Vimentin Expression in Renal Cell Carcinoma and It's Association with Histological Grade and Pathological Stage

*Adhikary P,[1] Begum S,[2] Ferdous JN,[3] Saha AK,[4] Papry A,[5] Emita U[6]

Abstract

Introduction: Renal cell carcinoma (RCC) is the most common type of kidney cancer and the incidence of it has been steadily increasing over the past few years. The precise prediction of prognosis is very important for planning an effective therapy and follow-up visits. There are some established prognostic factors, like- histological grade, pathological stage and tumor subtypes, but the tumor behavior is difficult to predict and may show different outcome. Immunohistochemical study of several molecular markers may be helpful in this regard.

Objectives: The aim of the present study was to evaluate the expression of vimentin in renal cell carcinoma and to determine it's association with histological types, grades and pathological stages.

Methods: This cross-sectional observational study was conducted with 35 cases of RCC for a period of 2 years in Department of Pathology, Sir Salimullah Medical College, Dhaka. In all of histopathologically diagnosed cases of renal cell carcinoma immunohistochemical staining was done. The collected data were organized and analyzed using Statistical Package for the Social Sciences (SPSS) version 25. Chi-square test, Fisher's exact test and Spearman's correlation test were used for statistical analysis. A p value of < 0.05 was considered as significant.

Results: The mean age of the study population was 53.17 ± 11.95 years & large number of patients (57.1%) were belonged to 41-60 years age group with male predominance (74.3%). Most of the patients (91.4%) presented with hematuria followed by 74.3% had flank pain and 51.4% had palpable renal mass. The most common subtype was clear cell carcinoma (68.6%) followed by papillary carcinoma (22.8%), chromophobe (5.7%) and collecting duct carcinoma (2.9%). Stage pT1 (40.0%) was the most frequent diagnosis of RCC followed by stage pT2 (34.3%) & Stage pT3 (25.7%). Grade 2 RCC was the most frequent diagnosis (40.6%) followed by 28.1% grade 3, 18.8% grade 4 and 12.5% grade 1 RCC. Vimentin expression was negative in chromophobe RCC but other subtypes showed variable degree of cytoplasmic expression of vimentin. Vimentin IHC expression was significantly high (≥8) with increase in the pathological T stage & WHO/ISUP grade of RCC (p-value 0.038 and 0.03 respectively).

Conclusion: Immunohistochemical expression of vimentin showed statistically significant association with the pathological T stage (pT) and histological grades (G) of renal cell carcinoma. Routine use of vimentin expression in conjunction with histopathological grading and staging may provide prognostic information to predict disease progression.

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1. **Dr. Priyanka Adhikary, MBBS, MD (Pathology), Junior consultant, Department of Pathology, Pirojpur District Hospital, Pirojpur, Bangladesh. dr.priyanka003@gmail.com. ORCID: https://orcid.org/0009-0007-0148-2733.*
2. *Dr. Shahnaj Begum, Professor & Head, Department of Pathology, Sir Salimullah Medical College, Dhaka, Bangladesh. dr.shahnaj68@gmail.com ORCID: https://orcid.org/0009-0005-8210-1327.*
3. *Dr. Jesmin Naz Ferdous, MBBS, MD(Pathology), Professor, Department of Pathology, Sir Salimullah Medical College, Dhaka, Bangladesh. limaf888@gmail.com ORCID: https://orcid.org/0009-0009-5412-3958.*
4. *Anjishu Kumar Saha, MSc (Statistics) Statistician, Khulna University, Khulna Bangladesh. anjishusaha9@gmail.com ORCID: https://orcid.org/0009-0004-2122-123X.*
5. *Dr. Afsana Papry, MBBS, MD, Lecturer, Department of Pathology, Sir Salimullah Medical College, Dhaka, Bangladesh. afsana.papry121.ap@gmail.com ORCID: https://orcid.org/0009-0003-6601-3955.*
6. *Dr. Umama-Tun-Nesa Emita, MBBS, MD, Pathologist, Khulna Medical College and Hospital, Khulna, Bangladesh. emita.rmc@gmail.com*

