



Immunohistochemical analysis of beta-catenin expression: a probable prognostic marker and potential therapeutic target in renal cell carcinoma

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Abstract

Background and aims. Renal cell carcinoma (RCC) seems to be the most aggressive type of genitourinary neoplasm. Down regulation of normal beta-catenin expression contributes to development of RCC, reflecting the role of beta-catenin/Wnt signaling pathway in pathogenesis. This study aims to evaluate the significance of beta-catenin expression and its correlation with the prognostic parameters.

Methods. A cross-sectional observational study was carried out in a tertiary care center on 58 RCC cases using variables like histological grade and type, tumor stage, necrosis. Formalin fixed, paraffin-embedded blocks were evaluated for beta-catenin expression by immunohistochemistry using scoring system. Data were analyzed by mean \pm SD, χ^2 test, Pearson's correlation test.

Results. Membranous score (MS) had a strong negative correlation with tumor stage ($r=-0.407$, $p=0.044$) and grade ($r=-0.787$, $p<0.001$). Mean membranous score difference between low (Stage 1 and 2) vs. high stage (Stage 3 and 4) and low (Grade 1 and 2) vs. high grade (Grade 3 and 4) was statistically significant ($p<0.001$). Cytoplasmic score (CS) had positive correlation with tumor stage ($r=0.586$; $p=0.002$). No significant correlation was evident between cytoplasmic scores and tumor grade, however the mean cytoplasmic score difference between low grade vs. high grade was statistically significant ($p < 0.001$).

Conclusion. Beta-catenin may play a crucial role in the pathogenesis of RCC and has a positive correlation with the biological behavior of this tumor. The important role of beta-catenin as a prognostic parameter and probably a critical evaluator of targeted chemotherapy cannot be overemphasized.

Keywords: renal cell carcinoma, neoplasm, beta-catenin, immunohistochemistry, chemotherapy

Background and aims

Kidney cancer has been identified as the twelfth most common cancer in the world, with 338,000 new cases diagnosed in 2012 [1]. Renal cell carcinoma (RCC), the most common kidney cancer, accounts for approximately 80–90% of all primary kidney neoplasms. It is a very aggressive type of genitourinary cancer due to its refractoriness to current treatment regimens, including chemotherapy and radiotherapy [2]. According to GLOBOCAN 2012, in

India 9658 new cases of kidney cancer were diagnosed, while 5973 deaths were reported in the same calendar year [1]. Survival at five years is >90% for stage I, and 20–30% for stage IV, which indicates the importance of stage as prognostic factor [3].

The current treatment algorithm comprises active surveillance for small incidental masses in the elderly and co-morbid patients, surgical resection for curative resectable masses and systemic therapy with or without cytoreductive

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surgery in patients with metastatic disease [4]. Common therapeutic regimes include tyrosine kinase inhibitors, antivascular endothelial growth factors, and monoclonal antibodies. As of now, limited efficacy has been reported for these drugs [5], and therefore more potent compounds targeting specific signaling pathways of RCC pathogenesis are required to improve the high rate of refractoriness [6].

To anticipate the clinical outcome of different RCC patients is not always possible with classic prognostic factors, staging and grading, which are primarily used to assess cancer prognosis [7,8]. Appropriate prognostic value may help in patient counseling and also in treatment with follow up [8].

Renal carcinogenesis involves the intervention of numerous signaling pathways. Multiple research reports bring solid evidence for the implication of Wnt/beta-catenin, PI3K/AKT/mTOR (phosphatidylinositol 3-kinases/protein kinase B/mammalian target of rapamycin), HGF/c-Met (hepatocyte growth factor/c-mesenchymal epithelial transition factor), and MAPK (mitogen-activated protein kinase) pathways, by alterations in one or more components of their structure [9,10]. Among all these beta-catenin signaling and targeted genes are known to actively participate in different biological processes during embryonic development and RCC [11].

