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# Prognostic Roles of Combined Twist-1 & E-Cadherin Tissue Protein Expression in Clear Cell Renal Cell Carcinoma (cc-RCC); An Immunohistochemical Study

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**Abstract:** ***Background; clear cell renal cell carcinoma (cc-RCC), which is the commonest type of renal cell carcinoma (RCC), is spreading mainly through the blood that leads to its poorer prognosis than other histopathological RCC subtypes. It is essential to identify the molecular pathogenesis of cc-RCC initiation, progression invasion and spread aiming at detection of novel targeted therapies for improving patients' outcome. Twist-1 that is considered a basic helix-loop-helix (bHLH) highly conserved transcription factor that is characterized by the presence of a basic domain which binds to DNA and such domain targets E-box sequence 59-CANNTG-39 and another helix-loop-helix domain. E-cadherin (E-cad) that is a cell adhesion factor which is normally found in the epithelial cell membrane. Aim of that work was to assess tissue protein markers Twist 1& E-cadherin expressions in both non-neoplastic and malignant tissue in cc-RCC patients, to evaluate relations between both markers in initiation of EMT in cc-RCC, and to clarify significance of their combined tissue protein expression, clinicopathological parameters of the tumor, cc-RCC progression, recurrence and patient survival. Methods; tissue protein markers Twist 1& E-cadherin combined expression using immunohistochemistry method was assessed samples from 40 patients with cc-RCC and 10 samples of adjacent non-neoplastic kidney tissue. We followed cc-RCC patients for 5 years, detected associations between the combined tissue protein markers expressions, clinicopathological parameters of the tumor, cc-RCC progression, recurrence and patient survival. Results; combination of up regulation of Twist 1anddown regulation of E- cadherin was noticed in cc-RCC tissues more than adjacent non-neoplastic kidney tissue (p=0.004 and p<0.001 respectively).up regulation of Twist 1 in cc-RCC tissues in addition to down regulation of E- cadherin was correlated with high grade (p=0.007&<0.001 respectively), advanced TNM stage (p=0.023&<0.001 respectively), presence of L.N spread (p=0.010 and <0.001 respectively), haematogenous spread (p=0.003 &0.035 respectively), shorter patients survival rates (p<0.001 and p=0.013 respectively). Conclusion; up regulation of Twist 1 and down regulation of E- cadherin are related to poor prognosis in cc-RCC patients.***

**Keywords:** cc-RCC, **Twist 1**, E- cadherin, immunohistochemistry, prognosis

## 1. Introduction

Renal cell carcinoma (RCC), is considered the most common cancer of the urinary tract, it forms 3.79% of all malignancies in adults and also considered the most fatal among all urinary tract tumors [**1**]. The commonest subtype of RCC is clear cell renal cell carcinoma (cc-RCC), which forms about 70-80% of all RCC cases and it is considered a very dangerous malignancy as regard high fatality, invasion, lymph node and blood spread and resistance to the currently used chemotherapeutic agents [**2**]. Cc-RCC is spreading mainly through the blood that leads to its poorer prognosis than other histopathological RCC subtypes [**3**].It is essential to identify the molecular pathogenesis of cc-RCC initiation, progression invasion and spread aiming at detection of novel targeted therapies for improving patients' outcome. To our knowledge no recent prognostic and therapeutic biomarker proved its accuracy clinically and could be used in the routine practice up till now [**4**].

The process and the genetic program that is responsible for cell movement, embryonic development and maintaining tissue homeostasis in adult tissue is called epithelial-mesenchymal transition (EMT) [**5**]. EMT have been much incriminated in malignant cells mobility, invasion, occurrence of lymphatic and distant metastases, additionally EMT activation in carcinoma cells leads to cancer stem cells activation which subsequently leads to malignant invasion and metastasis [**6**]. EMT and factors controlling it have been extensively discussed in previous studies the most commonly studied factor is Twist-1 that is considered a basic helix-loop-helix (bHLH) highly conserved transcription factor. Twist-1 is characterized by the presence of a basic domain which binds to DNA and such domain targets E-box sequence 59-CANNTG-39 and another helix-loop-helix domain [**7].**

Another EMT related factor is E-cadherin (E-cad) that isa cell adhesion factor which is normally found in the epithelial cell membrane. E-cad binds to β-catenin and it forms a protein complex that is attached to the actin cytoskeleton. It was found that any disturbances in components of the adherens junction molecules lead to EMT process initiation which will subsequently lead to malignant invasion and metastases **[8].**

There are several researchers have tried to clarify relations between Twist-1 and E-cad in malignant tissue **[9],** but the studies that focused on the association between both biomarkers, clinical and prognostic parameters and tissue proteins expression in non- neoplastic kidney tissues and in cc-RCC are limited.

