Research Article

Immunohistochemical Expression of Survivin and B٧- H١ in Renal Cell Carcinoma

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Abstract

Background: Kidney cancer is the ninth most common cancer in developed countries. According to last registries of Egyptian National Cancer Institute, renal cell carcinoma represents ٥٦.٧٨٪ of all malignant renal tumors. Survivin is a member of the inhibitor of apoptosis protein family that inhibits apoptosis and play a critical role in regulating mitosis and microtubule stability. B٧-H١ (also known as PD-L١) is a ligand that inhibits T cell – mediated immunity and has been implicated as a potent negative regulator of antitumor immunity. **Methods:** The aim of the current study was to investigate the immunohistochemical expression and the relevant clinicopathological significance of survivin and B٧-H١ and to study the relationship between the two markers in one hundred cases of RCC tumors including histologically confirmed ٧٠ case of clear renal cell carcinoma, ١٠ cases of chromophobe renal cell carcinoma, ١٠ cases of papillary renal cell carcinoma, ٢ cases of mixed renal cell carcinoma, ٣ cases of granular renal cell carcinoma and ٥ cases of sarcomatoid renal cell carcinoma. **Results:** A significant association was found between nuclear survivin expression and different clinicopathological features including (primary tumor classification, regional lymph involvement, advanced tumor stage, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score ($p<٠.٠٠١$ for each), tumor size ($p=٠.٠٠١$), nuclear grading ($p=٠.٠٠٢$) and coagulative tumor necrosis ($p=٠.٠٠٢$). However, no significant association between cytoplasmic survivin expression and any of clinicopatho-logical features. Regarding B٧-H١expression, the present study showed positive B٧-H١expression in ٢٩٪ of RCC tumors. A significant association was found between B٧-H١ expression and different clinicopathological features including, primary tumor classification, regional lymph involvement, advanced tumor stage, nuclear grading, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score ($p<٠.٠٠١$) and tumor size ($p=٠.٠٠١$).Combined expression patterns of both markers revealed ٤ immunophenotypes, including ٥٧(٥٧٪) survivin^{Low}/B٧-H١$^{-}$ tumors, ١٤ (١٤٪) survivin^{Hi}/B٧-H١$^{-}$ tumors, ٩(٩٪) survivin^{Low}/B٧-H١$^{+}$ tumors, and ٢٠ (٢٠٪) survivin^{Hi}/B٧-H١$^{+}$ tumors. Among them, the survivin^{Hi}/B٧-H١$^{+}$immunoprofile showed a strong significant association with the more aggressive clinicopathological features including advanced primary tumor classification, regional lymph involvement, advanced tumor stage, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, higher SSIGN score and MR score ($p<٠.٠٠١$) and higher nuclear grading ($p=٠.٠٠٢$). On studying the differential expression of both markers in primary RCC tumors and their corresponding LN metastasis, no significant differences were noticed between primary tumors and their corresponding LN metastasis with high concordance rates were found between both locations. **Conclusion**: Taken together, it can be speculated that dual expression of survivin and B٧-H١can be used to predict RCC tumor aggressiveness.

Key words: Survivin– B٧-H١ – RCC – Immunohistochemistry

Introduction

Kidney cancer is the ninth most common cancer in developed countries(١). It constitutes about ٣٪ of all solid neoplasms and ranks ١٠th as the leading cause of cancer

mortality[٢]. In Egypt, renal cell carcinoma represents nearly ٠.٦٨٪ of total adult malignancies and accounts for ٥٦.٧٨٪ of all malignant renal tumors[٣]. Eighty percent of renal cell cancers are clear cell adeno-carcinomas, the remainder being papillary (١٥٪), chromophobe (٥٪), and collecting duct carcinomas (<١٪)[١].

There are different factors influencing RCC prognosis which include anatomical, histo-logical, prognostic nomograms and mole-cular factors[٤,٥]. Regarding the anatomical factors, they include tumor size, venous invasion, renal capsule invasion, adrenal involvement, lymph node, and distant metastasis. These factors are commonly gathered together in the universally used TNM staging classification system[٤,٦,٧,٥], while the histological factors include Fuhrman grade, RCC subtype, sarcomatoid features and tumor necrosis and lympho-cytic infiltrate[٨,٩,٥,١٠]. Several clinic-patho-logic scoring systems (also referred to as nomograms or algorithms) have been reported to predict outcomes for surgically treated RCC patients. Such algorithms include the ٢٠٠٢ American Joint Committee on Cancer (AJCC) TNM stage groupings, the (UCLA) University of California Los Angeles Integrated Scoring System (UISS), nomograms from Memorial Sloan-Kettering Cancer Center, and the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score[١١] . These scoring systems alone do not fully account for the varied outcomes associated with RCC, and fail to reveal the molecular basis for RCC aggressiveness or rational targets for therapy[١٢]. As a result, there is considerable interest in the identification of tumor-associated biomarkers that might enhance RCC prognostication and guide development of new therapies[١٣].

Survivin is a member of the inhibitors of apoptosis (IAP) family of antiapoptoti-cproteins[١٤]. Survivin has attracted attention as a unique member of the IAP gene family with a potential dual role in apoptosis inhibition and regulation of mitosis[١٥].In fact, although survivin is undetectable in most adult tissues, it is demonstrated a strong survivin expression in most human solid tumor types as lung, colon, breast, pancreas, liver cancer, as well as in hematologic malignancies. They also showed that high levels of the protein were predictive of tumor progression in terms of either disease-free or overall survival[١٦,١٧,١٨,١٩,٢٠]. Several studies suggests that those RCC patients who present with tumors that expresshigh levels of survivin are at increased risk of cancer progression-nand RCC death [٢١, ١٩, ٢٢, ٢٣, ٢٤].

B٧ homolog ١ (B٧-H١) also known as Prog-rammed cell death ligand ١ (PD-L١) or cluster of differentiation (CD٢٧٤), is a protein is encoded by the CD٢٧٤ gene in humanswhich belongs to group II B٧ family[٢٥]. B٧-H١ on tumor cells promotes immune suppression by binding to PD-١ on activated T cells, thereby sustaining tumor growth inhibit tumor-specific T cell–mediated immunity, through binding to the T-cell PD-١ (or a putative non–PD-١) receptor, inducing T cell apoptosis, impair-ring cytokine production, and diminishing the cytotoxicity of activated T cells[٢٦].Many studies revealed thatB٧-H١ is highly expr-essed in most human solid cancers including breast, colon, esophageal, gastric, head and neck squamous cell, kidney, liver, lung, ovarian, pancreatic, salivary and urothelial carcinomas, as well as in glioblastoma, wilms' tumor and melanoma[٢٧,٢٨,٢٩,٣٠]. In RCC many studies showed that RCC patients harboring tumors expressing B٧-H١ are at significantly increased risk for progression and mortality[٣١,٣٢,٢٢].

Both survivin and B٧-H١ may promote RCC tumor progression: the former by promoting tumor cell immortalization and the latter through evasion of the immune system. Because both of these molecules act via very different mechanisms to preserve tumor cell viability, one might anticipate that RCC tumors expressing both of these molecules might behave significantly more aggressively than RCC tumors that express either marker alone. Alternatively, one might just as easily predict that these two molecules are randomly produced by increasingly dysplastic cells, overlapping as prognostic variables and acting as surrogate biomarkers for one another. Hence, we examined the clinical effect of combined

survivin and B٧-H١ expression in RCC tumors obtained from one hundreds surgically treated patients.

Material and Methods

Cases Selection: The present study comprised one hundred case randomly selected from formalin – fixed paraffin embedded cases of renal cell carcinomas which were chosen from the archive of histopathological laboratories of Minia University Hospital and National Cancer Institute (NCI) of Cairo (In the period between ٢٠٠٥ and ٢٠١١). The cases included; ٧٠ case of clear renal cell carcinoma, ١٠ cases of chromophobe renal cell carcinoma, ١٠ cases of papillary renal cell carcinoma, ٢ cases of mixed renal cell carcinoma, ٣ cases of granular renal cell carcinoma and ٥ cases of sarcomatoid renal cell carcinoma.

Clinical and pathological features: The available clinicopathological data were obtained from the pathology reports of the cases. This data includes patients' age and sex, tumor localization, tumor size, tumor necrosis, perinephric fat invasion, sarcomatoid differentiation, tumor type (tumor classification was performed according to the WHO criteria)[٣٣], nuclear grade was revised according to Fuhrman nuclear grading system and subdivided into ٤ grades; ١, ٢, ٣ and ٤ respectively[٣٤], tumor stage and lymph node metastasis was estimated according to TNM staging classification system[٦]. The key clinicpathological data of the patients are summarized in Table (١).

Regarding clear cell type, assessment of lymphocytic infiltrate was done. T lymphocytes cell infiltration is then categorized according to the density as: grade ٠, absent; grade ١, focal infiltration (scattered lymphoid aggregates); grade ٢, moderate infiltration; grade ٣, marked infiltration[٨].

SSIGN score and Metastatic Risk score were also estimated according to the data obtained from the pathology reports in each case. All primary tumors and ١٧/٢٣ (available blocks) of metastatic malignant lymph nodes were prepared and stained with haematoxylin and eosin stain to revise the histological findings of all the cases.

Immunohistochemistry for survivin and B٧-H١: Immunohistochemistry was carried out using the avidinbiotin peroxidase complex method. Two, ٣-µm sections thickness from representative paraffin-embedded tissue blocks were sectioned for each case of the cohort and its available corresponding lymph node. One slide was stained with anti-survivin (Monoclonal mouse antibody, clone١٢ C٤, ٠.٢ ml concentrated, Dako; ١:٥٠ dilution, incubated for one hour) using standard techniques. The second slide was stained with ٥H١, a mouse anti-human monoclonal antibody specific for B٧-H١ (Polycolonal rabbit antibody, ٠.١ ml concentrated, US Biological; ١:٧٠٠ dilution, incubated overnight).