**For correspondence*

Introduction

Renal cell carcinoma (RCC) accounts for approximately 3% of adult cancers and constitutes 80-90% of all primary malignant renal tumors.[1] Though RCC accounts for 2% of global cancer diagnoses and deaths, it has more than doubled in incidence in the developed world over the past half century According to GLOBOCAN 2022 estimated new cases in Bangladesh for RCC were 1440, whereas deaths were 969 and prevalence rate was 2.1% in Bangladesh.[2,3] More than 90% of the renal cell carcinoma arises from the renal tubules.[4]

Formally, RCC was divided into 5 main histologic subtypes: Clear cell, Papillary, Chromophobe, collecting duct and unclassified RCC.[5,6] Clear cell, Papillary and Chromophobe RCC are the three most common types, comprising 70-80%, 14-17% and 4-8% of all RCCs, respectively. Collecting duct carcinoma is the rarest type of RCC (<1/%). Most cases of RCC are discovered incidentally on imaging and survival is highly dependent on the stage at diagnosis.[2] Up to 30% of patients of RCC present with metastatic disease. The median overall survival in patients with metastatic disease is 12 months.[7] Surgery is the mainstay of treatment.[8]

Tumor stage and nuclear grade are usually considered as the main pathological prognostic factors for patients with RCC. However, in many cases, these parameters are insufficient to predict the clinical behavior of these tumors. Because they are helpful in making diagnosis of RCC but most of the time they can not predict the long term outcome of RCC or can not give proper guidance regarding therapy. So, attempts to find out better prognostic criteria remain under investigation.[1] There are several methods for the precise diagnosis of RCC. In addition to pathological diagnosis, various molecular biomarkers like vimentin have been considered as effective diagnostic markers for RCC.[9,10] Vimentin is involved in the development of RCC, and their expression levels are related to the clinical stage, histological grade and tumor size. High expression level of Vimentin is presented in carcinoma tissue with high clinical stage, late histological grade and large tumor.[11] In RCC, Vimentin overexpression is one of the independent predictors of poor clinical outcome and may serve as a useful adjunct in differentiating different pathological types. By virtue of its unique expression pattern in RCC, Vimentin may serve as an attractive target for RCC therapy.[9,10]

The aim of the present study is to evaluate the expression of Vimentin in renal cell carcinoma and to determine it's association with histological types, grades and pathological T stages in order to find out the role of Vimentin in tumor progression, prognosis & whether it can be used as potential target for RCC therapy.

Methods

It was a cross-sectional purposive type of observational study conducted at the Department of Pathology, Sir Salimullah Medical College, Dhaka. The study population was histopathologically diagnosed cases of renal cell carcinoma of any grade and stage. Patients diagnosed as renal cell carcinoma and received pre-operative chemo/radiotherapy, sample obtained by needle biopsy and poorly preserved sample were excluded.

Study procedure

A structured case record form was developed to collect data from the patients. Data was recorded on variables of interest by interview and also from patient's requisition form using the structured questionnaire after taking properly informed written consent from the patient. All the cases were numbered chronologically and the same number was given to histopathological as well as in immunohistochemical slides.

The cases were collected from the Department of Pathology, SSMC and the Department of Pathology, Bangladesh Medical University. Among 35 cases, 16 cases were formalin fixed specimens and 19 cases were paraffin blocks.

After receiving fresh nephrectomy samples, gross examination was done as per standard procedure. All the tissue blocks (16) were submitted for routine processing, paraffin embedding and slides were stained with routine H & E stain. The collected paraffin blocks were given re-cut and subsequent staining was done.

Immunohistochemistry was done in the Department of Pathology, BMU, Dhaka. 3-4 micrometer thick sections of formalin fixed; paraffin-embedded tissues were used.