**Aim of that work** was to assess tissue protein markers **Twist 1**& E-cadherin expressions in both non-neoplastic

and malignant tissue in cc-RCC patients, to evaluate relations between both markers in initiation of EMT in cc-RCC, and to clarify significance of their combined tissue protein expression, clinicopathological parameters of the tumor, cc-RCC progression, recurrence and patient survival.

**Patients, tissue samples and methods**

This is a retrospective cohort study, in which we included samples from 40 patients with cc-RCC and 10 samples of adjacent non-neoplastic kidney tissue. The treatment of patients with cc-RCC was, according to the stage, by total nephrectomy or radical-nephrectomy with pelvic-lymphadenectomy, in General surgery department, faculty of medicine, Zagazig University. Samples from the included 40 operated patients have been processed, diagnosed, graded using WHO grading system of urinary tumors [**10]** and staged using TNM staging system of RCC in Pathology Department, Faculty-of Medicine, Zagazig University **[11].** We have followed our 40 patients for five years, in the period from May 2013 and May 2018inOncology Departments.

**Immunohistochemical staining:**

Sections from fifty paraffin blocks of formalin-fixed samples that were retrieved from forty cc-RCC patients and ten adjacent non-neoplastic kidney tissues were included in our study. We have evaluated tissue protein expression of both Twist-1& E-cad markers in the 50 samples using the immunohistochemistry method [**12 & 13].** We incubated all sections with primary Rabbit polyclonal anti-Twist antibody (ab50581) and anti-E-cad antibody (ab15148) (Abcam, Cambridge, UK dilution 1; 100), followed by hematoxylincounter staining, thyroid tumors were used as positive control for twist-1 and sections from normal skin were used as positive control for E-cad [Buehler et al., 2013), we made negative controls by removal of the primary antibodies replacing them with normal saline.

**Evaluation of Twist 1& E-cadimmunohistochemical-staining:**

We have considered positive nuclear stain of various levels as positive for Twist-1 while membranous stain was considered positive for E-cad. The final stain score of both markers is calculated semi-quantitatively by summation of scores of stain intensity and extent which gave us scores from 0-7. The scores of stain intensity in the nuclei and membranes of Twist-1 and E-cad respectively, were graded as: 0, completely negative stain; 1, weak faint brown; 2, moderated stain; and 3, strong dark brown stain, the scores of stain extent in the nuclei and membranes of Twist-1 and E-cad respectively was graded as: 0, 0% of tissues were stained; 1, 1-25% tissues were stained; 2, 26-50% tissues were stained; 3, 51-75% tissues were stained; and 4, 76-100% tissues were stained. We have considered a final staining score of 0-3as low tissue protein markers expression and score of more than 3 were as high protein markers expression of both markers **[9].**

**Statistical Analysis**

Data were evaluated, collected, computerized and analyzed statistically using Statistical Package for Social Science (SPSS) program.

Qualitative data were put as frequencies and percentages. Either Chi square test ($\chi 2$) or Fisher exact test were used for assessments of differences between qualitative variables, while Spearman's correlation test for correlating other variables.We considered a p-value$\leq$ 0.05 as significant, p <0.001 as highly significant, while, P> 0.05 as non-significant differences. We have used Kaplan and Meier curves to calculate and present overall (OS) and progression free survival (PFS) rates.

## 2. Results

The demographic results of our included patients are found in Table 1.

- We have included 40 cases of cc-RCC and10 samples from adjacent non-neoplastic renal tissue.
- 19 (34.3%) patients aged less than fifty-five years old and 21 (65.7%) patients aged more than fifty-five years old.
- We have included 32 (80%) males and 8 (20%) females.

