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Original Research Article

Expression of proliferating cell nuclear antigen and Ki-67 in renal cell carcinoma in eastern Indian patients

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ABSTRACT

Background: Several molecular markers play important role in development and prognosis of renal cell carcinoma (RCC). Proliferating cell nuclear antigen (PCNA) and Ki-67 are such kind of molecular markers which may have prognostic significance in RCC and need to be studied about. Estimation of proliferation index by immunohistochemical expression analysis of PCNA and Ki-67 at different clinical stages of RCC samples and correlations of expression of the genes with different clinicopathological parameters between tumour tissue cells and adjacent normal tissue cells were the objectives.
Methods: Thirty two patients of RCC who had been operated at one tertiary care institute of eastern India were taken for the study. Histopathological and immunohistochemistry analysis of PCNA and Ki-67 from tumour tissue and normal tissue were done. Patients who received radiotherapy, chemotherapy etc. before operation and who had benign tumours of the kidney in histopathological examination were excluded from the study.
Results: Mean PCNA expression in normal renal tissue is 4.54%; whereas the clear cell RCC, papillary RCC and chromophobe RCC showed 49.81%, 50.75% and 66.50% of mean PCNA expression respectively. Mean expression of Ki-67 in normal tissues was 1.75%. Whereas the clear cell RCC, papillary RCC and chromophobe RCC showed 23.96%, 24.75% and 31% of mean Ki-67 expression respectively. Both molecular markers were positively correlated overall.
Conclusions: PCNA and Ki-67 expression is increased in RCC when compared with normal tissues and it increases with Stage of RCC. PCNA expression is positively correlated with Ki-67 in different stages and histopathological groups of RCC.

Keywords: PCNA, Ki-67, Renal cell carcinoma

INTRODUCTION

Renal cell carcinoma (RCC) is an adenocarcinoma, accounting for 3% of all adult malignancies.[1] The incidence of advanced stage tumours and the mortality arising from renal cell carcinoma have been increasing.[2] Male-to-female ratio is of 3:2.[2] It is a disease of older adults, with usual presentation between 50 and 70 years of age.[3] Patients who have disease in early stage are cured by surgical resection, but metastatic disease remains almost incurable. Hence the identification of prognostic markers in targeted therapy is required.

Most of the cases of RCC are sporadic; 2% to 3% are proven to be familial.[4]

Clear cell carcinoma (ccRCC) is the most common histologic type of renal cell carcinoma accounting for approximately 70%- 80% of renal neoplasm, and arises from the proximal convoluted tubule.[4] However, other types of RCC like papillary (10%), chromophobe (5%) and oncocytoma (5%) are found to be relatively rare.[5]

Patient's prognosis depends on multiple clinico-pathological factors such as TNM stage, Fuhrman nuclear grade, tumour size, and other haematological indices.[6]

In addition to clinicopathological prognostic features, several molecular markers play important role in predicting prognosis of renal cell carcinoma.[7] It is evident that proliferation index is associated with its stage and grade. For that purpose, it is pertinent to analyze the proliferation status during development of RCC. This may have diagnostic and prognostic implications. Here comes the importance of PCNA (Proliferating cell nuclear antigen).

Proliferating cell nuclear antigen (PCNA) is an essential element for replication and is basically a DNA clamp acting as a DNA polymerase δ processivity factor in cells. PCNA encircles DNA and operate as scaffold for recruiting proteins which are involved in replication and repair of DNA, epigenetics and chromatin remodelling.[8]

A number of proteins have interaction with PCNA by means of AlkB homologue 2 PCNA interacting motif and PCNA interacting peptide box.[9,10] Proteins which bind to PCNA through these two PCNA interacting motifs are required in the process of DNA replication and management of genotoxic stress.[11]

Antibody labelling of nuclear PCNA distinguishes between different S phases of cell cycle.[12] PCNA has got the potential to become a therapeutic target in management of cancer.[13]

KI-67 is an antigen encoded by MKI67 gene which is identified with help of monoclonal antibody against Ki-67.[14,15] It is a type of nuclear protein associated with cellular proliferation. It is also associated with transcription of ribosomal RNA.[15] KI-67 inactivation causes inhibition of the synthesis of ribosomal RNA.[16]

Ki-67 is also a cellular proliferation marker.[17] It is present throughout G_1 phase, S phase, G_2 phase and mitosis of cell cycle and is absent in G_0 phase.