Positive and negative control: One negative control tissue was processed for each run by omitting the specific primary antibody from the staining procedure and replaced with PBS. Regarding survivin, the positive control was sections of prostate adenocarcinoma, while sections of human tonsillar tissue were used as positive control for B٧-H١ expression.

Scoring system: To assess positive staining for survivin, the entire tissue section was screened for positive tumour cells, defined as cells with nuclear and /or cytoplasmic staining. Survivin nuclear and cytoplasmic staining was evaluated separately for each case.

- Nuclear survivin expression was evaluated as the percentage of tumor cells stained positive by counting numbers of survivin-positive (versus total) tumor cells in five representative high-powered fields (X ٤٠٠ magnification). Cases were then stratified into low expression ($Survivin^{low}$) and high expression ($Survivin^{Hi}$**)** corresponding to <١٥ positive cell per $mm^{٢}$ and ≥ ١٥positive cells per $mm^{٢}$ according to Parker et al.,[٢٣]
- Cytoplasmic survivin expression was evaluated with each slide as the percentage of positively stained cells in five high power fields (X ٤٠٠ magnification). Cases were

considered +ve when ≥١٠٪ of tumor cells showed cytoplasmic survivin expression according to Byun et al.,[٣٥]

Regarding B٧-H١ Expression, tissue section was screened for positive tumor cells, defined as cells with memberanous and/or cytoplasmic staining. Cases were considered +ve when ≥١٠٪ of tumor cells showed memberanous and/or cytoplasmic B٧-H١ expression according to Thompson et al.,[٣٦].

Statistical analysis

All statistical analysis was done using stastical package of social science (SPSS® Release ١٦) (SPSS, Inc.) soft ware. Association between immune reactivity and different clinicopathological data were done by Chi-square test. Spearman's rho coefficient was used for continuous variables to assess the corre-lation between the two markers. McNemer test was used to compare expression of survivin and B٧-H١ in primary tumors and their corresponding LN metastasis. Statistical significance was determined at p value of ≤٠.٠٥.

Results

On studying the expression of survivin, we found that both nuclear and cytoplasmic survivin expressions were widely expressed in tumor cells. In contrast, it was undetectable in normal renal tublar cells.

For clear RCC cases, the association between lymphocytic infiltrate and SSIGN score was summarized in Table (٢). A significant association was observed between lymphocytic infiltrate and SSIGN score (p<٠.٠٠١). The frequency of marked lymphoctic infiltrate was much higher in cases with high SSIGN score (٠-٤٧٪ ٥y SR) reaching up to ٩٠٪ compared to only one case with marked lymphocytic infiltrate was observed in whom with low SSIGN score (١٠٠٪ ٥y SR).

The association between nuclear survivin expression and different clinicopathological featureswas summarized in table (٣). A significant positive association was observed with different clinicopathological data including primary tumor classification, regional lymph involvement, advanced tumor stage, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score (p<٠.٠٠١ for each), tumor size (p=٠.٠٠١), nuclear grading (fig. ٢-A,B) (p=٠.٠٠٢) and coagulative tumor necrosis (p=٠.٠٠٢).

In the current study, we noticed that survivin was expressed in all histologic RCC types with a higher survivinimmunostaining scores in SRCC type(fig. ٢-C) and GRCC type as compared to clear cell type, papillary type and chromopobe type, Moreover, we demonstrated a significant association between survivin nuclear expression and lymphocytic infiltrate in ccRCC cases. On the other hand, no significant association was noticed between cytoplasmic survivin expression and any of clinicopathological features as shown in table (٤).

Seventeen pairs of primary RCC and their corresponding LN metastasis were compared for nuclear and cytoplasmic survivin expression, which was summarized in table (٥). Regarding both nuclear and cytoplasmic survivin, no significant difference were found between primary RCC and their corresponding LN metastasis (p=١.٠٠٠).

With respect to B٧-H١expression, we reported a positive B٧-H١expression in ٢٩٪ of RCC tumors. The associationbetween B٧-H١ expression and different clinicpathological features was summarized in table (٦). A significant positive association was observed with different clinicpathological data including, primary tumor classification, regional lymph involvement, advanced tumor stage, nuclear grading, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score (p<٠.٠٠١ for each) and tumor size (p=٠.٠٠١).

Here in, we noticed that B٧-H١expressed in all histologic RCC types (fig.٣-A,B,C). No significant difference was noticed among different histological subtypes of RCC, although a higher immunostaining scores were noticed more frequently in SRCCtype and GRCC type as compared to other

histological subtypes. Our results showed a significant association between positive B٧-H١ expression and lymphocytic infiltrate.

Primary RCC tumors and their corresponding LN metastasis were compared for B٧-H١ expression, which was summarized in table (٧). No significant difference between primary RCC and their corresponding LN metastasis (p=.٦٢٥).

As the cytoplasmic expression of survivin showed no significant association with any of clinicopathological features as compared to its nuclear expression that showed a significant association with many of clinicopathological features, so we assessed the combined expression between B٧-H١ and the nuclear type of survivin expression.

According to the combined expression patterns of both markers in patients with RCCs, ٤ immunophenotypes were identified, including ٥٧(٥٧٪) survivin^{Low}/B٧-H١$^{-}$ tumors, ١٤(١٤٪) survivin^{Hi}/B٧-H١$^{-}$ tumors, ٩(٩٪) survivin^{Low}/B٧-H١$^{+}$ tumors, and ٢٠ (٢٠٪) survivin^{Hi}/B٧-H١$^{+}$ tumors. The association of theses immunoprofiles with different clinicopathological variables was shown in table (٨).

In this study, our findings demonstrated a strong association of survivin^{Hi}/B٧-H١$^{+}$ immunoprofile and the more aggressive clinicopathological including advanced primary tumor classification, regional lymph involvement, advanced tumor stage, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, higher SSIGN score and MR score (p<٠.٠٠١ for each) and higher nuclear grading (p =٠.٠٠٢). We also observed a high level of lymphocytic infiltrate within ccRCC cases with survivin^{Hi}/B٧-H١$^{+}$ expression.Finally, the present study identified a moderate positive significant correlation between nuclear survivin and B٧-H١ expression levels (Sperman's rank correlation p<٠.٠٠١, r=٠.٧٣) was identified (Fig. ١).

Table١: Clinicopathological features for patients with RCC (n=١٠٠)

Clinicopathologicalfeatures	No. (%)
Age at Surgery, y	
<٦٥	٧٦ (٧٦٪)
≥٦٥	٢٤ (٢٤٪)
Sex	
Male	٥٣ (٥٣٪)
Female	٤٧ (٤٧٪)
Localization	
Right	٥٠ (٥٠٪)
Left	٥٠ (٥٠٪)
Histological subtypes	
Clear RCC	٧٠ (٧٠٪)
Papillary RCC	١٠ (١٠٪)
Chromophobe RCC	١٠ (١٠٪)
Granular RCC	٣ (٣٪)
Mixed RCC	٢ (٢٪)
Sarcomatoid RCC	٥ (٥٪)
Primary tumor classification	
T١a	١٥ (١٥٪)
T١b	١٨ (١٨٪)
T٢a	١٣ (١٣٪)
T٢b	٢٤ (٢٤٪)
T٣a	٢٥ (٢٥٪)
T٣b	٢ (٢٪)
T٤	٣ (٣٪)
Regional lymph node involvement	
N٠	٧٧ (٧٧٪)
N١	٢١ (٢١٪)
N٢	٢ (٢٪)

TNM stage groupings	
I	٣٢ (٣٢٪)
II	٣٣ (٣٣٪)
III	٣٠ (٣٠٪)
IV	٥ (٥٪)
Tumor size, cm	
<٥	١٧ (١٧٪)
٥ to< ٧	٢٢ (٢٢٪)
٧ to< ١٠	٢٠ (٢٠٪)
≥١٠	٤١ (٤١٪)
Nuclear grade	
١	١٩ (١٩٪)
٢	٤٣ (٤٣٪)
٣	٢٨ (٢٨٪)
٤	١٠ (١٠٪)
Coagulative tumor necrosis	
-ve	٤٨ (٤٨٪)
+ve	٥٢ (٥٢٪)
Perinephric fat invasion	
-ve	٧٣ (٧٣٪)
+ve	٢٧ (٢٧٪)
Sarcomatoid differentiation	
-ve	٩٢ (٩٢٪)
+ve	٨ (٨٪)
Lymphocytic infiltrate*	
-ve	٣٩ (٥٥.٧٪)
Focal	١٤ (٢٠٪)
Moderate	١١ (١٥.٧٪)
Marked	٦ (٨.٦٪)
SSIGN score*	
٥y survival rate ١٠٠٪	٢٢ (٣١.٤٪)
٥y survival rate ٩٠٪	١٧ (٢٤.٣٪)
٥y survival rate ٦٤٪	١٥ (٢١.٤٪)
٥y survival rate ٤٧٪	١٣ (١٨.٦٪)
٥y survival rate ٠٪	٣ (٤.٣٪)
MR score*	
Low risk	٢١ (٣٠٪)
Moderate risk	٣٣ (٤٧.١٪)
High risk	١٦ (٢٢.٩٪)

***** Variable specific only for clear cell type group **(**n=٧٠)
SSIGN: Mayo Clinic's Stage, Size, Grade and Necrosis scoring system
MR *:* Mayo scoring system for metastatic risk

Table (٢): Association of lymphocytic infiltrate and SSIGN score for patients with clear RCC (n=٧٠)

