent (%)	46	16 (34.8%)	19 (41.3%)	11 (23.9%)	0.0001**
	Present (%)	20	1 (5%)	3 (15%)	16 (80%)	
Renal vein thrombosis	Absent (%)	52	17 (32.7%)	19 (36.5%)	16 (30.8%)	0.003**
	Present (%)	14	0 (0%)	3 (21.4%)	11 (78.6%)	
Lymph node status	N0 (%)	48	16 (33.3 %)	19 (39.6%)	13 (27.1%)	0.001**
	N1 (%)	18	1 (5.5%)	3 (16.7%)	14 (77.8%)	
Stage group	Stage I (%)	24	10 (41.6%)	13 (54.2%)	1 (4.2%)	0.0001**
	Stage II (%)	19	6 (31.6%)	6 (31.6%)	7 (36.8%)	
	Stage III (%)	18	1 (5.6%)	2 (11.1%)	15 (83.3%)	
	Stage IV (%)	5	0 (0%)	1 (20%)	4 (80%)	

N: Number, *: Significant, **: Highly significant, ccRCC: clear cell renal cell carcinoma, pRCC: papillary renal cell carcinoma, ChRCC: chromophobe renal cell carcinoma, FOXP3: Forkhead box protein 3.

Table (4) Relation between FOXP3 IHC expression in Treg lymphocytes and histo-pathological features of studied RCC cases:

		N	FOXP3+Treg		P-value
			Negative	Positive	
RCC subtype	ccRCC (%)	30	23 (76.7%)	7 (23.3%)	0.568
	pRCC (%)	20	15 (75%)	5 (25%)	
	ChRCC (%)	16	10 (62.5%)	6 (37.5%)	
Histologic grade	Grade I (%)	11	11 (100%)	0 (0%)	0.0001**
	Grade II (%)	24	23 (95.8%)	1 (4.2%)	
	Grade III (%)	9	4 (44.4%)	5 (55.6%)	
	Grade IV (%)	6	0 (0%)	6 (100%)	
Tumor Size	≤ 4cm (%)	14	12 (85.7%)	2 (14.3%)	0.034*
	> 4 to ≤ 7cm (%)	20	18 (90%)	2 (10%)	
	> 7 to ≤ 10cm (%)	22	12 (54.5%)	10 (45.5%)	
	>10cm (%)	10	6 (60%)	4 (40%)	
Capsular invasion	Absent (%)	60	46 (76.7%)	14 (23.3%)	0.023*
	Present (%)	6	2 (33.3%)	4 (66.7%)	
Renal sinus invasion	Absent (%)	46	37 (80.4%)	9 (19.6%)	0.033*
	Present (%)	20	11 (55%)	9 (45%)	
Renal vein thrombosis	Absent (%)	52	40 (76.9%)	12 (23.1%)	0.140
	Present (%)	14	8 (57.1%)	6 (42.9%)	
Lymph node status	N0 (%)	48	38 (79.2 %)	10 (20.8%)	0.06
	N1 (%)	18	10 (55.6%)	8 (44.4%)	
Stage group	Stage I (%)	24	23 (95.8%)	1 (4.2%)	0.002**
	Stage II (%)	19	13 (68.4%)	6 (31.6%)	
	Stage III (%)	18	11 (61.1%)	7 (38.9%)	
	Stage IV (%)	5	1 (20%)	4 (80%)	

N: Number, *: Significant, **: Highly significant, ccRCC: clear cell renal cell carcinoma, pRCC: papillary renal cell carcinoma, ChRCC: chromophobe renal cell carcinoma, FOXP3: Forkhead box protein 3, Treg: Regulatory T cells

Table (5): Correlation of TDO2 expression with FOXP3 expression in tumor cells and FOXP3+ Treg in studied RCC cases.

TDO2 expression	N	FOXP3 expression in tumor cells			P value	FOXP3+Treg		P value
		Negative	**Weak positive**	**Strong positive**		**Negative**	**Positive**	
Negative	**11 (100%)**	**4 (36.4%)**	**7 (63.6%)**	**0 (0%)**		**11 (100%)**	**0 (0%)**	
Weak positive	**24 (100%)**	**8 (33.3%)**	**7 (29.2%)**	**9 (37.5%)**	**<0.01****	**20 (83.3%)**	**4 (16.7%)**	**<0.01****
Strong positive	**31 (100%)**	**5 (16.1%)**	**8 (25.8%)**	**18 (58.1%)**		**17 (54.8%)**	**14 (45.2%)**	

N: Number, **: Highly significant, TDO2: Tryptophan 2, 3-dioxygenase, FOXP3: Forkhead box protein 3, Treg: Regulatory T cells.