Ki-67 is also an outstanding marker for growth of a population of cells. Labelling index of Ki-67 (fraction of tumour cells which are Ki-67 positive) correlates well with clinical progress of the cancer.[18]

Thus, PCNA and Ki67 dual markers may have important diagnostic and prognostic roles in RCC. However, there is limited study in this regard in Indian patients. Hence the authors decided to study the expression of PCNA and Ki-67 in RCC patients of Eastern India.

METHODS

This prospective study was done from August 2016 to August 2018 at the Urology Department of one tertiary care institute of eastern India (IPGMER, Kolkata) and at the Department of Oncogene Regulation of another premier cancer research institute of eastern India (CNCI, Kolkata). Prior approval was taken from the Institutional Ethical Committee. A number of thirty two patients who got operated for renal mass with histologically proven renal cell carcinoma were taken for the study after taking written consent from them. Subject inclusion criteria incorporated renal tumour that had not received any treatment like radiotherapy, chemotherapy etc. before operation.

Exclusion criteria included renal cancer patients who received radiotherapy, chemotherapy etc. before operation and benign tumours of the kidney in histopathological examination.

Part of freshly operated renal cancer tissues was collected from urology operation theatre of that institute. For control sample, normal renal tissue adjacent to the tumour was taken. The RCC samples with normal tissue samples were fixed in 10% phosphate buffered formalin and embedded in paraffin. Sections were stained by haematoxylin and eosin for routine histopathological analysis according to standard protocol.

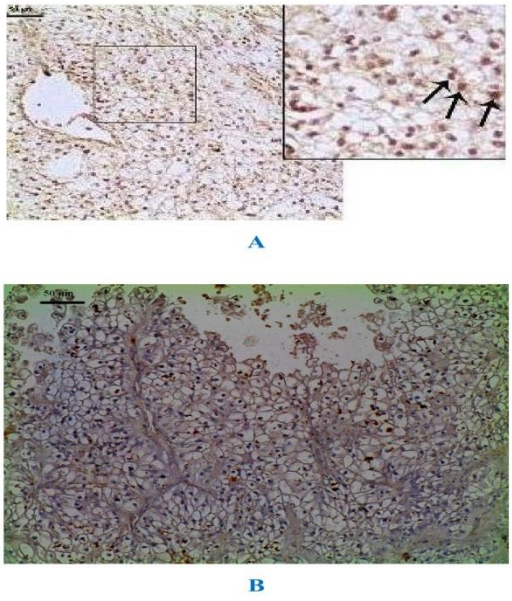

Figure 1: (A) Photomicrographs of expression of PCNA in ccRCC sample, magnification: 20X, inset magnification: 40X, scale bar 50 µM. Arrows indicate PCNA positive nuclei; (B) representative photograph of Ki-67 expression in ccRCC sample, magnification: 20X, scale bar 50 µM.

Immunohistochemical (IHC) expression analysis of PCNA and Ki-67 in tumour / normal tissue samples was done. The particular protein expression in test sample was compared with the expression of normal. The staining intensity and the percentage of immunopositive cells were determined (Figure 1).

RESULTS

Thirty two patients were included in the study. Their normal and tumour tissues were analysed by histopathological and immunohistochemical examination.

Statistical analysis for expression of PCNA and Ki-67 was done with EPI INFO (TM) 7.2.2.2. Chi square (χ^2) test was done to determine association of variables with different study groups. Significant difference between two proportions was tested with Z-test (Standard Normal Deviate). Means were compared using T-test. One way analysis of variance (ANOVA) was used to compare more than two means at a time and also Tukeys test followed by one way ANOVA were used to calculate critical difference (CD) to compare the means pair wise. P<0.05 was considered as statistically significant.

Amongst 32 patients, 34.4% were below or equal to 50 years and 65.6% were more than 50 years. Average age of patients was 54 years with standard deviation of 10.4.

