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The Immunohistochemical Staining of Vimentin and E-Cadherin in Bladder Cancer Patients Infected with Hepatitis C Virus

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Abstract

The invasion and spread of cancer cells are two of the most notable characteristics of malignant tumors. Recent studies suggest that the epithelial-mesenchymal transition (EMT) has been linked to this significant occurrence. It is linked to the absence of the epithelial brow and the presence of mesenchymal facial hair. The aims of the present study were to explore the immunohistochemical staining of vimentin and E-cadherin ex vivo as EMT markers and assess their potential as predictive biomarkers for transitional cell cancer (TCC). In this study, 55 paraffin-embedded biopsies from TCC patients and 10 autopsies that appeared to be normal were included. Immunohistochemistry was used to produce patterns of vimentin and E-cadherin expression. When compared to female patients, the expression of E-cadherin and vimentin significantly increased with increasing age in male patients (> 50 years). In contrast to the considerable rise in vimentin expression in higher grades and stages of the tumor, E-cadherin expression was significantly reduced with tumor stage and grade. The findings of this study reveal that elevated vimentin and reduced E-cadherin are important indicators associated with a poor prognosis for TCC.

Keywords: HCV, Vimentin, E-cadherin, Bladder Cancer, IHC

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INTRODUCTION

Human urinary bladder carcinoma (UBC) is one of the top 10 most common cancer types globally. The majority of bladder cancers are transitional cell carcinomas (TCC).[1] The urothelial cells that generally line the inside of the bladder are where bladder cancer first develops.[2] Other but less typical kinds of bladder inner lining cancer include squamous cell carcinoma and adenocarcinoma.[3] Bladder cancer is one of the most prevalent health issues in the world, with industrialized nations having the greatest incidence rates.[4-6] In the US, TCC ranks fourth among men's cancers and tenth among women's cancers, accounting for almost 95% of bladder malignancies.[7] The grading of cellular differentiation was used to classify the cellular morphology of TCC; well-differentiated cells were graded as 1, moderately-differentiated cells as grade 2, and poorly-differentiated cells as grade 3. The bladder cancer stage is determined by the degree of tumor infiltration into the bladder wall; stages Tis, Ta, and T1 represent noninvasive types that are only present in the inner lining of the bladder epithelium, tumor invasion of the muscle, or perivesical invasion.[2] Bladder cancer risk factors include smoking, which is a major factor, occupational exposure to carcinogens, dietary risk factors, pollution, sex, race, and medical conditions. However, for a more accurate assessment of the effects, each risk factor must be taken into account in the context of genetic-environmental interactions.[8,9]

Hepatitis C virus (HCV), a member of the genus Hepacivirus in the family Flaviviridae, is present in around 160 million people, and a significant portion of this population develops hepatocellular carcinoma (HCC).[10-12] Moreover, HCV infection has been linked to several malignancies, including head and neck cancer and oral squamous cell carcinoma.[13] According to Gordon et al., those with chronic hepatitis C have nearly double the risk of acquiring renal cell carcinoma.[14] Although it was discovered that HCV may be responsible for the impairment of the DNA damage repair system through its propensity to interfere with cellular processes involved in identifying and responding to DNA damage, the chronic inflammatory microenvironment of HCV may be the cause of such tumors.[12,15] The membrane-associated glycoproteins known as epithelial cadherins (E-cad), usually referred to as "classical" cadherins of type I, are produced by epithelial cells and play a crucial role in tissue morphogenesis and remodeling. They are a part of the broad cadherin family that regulates cell-to-cell adhesion.[16] It has been demonstrated that the majority of epithelial malignancies lose E-cad partially or completely by mutation, epigenetic silencing, or increased expression of non-epithelial cadherins, as in cases of gastric cancer (GC), breast cancer (BC), and colorectal cancer (CRC).[17]