Beta-catenin is both a structural component of cell-cell contact sites and a signaling protein that activates the Wnt survival pathway. Wnt, the mammalian homologue, is a family of secreted glycoproteins that control cell proliferation, differentiation, migration and survival. Beta-catenin activates target genes such as the MYC (v-myc avian myelocytomatosis viral oncogene homolog) oncogene [12]. MYC depicts copy number amplification in a subset of primary clear cell RCC [13] and papillary RCC [14]. Loss of VHL results in reduced levels of Jade-1 and consequent increased levels of beta-catenin, providing one mechanism by which VHL loss promotes renal tumorigenesis. MET phosphorylation also induces tyrosine phosphorylation of beta-catenin causing beta-catenin dissociation from E-cadherin followed by nuclear translocation of beta-catenin and transcription activation [15]. VHL expression in RCC cells suppresses HGF (Hepatocyte Growth Factor) stimulated beta-catenin signaling and thus loss of VHL in RCC could validate HGF-driven oncogenic beta-catenin signaling [16]. Downregulation of normal beta-catenin expression, contributes to the malignant character and tumor progression in RCC, shows the role of beta-catenin/wnt signaling pathway in RCC. Aberrant staining correlated with an advanced tumor grade and stage [17]. Cytoplasmic beta-catenin corresponds with poor prognosis and reduced survival in RCC [18]. Decreased membranous and increased cytoplasmic expression is also individually related with adverse histopathological features [19].

In this scenario, an institutional based cross sectional observational study was done among the different types

of RCC cases with the purpose to assess the beta-catenin expression and to study the possibility of a correlation between histological grade, stage and other prognostic parameters of RCC by immunohistochemistry.

Methods

This was a cross-sectional observational study conducted in the Department of Pathology in collaboration with Department of Urology of Nil Ratan Sircar medical college and hospital, Kolkata, from February 2016 to January 2017. The work was initiated after obtaining ethical clearance from Institutional Ethical Committee and informed consent from the study population. Study group included 58 cases of histologically diagnosed RCC, subjected to nephrectomy. Data were collected using a pre-designed, pretested semi-structured schedule on dependent variables like beta-catenin expression and independent variables like histological grade, type, stage and other prognostic parameters.

Histopathology

Tissue slices were taken from different areas of the tumoral properly fixed nephrectomy specimen and processed. Sections (4-5 microns) were stained with routine hematoxylin-eosin stain for histopathological examination. After histological confirmation of the diagnosis, the following parameters were analyzed: histological type, tumor grade (WHO/ International Society of Urological Pathology (ISUP) grading system), lymphovascular and perineural invasion, nodal involvement, necrosis, sarcomatoid or rhabdoid differentiation, staging (American Joint Committee on Cancer 2010).

Immunohistochemistry (IHC)

For IHC staining, 3 µm thick sections from formalin fixed paraffin embedded tissues were taken on poly L Lysine coated slides. IHC was done manually using rabbit monoclonal beta-catenin antibody. Normal colorectal tissue was taken as control for beta-catenin immunohistochemistry. The criterion for a positive immune reaction (in control) was brown membranous staining. Positive findings were evaluated in four fields (1000 cells) under a light microscope at 400X magnification, without knowledge of the clinical outcome.

Scoring of immunohistochemistry

The staining intensity was scored as follows: 0 = no expression, 1+ = weak expression, 2+ = moderate expression and 3+ = strong expression. The final score was depicted as IHC score by multiplying the percentage of positive cells with the staining intensity according to Mauri et al [20]. Nuclear β-catenin expression was evaluated as either present or absent [18].

Statistical analysis

Data was entered in MS excel. For descriptive purposes mean ± SD, range and percentage were used while for testing the relationship between variables chi-

square (χ^2) test and Pearson's correlation test was used using SPSS Version 20.0. Armonk, NY: IBM Corp. and GraphPad Prism version 7 La Jolla California USA. Significance level was considered at p value < 0.05.

Results

A total of 58 cases were included: Clear cell RCC (CCRCC) [n=44], Papillary RCC (PRCC) [n=8], Chromophobe RCC (ChRCC) [n=4], Mucinous and Tubular Spindle Cell Carcinoma (MTSCC) [n=1] and Multilocular cystic Renal Neoplasm Of Low Malignant Potential (MRNLMP) [n=1]. According to WHO/ISUP grading system, grade 1, 2 and 3 share equal number of cases (26%). 14% cases showed sarcomatoid differentiation whereas 7% cases showed rhabdoid differentiation. Among the 58 cases 32 (55.2%) showed microscopic evidence of lymphovascular invasion, and 15 (25.9%) presented with microscopic evidence of perineural invasion. There were 43% cases stage I, 12% cases stage II, 41% stage III and 4% stage IV disease. The clinical and pathological characteristics of the study population are shown in table I. Different types of RCC showed different patterns of beta-catenin expression. In clear cell RCC, low grade and stage cases showed predominant membranous expression whereas high grade and stage cases showed predominantly cytoplasmic expression (Figure 1A,B,C and D).