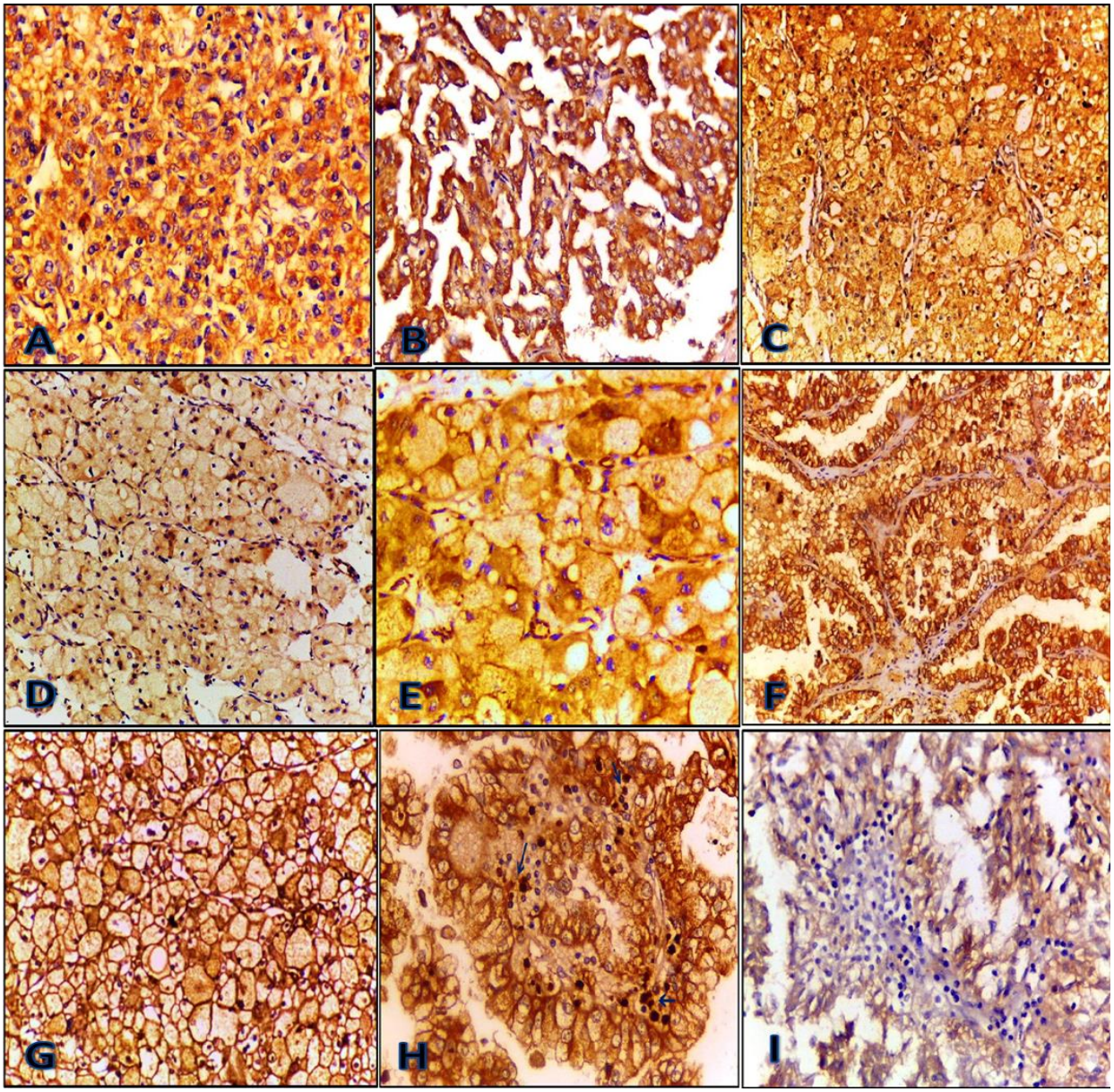

Figure 1:

A. RCC, Clear cell subtype, Grade 3 with strong cytoplasmic TDO2 expression (ABC, x400)

B. RCC, Papillary subtype, Grade 3, with strong cytoplasmic TDO2 expression (ABC, x400)

C. RCC, Chromophobe subtype, with strong cytoplasmic TDO2 expression (ABC, x200)

D. RCC, Clear cell subtype, Grade 1, with weak cytoplasmic TDO2 expression (ABC, x200)

E. RCC, Clear cell subtype, Grade 3, with strong cytoplasmic FOXP3 expression in tumor cells (ABC, x400)

F. RCC, Papillary subtype, Grade 3, with strong cytoplasmic FOXP3 expression in tumor cells (ABC, x200)

G. RCC, Chromophobe subtype, with strong cytoplasmic FOXP3 expression in tumor cells (ABC, x200)

H. RCC, Papillary subtype, Grade 3, with positive nuclear FOXP3 expression in Treg cells (arrows) and strong cytoplasmic FOXP3 expression in tumor cells (ABC, x400)