Vimentin, a member of type III intermediate filament protein group, is one of the most commonly expressed proteins. It participates in intracellular conformational changes and mechanoprotection in cells of mesenchymal origin, such as leukocytes, endothelial cells, and smooth muscle cells.[18,19] The excessive exposure of the apoptotic cell to vimentin could form vimentin autoantibodies (AVA) and consequently induce platelet activation as well as white blood cells.[20,21] In many epithelial cancers, including colorectal and prostate cancers, vimentin was found to be overexpressed. This overexpression has been linked to tumor invasion, proliferation, and a poor prognosis. The upregulation of vimentin levels also encourages cellular motility, which signals the initiation of EMT.[4,22] The primary characteristics of EMT are the loss of cell-cell contact and the partitioning of intracellular tight junctions, which result in the loss of epithelial characteristics and the acquisition of mesenchymal phenotypes,[23] and the release of subpopulations of cells capable of migrating and forming metastatic colonies.[24] Vimentin displayed a different pattern of expression in normal transitional epithelium compared to bladder cancer. It was detected in 43% of bladder malignancies but was not observed in all normal urothelial cells.[25] There is a dearth of local research addressing the issue concerning patients with TCC carcinoma and a history of HCV. The present study was aimed at evaluating the expression of vimentin and E-cadherin as biomarkers with a link to tumor stage and grade in transitional cell carcinoma of the bladder in Iraqi patients.

MATERIALS AND METHODS

Biopsy Samples

The study samples comprised 55 paraffin-embedded tissues from transitional cell carcinoma patients whose ages ranged from 36 to 70 years, with a mean age of 57 years. Thirteen of the patients were under 50 years old. Men made up about 75% of the samples from the 55 cases. The tissue samples were obtained from the histopathology laboratory archives in Baghdad, Iraq. Afterward, tissue blocks were classified primarily using the histological records of the bladder biopsy specimens. Grades and stages of bladder cancer were assigned based on the tumor, node, and metastasis (TNM) grading system into (GI, GII, and GIII) and (Ta, T1, and T2, respectively).[26] A total of 25 patients have tumor grades GI, 18 with GII, and 12 with GIII, while 12, 24, and 19 patients were with Ta, T1, and T2, respectively. A qualified pathologist histopathologically re-examined each tissue sample that was collected to confirm the record information and select the best tissue sections. A total of 10 seemingly normal bladder autopsies from the Institute of Forensic Medicine were obtained after receiving the consent of the deceased's next of kin. The patient group was age-matched with eight males and two females. Using a microtome, sections of formalin-fixed, paraffin-embedded blocks were cut at a thickness of 4 mm. One section from each block was stained with hematoxylin and eosin (H&E), and four more sections from the same tissue block were placed on glass slides. Using immunohistochemical staining, 4 additional sections of the same tissue block were put on positively charged glass slides to identify vimentin and E-cadherin.

Immunohistochemical Analysis

The paraffin-embedded sections were deparaffinized with xylene and then rehydrated in successively lower alcohol concentrations. To obtain the antigen (epitope), a heating technique was used. As a result, the slides were rinsed in citrate buffer (0.01 mol/L, pH 6.0) and heated at 100°C in the oven. The slides were pre-treated with 3% of H_2O_2 for 15 min at room temperature to stop the endogenous peroxidase activity and then rinsed with phosphate-buffered saline (PBS) for 3-5 min. E-cadherin monoclonal antibody (NCH-38) and anti-vimentin monoclonal antibody, clone V9 (DAKO, USA), were used in the process. Using a 1:100 dilution of the primary antibody, tissue samples were incubated for two hours at 4°C in a humid environment. Sections were washed with PBS before being incubated with the secondary antibody for 45 minutes. Sections were then twice-washed with PBS for 5 minutes, stained with hematoxylin and diaminobenzidine (DAB) chromogen, dried, and covered with a cover slip. Samples of negative control were prepared simultaneously in duplicate, except for the primary antibody, which was replaced with PBS. The positive controls were provided with the kits mentioned above.

Statistical Analysis

The statistical analysis system (SAS; 2012, version 9.1) was utilized in this study to examine how various factors affected the study variables. The Chi-square test was performed to compare the number of positive samples.[27]

Table 1. HCV incidence in TCC samples according to grades and stages

Grade	TCC Cancer Patients	HCV Positive	Stage	TCC Cancer Patients	HCV Positive
G1	25	4	Ta	12	1
G2	18	9	T1	24	10
G3	12	11	T2	19	13
Total	55	24	Total	55	24
		p<0.001			p<0.001