Table I. Clinicopathological profile of renal cell carcinoma cases (n=58).

Clinicopathological parameters	Number (%)		
Age in years	<39	6 (10.5)	
	40-59	39 (67.24)	
	>.60	13 (22.41)	
Sex	Male	42 (72.4)	
	Female	16 (27.6)	
Histological type	CCRCC.	44 (75.9)	
	PRCC	8 (13.8)	
	ChRCC	4 (6.9)	
	MTSCC	1 (1.7)	
Histological grade	Low grade	1	15 (25.9)
		2	15 (25.9)
	High grade	3	15 (25.9)
		4	13 (22.4)
Stage	Low stage	I	25 (43.1)
		II	7 (12.1)
	High stage	III	24 (41.4)
		IV	2 (3.4)
LVSI	Found	32 (55.2)	
	Not found	26 (44.8)	
PNI	Found	15 (25.9)	
	Not found	43 (74.1)	
Necrosis	Present	34 (58.6)	
	Absent	24 (41.4)	
Sarcomatoid Differentiation	Present	8 (13.8)	
	Not found	50 (86.2)	
Rhabdoid Differentiation	Present	4 (6.9)	
	Not found	54 (93.1)	

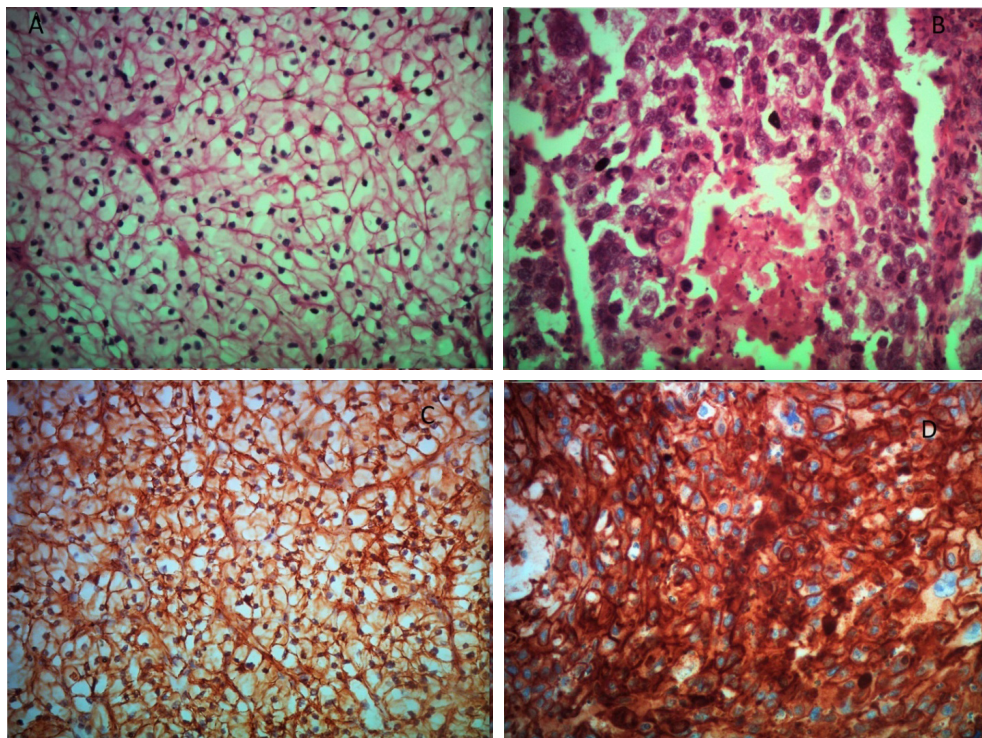


Figure 1. Clear cell RCC H&E, X400: low grade and stage (A) & high grade and stage (B). IHC with beta-catenin (DAB, X400): predominantly membranous in low grade and stage (C) & predominantly cytoplasmic with focal nuclear in high grade and stage (D).