I. RCC, Papillary subtype, Grade 2, with weak cytoplasmic FOXP3 expression in tumor cells and negative nuclear FOXP3 expression in Treg cells (ABC, x400)

Discussion

Renal cell carcinoma (RCC) accounts 2.2% of all neoplasms and 1.6% of all cancer-related mortality worldwide (**1).** Renal cell carcinoma characterized by reprogramming of metabolic pathways, like tryptophan metabolism **(16).** Tyrptophan 2,3-dioxygenase (TDO2) catabolizes tryptophan (Trp) into kynurenine (Kyn) **(3)**.

There was statistically significant difference in TDO2 expression among our studied cases (P value < 0.01), with 83.3 % of RCC cases being positive, out of which 47% showed strong cytoplasmic expression and 36.3% showed weak expression, and 16.7% were negative, while all non-neoplastic cases (control group) (100%) were negative**.** This result was close to other studies that found significant higher expression of TDO2 in RCC tissue than in normal kidney tissue (**2 &17).** This could indicates that TDO2 has role in tumorigenesis. While, other study reported that TDO2 mRNA expression was not different between normal kidney and RCC tissues **(18).**

Our result reported insignificant relation of TDO2 expression with RCC subtypes (P=0.940), that was in line with other study **(2)**.While, other authors **(17)** reported that TDO2 was strongly expressed in papillary RCC and clear RCC more than other types, which may be caused by variation in immunostaining protocols and sample sizes of RCC subtypes.

The findings of our work indicated a statistically significant association among tumor grade and the expression of TDO2 (P value= 0.03), which was consistent with prior researches **(2, 19, 20).** This may be attributed to the fact that an increase in TDO2 expression results in an increase in kynurenine synthesis (Kyn), which in turn enhances cancer cells malignant properties and aggressiveness of tumors **(21).**

In our study, TDO2 expression in RCC cases was statistically significantly correlated with the stage group (P= 0.0001), tumor size (P= 0.0001), and renal sinus invasion (P= 0.005) and nodal metastasis (P= 0.018). Renal vein thrombosis (P=0.129) and capsular invasion (P=0.160) did not exhibit a statistically significant correlation. Our study results were parallel to other research **(2).** These results suggest that TDO2 is important in RCC progression.

This could be supported by other studies for the role of TDO2 in other tumors as hepatocellular carcinoma **(13),** breast cancer (**3),** esophageal squamous cell carcinoma **(22),** glioma **(23),** ovarian cancer **(24)**, gastric cancer **(25)**, colon cancer **(26),** and lung adenocarcinoma **(27).**

The function of TDO2 in tumors development and progression, clarified by the following: TDO2 activates STAT3/NF-kB, Akt, and mTOR signaling to promote tumor proliferation and growth **(28)**, also, it promotes cancer cells survival by increasing expression of antiapoptotic proteins **(29),** and has a role in tumor neovascularization by increasing vascular endothelial growth factor

expression **(30).** In addition, TDO2 induces metastasis and invasiveness, via upregulation of matrix metalloprotease 7 expression and promotes the epithelial mesenchymal transition **(31).**

The biological characteristics of renal cell carcinoma are significantly influenced by the immune microenvironment components, and characterized by high immune infiltration **(32)**. FOXP3 expression is essential for the tumor promoting regulatory T cells function and required for regulation of suppressive signals **(6).**

Expression of FOXP3 found in tumor cells as well as Treg cells in RCC cases. For tumoral FOXP3 expression, highly significant statistical difference of FOXP3 expression in tumor cells in studied cases (P value < 0.01), in RCC, 74.3% exhibited positive tumoral FOXP3 expression, out of which 41% exhibited strong cytoplasmic expression and 33.3% exhibited weak expression, and 25.7 % were negative, while all non-neoplastic cases (control group) were negative**.** This result was consistent with another study that demonstrated that the expression of FOXP3 was higher in RCC tissue than benign tissues **(33)**. This may be attributed to the oncogenic function of FOXP3 in development of tumors.