**Immunohistochemical results**

**Twist 1expression: Figs 1; tables 2& 3**

- High Twist-1expression was found in 23 (47.1%) of all cases, 19 (58.0%) of the cc-RCC cases and8 (80.0%) out of the 10 adjacent non-neoplastic renal tissue
- Twist 1up-regulation was found in samples from cc-RCC patients more than adjacent non-neoplastic renal tissueand the differences were statistically significant p=0.004.
- In samples from cc-RCC patients Twist 1 up-regulation was significantly positively associated with higher grade (0.006), advanced TNM stage (p=0.013), higher incidence of L.N metastases (p=0.002) and higher incidence of presence of distant metastases (p=0.001), no significant association of Twist-1 over expression age, sex of the patient or size of the cancer.

**E- cad expression: Figs 2; tables 2& 3**

- **E- cad** low expression was detected in in 33 (47.1%) of all cases, 33 (58.0%) of the cc-RCC cases and 0 (00.0%) out of the 10 adjacent non-neoplastic renal tissue
- **E- cad was down regulated** in cc-RCC less than adjacent non-neoplastic renal tissue and the differences between both groups was highly significant <0.001.
- In samples from cc-RCC patients **E-cad** down-regulation was significantly positively associated with higher grade (0.006), advanced TNM stage (p=0.013), higher incidence of L.N metastases (p=0.002) and higher incidence of presence of distant metastases

(p=0.001), no significant association of **E-cad** down-regulation age, sex of the patient or size of the cancer.

**Correlations between Twist 1and E-cad expression in cc-RCC and in** adjacent non-neoplastic renal tissue**;**

- Twist-1 expression is negatively correlated with E-cad expression in cc-RCC samples and in adjacent non-neoplastic renal tissuer= -0.376 (p=0.007).

**Progression and survival analysis in relation to Twist 1& E-cad expression; Fig 5; tables 4& 5**

Combination of up regulation of **Twist 1** and down regulation **of** E- cadherin was associated with progression of the tumor, shorter patients OS& PFS rates (p<0.001 and p=0.013 respectively).

**Table 1:** Demographic data of all included patients

| | | Total N=50 N (%) | CCRCC N=40 N (%) |
|---|---|---|---|
| **Age group** | <55y | 19 (38.0%) | 19 (38.0%) |
| | >55y | 21 (62.0%) | 21 (62.0%) |
| **Histopathology** | cc-RCC | 40 (71.4%) | |
| | Non-neoplastic | 10 (28.6%) | |
| **Sex** | Male | 32 (84.0%) | 32 (84.0%) |
| | Female | 8 (16.0%) | 8 (16.0%) |
| **Grade** | 1 | 10 (24.0%) | 10 (24.0%) |
| | 2 | 15 (36.0%) | 15(36.0%) |
| | 3 | 11 (30.0%) | 11 (30.0%) |
| | 4 | 4 (10.0%) | 4 (10.0%) |
| **Size** | <7cm | 13 (26.0%) | 13 (26.0%) |
| | >7cm | 27 (74.0%) | 27 (74.0%) |
| **T** | 1 | 10 (26.0%) | 10 (26.0%) |
| | 2 | 14 (32.0%) | 14 (32.0%) |
| | 3 | 10 (26.0%) | 10 (26.0%) |
| | 4 | 6 (16.0%) | 6 (16.0%) |
| **N** | 0 | 30 (56.0%) | 30 (56.0%) |
| | 1 | 10 (44.0%) | 10 (44.0%) |
| **M** | 0 | 32 (78.0%) | 32 (78.0%) |
| | 1 | 8 (22.0%) | 8 (22.0%) |
| **Stage** | I | 7 (22.0%) | 7 (22.0%) |
| | II | 15 (36.0%) | 15 (36.0%) |
| | III | 10 (22.0%) | 10 (22.0%) |
| | IV | 8 (20.0%) | 8 (20.0%) |

**Table 2:** Twist-1& E-cad expression in all the studied cases

| | | Total N=50 N (%) | CCRCC N=40 | Non-neoplastic N=10 |
|---|---|---|---|---|
| | | | N (%) | N (%) |
| **Twist-1** | Low | 27 (52.9%) | 19 (38.0%) | 8 (80.0%) |
| | High | 23 (47.1%) | 21 (62.0%) | 2 (10.0%) |
| **E-CAD** | Low | 33 (61.4%) | 33 (76.0%) | 0 (00%) |
| | High | 17 (38.6%) | 7 (24.0%) | 10 (100.0%) |