HCV: Hepatitis C virus; TCC: Transitional cell carcinoma

RESULTS

The present study discovered a significant increase in the number of TCC carcinoma patients with HCV, as shown in Table 1. According to the grade of cancer, which increased from 4 patients only with G1 to 11 patients with G3, as well as the stage of cancer, where only 1 patient had Ta and 13 had T2. According to the tumor grade, the cytoplasmic expression of E-cadherin was detected in 40% of TCC patients (Figure 1). Concerning E-cadherin, the results obtained showed that there is an inversely significant relationship between the number of TCC carcinoma patients whose samples were positive for E-cadherin and the grade of the disease. This was evident by a decrease in the number as the grade progressed. There was a reduction from 15 patients in G1 to only 2 patients in G3 patients. As shown in Table 2 the number of E-cadherin-positive samples decreased significantly and inversely with progression in disease stages. On the other hand, 40% of TCC patients showed vimentin expression (Figure 2).

The number of patients with positive samples for this marker increased with the increase in both the grade and stage of the disease, from 6 to 9 patients in G1 and G3, respectively, and from 3 patients with Ta TCC to 7 patients with T2 (Table 2). Additionally, vimentin expression recorded a significant positive relationship with TCC development.

The relationship between markers under study with the age and gender of TCC patients from whom samples were obtained was analyzed. In this situation, patients with positive results were divided into two age groups based on their age: patients under 50 included 13 patient samples, while patients over 50 contained 42 patient samples. In terms of HCV incidence, E-cadherin expression, and vimentin staining, the results showed a significant difference between the above groups. The number of patients rose in the group of people over 50 in terms of HCV incidence, E-cadherin, and vimentin expression. However, the gender-based analysis revealed that females were more likely than males to be infected with

Table 2. Expression of E-cadherin and vimentin in TCC samples according to grades and stages

Grade	TCC Cancer Patients	E-cadherin Positive	Vimentin Positive	Stage	TCC Cancer Patients	E-cadherin Positive	Vimentin Positive
G1	25	15	6	Ta	12	8	3
G2	18	5	7	T1	24	9	7
G3	12	2	9	T2	19	3	7
Total	55	24	22	Total	55	20	17
		p<0.001	p<0.001			p<0.001	p<0.001

TCC: Transitional cell carcinoma
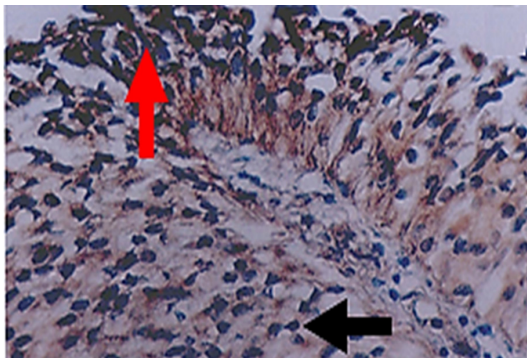
Figure 1. Positive (red pointer) and negative (black pointer) cytoplasmic expression of E-cadherin in transitional cell carcinoma (40x)
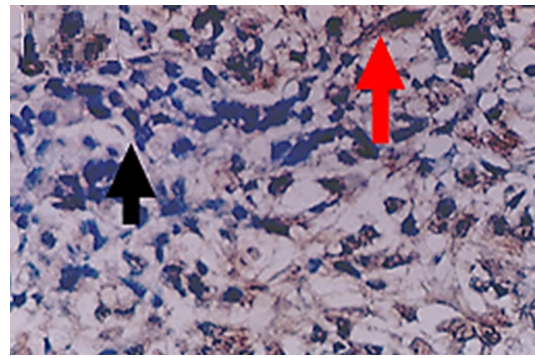
Figure 2. Positive (red pointer) and negative (black pointer) cytoplasmic expression of vimentin in transitional cell carcinoma (40x)

HCV and that vimentin expression was higher in positive samples from females than from males. In contrast, E-cadherin expression showed an inverse relationship with the number of positive samples, with males recording a higher number of positive samples than females (Table 3).