Significantly high correlation had been observed between the tumor's grade and expression of FOXP3 in tumor cells (P=0.0001), which was in contrast to other study (**33)**. This discrepancy may be attributable to different number of cases and scoring criteria used to evaluate FOXP3 expression. The current work found a statistically significant correlation between the expression of tumoral FOXP3 and invasion of the renal sinus (P=0.0001), lymph node metastasis (P=0.001), renal vein thrombus (P=0.003), and stage group (P=0.0001) of the RCC cases. That was close to study reported significant relation between high stage and tumoral FOXP3 expression **(33)**. This could imply that FOXP3 involved in progression of tumors. Insignificant correlation was observed between FOXP3 expression and capsular invasion (P=0.076), tumor size (P=0.066), and RCC subtypes (P=0.102).

According to the nuclear expression of FOXP3 in Treg cells in our RCC cases; (27.3%) cases exhibited FOXP3+Treg (high infiltration), while (72.7%) cases were negative (absent or low infiltration). This was close to other study in which 25.3% of studied RCC cases have $FOXP3^{+}$ Treg cells **(34)**. The statistical relationship between FOXP3 expression in Treg lymphocytes and the histological type of RCC cases was insignificant (P = 0.568), which was consistent with the findings of other authors **(35)**.

In our result, tumor grade and FOXP3+ Treg exhibited a statistically significant relation (P=0.0001). Additionally, the correlation between FOXP3+Treg and tumor size (P=0.034), capsular invasion (P=0.023), renal sinus invasion (P=0.033), and stage group (P=0.002)- was statistically significant. While, an insignificant correlation observed with

renal vein thrombus (P=0.140) and lymph node metastasis (P=0.06). These findings were in line with other prior researches (**36, 37, 38).**

The oncogenic function of tumoral FOXP3 and FOXP3+Treg in other malignancies has been clarified by a variety of studies, such as gastric cancer **(39, 40)**, breast cancer **(41**), pancreatic adenocarcinoma **(42, 43)**, colorectal cancer **(44, 45).**

We found that the statistical correlation between FOXP3 expression in tumor cells and FOXP3+Treg- was highly significant (P value < 0.01), which was consistent with the findings of other study **(33)**. This is elucidated by the fact that renal cancer cells express FOXP3, which helps them to evade the immune system by attracting more Treg cells into the tumor microenvironment. Conversely, other authors demonstrated that expression of FOXP3 in colon cancer cells was inversely correlated with the expression of FOXP3+ Treg cells (**46).**

The function of FOXP3 in the development and progression of malignancies clarified by the following mechanisms: The immune evasion of tumors is facilitated by the FOXP3+Treg, which suppress the activities of T effector cells (Teffs). The utilization of IL-2 by FOXP3+Tregs benefits their own survival and deprives Teffs of IL-2, thereby suppressing Teffs' function **(47).** Treg causes cytolysis of Teffs by releasing granzyme B and perforin **(48).** Furthermore, by increasing matrix metalloprotease expression, activating the TGF-B, Wnt/β-catenin, and epithelial mesenchymal pathways-FOXP3 has the capacity to facilitate cancer migration, invasion, and metastasis **(44)**.

The biofunction of FOXP3 in various malignancies remains a topic of controversy, as indicated by published reports **(33).** In tumorigenesis, the functions of FOXP3 are diverse and contingent upon the type of tumor **(49)**. Contrast studies about various cancers (**50, 51, 52, 53)** reported that favorable prognosis is associated with FOXP3 expression, and it may serve as a tumor suppressor gene.

In our study, the statistical correlation of TDO2 with tumoral FOXP3 and FOXP3+Treg expression- was highly significant (P value < 0.01), which was consistent with a previous study (**17)**. The significant correlation can be attributed to TDO2's role in conversion of tryptophan to kynurenine, which promotes FOXP3+ regulatory T cells differentiation and inhibits cytotoxic T-cells (Tc) and T helper cells (Th) **(54, 4).** In naive T cells, kynurenine activates the aryl hydrocarbon receptor (AHR), which subsequently activates FOXP3. This process results in the selective proliferation of Tregs **(4).** In addition, the activation of cytochrome c and caspase-8 by 3-Hydroxyanthranilic acid, which is a product of kynurenine, results in the apoptosis of Th1 and Tc cells **(55).**

Conclusion:

Our results demonstrated that TDO2, tumoral FOXP3, and FOXP3+ Treg-could be important markers for development and progression in RCC. The significant correlation between

these markers illustrated their role in promoting immunosuppressive micro-environment in RCC. In RCC, TDO2 and FOXP3- may serve as potential markers for targeted therapy and to enhance the immune therapy's response.

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