**Table 3:** Correlations between Twist-1, E-cad expression and histopathology of the studied cases

| | | Twist-1 | | P | E-CAD | | P |
|---|---|---|---|---|---|---|---|
| | | Low N=27 | High N=23 | | Low N=33 | High N=17 | |
| **Age group** | <55y | 10 (32.4%) | 10 (36.4%) | 0.729 | 13 (32.6%) | 10 (37.0%) | 0.701 |
| | >55y | 17 (67.6%) | 12 (63.6%) | | 20 (67.4%) | 7 (63.0%) | |
| **Histopathology** | ccRCC | 19 (51.4%) | 21 (93.9%) | <0.001 | 33 (88.4%) | 7 (44.4%) | <0.001 |
| | Non-neoplastic | 8 (48.6%) | 2 (6.1%) | | 0 (11.6%) | 10 (55.6%) | |
| **Sex** | Male | 17 (73.0%) | 20 (90.9%) | 0.054 | 26 (83.7%) | 11 (77.8%) | 0.534 |
| | Female | 10 (27.0%) | 3 (9.1%) | | 7 (16.3%) | 6 (22.2%) | |

**Table 4:** Correlations between Twist-1, E-cad expression and Survival of patients in the studied cases

| ccRCC | | Total N=40 | | | | Twist-1 | | P | E-CAD | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Low N=19 | High N=21 | | Low N=33 | High N=7 | |
| **Progression** | Absent | 22 (44.0%) | | | | 17 (89.5%) | 5 (16.1%) | **<0.001** | 13 (34.2%) | 9 (75.0%) | **0.013** |
| | Present | 18 (56.0%) | | | | 2 (10.5%) | 16 (83.9%) | | 15 (65.8%) | 3 (25.0%) | |
| **Survival status** | Censored | 29 (58.0%) | | | | 19 (100.0%) | 10 (32.3%) | **<0.001** | 24 (50.0%) | 5 (83.3%) | **0.041** |
| | Died | 11 (42.0%) | | | | 0 (0.0%) | 11 (67.7%) | | 9 (50.0%) | 2 (16.7%) | |

**Table 5:** Correlations between Twist-1, E-cad expression and clinicopathological features in the studied cases

| RCC | | CCRCC N=40 | Twist-1 Low 19 | Twist-1 High 21 | P | E-CAD Low 33 | E-CAD High 7 | P |
|---|---|---|---|---|---|---|---|---|
| **Age group** | <55y | 19 (38.0%) | 9 (47.4%) | 10 (32.3%) | 0.285 | 14 (36.8%) | 5 (41.7%) | 0.51 |
| | >55y | 21 (62.0%) | 10 (52.6%) | 11 (67.7%) | | 14 (63.2%) | 7 (58.3%) | |
| **Sex** | Male | 32 (84.0%) | 14 (73.7%) | 18 (90.3%) | 0.119 | 21 (81.6%) | 11 (91.7%) | 0.406 |
| | Female | 8 (16.0%) | 5 (26.3%) | 3 (9.7%) | | 7 (18.4%) | 1 (8.3%) | |
| **Grade** | 1 | 10 (24.0%) | 8 (47.4%) | 2 (9.7%) | 0.007 | 2 (5.3%) | 8 (83.3%) | <0.001 |
| | 2 | 15(36.0%) | 7 (36.8%) | 8 (35.5%) | | 14 (42.1%) | 2 (16.7%) | |
| | 3 | 11 (30.0%) | 3 (15.8%) | 10 (38.7%) | | 11 (39.5%) | 0 (0.0%) | |
| | 4 | 4 (10.0%) | 0 (0.0%) | 4 (16.1%) | | 4 (13.2%) | 0 (0.0%) | |
| **Size** | <7cm | 13 (26.0%) | 7 (36.8%) | 6 (19.4%) | 0.15 | 3 (7.9%) | 10 (83.3%) | <0.001 |
| | >7cm | 27 (74.0%) | 12 (63.2%) | 15 (80.6%) | | 25 (92.1%) | 2 (16.7%) | |
| **T** | 1 | 10 (26.0%) | 7 (36.8%) | 3 (19.4%) | 0.056 | 3 (7.9%) | 7 (83.3%) | <0.001 |
| | 2 | 14 (32.0%) | 8 (42.1%) | 6 (25.8%) | | 12 (36.8%) | 2 (16.7%) | |
| | 3 | 10 (26.0%) | 2 (21.1%) | 8 (29.0%) | | 10 (34.2%) | 0 (0.0%) | |
| | 4 | 6 (16.0%) | 0 (0.0%) | 6 (25.8%) | | 6 (21.1%) | 0 (0.0%) | |
| **N** | 0 | 30 (56.0%) | 15 (78.9%) | 15 (41.9%) | 0.01 | 23 (42.1%) | 7 (100.0%) | <0.001 |
| | 1 | 10 (44.0%) | 4 (21.1%) | 6 (58.1%) | | 10 (57.9%) | 0 (0.0%) | |
| **M** | 0 | 32 (78.0%) | 19 (100.0%) | 13 (64.5%) | 0.003 | 30 (71.1%) | 2 (100.0%) | 0.035 |
| | 1 | 8 (22.0%) | 0 (0.0%) | 8 (35.5%) | | 3 (28.9%) | 5 (70.0%) | |
| **Stage** | I | 7 (22.0%) | 7 (36.8%) | 0 (0 %) | 0.023 | 1 (2.6%) | 6 (83.3%) | <0.001 |
| | II | 15 (36.0%) | 5 (42.1%) | 10 (32.3%) | | 14 (42.1%) | 1 (16.7%) | |
| | III | 10 (22.0%) | 4 (21.1%) | 6 (22.6%) | | 10 (28.9%) | 0 (0.0%) | |
| | IV | 8 (20.0%) | 0 (0.0%) | 8 (32.3%) | | 8 (26.3%) | 0 (0.0%) | |