Papillary and chromophobe RCC showed predominantly cytoplasmic beta-catenin expression whereas mucinous tubular and spindle cell carcinoma

and multilocular cystic renal neoplasm of low malignant potential showed membranous beta-catenin (Figure 2 A,B,C and D and Figure 3 A,B,C and D).

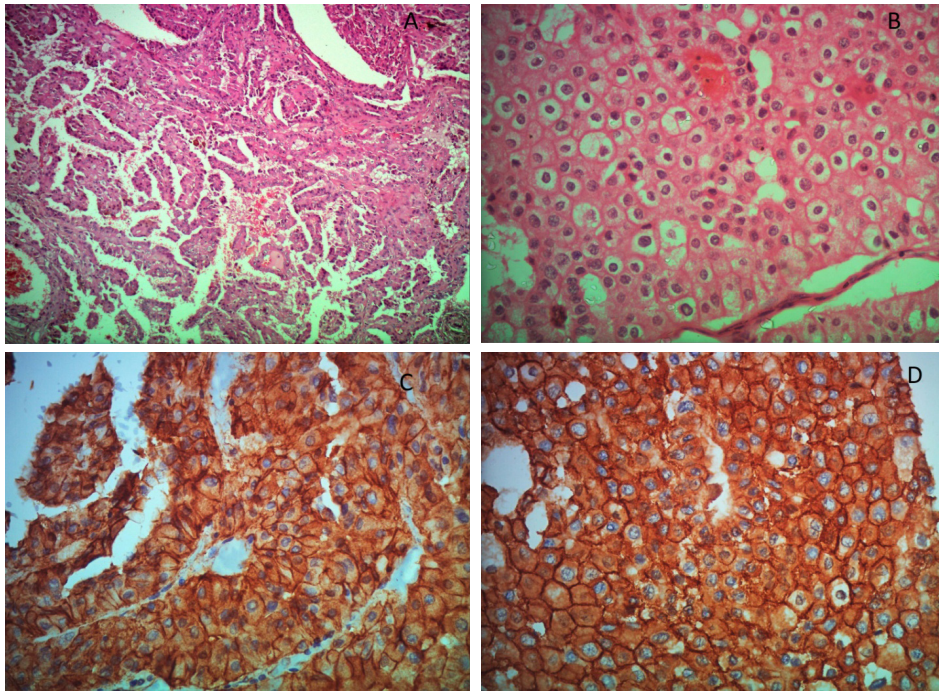


Figure 2. Papillary RCC:H&E, X100 (A). Chromophobe RCC: H&E, X400 (B). IHC with beta-catenin (DAB, X400): predominantly cytoplasmic in papillary (C) & chromophobe (D) RCC.