SSIGN	Total No. ٧٠	Lymphocytic infiltration				P value
		Absent No.٣٩	Focal No.١٤	Moderate No.١١	Marked No.٦	
٥y. survival R.١٠٠٪	٢٢	١٥(٦٨.٢٪)	٦(٢٧.٣٪)	٠(٠٪)	١(٤.٥٪)	<٠.٠٠١*
٥y. survival R.٩٠٪	١٧	١٣(٧٦.٥٪)	٣(١٧.٦٪)	١(٥.٩٪)	٠(٠٪)	
٥y. survival R.٦٤٪	١٥	٨(٥٣.٣٪)	٣(٢٠٪)	٤(٢٦.٧٪)	٠(٠٪)	
٥y. survival R.٤٧٪	١٣	٢(١٥.٤٪)	٢(١٥.٤٪)	٦(٤٦.٢٪)	٣(٢٣.١٪)	
٥y. survival R.٠٪	٣	١(٣٣.٣٪)	٠(٠٪)	٠(٠٪)	٢(٦٦.٧٪)	

Test of significance: Chi- square- test *P - value ≤ ٠.٠٥ are considered statistically significant
Low SSIGN: ٥y. survival R.١٠٠٪, **Moderate SSIGN**: ٥y. survival R. ٦٤- ٩٠٪,

High SSIGN: ٥y. survival R.٠-٤٧٪

Table (٣): Association of nuclear survivin expression and clinicopathological features for patients with RCC (n=١٠٠)

Clinicopathological features	Total ١٠٠ (١٠٠٪)	Nuclear survivin		P value
		<١٥ Low expression No. (%) ٦٦(٦٦٪)	≥١٥ High expression No. (%) ٣٤(٣٤٪)	
Age at Surgery, y				
<٦٥	٧٦(٧٦٪)	٥٠(٦٥.٨٪)	٢٦(٣٤.٢٪)	٠.٩
≥٦٥	٢٤(٢٤٪)	١٦(٦٦.٧٪)	٨(٣٣.٣٪)	
Sex				
Male	٥٣(٥٣٪)	٣٦(٦٧.٩٪)	١٧(٣٢.١٪)	٠.٦
Female	٤٧(٤٧٪)	٣٠(٦٣.٧٪)	١٧(٣٦.٢٪)	
Localization				
Right	٥٠(٥٠٪)	٣٣(٦٦٪)	١٧(٣٤٪)	١.٠٠
Left	٥٠(٥٠٪)	٣٣(٦٦٪)	١٧(٣٤٪)	
Histological type				
Clear RCC	٧٠(٧٠٪)	٤٧(٦٧.١٪)	٢٣(٣٢.٩٪)	٠.٢
Papillary RCC	١٠(١٠٪)	٧(٧٠٪)	٣ (٣٠٪)	
Chromophobe RCC	١٠(١٠٪)	٨(٨٠٪)	٢(٢٠٪)	
Granular RCC	٣(٣٪)	١(٣٣.٣٪)	٢(٦٦.٧٪)	
Mixed RCC	٢(٢٪)	٠(٠٪)	٢(١٠٠٪)	
Sarcomatoid RCC	٥(٥٪)	٣(٦٠٪)	٢(٤٠٪)	
Primarytumor classification				
T١a	١٥(١٥٪)	١٥(١٠٠٪)	٠(٠٪)	<٠.٠٠١*
T١b	١٨(١٨٪)	١٧(٩٤.٤٪)	١(٥.٦٪)	
T٢a	١٣(١٣٪)	١٣(١٠٠٪)	٠(٠٪)	
T٢b	٢٤(٢٤٪)	١٥(٦٢.٥٪)	٩(٣٧.٥٪)	
T٣a	٢٥(٢٥٪)	٤(١٦٪)	٢١(٨٤٪)	
T٣b	٢(٢٪)	١(٥٠٪)	١(٥٠٪)	
T٤	٣(٣٪)	١(٣٣.٣٪)	٢(٦٦.٧٪)	
Regional lymph node involvement				
N٠	٧٧(٧٧٪)	٦٤(٨٣.١٪)	١٣(١٦.٩٪)	<٠.٠٠١*
N١	٢١(٢١٪)	٢(٩.٥٪)	١٩(٩٠.٥٪)	
N٢	٢(٢٪)	٠(٠٪)	٢(١٠٠٪)	
TNM stage groupings				
I	٣٢(٣٢٪)	٣٢(١٠٠٪)	٠(٠٪)	<٠.٠٠١*
II	٣٣(٣٣٪)	٢٧(٨١.٨٪)	٦(١٨.٢٪)	
III	٣٠(٣٠٪)	٦(٢٠٪)	٢٤(٨٠٪)	
IV	٥(٥٪)	١(٢٠٪)	٤(٨٠٪)	
Tumor size, cm				
<٥	١٧(١٧٪)	١٦(٩٤.١٪)	١(٥.٩٪)	<٠.٠٠١*
٥ to < ٧	٢٢(٢٢٪)	١٨(٨١.١٪)	٤(١٨.٢٪)	
٧ to < ١٠	٢٠(٢٠٪)	١٥(٧٥٪)	٥(٢٥٪)	
≥١٠	٤١(٤١٪)	١٧(٤١.٥٪)	٢٤(٥٨.٥٪)	
Nuclear grade				
G١	١٩(١٩٪)	١٧(٨٩.٥٪)	٢(١٠.٥٪)	٠.٠٠٢*
G٢	٤٣(٤٣٪)	٣٢(٧٤.٤٪)	١١(٢٥.٦٪)	
G٣	٢٨(٢٨٪)	١٤(٥٠٪)	١٤(٥٠٪)	
G٤	١٠(١٠٪)	٣(٣٠٪)	٧(٧٠٪)	

Coagulative tumor necrosis				
-ve	٤٨(٤٨٪)	٤٢(٨٧.٥٪)	٦(١٢.٥٪)	<٠.٠٠١*
+ve	٥٢(٥٢٪)	٢٤(٤٦.٢٪)	٢٨(٥٣.٨٪)	
Perinephric fat invasion				
-ve	٧٣(٧٣٪)	٦١(٨٣.٦٪)	١٢(١٦.٤٪)	<٠.٠٠١*
+ve	٢٧(٢٧٪)	٥(١٨.٥٪)	٢٢(٨١.٥٪)	
Sarcomatoid differentiation				
-ve	٩٢(٩٢٪)	٦٣(٦٨.٥٪)	٢٩(٣١.٥٪)	٠.٠٧
+ve	٨(٨٪)	٣(٣٧.٥٪)	٥(٦٢.٥٪)	
Lymphocytic infiltrate*				
-ve	٣٩ (٥٥.٧٪)	٣٢ (٨٢.١٪)	٧ (١٧.٩٪)	<٠.٠٠١*
Focal	١٤ (٢٠٪)	١١ (٧٨.٦٪)	٣ (٢١.٤٪)	
Moderate	١١(١٥.٧٪)	٢ (١٨.٢٪)	٩ (٨١.٨٪)	
marked	٦ (٨.٦٪)	٢ (٣٣.٣٪)	٤ (٦٦.٧٪)	
SSIGN score*				
٥y SR ١٠٠٪	٢٢ (٣١.٤٪)	٢٢ (١٠٠٪)	٠ (٠٪)	
٥y SR ٩٠٪	١٧ (٢٤.٣٪)	١٤ (٨٢.٤٪)	٣ (١٧.٦٪)	<٠.٠٠١*
٥y SR ٦٤٪	١٥ (٢١.٤٪)	١٠ (٦٦.٧٪)	٥ (٣٣.٣٪)	
٥y SR ٤٧٪	١٣ (١٨.٦٪)	١ (٧.٧٪)	١٢ (٩٢.٣٪)	
٥y SR ٠٪	٣ (٤.٣٪)	٠ (٠٪)	٣ (١٠٠٪)	
MR score*				
Low risk	٢١(٣٠٪)	٢١ (١٠٠٪)	٠ (٠٪)	<٠.٠٠١*
Moderate risk	٣٣ (٤٧.١٪)	٢٥ (٧٥.٨٪)	٨ (٢٤.٢٪)	
High risk	١٦ (٢٢.٩٪)	١ (٦.٢٪)	١٥ (٩٣.٨٪)	

Test of significance: Chi- square- test * P - value ≤ ٠.٠٥ are considered statistically significant
***** variable specific only for clear cell type group **(**n=٧٠)

Table (٤): Association of cytoplasmic survivin expression and clinicopathological features for patients with RCC (n=١٠٠)

Clinicopathological features	***Total*** ١٠٠ (١٠٠٪)	**Cytoplasmic survivin**		**P value**
		(-ve) <١٠ No. (%) ٥٣(٥٣٪)	**(+ve) ≥١٠ No. (%)** ٤٧(٤٧٪)	
Age at Surgery, y				
<٦٥	٧٦(٧٦٪)	٤٢(٥٥.٣٪)	٣٤(٤٤.٧٪)	٠.٤
≥٦٥	٢٤(٢٤٪)	١١(٤٥.٨٪)	١٣(٥٤.٢٪)	
Sex				
Male	٥٣(٥٣٪)	٣٢(٦٠.٤٪)	٢١(٣٩.٦٪)	٠.١
Female	٤٧(٤٧٪)	٢١(٤٤.٧٪)	٢٦(٥٥.٣٪)	
Localization				
Right	٥٠(٥٠٪)	٢٥(٥٠٪)	٢٥(٥٠٪)	٠.٥
Left	٥٠(٥٠٪)	٢٨(٥٦٪)	٢٢(٤٤٪)	
Histological type				
Clear RCC	٧٠(٧٠٪)	٣٧(٥٢.٩٪)	٣٣(٤٧.١٪)	
Papillary RCC	١٠(١٠٪)	٥(٥٠٪)	٥(٥٠٪)	٠.٩
Chromophobe RCC	١٠(١٠٪)	٥(٥٠٪)	٥(٥٠٪)	
Granular RCC	٣(٣٪)	٢(٦٦.٧٪)	١(٣٣.٣٪)	
Mixed RCC	٢(٢٪)	١(٥٠٪)	١(٥٠٪)	
Sarcomatoid RCC	٥(٥٪)	٣(٦٠٪)	٢(٤٠٪)	
Primary tumor classification				
T١a	١٥(١٥٪)	٥(٣٣.٣٪)	١٠(٦٦.٧٪)	
T١b	١٨(١٨٪)	١١(٦١.١٪)	٧(٣٨.٩٪)	
T٢a	١٣(١٣٪)	١٠(٧٦.٩٪)	٣(٢٣.١٪)	٠.٠٦