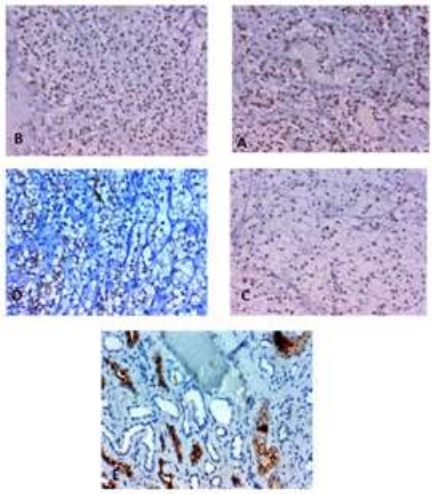

**Figure 1:** Immunohistochemical Twist-1 expression in clear cell renal cell carcinoma (cc-RCC) :(A) Nuclear over expression in high grade cc-RCC stage IVx400 (B) Nuclear over expression in high grade cc-RCC stage IIIx400 (C) Nuclear low expression in low grade cc-RCC grade I stage IIx200 (D) Nuclear low expression in low grade cc-RCC stage IIx400 (E) Nuclear low expression in proximal convoluted tubules of adjacent non-neoplastic kidney tissue.

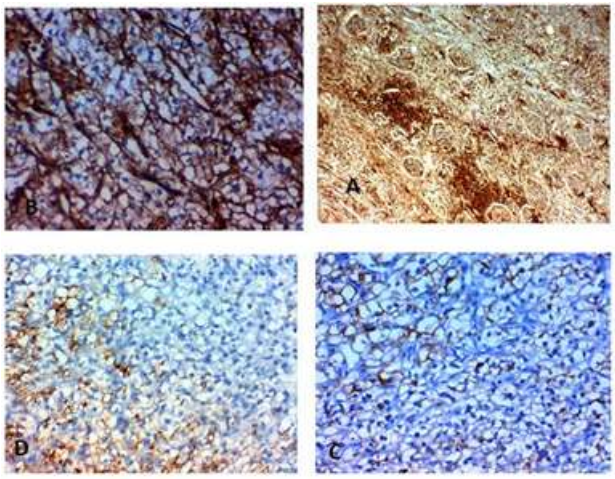

**Figure 2:** Immunohistochemical E-cad expression in clear cell renal cell carcinoma (cc-RCC) : (A) membranous up-regulation in proximal convoluted tubules of adjacent non-neoplastic kidneyx100 (B) membranous up- regulation in low grade cc-RCC stage I x400 (C) membranous down regulation in high grade cc-RCC stage IIx400 (D) membranous down regulation in high grade cc-RCC stage IIX400