75% patients were found to be male and 25% were female. Male to female sex ratio was 3:1. 78.1% of patients (25 in number) were found to be exposed to some form of substance abuse only. 21.9% patients were having no substance abuse. 68.75% patients were symptomatic at the time of presentation. Only 31.2% of patients were incidentally diagnosed.

62.5% of patients were presented with hypertension in which 40% had only hypertension and 20% were associated with diabetes. Hypertension was the most prevalent co-morbidity in our study. Clear cell carcinoma is the most common (81.2%) histopathological finding among patients of renal cell carcinoma. According to polarity lower pole was the most common site of tumour occurrence in our study (either involving only mentioned site or >50% of the tumour was situated in that region). 53% patients presented with right sided tumour. 40.6% of the patients presented with stage 1 and 2 each. Only 18.8% of patients had stage 3 renal malignancy.

Mean expression of PCNA in normal tissues was 4.54%. Whereas the clear cell RCC, papillary RCC and chromophobe RCC showed 49.81%, 50.75% and 66.50% of mean PCNA expression respectively (Table 1).

One ANOVA showed that there were significant differences in mean expression of PCNA of the patients for different types of HPE of the patients ($F_{3,56}$=129.75; p<0.001). As per Tukeys critical difference (CD) the mean expression of PCNA of the patients with Chromophobe tumour was significantly highest of all and that was the lowest of all for normal cases (p<0.001). However, no significant difference was found between the mean expression of PCNA of the tumours with ccRCC and papillary renal cell carcinoma (p>0.05).

Table 1: Comparison of expression of PCNA of the patients according to the findings of HPE of the patients.

Findings of HPE	Mean±S.D.	Median	Range	F-value	P value
Normal (n=28)	4.54±2.20	4	1-9	$F_{3,56}$=129.75	<0.0001 (statistically significant)
ccRCC (n=26)	49.81±13.43	48	24-79		
Papillary (n=4)	50.75±4.43	51	46-55		
Chromophobe (n=2)	66.50±2.12	66.5	65-68		

Table 2: Comparison of expression of Ki-67 of the patients according to the findings of HPE of the patients.

Findings of HPE	Mean±S.D.	Median	Range	F-value	P value
Normal (n=28)	1.75±0.65	2	1-3	$F_{3,56}$=169.96	<0.0001 (statistically significant)
ccRCC (n=26)	23.96±5.52	23	15-34		
Papillary (n=4)	24.75±4.19	25.5	19-29		
Chromophobe (n=2)	31.00±5.66	31	27-35		

Mean expression of Ki-67 in normal tissues was 1.75%. Whereas the clear cell RCC, papillary RCC and chromophobe RCC showed 23.96%, 24.75% and 31% of mean Ki-67 expression respectively (Table 2).

One ANOVA showed that there were significant differences in mean expression of KI-67 of the patients for different types of HPE of the patients ($F_{3, 56}$=129.75; p<0.001). As per Tukeys critical difference (CD) the mean expression of KI-67 of the patients with Chromophobe tumour was significantly highest of all and that was the lowest of all for normal cases (p<0.001). However, no significant difference was found between the mean expression of Ki-67 of the tumours with ccRCC and papillary (p>0.05).

Stage I, II and III of RCC showed 39.62%, 53.23% and 70.67% of mean PCNA expression respectively (Table 3).

Table 3: Comparison of expression of PCNA of the patients according to the stage of cancer of the patients.

Stage	Mean±S.D.	Median	Range	F-value	P value
Normal (n=28)	4.54±2.20	4	1-9	$F_{3,56}$=586.80	<0.0001 (statistically significant)
Stage I (n=13)	39.62±7.27	40	24-50		
Stage II (n=13)	53.23±4.66	54	46-60		
Stage III (n=6)	70.67±5.05	69	65-79		

Table 4: Comparison of expression of Ki-67 of the patients according to the stage of cancer of the patients.