T٢b	٢٤(٢٤٪)	١٦(٦٦.٧٪)	٨(٣٣.٣٪)	
T٣a	٢٥(٢٥٪)	١٠(٤٠٪)	١٥(٦٠٪)	
T٣b	٢(٢٪)	١(٥٠٪)	١(٥٠٪)	
T٤	٣(٣٪)	٠(٠٪)	٣(١٠٠٪)	
Regional lymph node involveme				
N٠	٧٧(٧٧٪)	٤١(٥٣.٢٪)	٣٦(٤٦.٨٪)	
N١	٢١(٢١٪)	١١(٥٢.٤٪)	١٠(٤٧.٦٪)	٠.٩
N٢	٢(٢٪)	١(٥٠٪)	١(٥٠٪)	
TNM stage groupings				
I	٣٢(٣٢٪)	١٥(٤٦.٩٪)	١٧(٥٣.١٪)	
II	٣٣(٣٣٪)	٢٢(٦٦.٧٪)	١١(٣٣.٣٪)	٠.١
III	٣٠(٣٠٪)	١٥(٥٠٪)	١٥(٥٠٪)	
IV	٥(٥٪)	١(٢٠٪)	٤(٨٠٪)	
Tumor size, cm				
<٥	١٧(١٧٪)	٧(٤١.٢٪)	١٠(٥٨.٨٪)	
٥ to< ٧	٢٢(٢٢٪)	١٢(٥٤.٥٪)	١٠(٤٥.٥٪)	٠.٥
٧ to < ١٠	٢٠(٢٠٪)	١٣(٦٥٪)	٧(٣٥٪)	
≥١٠	٤١(٤١٪)	٢١(٥١.٢٪)	٢٠(٤٨.٨٪)	
Nuclear grade				
G١	١٩(١٩٪)	١٢(٦٣.٢٪)	٧(٣٦.٨٪)	
G٢	٤٣(٤٣٪)	٢١(٤٨.٨٪)	٢٢(٥١.٢٪)	٠.٧
G٣	٢٨(٢٨٪)	١٤(٥٠٪)	١٤(٥٠٪)	
G٤	١٠(١٠٪)	٦(٦٠٪)	٤(٤٠٪)	
Coagulative tumor necrosis				
-ve	٤٨(٤٨٪)	٢٧(٥٦.٢٪)	٢١(٤٣.٨٪)	٠.٥
+ve	٥٢(٥٢٪)	٢٦(٥٠٪)	٢٦(٥٠٪)	
Perinephric fat invasion				
-ve	٧٣(٧٣٪)	٤٤(٦٠.٣٪)	٢٩(٣٩.٧٪)	
+ve	٢٧(٢٧٪)	٩(٣٣.٣٪)	١٨(٦٦.٧٪)	٠.٠٧
Sarcomatoid differentiation				
-ve	٩٢(٩٢٪)	٤٩(٥٣.٣٪)	٤٣(٤٦.٧٪)	٠.٨
+ve	٨(٨٪)	٤(٥٠٪)	٤(٥٠٪)	
Lymphocytic infiltrate*				
-ve	٣٩(٥٥.٧٪)	٢١ (٥٣.٨٪)	١٨ (٤٦.٢٪)	
Focal	١٤(٢٠٪)	٦ (٤٢.٩٪)	٨ (٥٧.١٪)	٠.٣
Moderate	١١(١٥.٧٪)	٥ (٤٥.٥٪)	٦ (٥٤.٥٪)	
Marked	٦ (٨.٦٪)	٥ (٨٣.٣٪)	١ (١٦.٧٪)	
SSIGN score*				
٥y SR ١٠٠٪	٢٢(٣١.٤٪)	٩ (٤٠.٩٪)	١٣ (٥٩.١٪)	
٥y SR ٩٠٪	١٧ (٢٤.٣٪	١٢ (٧٠.٦٪)	٥ (٢٩.٤٪)	٠.٤
٥y SR ٦٤٪	١٥ (٢١.٤٪	٧ (٤٦.٧٪)	٨ (٥٣.٣٪)	
٥y SR ٤٧٪	١٣ (١٨.٦٪	٧ (٥٣.٨٪)	٦ (٤٦.٢٪)	
٥y SR ٠٪	٣ (٤.٣٪)	٢ (٦٦.٧٪)	١ (٣٣.٣٪)	
MR score*				
Low risk	٢١ (٣٠٪)	٩ (٤٢.٩٪)	١٢(٥٧.١٪)	
Moderate risk	٣٣ (٤٧.١٪)	١٩ (٥٧.٦٪)	١٤ (٤٢.٤٪)	٠.٥
High risk	١٦(٢٢.٩٪)	٩ (٥٦.٢٪)	٧ (٤٣.٨٪)	

Test of significance: Chi- square- test P - value ≤ ٠.٠٥ are considered statistically significant
***** variable specific only for clear cell type group (n=٧٠)

Table (٥): Comparsion for nuclear and cytoplasmic survivin expression among ١٧ pairs of primary RCC and their corresponding LN metastasis.

	Positive Expression Rate		Change in Expression Pattern			
	Primary (n=١٧)	Metastasis (n=١٧)	P = M	P > M	M > P	P value
Nuclear Survivin	١٥(٨٨.٢٪)	١٤(٨٢.٤٪)	١٤(٨٢.٤٪)	٢(١١.٨٪)	١(٥.٩٪)	١.٠٠٠
Cytoplasmic Survivin	٧(٤١.٢٪)	٦(٣٥.٣٪)	١٦(٩٤.١٪)	١(٥.٩٪)	٠(٠٪)	١.٠٠٠

P: primary renal cell carcinoma tumor M: metastatic malignant LN
Test of significance: McNemar Test

Table (٦): Association of B٧-H١ expression and clinicopathological features for patients with RCC (n=١٠٠)

Clinicopathological features	*Total* ١٠٠ (١٠٠٪)	B٧-H١ expression		P value
		-ve (<١٠) No. (%) ٧١(٧١٪)	+ve (≥١٠) No. (%) ٢٩(٢٩٪)	
Age at Surgery, y				
<٦٥	٧٦(٧٦٪)	٥٦(٧٣.٧٪)	٢٠(٢٦.٣٪)	٠.٧
≥٦٥	٢٤(٢٤٪)	١٥(٦٢.٥٪)	٩(٣٧.٥٪)	
Sex				
Male	٥٣(٥٣٪)	٣٧(٦٩.٨٪)	١٦(٣٠.٢٪)	٠.٤
Female	٤٧(٤٧٪)	٣٤(٧٢.٣٪)	١٣(٢٧.٧٪)	
Localization				
Right	٥٠(٥٠٪)	٣٥(٧٠٪)	١٥(٣٠٪)	٠.٨
Left	٥٠(٥٠٪)	٣٦(٧٢٪)	١٤(٢٨٪)	
Histological type				
Clear RCC	٧٠(٧٠٪)	٥٠(٧١.٤٪)	٢٠(٢٨.٦٪)	
Papillary RCC	١٠(١٠٪)	٨(٨٠٪)	٢(٢٠٪)	٠.٣
Chromophobe RCC	١٠(١٠٪)	٨(٨٠٪)	٢(٢٠٪)	
Granular RCC	٣(٣٪)	٢(٦٦.٧٪)	١(٣٣.٣٪)	
Mixed RCC	٢(٢٪)	٠(٠٪)	٢(١٠٠٪)	
Sarcomatoid RCC	٥(٥٪)	٣(٦٠٪)	٢(٤٠٪)	
Primary tumor classification				
T١a	١٥(١٥٪)	١٥(١٠٠٪)	٠(٠٪)	
T١b	١٨(١٨٪)	١٧(٩٤.٩٪)	١ (٥.٦٪)	
T٢a	١٣(١٣٪)	١٢(٩٢.٣٪)	١(٧.٧٪)	
T٢b	٢٤(٢٤٪)	١٨(٧٥٪)	٦(٢٥٪)	<٠.٠٠١*
T٣a	٢٥(٢٥٪)	٩(٣٦٪)	١٦(٦٤٪)	
T٣b	٢(٢٪)	٠(٠٪)	٢(١٠٠٪)	
T٤	٣(٣٪)	٠(٠٪)	٣(١٠٠٪)	
Regional lymph node involvement				<٠.٠٠١*
N٠	٧٧(٧٨.٦٪)	٦٦(٨٥.٧٪)	١١(١٤.٣٪)	
N١	٢١(٢٠٪)	٥(٢٣.٨٪)	١٦(٧٦.٢٪)	
N٢	٢(١.٤٪)	٠(٠٪)	٢(١٠٠٪)	
TNM stage groupings				
I	٣٢(٣٢٪)	٣٢(١٠٠٪)	٠(٠٪)	
II	٣٣(٣٣٪)	٢٨(٨٤.٨٪)	٥(١٥.٢٪)	<٠.٠٠١*
III	٣٠(٣٠٪)	١١(٣٦.٧٪)	١٩(٦٣.٣٪)	
IV	٥(٥٪)	٠(٠٪)	٥(١٠٠٪)	