Immunohistochemical analysis of Vimentin

Primary antibody was Dako FLEX Monoclonal Mouse Anti-Vimentin Clone V9 Ready to use and secondary antibody was EnVision Flex HRP. Positive control was leiomyoma of uterus and sections were examined under x40 magnifications. Distinct granular cytoplasmic staining was taken as positive. Five independent areas of each slide were examined under a microscope & Immuno score was calculated for vimentin-stained slides according to formula.[12,13]

Table I: Immunoscore (Point obtained by % of positive cells) x (staining intensity)

Percentage of positive cells	Points	Staining intensity	Points	Immuno score (cut-off by median)	
≤10%	1	Negative	0	Negative	0
11-40%	2	Weakly positive	1	Low expression	<8
41-75%	3	Moderately positive	2	High expression	≥8
>75%	4	Strongly positive	3		

Statistical analysis

The statistical analysis was carried out using the Statistical Package for Social Sciences version 25 for Windows (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics (frequencies and percentages) were used to summarize the patient's demographic characteristics and presented in tables, figures, charts & diagrams. The frequencies of different entities were expressed as percentages. Fisher Exact test and Chi-square tests were used to analyze the association between different categorical variables. Spearman's correlation test was used to analyze the correlation between categorical variables.

Results

Out of 35 cases, grading was observed in 32 cases of clear cell RCC and papillary RCC. Demographic and histopathological variables (age, sex, morphological variants, tumour stage, and tumour grade) were assessed and immunohistochemical expression of Vimentin was observed. Statistical analysis was performed to establish the association

between histopathological parameters (morphological variant, tumour stage, tumour grade) and immunohistochemical expression of Vimentin.

Table II: Baseline patients' characteristics with histological variables (n=35)

Parameters	Frequency (n)	Percentage (%)
Age groups (years)		
Less than 40	7	20.0
40-60	20	57.1
More than 60	8	22.9
	Mean 53.17±11.95, Range 18-72	
Sex		
Male	26	74.3
Female	9	25.7
	Male: Female - 2.9:1	
Clinical Presentation		
Hematuria	32	91.4
Flank pain	26	74.3
Palpable renal mass	18	51.4
Laterality of tumor		
Right	24	68.6
Left	21	31.4
Pole of tumor		
Upper pole	14	40.0
Lower pole	16	45.7
Whole kidney	5	14.3
Morphological Variants (n-35)		
Clear cell RCC	24	68.6
Papillary RCC	08	22.8
Chromophobe RCC	02	5.7
Collecting duct RCC	01	2.9
TNM stage of RCC (n-35)		
pT3	14	40.0
pT2	12	34.3
pT3	09	25.7
Tumor grade (n-32)		
Grade 1	04	12.5
Grade 2	13	40.6
Grade 3	09	28.1
Grade 4	06	18.8

The mean age of the study population was 53.17 ± 11.95 years and large number of the patients (57.1%) were belonged to 41-60 years age group. Male (74.3%) was predominantly higher than female (25.7%) with male to female ratio of 2.9:1. Among 35 cases, 32(91.4%) patients had hematuria, 26(74.3%) had flank pain and 18(51.4%) had palpable renal mass. 68.6% tumors were in right kidney followed by 31.44% tumors in left kidney. 40% tumors were located in upper pole, 45.7% were in lower pole & 14.3% were in whole kidney. Most were clear cell carcinoma (24 cases, 68.6%), followed by papillary carcinoma (22.8%) chromophobe type (5.7%) and collecting duct carcinoma (2.9%). Stage pT1 (40.0%) was the most frequent diagnosis of RCC followed by stage pT2 (34.3%) and Stage pT3 (25.7%) (shown in table-2). 40.6% cases were grade 2 RCC followed by 28.1% were grade 3 RCC, 18.8%

were grade 4 RCC and 12.5% cases were grade 1 RCC (shown in table-II).

Table III: Distribution of study cases by Vimentin IHC expression (n=35)

Vimentin expression	Frequency (n)	Percentage (%)
Negative	2	5.7
Low expression (score <8)	14	40.0
High expression (score ≥8)	19	54.3
Total	35	100

Immunohistochemical expression of Vimentin were assessed in all cases. 2(5.7%) cases were negative, low expression was observed in 14(40.0%) cases and high expression in 19(54.3%) cases. Shown in Table III.