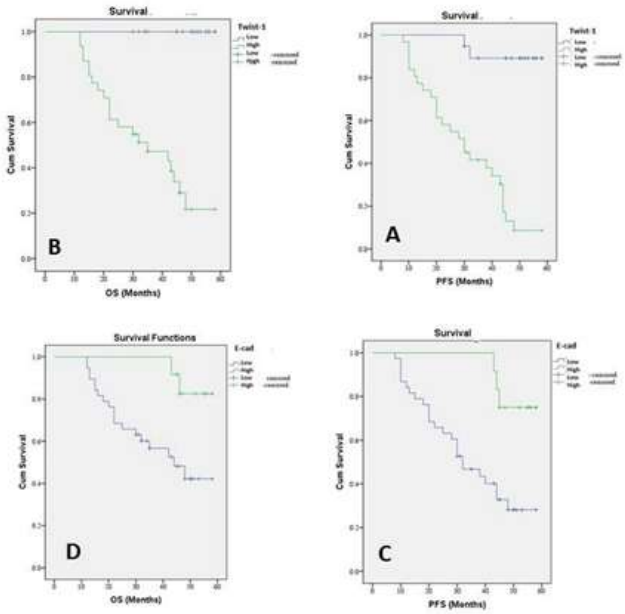


**Figure 3:** Kaplan Meir curves of progression free survival (PFS) and overall survival rates (OS) of cc-RCC patients (A) PFS rate of cc-RCC cases in relation to Twist-1 (B) OS rate of cc-RCC cases in relation to Twist-1 expression (C) PFS rate of cc-RCC cases in relation to E-cad expression (D) OS rate of cc-RCC cases in relation to E-cad expression

## 3. Discussion

In this study we have tried to assess the prognostic values of 2 of the EMT markers in malignant tissues of cc-RCC and adjacent non-neoplastic renal tissue aiming to detect novel therapeutic targets for this aggressive urological cancer. We have proved that Twist 1up-regulation was found in samples from cc-RCC patients more than adjacent non-neoplastic renal tissue and in cc-RCC patients Twist 1 up-regulation was significantly positively associated with higher grade, advanced TNM stage, higher incidence of L.N metastases and higher incidence of presence of distant metastases. Additionally Twist-1 over expression in cc-RCC was associated with progression of the disease and poor PFS and OS rates. We have found no significant association of Twist-1 over expression age, sex of the patient or size of the cancer.

Our results were similar to results of **Kojiro Ohba, et al., [14]** who proved that increased Twist-1 tissue protein expression in RCC was positively correlated with higher grade advanced pT stage, and higher incidence of metastasis. Several studies have assessed the value of Twist-1 expression in cancer and proved results similar to ours as the meta-analysis done by **Wushou et al., [15],** have concluded thatTwist-1 expression, as assessed by IHC has been associated with a worse prognosis in carcinomas of various types, there results encourage trying to develop therapeutic targets against Twist-1 to improve the treatment outcome and patients prognosis. Results of our study were similar to results that are found by previous studies demonstrated the role Twist-1 role in EMT induction in RCC cells [**16].**Moreover **Harada et al., [17],** have proved similar results to us that Twist-1 expression is associated with dismal outcome in RCC patients**.** The novel part of our study that has not been previously assessed is Twist-1 decreased expression in renal tissues that are adjacent to the tumor in kidney tissue which points to the initiating role of Twist-1 in renal carcinogenesis.