Tumor size, cm				
<٥	١٧(١٧٪)	١٥(٨٨.٢٪)	٢(١١.٨٪)	٠.٠٠١*
٥ to < ٧	٢٢(٢٢٪)	٢٠(٩٠.٩٪)	٢(٩.١٪)	
٧ to< ١٠	٢٠(٢٠٪)	١٦(٨٠٪)	٤(٢٠٪)	
≥١٠	٤١(٤١٪)	٢٠(٤٨.٨٪)	٢١(٥١.٢٪)	
Nuclear grade				
G١	١٩(١٩٪)	١٦(٨٤.٥٪)	٣(١٥.٣٪)	<٠.٠٠١*
G٢	٤٣(٤٣٪)	٣٧(٨٦٪)	٦(١٤٪)	
G٣	٢٨(٢٨٪)	١٥(٥٣.٦٪)	١٣(٤٦.٤٪)	
G٤	١٠(١٠٪)	٣(٣٠٪)	٧(٧٠٪)	
Coagulative tumor necrosis				
-ve	٤٨(٤٨٪)	٤٣(٨٩.٦٪)	٥(١٠.٤٪)	<٠.٠٠١*
+ve	٥٢(٥٢٪)	٢٨(٥٣.٨٪)	٢٤(٤٦.٢٪)	
Perinephric fat invasion				
-ve	٧٣(٧٣٪)	٦٣(٨٦.٣٪)	١٠(١٣.٧٪)	<٠.٠٠١*
+ve	٢٧(٢٧٪)	٨(٢٩.٦٪)	١٩(٧٠.٤٪)	
Sarcomatoid differentiation				
-ve	٩٢(٩٢٪)	٦٧(٧٢.٨٪)	٢٥(٢٧.٢٪)	٠.١
+ve	٨(٨٪)	٤(٥٠٪)	٤(٥٠٪)	
Lymphocytic infiltrate*				
-ve	٣٩(٥٥.٧٪)	٣٥(٨٩.٧٪)	٤(١٠.٣٪)	
Focal	١٤(٢٠٪)	١١(٧٨.٦٪)	٣(٢١.٤٪)	<٠.٠٠١*
Moderate	١١(١٥.٧٪)	٢(١٨.٢٪)	٩(٨١.٨٪)	
marked	٦(٨.٦٪)	٢(٣٣.٣٪)	٤(٦٦.٧٪)	
SSIGN score*				
٥y SR ١٠٠٪	٢٢(٣١.٤٪)	٢٢(١٠٠٪)	٠(٠٪)	
٥y SR ٩٠٪	١٧(٢٤.٣٪)	١٦(٩٤.١٪)	١(٥.٩٪)	<٠.٠٠١*
٥y SR ٦٤٪	١٥(٢١.٤٪)	٩(٦٠٪)	٦(٤٠٪)	
٥y SR ٤٧٪	١٣(١٨.٦٪)	٣ (٢٣.١٪)	١٠(٧٦.٩٪)	
٥y SR ٠٪	٣(٤.٣٪)	٠(٠٪)	٣(١٠٠٪)	
MR score*				
Low risk	٢١(٣٠٪)	٢١(١٠٠٪)	٠(٠٪)	<٠.٠٠١*
Moderate risk	٣٣(٤٧.١٪)	٢٦(٧٨.٨٪)	٧(٢١.٢٪)	
High risk	١٦(٢٢.٩٪)	٣ (١٨.٨٪)	١٣(٨١.٢٪)	

Test of significance: Chi- square- test * P - value ≤ ٠.٠٥ are considered statistically significant.
***** Variables specific only for clear cell type group (n=٧٠)

Table (٧): Comparsion for B٧-H١ expression among ١٧ pairs of primary RCC and their corresponding LN metastasis.

	Positive Expression Rate		**Change in Expression Pattern**			
	Primary (n=١٧)	**Metastasis (n=١٧)**	**P = M**	**P > M**	**M > P**	**P value**
B٧-H١ Expression	١٥(٨٨.٢٪)	١٣(٧٦.٥٪)	١٣(٧٦.٥٪)	٣(١٧.٦٪)	١(٥.٩٪)	.٦٢٥

P: primary renal cell carcinoma tumor M: metastatic malignant LN
Test of significance: McNemar Test

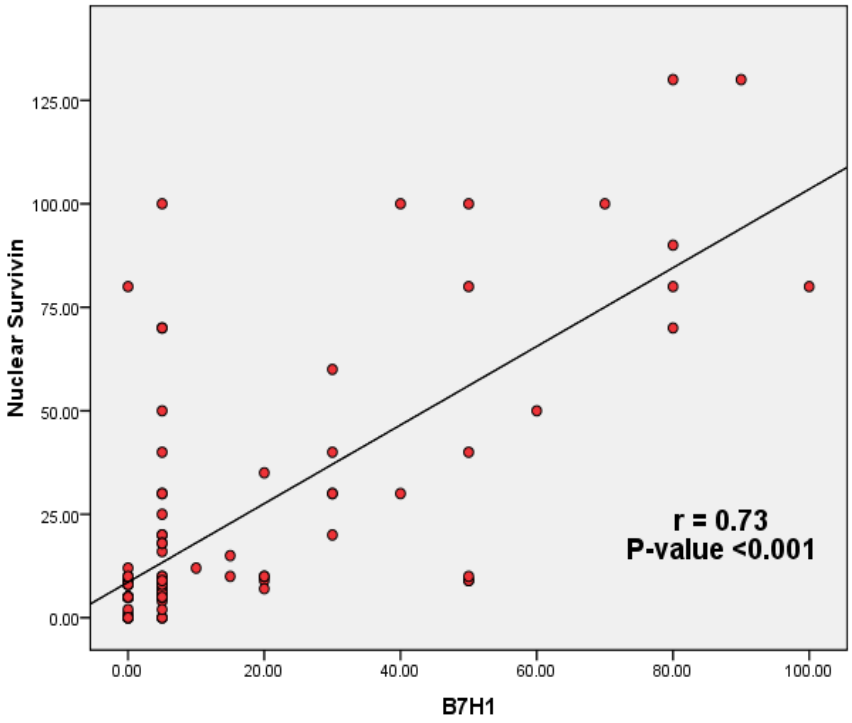
Table (٨): Association of combination of nuclear survivin expression &B٧-H١ expression and clinicopathological features for patients with RCC (n=١٠٠)