Table IV: Association between Vimentin IHC expression and morphological variants of RCC (n=35)

Morphological Variants (n)	Negative n(%)	Low expression n(%)	High expression n(%)	p-value
Clear Cell RCC (24)	0(0)	11(45.8)	13(54.2)	
Papillary RCC (8)	0(0)	3(37.5)	5(62.5)	
Chromophobe RCC (2)	2(100)	0(0)	0(0)	**0.009[s]**
Collecting duct RCC (1)	0(0)	0(0)	1(100)	
Total (35)	2	14	19	

s-significant, p value was determined by Fisher's exact test

In the present study, Vimentin IHC expression were found in all variants of RCC except Chromophobe RCC. Among them 54.2% clear cell RCC, 62.5% papillary RCC & 100% collecting duct RCC showed high expression of Vimentin, that was statically significant (p=0.009). Shown in Table-IV

Table V: Association between Vimentin IHC expression and pathological T stage of RCC (n=35)

Pathological stage (n)	Negative n(%)	Low expression n(%)	High expression n(%)	p-value
pT1(14)	1(7.1)	9(71.5)	4(21.4)	**0.038[s]**
pT2(12)	0(0)	4(33.3)	8(66.7)	
pT3(9)	1(11.1)	1(11.1)	7(77.8)	
Total (35)	2	14	19	

s-significant, p value was determined by Fisher's exact test

Vimentin IHC expression became progressively higher with increase in the pathological stage of RCC. High expression was observed in 21.4% of pT1, 66.7% of pT2 & 77.8% of pT3 RCC that was statistically significant (p-value 0.038). Shown in Table-V

Table VI: Association between vimentin IHC expression and grade of RCC (n=32)

Grade of RCC	Low expression n (%)	High expression n (%)	p-value
Grade 1	3(75.0)	1(25.0)	**0.03**[s]
Grade 2	8(61.5)	5(38.5)	
Grade 3	3(33.3)	6(66.7)	
Grade-4	0(0)	6(100)	
Total	14	18	

s-significant, p value was determined by Chi square test

IHC expression of Vimentin significantly increased as the grade of RCC increased (p-value 0.03). High expression was found in 25.0% of grade 1, 38.5% of grade 2, 66.7% of grade 3 and 100% of grade 4 RCC. Shown in Table-VI.

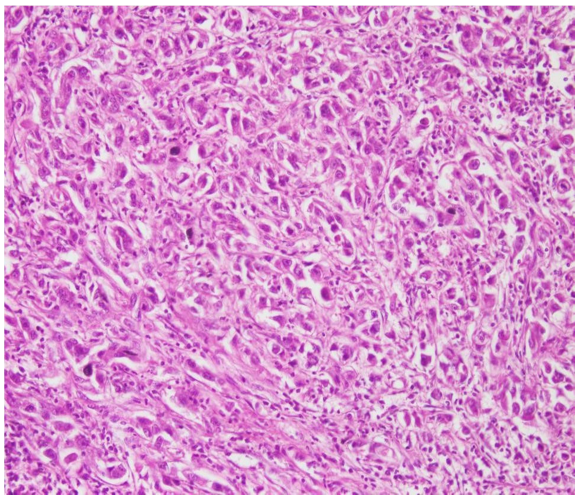

Figure 1. Clear Cell RCC with sarcomatoid features, histological grade 4 (H & E, x20)

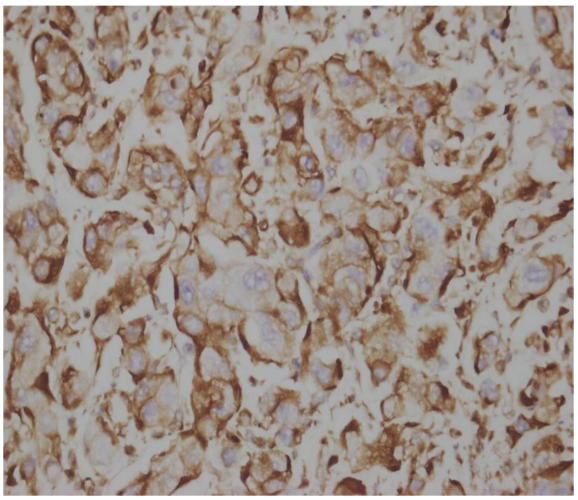

Figure 2. Clear Cell RCC (G4) with high Vimentin expression (IHC, x40)