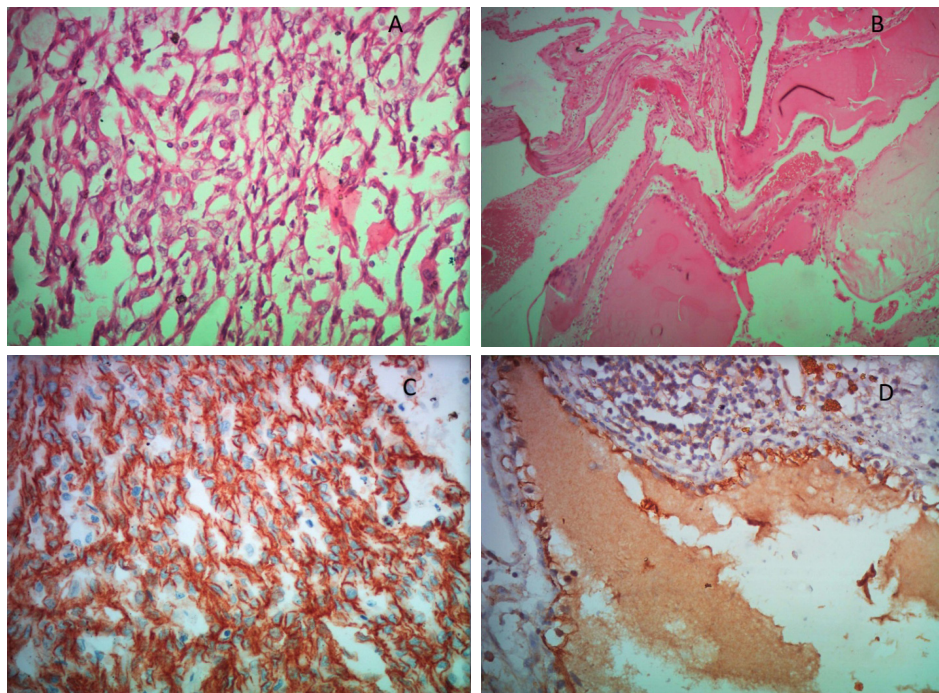


Figure 3. Mucinous tubular and spindle cell carcinoma: H&E, X400 (A). Multilocular cystic renal neoplasm of low malignant potential: H&E, X100 (B). IHC with beta-catenin (DAB, X400): predominantly membranous in Mucinous tubular and spindle cell carcinoma (C) & Multilocular cystic renal neoplasm of low malignant potential (D) RCC.

Table II. Cytoplasmic and membranous Scores among different types of RCC.

Type of CA	C score Median (IQR)	p-value	M score Median (IQR)	p-value
CCRCC (n=44)	69 (40-215)	<0.0001	127 (20-154.5)	<0.0001
PRCC (N=8)	232 (191-271.5)		28.5 (5-33.5)	
ChRCC (N=4)	262 (236.5-287.5)		27.5 (8.25-47.5)	
MTSCC (N=1)	29		150	
MRNLMP (N=1)	0		153	

Median cytoplasmic beta-catenin score was found to be lowest in clear cell (value was still lower in one single case of MTSCC), higher in papillary and highest in chromophobe RCC. Median score differences were found to be statistically significant by chi-square test for trend analysis (p value <0.0001). A similar trend was found in case of median membranous score (Table II).

There was a strong negative correlation between membranous score and tumor grade ($r=-0.787$, $p<0.001$). Mean membranous score difference between low grade (Grade 1 and 2) vs. high grade (Grade 3 and 4) was found to be statistically significant ($p<0.001$). No significant correlation was found between cytoplasmic score and tumor grade. But mean cytoplasmic score difference between low grade (grade 1 and 2) vs. high grade (grade 3 and 4) was statistically significant ($p<0.001$) (Figure 4a and 4b).

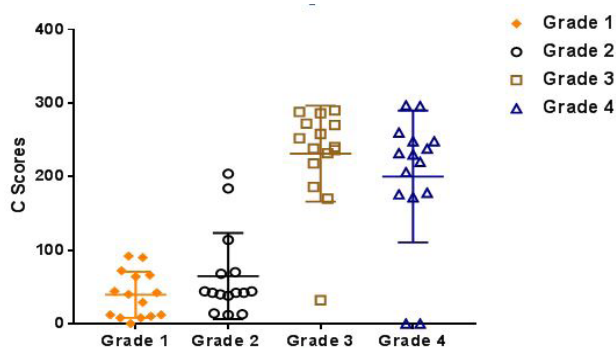


Figure 4a. Scatterplot - Membranous score vs different grades.

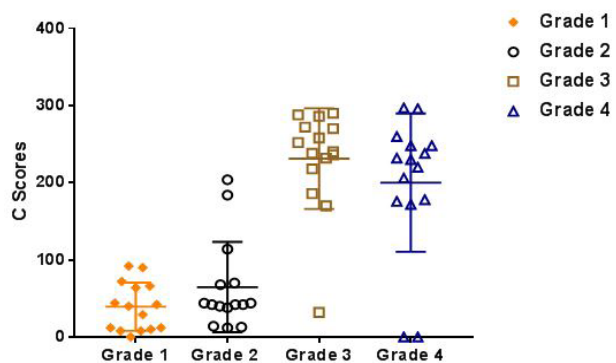


Figure 4b. Scatter plot - Cytoplasmic score vs different grades.

Both mean cytoplasmic and membranous score difference between necrosis, sarcomatoid differentiation (S/D), rhabdoid differentiation (R/D), lymphovascular invasion (LVI) and perineural invasion (PNI) present and absent cases were statistically significant ($p<0.001$) (Figure 5a and 5b).