DISCUSSION

Several studies have shown that the prevalence of TCC increases with age and is more common in men than women.[7,28-30] This observation is consistent with the results of the present study, which found that 76% (42 patients out of 55) of the study samples belonged to patients over the age of 50, and 75% of TCC patients were men. This may be caused by a variety of factors, including aging, which increases the likelihood that cellular processes that could lead to neoplastic transformation will intensify and manifest, cumulative exposure to carcinogens in the environment, especially if they smoke, and decreased ability to empty the bladder.[31] In addition, men's higher exposure to environmental carcinogens including cigarettes and industrial pollution has been linked to the gender discrepancy in bladder cancer incidence.[32] Also, other factors such as those of a genetic and hormonal nature may make women more susceptible to the disease.[33]

The Hepatitis C virus is considered one of the oncoviruses that are connected to the development of cancer. These extrahepatic cancers include lymphoma,[12] renal cell carcinoma (RCC),[14] TCC, and head and neck squamous cell carcinoma.[34] Consequently, the current study revealed a high proportion of HCV-positive cases in the TCC samples (24/54). The current study's results support the findings of Hemmaid et al.

regarding the association between HCV infection and more advanced bladder TCC, including higher grades and stages as well as being more invasive.[34] Although, the exact mechanism by which HCV causes bladder cancer is still controversial, some hypotheses have emerged, that suggest that hyper-telomerase expression as well as activity in bladder cancer tissue occur in correlation with HCV.[34] Also, it was hypothesized that HCV disrupts the mechanism of DNA damage repair by binding to the host protein RAD51AP1 and disrupting its function in mammalian cells.[35] Moreover, HCV may interfere with cytotoxic T-cell mediated apoptosis, which is vital for the maintenance of host immunity and normal tissue, and can lead to renal oncogenesis.[36]

Although bladder cancer treatment approaches have advanced considerably, unfavorable biological processes, particularly those that are prone to invasion and metastasis, continue to complicate clinical treatment, resulting in ineffective therapy and a bad prognosis.[37] Several molecular markers are employed to detect bladder cancer using the immunohistochemical technique since they show great potential for predicting prognosis in TCC.[38,39] E-cadherin and vimentin, two markers associated with EMT, were examined in the current study to examine their expression and potential as prognostic indicators for TCC. The cytoplasmic expression of E-cadherin decreased with the progression of the stages and grades of the disease, whereas the expression of vimentin was demonstrated to increase, according to the stage and grade of TCC. These differences were statistically significant. The results are consistent with several studies.[4,38,40-42]

According to Zhao et al., the expression of E-cadherin was inversely correlated with the progression of tumor grade. As tumor stage

Table 3. Expression of the study markers in term of age and gender

Parameter	Age		P-value	Gender		P-value
	<50 years (N=13)	≥ 50 years (N=42)		Male (N=41)	Female (N=14)	
HCV	6	18	p<0.01	16	8	p<0.01
E-cadherin	5	15	p<0.01	18	4	p<0.001
Vimentin	7	10	p<0.001	15	6	p<0.001

HCV: Hepatitis C virus

increased, E-cadherin expression significantly decreased, whereas vimentin showed an opposite expression distribution to that of E-cadherin and significantly increased with the tumor stage.[40] Another study demonstrated a statistically significant correlation between lower expression of E-cadherin and unfavorable clinicopathological parameters, indicating that expression of E-cadherin may be one of the beneficial prognostic markers in individuals with upper tract urothelial cancer.[43] With a positive correlation with the increase in disease stage, vimentin expression was observed to be overexpressed in around 69% of TCC patients, rising from 43% in patients with G1 to nearly 94% in patients with G3 TCC.[4] Several studies have demonstrated that decreased E-cadherin expression and increased vimentin are linked to a poor prognosis for individuals with bladder,[38,40] colorectal cancer,[44] and breast carcinoma.[45] It is commonly accepted that EMT is necessary for tumor invasion and metastasis because it gives cells improved properties for motility and invasion.[5,42] During such EMT-related events, the expression of cadherin switches from epithelial to mesenchymal.[46] Finally, molecular techniques have been recommended to identify infectious diseases[47-56] and cancers.[57-65]

CONCLUSION

Since the expression of vimentin and E-cadherin in TCC tissue showed a substantial relationship with tumor grade and stage, the findings of this study reveal that employing these markers in combination as prognostic markers for TCC may be of interest.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

DATA AVAILABILITY
The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT
This study was approved by the Institutional Ethics Committee, University of Baghdad, Baghdad, Iraq, with reference number CSEC/0322/0032 dated March 20, 2022.

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