Clinicopathological features	*Total* ١٠٠(١٠٠٪)	Combination of Nuclear survivin& B٧-H١ expression				P value
		Low S/ -ve B٧-H١ No. (%) ٥٧(٥٧٪)	High S/ -ve B٧-H١ No. (%) ١٤(١٤٪)	Low S/ +ve B٧-H١ No. (%) ٩(٩٪)	High S/ +ve B٧-H١ No. (%) ٢٠(٢٠٪)	
Age at Surgery, y						
<٦٥	٧٦(٧٦٪)	(٥٧.٩٪)	(١٥.٨٪)	(٧.٩٪)	٤(١٨.٤٪)	٠.٦
≥٦٥	٢٤(٢٤٪)	١٣(٥٤.٢٪)	٢(٨.٣٪)	٣(١٢.٥٪)	٦(٢٥٪)	
Sex						
Male	٥٣(٥٣٪)	٣١(٥٨.٥٪)	٦(١١.٣٪)	٥(٩.٤٪)	١١(٢٠.٨٪)	٠.٨
Female	٤٧(٤٧٪)	٢٦(٥٥.٣٪)	٨(١٧٪)	٤(٨.٥٪)	٩(١٩.١٪)	
Localization						
Right	٥٠(٥٠٪)	٢٩(٥٨٪)	٦(١٢٪)	٤(٨٪)	١١(٢٢٪)	٠.٨
Left	٥٠(٥٠٪)	٢٨(٥٦٪)	٨(١٦٪)	٥(١٠٪)	٩(١٨٪)	
Histological type						
Clear RCC	٧٠(٧٠٪)	٤٠(٥٧.١٪)	(١٤.٣٪)	٧(١٠٪)	١٣(١٨.٦٪)	
Papillary RCC	١٠(١٠٪)	٧(٧٠٪)	١(١٠٪)	٠(٠٪)	٢(٢٠٪)	
Chromophobe RCC	١٠(١٠٪)	٧(٧٠٪)	١(١٠٪)	١(١٠٪)	١(١٠٪)	٠.٥
Granular RCC	٣(٣٪)	١(٣٣.٣٪)	١(٣٣.٣٪)	٠(٠٪)	١(٣٣.٣٪)	
Mixed RCC	٢(٢٪)	٠(٠٪)	٠(٠٪)	٠(٠٪)	(١٠٠٪)	
Sarcomatoid RCC	٥(٥٪)	٢(٤٠٪)	١(٢٠٪)	١(٢٠٪)	١(٢٠٪)	
Primary tumor classification						
T١a	١٥(١٥٪)	١٥(١٠٠٪)	٠(٠٪)	٠(٠٪)	٠(٠٪)	
T١b	١٨(١٨٪)	١٧(٩٤.٤٪)	٠(٠٪)	٠(٠٪)	١(٥.٦٪)	<٠.٠٠١*
T٢a	١٣(١٣٪)	١٢(٩٢.٣٪)	٠(٠٪)	١(٧.٧٪)	٠(٠٪)	
T٢b	٢٤(٢٤٪)	١٢(٥٠٪)	٦(٢٥٪)	٣(١٢.٥٪)	٣(١٢.٥٪)	
T٣a	٢٥(٢٥٪)	١(٤٪)	٨(٣٢٪)	٣(١٢٪)	١٣(٥٢٪)	
T٣b	٢(٢٪)	٠(٠٪)	٠(٠٪)	١(٥٠٪)	١(٥٠٪)	
T٤	٣(٣٪)	٠(٠٪)	٠(٠٪)	١(٣٣.٣٪)	٢(٦٦.٧٪)	
Regional lymph node involvement						
N٠	٧٧(٧٧٪)	٥٥(٧١.٤٪)	١١(١٤.٣٪)	٩(١١.٧٪)	٢(٢.٦٪)	<٠.٠٠١*
N١	٢١(٢١٪)	٢(٩.٥٪)	٣(١٤.٣٪)	٠(٠٪)	١٦(٧٦.٢٪)	
N٢	٢(٢٪)	٠(٠٪)	٠(٠٪)	٠(٠٪)	٢(١٠٠٪)	
TNM stage groupings						
I	٣٢(٣٢٪)	٣٢(١٠٠٪)	٠(٠٪)	٠(٠٪)	٠(٠٪)	
II	٣٣(٣٣٪)	٢٣(٦٩.٧٪)	٥(١٥.٢٪)	٤(١٢.١٪)	١(٣٪)	<٠.٠٠١*
III	٣٠(٣٠٪)	٢(٦.٧٪)	٩(٣٠٪)	٤(١٣.٣٪)	١٥(٥٠٪)	
IV	٥(٥٠٪)	٠(٠٪)	٠(٠٪)	١(٢٠٪)	٤(٨٠٪)	
Tumor size, cm						
<٥	١٧(١٧٪)	١٥(٨٨.٢٪)	٠(٠٪)	١(٥.٩٪)	١(٥.٩٪)	<٠.٠٠١*
٥ to< ٧	٢٢(٢٢٪)	١٧(٧٧.٣٪)	٣(١٣.٦٪)	١(٤.٥٪)	١(٤.٥٪)	
٧ to<١٠	٢٠(٢٠٪)	١٢(٦٠٪)	٤(٢٠٪)	٣(١٥٪)	١(٥٪)	
≥١٠	٤١(٤١٪)	١٣(٣١.٧٪)	٧(١٧.١٪)	٤(٩.٨٪)	١٧(٤١.٥٪)	
Nuclear grade						
G١	١٩(١٩٪)	١٥(٧٨.٩٪)	١(٥.٣٪)	٢(١٠.٥٪)	١(٥.٣٪)	
G٢	٤٣(٤٣٪)	٣٠(٦٩.٨٪)	٧(١٦.٣٪)	٢(٤.٧٪)	٤(٩.٣٪)	٠.٠٠٢*
G٣	٢٨(٢٨٪)	١٠(٣٥.٧٪)	٥(١٧.٩٪)	٤(١٤.٣٪)	٩(٣٢.١٪)	
G٤	١٠(١٠٪)	٢(٢٠٪)	١(١٠٪)	١(١٠٪)	٦(٦٠٪)	

Coagulative tumor necrosis						<٠.٠٠١*
-ve	٤٨(٤٨٪)	٣٩(٨١.٢٪)	٤(٨.٣٪)	٣(٦.٢٪)	٢(٤.٢٪)	
+ve	٥٢(٥٢٪)	١٨(٣٤.٦٪)	١٠(١٩.٢٪)	٦(١١.٥٪)	١٨(٣٤.٦٪)	
Perinephric fat invasion						
-ve	٧٣(٧٣٪)	٥٦(٧٦.٧٪)	٧(٩.٦٪)	٥(٦.٨٪)	٥(٦.٨٪)	<٠.٠٠١*
+ve	٢٧(٢٧٪)	١(٣.٧٪)	٧(٢٥.٩٪)	٤(١٤.٨٪)	١٥(٥٥.٦٪)	
Sarcomatoid differentiation						
-ve	٩٢(٩٢٪)	٥٥(٥٩.٨٪)	١٢(١٣٪)	٨(٨.٧٪)	١٧(١٨.٥٪)	٠.٢
+ve	٨(٨٪)	٢(٢٥٪)	٢(٢٥٪)	١(١٢.٥٪)	٣(٣٧.٥٪)	
Lymphocytic infiltrate*						
-ve	٣٩(٥٥.٧٪)	٣٠(٧٦.٩٪)	٥(١٢.٨٪)	٢(٥.١٪)	٢(٥.١٪)	<٠.٠٠١*
Focal	١٤(٢٠٪)	٩(٦٤.٣٪)	٢(١٤.٣٪)	٢(١٤.٣٪)	١(٧.١٪)	
Moderate	١١(١٥.٧٪)	٠(٠٪)	٢(١٨.٢٪)	٢(١٨.٢٪)	٧(٦٣.٦٪)	
marked	٦(٨.٦٪)	١(١٦.٧٪)	١(١٦.٧٪)	١(١٦.٧٪)	٣(٥٠٪)	
SSIGN score*						
٥y SR ١٠٠٪	٢٢(٣١.٤٪)	٢٢(١٠٠٪)	٠(٠٪)	٠(٠٪)	٠(٠٪)	
٥y SR ٩٠٪	١٧(٢٤.٣٪)	١٣(٧٦.٥٪)	٣(١٧.٦٪)	١(٥.٣٪)	٠(٠٪)	<٠.٠٠١*
٥y SR ٦٤٪	١٥(٢١.٤٪)	٥(٣٣.٣٪)	٤(٢٦.٧٪)	٥(٣٣.٣٪)	١(٦.٧٪)	
٥y SR ٤٧٪	١٣(١٨.٦٪)	٠ (٠٪)	٣(٢٣.١٪)	١ (٧.٧٪)	٩ (٦٩.٢٪)	
٥y SR ٠٪	٣(٤.٣٪)	٠(٠٪)	٠(٠٪)	٠(٠٪)	٣(١٠٠٪)	
MR score*						
Low risk	٢١(٣٠٪)	٢١(١٠٠٪)	٠(٠٪)	٠(٠٪)	٠(٠٪)	
Moderate risk	٣٣(٤٧.١٪)	١٩(٥٧.٦٪)	٧(٢١.٢٪)	٦(١٨.٢٪)	١(٣٪)	<٠.٠٠١*
High risk	١٦(٢٢.٩٪	٠ (٠٪)	٣(١٨.٨٪)	١ (٦.٢٪)	١٢ (٧٥٪)	

Test of significance: Chi- square- test *P - value ≤ ٠.٠٥ are considered statistically significant ***** variables specific only for clear cell type group

Figure (١): Correlation of nuclear survivin and B٧-H١ expression



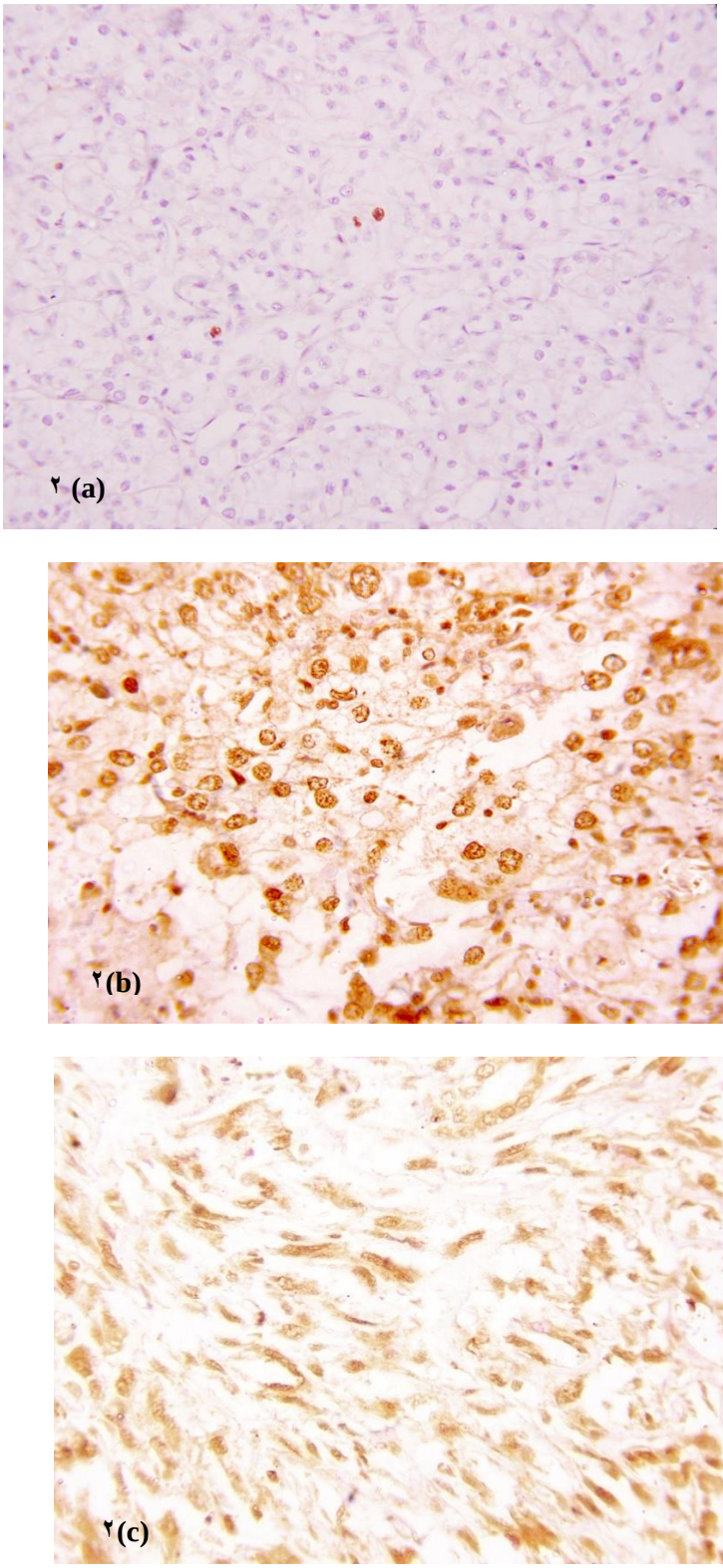


Fig. (٢-A, B, C): Survivinimmunostaining in representative sections of RCCs with (A) low nuclear survivin expression in grade ١ clear cell RCC, (B) high nuclear survivin expression in grade ٣ clear cell RCC and (C) high nuclear and positive cytoplasmic survivin expression in sarcomatoid RCC(X٤٠٠).