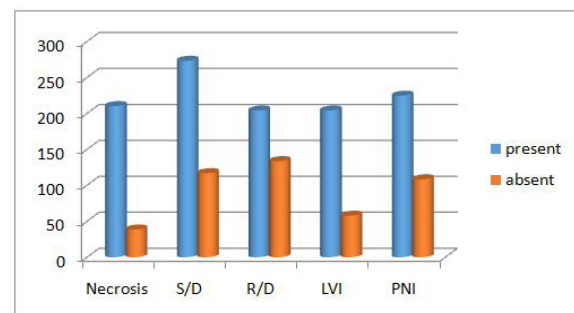


Figure 5a. Bar diagram of cytoplasmic score vs different parameters.

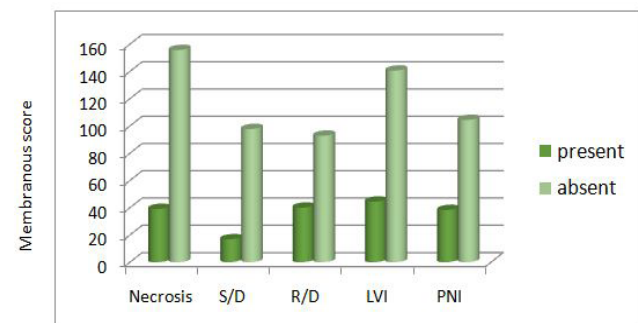


Figure 5b. Bar diagram of membranous score vs different parameters.

In the present study significant negative correlation was detected between beta-catenin membranous immunoscore (M score) and stage ($r=-0.407$, $p=0.044$). Very significant positive correlation was found between beta-catenin cytoplasmic immunoscore (C score) and stage ($r=0.586$; $p=0.002$). Mean membranous and cytoplasmic score difference between low stage (Stage 1 and 2) vs. High stage (Stage 3 and 4) is statistically significant ($p<0.001$) (Figure 6a and 6b).

In the present study, only 7 (12%) cases were found to express nuclear positivity and percentage of cells show nuclear positivity was less than 10%.

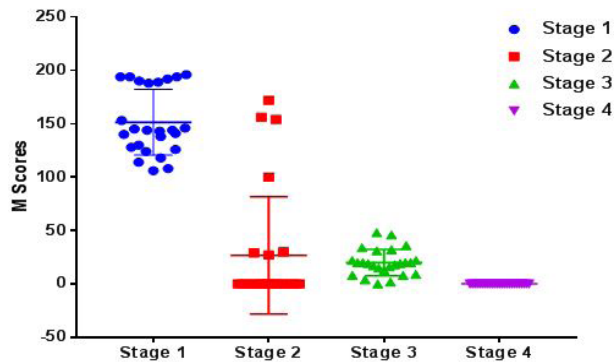


Figure 6a. Scatter plot - membranous score vs different stages.

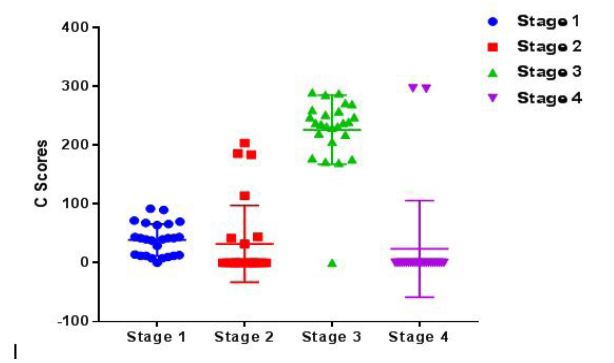


Figure 6b. Scatter plot - cytoplasmic score vs different stages.

Discussion

The most important prognostic factor of RCC is the stage of the lesion [21,22]. Tumor grade, necrosis, sarcomatoid and rhabdoid differentiation are also considered to assess the prognosis. Prognostic molecular markers add valuable information to the standard pathological variables. There is no such established molecular marker in clinical management of RCC. Such markers play a significant role in decision making, namely optimization of treatment protocol after surgery. Studies were carried out to find out the significance of beta-catenin in tumorigenesis and their quantitative and qualitative changes have been linked to tumor progression and prognosis in urological cancers [23-25]. For RCC there are contradictory reports of beta-catenin expression and its association with stage, grade and other prognostic factors. No one, to our knowledge, has attempted to find out correlation between beta-catenin expression and stage and grade.