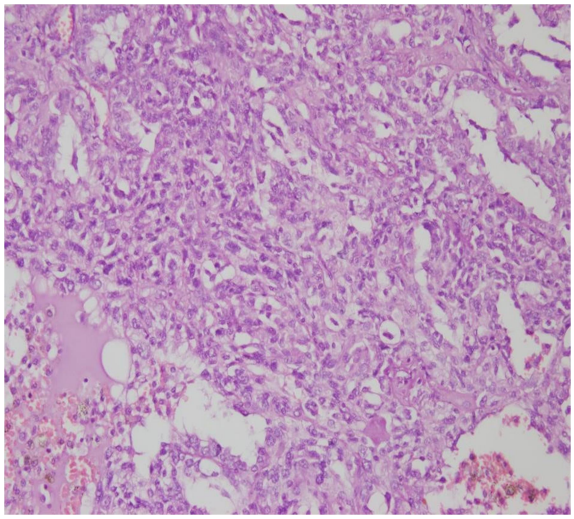

Figure 3. Papillary RCC, histological grade 4 (H & E, x20)

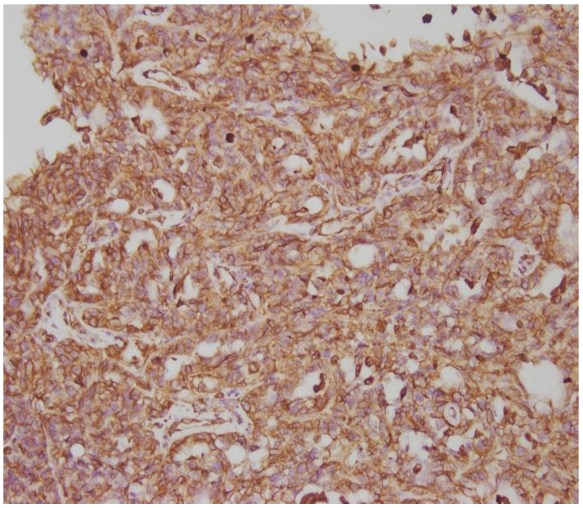

Figure 4. Papillary RCC (G4) with high Vimentin expression (IHC, x20)

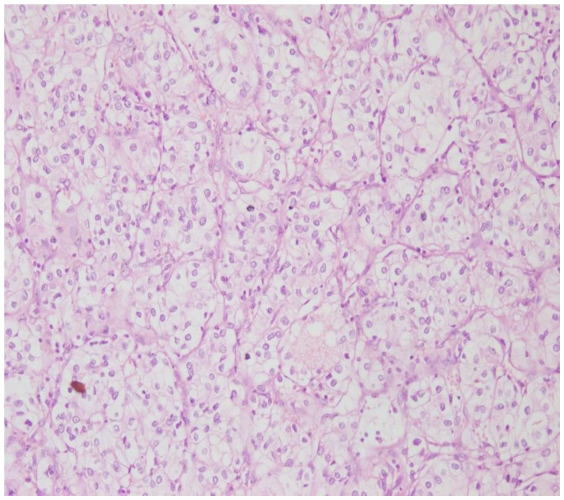

Figure 5. Clear cell RCC, histological grade 2(H & E, x20)