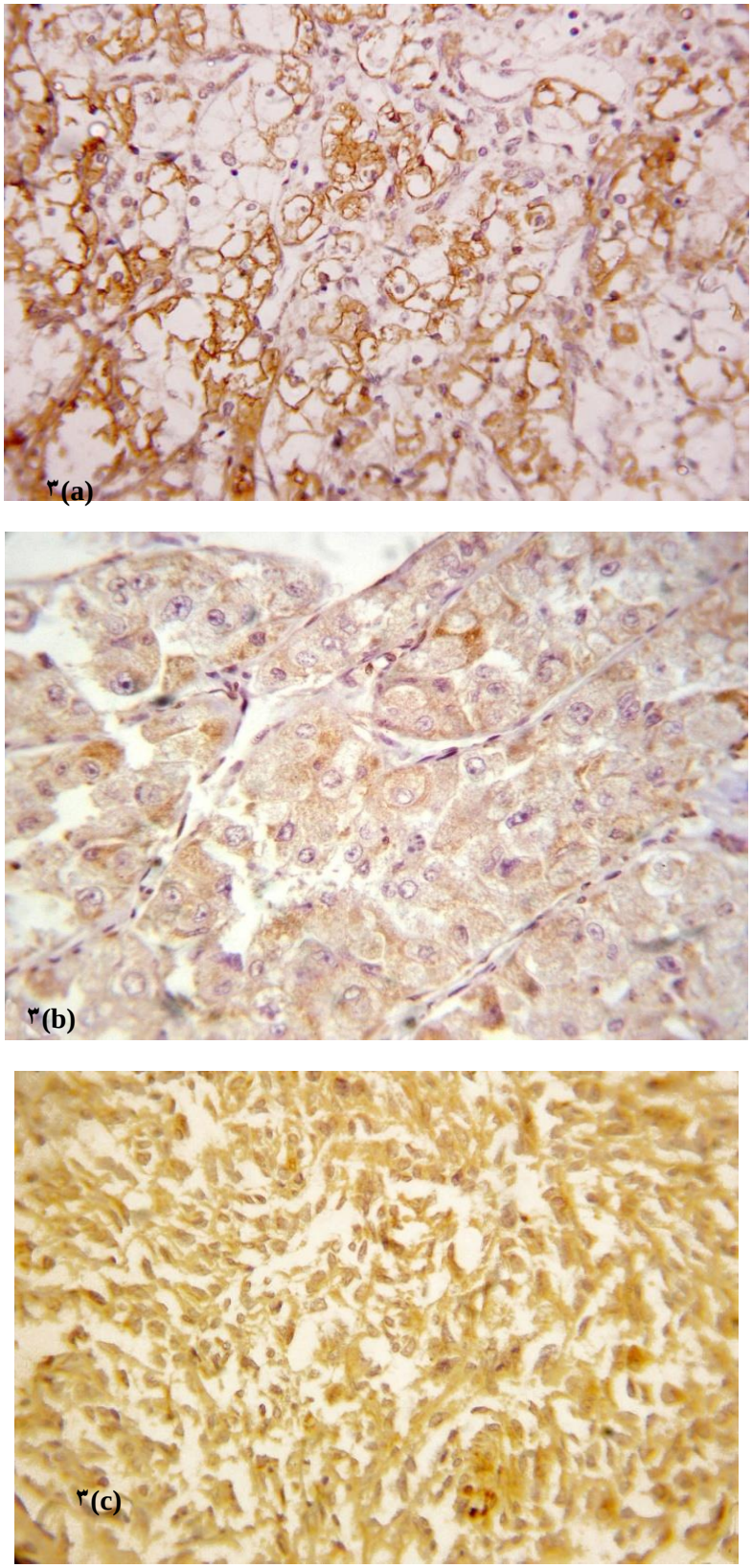


Fig. (٣-A, B, C): B٧−H١ immunostaining in representative sections of RCCs with (A) positive membranous B٧−H١ expression in grade ١ clear cell RCC, (B) positive cytoplasmic B٧−H١ expression in grade ٣clear cell RCC and (C) positive cytoplasmic B٧−H١ expression in sarcomatoid RCC(X٤٠٠).

Discussion

Renal cell carcinoma is recognized as a group of cancers that originate from the renal tubular epithelium and have distinct genetic and molecular backgrounds, unique morphological features and a characteristic clinical course[٣٧]. The molecular mechanisms underlying the development of RCC are still poorly understood. Therefore, it is crucial to exploit markers that can accurately represent biological features of tumors and predict the outcome, which will help us to perform tailored therapy for individual cases.

The present work was conducted to study the immunohistochemical expression of survivin and B٧-H١ in different types of RCC tumors, in order to evaluate their expression patterns and examine their association with various clinicopathological features and finally to investigate the presence of a possible relationship between both markers in RCC tumors.

In the current study, survivin expression was observed in both nuclei and cytoplasm of tumor cells, this was in line with Emaetig et al.,[٢٤]who reported both nuclear and cytoplasmic localization of survivin. Other studies reported survivin expression restricted to the nuclei of tumor cells[٤١,٣٨,٣٩,٢٣], while others demonstrated its cytoplasmic expression[٣٥,٤٠,٣٧]. These variations in survivin subcellular localization reported by different studies could be attributed to the different types of survivin antibody clones used which are specific for certain survivin localization. In contrast to RCC cells, survivin (nuclear and cytoplasmic) was undetectable in the adjacent normal renal tubular cell. Previous studies have shown the absence of survivin expression in normal renal tubular cells using immunehistochemical and reverse-transcription polymerase chain reaction assays[٣٥,٢٣,٣٧,٢٤]. Therefore, one of the most significant features of survivin is its differential distribution in cancer compared with normal tissues. This sharp differential expression in cancer versus normal tissues is one the most intriguing features of survivin and sets it apart from other members of the IAP family; this can be helpful particularly in therapeutic intervention[٢١].

The present study demonstrated a significant association between nuclear survivin expression and different clinicpathological features including primary tumor classification, regional lymph involvement, advanced tumor stage, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score, tumor size, nuclear grading and coagulative tumor necrosis.

In the current study, we noticed that survivin was expressed in all histologic RCC types with a higher survivingimmunostaining scores in SRCC type and GRCC type as compared to clear cell type, papillary type and chromopobe type, suggesting the expected role of survivin in more advanced renal cell carcinoma types. Moreover, we demonstrated a significant association between survivin nuclear expression and lymphocytic infiltrate in ccRCC cases. The CTL recognition of survivin possibly contributes to the significant increase in lymphocytic infiltration that is observed within survivin^{Hi} tumors, a hypothesis that enforce that ccRCCs is regarded as an immunogenic malignancy.

On studying the association between the SSIGN score and MR score with nuclear survivin expression, a significant association became evident between the nuclear survivin expression and SSIGN score and MR category. These results are in accordance with previous studies confirmed a significant association with death from RCC in cases with survivin^{Hi} expression after adjusting for the Mayo Clinic SSIGN score in relation to metastatic risk category[٤١,٣٨,٣٩,٢٣]. As regard the cytoplasmic expression of survivin, we noticed that the positive cytoplasmic survivin expression in ٤٧٪ of RCC tumors. This was comparable with previous studies that reported expression rates ranged from ٥٢.٣٪ up to ٧٩٪[٣٥,٣٧]. This wide difference in the expression rate could be related to biased case selection, different antibodies, scoring systems and different cutoff points for definition of positivity used by different

studies.Our results were similar to the findings that reported by Wang et al.,[٤٠] who confirmed a higher cytoplasmic survivinimmunostaining score in SRCC and GRCC but not in ccRCC, suggesting an important role of survivin in more aggressive subtypes of renal cell carcinoma. On studying the association of cytoplasmic survivin expression with different clinic-pathological features, no significant association was observed between cyto-plasmic survivin expression and any of clinicopathological features. Similarly, Wang et al.,[٤٠] reported that the cytoplasmic immunostaining score of survivin in RCC tumors did not significantly correlate with clinicopathological features including nuclear grading and staging that explained by low expression rate.On the contrary, other studies reported that a high level of cytoplasmic survivin expression was significantly correlated with tumor pathological stage, grade, and lymph node metastasis[٣٥,٣٧], but come in line with our results in the point of no significant association with other clinicopathological factors including age, sex, tumor size, histological type of RCC patients. The difference could be attributed to biased case selection, different scoring systems and different cutoff points for positivity used by different studies

On comparing the role of nuclear and cytoplasmic survivin expression in this cohort, nuclear survivin expression was significantly associated with aggressive tumor features, while no significant relations were noticed between cytoplasmic survivin expression and these features, although both nuclear and cytoplasmic survivin expression coexist in a consider-able proportion of tumors.