Kim et al. found cytoplasmic beta-catenin in 27%

clear cell RCC case, but not in papillary and chromophobe RCC [26]. In contrast, Guo et al. observed that positive frequency and staining score of beta-catenin in papillary and chromophobe RCC was significantly higher than conventional RCC [27].

Krabbe et al. found decreased membranous and increased cytoplasmic beta-catenin expression found to be separately associated with higher pathological stage, higher Fuhrman grade and adverse pathological features including sarcomatoid differentiation, necrosis, lymphovascular invasion in clear cell RCC ($p < 0.001$) [19]. Kruck et al. had observed that in clear cell RCC high membranous beta-catenin was associated with advanced stage, vascular invasion and tumor necrosis ($p \leq 0.01$) and high cytoplasmic beta-catenin associated with higher stage and grade, vascular invasion and sarcomatoid differentiation ($p \leq 0.01$) [18]. Kovacs et al. have demonstrated that cytoplasmic expression of beta-catenin conventional RCCs significantly associated with the progression of disease from low grade to high grade ($p < 0.001$) [28]. Guo et al. had observed that in conventional RCCs the positive frequency and staining score of beta-catenin showed a significant difference between Fuhrman nuclear grades I/II and grade III ($p < 0.05$) [27]. Bilim et al. noticed that the loss of membranous beta-catenin was associated with tumor stage 3 ($p < 0.05$, $\chi^2 = 4.8$) and nodal metastasis ($P \leq 0.05$, $\chi^2 = 5.86$) and preserved staining associated with tumor grade 1 ($p = 0.005$, $\chi^2 = 7.8$) [17]. Aaltoma et al. noticed that weak expression intensity of beta-catenin on cell membranes was related to venous growth inside the tumor ($p = 0.02$), extra tumoural venous growth ($p = 0.03$) and to perineural growth ($p < 0.001$) [29].

In the present study the membranous score was found to be gradually decreasing with the progression of tumor from low stage to high stage. It had a negative correlation with stage of RCC ($r = -0.407$, $p = 0.044$). This observation was found to be concordant with Bilim et al. [17], Krabbe et al. [19] and discordant with Kruck et al [18]. Cytoplasmic score was found to be gradually increasing with progression of tumor from low stage to high stage. Cytoplasmic score was positively correlated with stage ($r = 0.586$, $p = 0.002$). Similar findings were observed by Kruck et al. [18], Kovacs et al. [28] and Krabbe et al [19]. In the present study the membranous score was found to be gradually decreasing with the progression of tumor from low grade to high grade. It had a strong negative correlation with grade of RCC ($r = -0.787$, $p < 0.001$). Krabbe et al. also found similar association with Fuhrman grading [19]. Mean cytoplasmic score difference between lower grade and higher grade was found to be statistically significant ($p < 0.001$). No significant correlation was found between cytoplasmic score and tumor grade. Krabbe et al. [19], Kruck et al. [18] and Kovacs et al [28] also found association between cytoplasmic score and Fuhrman grade of RCC, but no one has further attempted to find out correlation. The small sample size may be the reason

for which no significant correlation was found between cytoplasmic score and grade in the present study.

Both cytoplasmic and membranous score were significantly associated with prognostic parameters like necrosis, sarcomatoid differentiation, rhabdoid differentiation, lymphovascular invasion and perineural invasion ($p < 0.001$). This observation was concordant with Krabbe et al [19].

It was observed that the decreased membranous expression and increased cytoplasmic expression of beta-catenin were separately correlated with pathological adverse features of RCC. This observation is supported by the current understanding of the beta-catenin pathway. Staining for beta-catenin might have important implications in patients undergoing tumor biopsy before treatment decisions either in the form of radical surgery or targeted chemotherapy are undertaken. It might also help in taking decision of postoperative adjuvant therapies. In addition, this identifies beta-catenin as a potentially targetable pathway in RCC that needs further investigation.

Conclusion

Considering the observations of the current study, it can be concluded that beta-catenin may play a crucial role in the carcinogenesis of RCC. Beta-catenin has the potential to be considered as a prognostic molecular marker in the near future. Moreover, beta-Catenin, one of the main component of Wnt signaling pathway can be a critical evaluator of targeted chemotherapy in RCC. As it was a time bound study of relatively small numbers of cases with limited scope of follow up, a wider evaluation involving large number of cases provided with proper follow up facility is recommended to validate its prognostic and therapeutic efficacy.

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