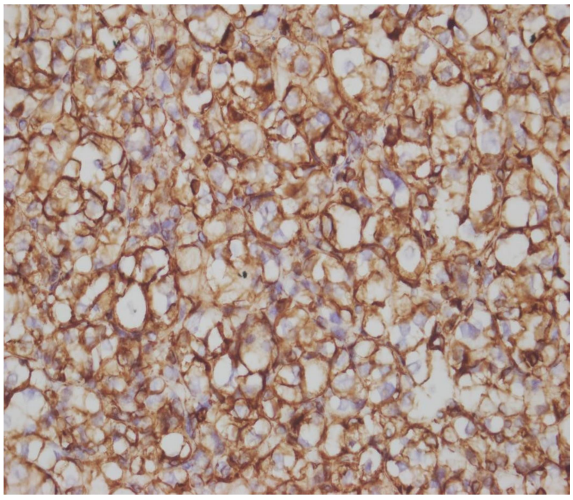

Figure 6. Clear cell RCC (G2) with high Vimentin expression (IHC, x20)

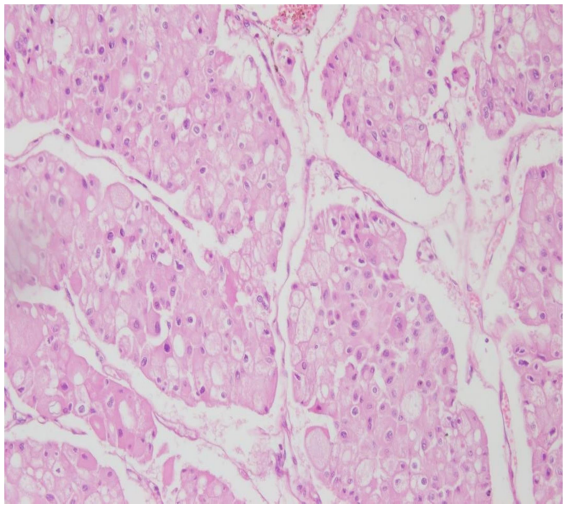

Figure 7. Chromophobe RCC (H & E, x20)

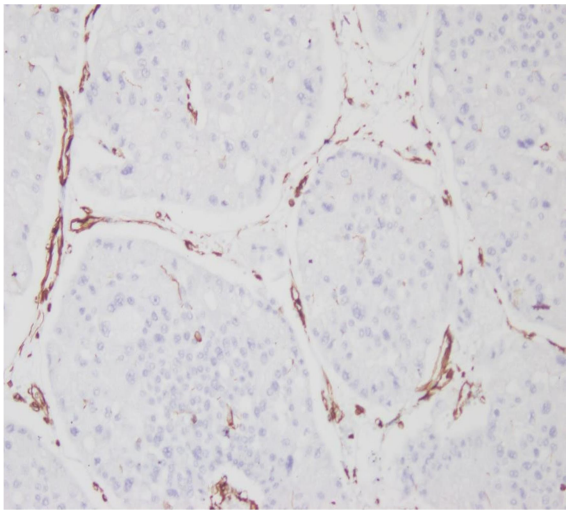

Figure 8. Chromophobe RCC with negative Vimentin expression (IHC, x20)

Discussion

The current study found four histological subtypes of renal cell carcinoma. The most common subtype was clear cell carcinoma (68.6%) followed by papillary carcinoma (22.8%), chromophobe (5.7%) and collecting duct carcinoma (2.9%) respectively. Similar findings were observed in many studies; i.e Likhiteswer Pallagan et al., 2021; Hossain et al., 2022; Du Plessis et al., 2020 and Gargi Mukherjee et al., 2016 studies where common subtype was clear cell carcinoma (68.8%,74.0%, 74.0%, 71.8% respectively).[14-17]

In present study, staging of each tumor was done by using 8th (AJCC) staging system of RCC. Here no regional lymph node metastasis and distant metastasis were seen. So staging was done only on primary tumour (pT). Stage pT1 (40.0%) was the most frequent diagnosis of RCC followed by stage pT2 (34.3%) & Stage pT3 (25.7%). This result is similar to Pramanik et al., 2019; Pehlivan et al., 2014; Esat KORĞALI et al., 2015 and Osman et al., 2015 studies where stage pT1 RCC was the most frequent diagnosis (40.6%, 34.2% 57.2% and 54.7% respectively).[18-21]