Survivin seems to exist in ٢ subcellular pools (cytoplasmic and nuclear)[٤٢]. This is consistent with its function in the regulation of both cell viability and cell division[٤٣]. One possibility is that the nuclear pool of survivin is involved in promoting cell proliferation in most (if not all) cases, whereas the cytoplasmic pool of survivin may participate in controlling cell survival but not cell proliferation. Alternatively, survivin has a number of splicing variants, which may differ in their subcellular localization and functions with respect to cell survival and cell division[٤٤].Survivin and survivin-٢B are predominantly cytoplasmic, whereas survivin-ΔEx-٣ is primarily nuclear. These different isoforms of survivin and their varied locations in the cell may represent a regulatory balance between apoptosis and inhibition of apoptosis[٤٥]. As it is possible immune-histochemically to distinguish two intracellular pools of survivin, a nuclear and a cytosolic one, the prognostic significance of the protein has been analyzed in some studies as a function of its intracellular localization and inconsistent and sometimes contrasting results have been obtained regarding the prognostic value of nuclear vs. cytoplasmic survivinexpression[٢١]. So the different prognostic value of survivin may reflect differential expression of survivin splice variants that exist.

In this study, a high concordance rate of survivin expression status was found between matched primary renal cell carcinoma and metastatic lymph node specimens. These results are in accordance with[٤٦] who reported survivin expression in both primary and metastatic lesions with no significant difference in between. These data along with our findings, suggest that survivin expression by tumor cells probably occurred before metastasis and that survivin bearing malignant cells have more ability to metastasize.

Our findings together with others suggests that nuclear survivin expression is an useful important biologic marker for aggressive RCCs, predicting prognosis in patients with RCC and for guiding the development of more effective methods for potential adjuvant therapy for high-risk patients.

Regarding B٧-H١ expression, in the current study we demonstrated a combined membranous and cytoplasmic expression. Some studies detected B٧-H١ cellular localization concentrated primarily within the cell membrane[٣٨,٢٣,٤٧], while others mentioned that equivalent staining was seen either in the cytoplasm or in the membrane

or even combined[٤٨,٣٦,٤٩]. It is worthwhile to mention that there are different antibodies that can identify different cellular compartments including thecell surface and the cytoplasm. In our study, the antibody that used was known to identify both cellular compartments, membranous and cytoplasmic.

The current study demonstrated significant associations between B٧-H١ expression and different clinicopathological features including, primary tumor classification, regional lymph involvement, advanced tumor stage, nuclear grading, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score and tumor size. Here in, we noticed that B٧-H١expressed in all histologic RCC types. No significant difference was noticed among different histological subtypes of RCC, although a higher immunostaining scores were noticed more frequently in SRCC type and GRCC type as compared to other histological subtypes, suggesting its role in these subtypes of renal cell carcinoma.

Our results showed a significant association between positive B٧-H١ expression and lymphocytic infiltrate. This may be attributed to the fact that the positive B٧-H١tumor cells might inhibit the function of tumor infiltrating T cells, either through the induction of apoptosis or anergy and contributes to the profile of immune-suppression observed in RCC patients that is responsible for immune-mediated tumor destruction for this treatment-refractory malignancy.

In this series, the expression rate of B٧-H١ was much higher in cases with high SSIGN score compared to those with moderate SSIGN score, Keeping up with our findings, similar results were demonstrated by previous studies that confirmed a significant association with death from RCC in cases with positive B٧-H١ expression after adjusting for the Mayo Clinic SSIGN score in relation to metastatic risk category[٣٨,٢٣]. Our results showed a significant association between positive B٧-H١ expression and lymphocytic infiltrate. A similar result was reported by other studies[٣١,٣٨,٣٦]**.** These results are supported by hypothesis that tumor express B٧-H١, might further inhibit the function of tumor infiltrating T cells, either through the induction of apoptosis or anergy. This contributes to the profile of immunosuppression observed in RCC patients. As such, blockade of B٧-H١ may theoretically permit immune-mediated tumor destruction for this treatment-refractory malignancy.

Based on its recognized ability to impair the function and survival of activated tumor-specific T cells, we infer that B٧-H١ expressed by RCC tumor cells and associated increased infiltrating lympho-cytes, may contribute to the profile of immunosuppresion that is observed in patients with RCC , so we further speculate that intratumoral B٧-H١ functions as a critical host determinant of treatment responses in patients who receive immune-therapy for management of advanced RCC (i.e., IL-٢, IFN vaccination, or T cell adoptive therapy).

Taken together, our previous findings suggest that positive B٧-H١ expression is a useful important biologic marker for aggressive RCCs, predicting prognosis in patients with RCC.

In the current study a high concordance rate of B٧-H١ expression status between matched primary renal cell carcinoma and metastatic lymph node specimens was found. This can be explained by the fact of positivity of tumor cells for B٧-H١ occurs early in the process of tumorogenesis that can later on metastatize.

One of the new modalities in immunothrapy is the use of cell surface signaling molecules (CSSMs) which make ideal targets for mAb immunotherapy. Antagonist mAbs targeting inhibitory CSSMs such as PD-١ and B٧-H١ promote immune activation against cancer may results in the generation of immune memory and, consequently, a durable response against cancer, which is of critical importance in immunotherapeutics[٥٠].

Herein, studying the combined expression patterns of survivin and B٧-H١ revealed a strong association of survivin^{Hi}/B٧-H١$^{+}$ immunoprofile and the more aggressive clinicopathological features. The frequency of survivin^{Hi}/B٧-H١$^{+}$ expression was higher in cases with T٤ and T٣ than those with both T٢ and T١ and in cases positive for lymph node metastases than those negative for lymph node metastases.

Our results also demonstrated that the highest incidences of grade ٤ and ٣ tumors were more frequently noticed in survivin^{Hi}/B٧-H١$^{+}$ cases and the rate of survivin^{Hi}/B٧-H١$^{+}$ expression in cases with perinephric fat invasion and coagulative tumor necrosis were much higher compared to those without perinephric fat invasionand coagulative tumor necrosis. Conversely, the survivin^{low}/ B٧-H١$^{-}$ phenotype was more frequently seen among stage I and stage II tumors with the highest incidences in grade ١ and ٢ tumors as well as small sized tumors. All these findings were agree with that reported by Krambeck et al.,[٣٨] who demonstrated that a combination of survivin^{Hi}/B٧-H١$^{+}$ expression was signify-cantly associated with several adverse clinicopathological features and form the most aggressive phenotype.

Furthermore, the current work demon-strated a highly significant association between SSIGN score and MR score with the combined survivin/B٧-H١ immuno-profile. The rate of survivin^{Hi}/B٧-H١$^{+}$ expression was higher in ccRCC cases with high SSIGN score compared to those with moderate SSIGN Score. None of tumors had low SSIGN score showed survivin^{Hi}/ B٧-H١$^{+}$ expression. Also a significant positive association was evident in relation to immunoprofile with MR score in which the rate of survivin^{Hi}/B٧-H١$^{+}$ expression was higher in cases with high risk category (٧٥٪) compared to those with intermediate (٣٪) and low risk category risk categories (٠٪).

Our findings showed that a combined survivin/B٧-H١ expression can provide a more significant degree of further stratify-cation among each category of ccRCC risk patients based upon SSIGN score. So this further stratification within the same risk category can help to provide additional prognostic information that contributes in the therapeutic interventions. This was in line with a similar study was conducted by Parker et al.,[٣٢] Owing to applicability of patient's follow up data records in their center, survival analysis could be conducted, so they could demonstrated that patients within the same risk category with combined expression are associated with poorer cancer-specific survival and the use of this combined expression can provide additional information to further stratify among patients initially predicted to be at intermediate risk and high risk by the SSIGN score with limited ability to be applied to low risk patients.

In our study, we observed high levels of lymphocytic infiltrate within survivin^{Hi}/B٧-H١$^{+}$ tumors relative to the survivin^{Low}/B٧-H١$^{-}$ tumors. So the combined effect of survivin^{Hi}/B٧-H١$^{+}$ on ccRCC tumor aggre-ssiveness occurs at the cellular level could be explained as the tumor cell survivin can be recognized by CTLs that lead to significant increase in lymphocytic infiltration, this significant increase in lymphocytic infiltration produce IFN-ɤ, which cause tumor cell B٧-H١ expression to be up-regulated. This up regulation inhibits the function of tumor-infiltrating T cells, either through the induction of apoptosis or anergy[٥١].

The current study demonstrated a moderate positive correlation between nuclear survivin and B٧-H١ expression. Also there was a distinctively positive correlation between combined nuclear survivin and B٧-H١ and clinicopathological features related to tumor progression indicating that these two markers may act in concert to mediate a more aggressive tumor behavior and poor outcome. Given that both survivin and B٧-H١ are widely expressed within human malignancies including RCC, we anticipate these observations will have broad imply-cations for improving prognostication and treatment of RCC and other malignancies.

Conclusion and Recommendations

Our results can confirm that RCC patients whose tumors exhibit high levels of nuclear survivin expression based on immune-histochemical (IHC) analysis are at markedly increased risk of cancer progression and poor prognosis from RCC relative to patients whose tumors express low levels of survivin suggesting that this biomarker lends meaningful prognostic information beyond standard clinical and pathologic indices. Our findings suggest that positive B٧-H١ expression is a useful important biologic marker for aggressive RCCs, predicting prognosis in patients with RCC, the basis for these associations may relate to the recognized ability of B٧-H١ to inhibit antitumor T-cell–mediated immunity. As such, B٧-H١ may represent a target for RCC immunotherapy and a potential biomarker to facilitate patient assignment to treatment, as well as aid in the determination of prognosis both before and after therapy.

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