Results of our study in cc-RCC are similar to results of previous studies in cancers of other organs **[18& 19],** Twist-1 over expression in carcinomas are related to poorer prognosis, higher grade, higher incidence of invasion and spread **[15].** Twist-1 over expression in squamous cell carcinoma predicted the higher incidence of occurrence of lymph node metastases and hematogenous spread to the lung as well as poor patient survival rates. Also increased expression of Twist-1 in lung cancer tissues have been considered a poor prognostic marker and was associated with poor survival and dismal patients outcome **Zeng et al., [20], sung et al., [21],** have proved similar results to ours in cancer stomach, **Buehler et al., [22],** have proved similar results in thyroid cancer **and Wallerand et al., [23],** have proved similar results in bladder cancer and prostatic cancer. Other previous studies have proved the same results in breast cancer, gliomas and liver cancer **[24-26],** the main role of Twist-1 in initiation and progression of carcinoma mainly relies on its ability to cause EMT which is the process by which epithelial malignant cells lose their epithelial characters and have acquire mesenchymal characters that allow malignant invasion and metastases **[27].**Hence our results regarding upregulation of Twist-1 in malignant renal tissue and relations to poor clinicopathological criteria and dismal outcome of patients so our results help to allow discovering novel therapeutic target which will help to decrease invasion and spread of such malignancy that was in agree with **[28],** as they have proved that Twist-1 inhibition in metastatic cells could induce inhibition of cancer cells invasiveness and spread which subsequently lead to decreased cancer progression.

EMT is the process that have been incriminated in induction of metastasis, gain of mesenchymal markers and loss of epithelial markers in addition to increased motility and invasiveness of malignant cells. Twist-1 is the commonest transcription factors which derived the EMT [**29, 30].**

As the main role proved by most studies that **Twist 1**inhibited "epithelialness" and initiate EMT by down-regulation of E-cad in addition to other epithelial markers down regulation, in this study we have evaluated the combined expression of Twist-1 and E-cad expression to prove their relation in cc-RCC initiation, invasion and metastases.

We have found that E- cad expression was down regulated in cc-RCC and upregulated in the adjacent non-neoplastic renal tissue and its down-regulation in cc-RCC tissues was positively associated with higher grade, advanced TNM stage, higher incidence of L.N metastases and higher incidence of presence of distant metastases, no significant association of E-cad down-regulation age, sex of the patient or size of the cancer. Our results in cc-RCC are similar to results of **Ma et al., [31],** who proved that E-cad

down regulation is related to higher grade, advanced stage and more liability for invasion of cervical carcinoma cells, additionally have stated that down regulation **of** E-cadherin was associated with progression of the tumor, shorter patients OS& PFS rates.

**Ma et al., [31],** found similar results in gastric carcinoma tissue that malignant invasion was associated with decreased membranous expression of E-cadherin**.** More over **Matsubaraet al., [32],** proved the same results regarding E-cad expression in non-small cell lung carcinoma and found similar results to ours. E-cad was proved to be a cell adhesion molecule which is found in the membranes of normal epithelial cells, it binds to β-catenin forming a protein complex that is connected to act in cytoskeleton. And disturbances of such adherens junction components will play a vital role in induction of EMT that is the main stay in malignant progression, invasion and metastases [**33**].

Similarly previous studies proved the role of E-cad down-regulation in EMT induction [**34& 35], they have found** that E-cadherin loss resulted in up-regulation of Twist-1 that initiate EMT malignant invasion and spread that explained our results, regarding inverse relation between E-cad expression and Twist-1 expression in malignant and non-neoplastic renal tissue.

Studies which studied the ability of **Twist 1** to decreased expression of E-cad by binding to E-box which is found on its promoter are recorded on the molecular level, while studies which focused on the clinical and prognostic implications of combined tissue protein expression in cc-RCC carcinogenesis and EMT are limited.

Regarding correlations between both markers in our study, we found inverse relation between both Twist-1 and E-cad expression in cc-RCC and also in adjacent non-neoplastic renal tissue, as we have found that Twist-1 initiate occurrence of metastases and its expression was related to dismal patients outcome via EMT stimulation, in contrast to E-cad increased expression which is associated with lower incidence of metastases and was related to good patients prognosis in cc-RCC via EMT inhibition **[9].**

## 4. Summary & Conclusions

We have proved that Twist-1 over expression in cc-RCC is increased more than adjacent non-neoplastic renal tissue and its expression in such cancer is related to poor clincopathological data, increasing the invasive capacity and patients' prognosis, this action of Twist-1 is mostly accomplished by EMT initiation and down regulation of E-cad that was negatively correlated with its expression in our study.

Molecular therapeutic targets that inhibit Twist-1 could improve outcome of patients having different cancer types.

## 5. Conflicts of Interest

Authors declared no conflicts of interest

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