WHO/ISUP (2022) grade is followed only in clear cell and papillary renal cell RCC. So, grading of chromophobe and collecting duct RCC were not considered here. Out of 35 cases, grading was observed in 32 cases of clear cell and papillary RCC. Observation of 32 cases revealed that, 40.6% cases were grade 2 RCC followed by 28.1% were grade 3, 18.8% were grade 4 and 12.5% cases were grade 1 RCC that is similar with Gargi Mukherjee et al., 2016 study where 3% of the RCC cases were grade 1 followed by 49% cases grade 2, 32% cases grade 3 and 16% cases were grade 4 RCC. Another study conducted by Esat KORĞALI et al., 2013 found 0.6% cases were grade 1, 42.2% were grade 2, 31.7% were grade 3 & 21.3% were grade 4 RCC which is also consistent with this study.[17,20]

Immunohistochemical expressions of vimentin were assessed in all cases of this study. Two (5.7%) cases were negative, low expression (<8) was observed in 14(40.0%) cases & high expression (≥8) in 19(54.3%) cases that is similar to the study conducted by Jia xi Yao et al., 2020 where 46.32% (n=107)

cases of mRCC were considered to have low vimentin expression, whereas 53.68% (n = 124) cases of mRCC were considered to have high vimentin expression.[22] Vimentin expression was found in all variants of RCC except Chromophobe type RCC. Among them 54.2% clear cell type RCC, 62.5% papillary type RCC & 100% collecting duct type RCC showed high expression of Vimentin, that was statistically significant (p=0.009). Gargi Mukherjee et al., 2016 had shown Vimentin was expressed in 100% Clear Cell and Papillary RCC and negative in Chromophobe RCC.[17]

Regarding association between Vimentin expression with pathological T stage of RCC in this study, Vimentin expression became progressively higher with increase in the pathological T stage of RCC. High expression was observed in 21.4% of stage pT1, 66.7% of stage pT2 & 77.8% of stage pT3 RCC that was statistically significant (p-value 0.038). A study conducted by Jia xi Yao et al., 2020 observed high vimentin expression in 48.4 % of early stage RCC & 58.1% of late stage RCC.[22] Liu Decan et al., 2018 found high expression level of Vimentin in RCC with higher clinical stage.[23] During observation of association between vimentin expression with WHO/ISUP Grade of RCC in this study, expression of Vimentin significantly increased as the grade of RCC increased (p-value 0.03). High expression was found in 25.0% cases of grade 1 RCC 59 followed by 38.5% cases of grade 2, 66.7% cases of grade 3 and 100% of grade 4 RCC. Similar findings were observed in many studies like Liu Decan et al., 2018 ; Gargi Mukherjee et al., 2016 and Jia xi Yao et al., 2020.[11,17,22] Jia xi Yao et al., 2020 study had shown high vimentin expression in higher grade.[22] Gargi Mukherjee et al., 2016 study found strong vimentin positivity in those cases having sarcomatoid changes (high grade) (P-value is <0.00001).[17] Liu Decan et al., 2018 conducted a study on "Expression of Vimentin and Ki-67 in renal carcinoma and its clinical significance" and found high expression level of Vimentin in RCC with late histological grade.[17]

Conclusion

Routine use of Vimentin IHC expression in conjunction with histopathological grading and staging in renal cell carcinoma may provide prognostic information to predict disease progression and outcome of patients with renal cell carcinoma.

Limitations

In this study, as regional metastasis & distant metastasis were not known, proper tumour staging (pTNM) could not be done. Follow-up of the patients could not be done, so, the clinical outcome of the patients could not be known.

Recommendations

Routine use of Vimentin immunostaining in RCC specimen with routine Hematoxylin & Eosin stain may help clinicians and patients regarding surgical management and advanced treatment such as adjuvant chemotherapy, radiotherapy, or targeted therapy.

Larger sample size, longer duration, multi-center studies and use of other biomarkers and molecular studies would bring out more precise representative data.

Long-term follow-up of the patients to correlate the expression of this molecular marker with the progression and recurrence of the disease and survival of the patients is needed to understand the prognostic role of this biomarker.

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