Stage	Mean±S.D.	Median	Range	F-value	P value
Normal (n=28)	1.75±0.65	2	1-3		<0.0001 S (statistically significant)
Stage I (n=13)	20.23±3.88	20	15-29		
Stage II (n=13)	25.62±3.93	26	19-31		
Stage III (n=6)	31.33±2.88	31	27-35		

One ANOVA showed that there were significant differences in mean expression of PCNA of the patients for different stages of cancer of the patients ($F_{3,56}$=586.80; p<0.001). As per Tukeys critical difference (CD) the mean expression of PCNA of the patients with Stage-III tumour was significantly highest of all and that was the lowest of all for normal cases (p<0.001). The expression of PCNA increased significantly with the increase in Stage of cancer.

Stage I, II and III of RCC showed 20.23%, 25.62% and 31.33% of mean Ki-67 expression respectively (Table 4).

One ANOVA showed that there were significant differences in mean expression of Ki-67 of the patients for different stages of cancer of the patients ($F_{3,56}$=368.99; p<0.001). As per Tukeys critical difference (CD) the mean expression of KI-67 of the patients with Stage-III tumour was significantly highest of all and that was the lowest of all for normal cases (p<0.001). The expression of Ki-67 increased significantly with the increase in stage of cancer.

Correlation between PCNA and Ki-67 for different HPE Findings: In overall, there was positive correlation between these two markers (r_{62} = 0.981; p<0.001). The values of PCNA and Ki-67 significantly positively correlated. Thus the values of PCNA significantly increased with the values of Ki-67 (Figure 2A).

For normal tissue, there was positive correlation between these two markers (r_{30}=0.488; p<0.001). The values of PCNA and Ki-67 significantly positively correlated. Thus the values of PCNA significantly increased with the values of Ki-67 (Figure 2B).

For ccRCC, there was positive correlation between these two markers (r_{24}= 0.878; p<0.001). The values of PCNA and Ki-67 significantly positively correlated. Thus the values of PCNA significantly increased with the values of Ki-67 (Figure 3A).

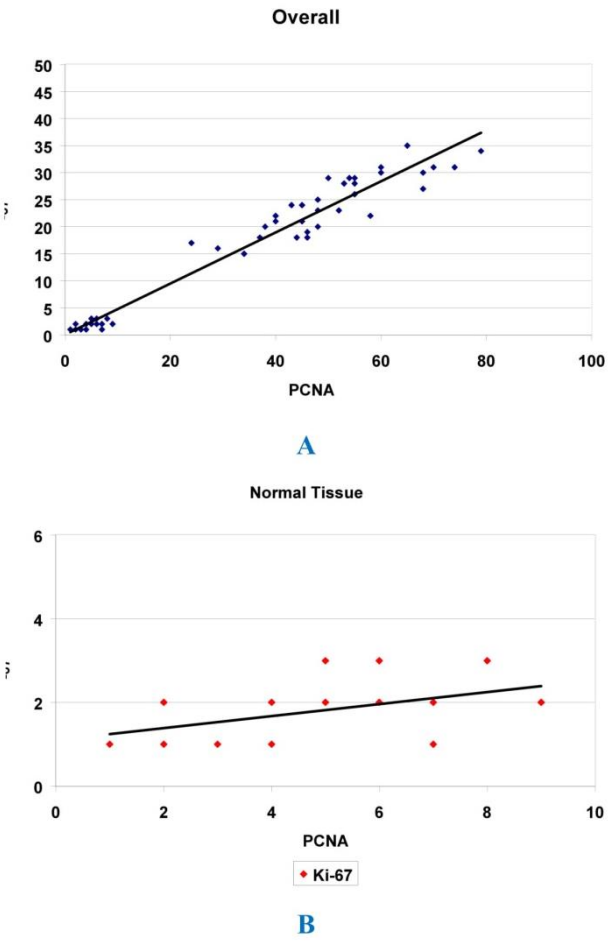

Figure 2: (A) Overall correlation between PCNA and Ki-67; (B) correlation between PCNA and Ki-67 for normal tissue.

For papillary RCC, there was positive correlation between these two markers (r_2 = 0.821; p<0.001). The values of PCNA and Ki-67 significantly positively correlated. Thus the values of PCNA significantly increased with the values of Ki-67 (Figure 3B).

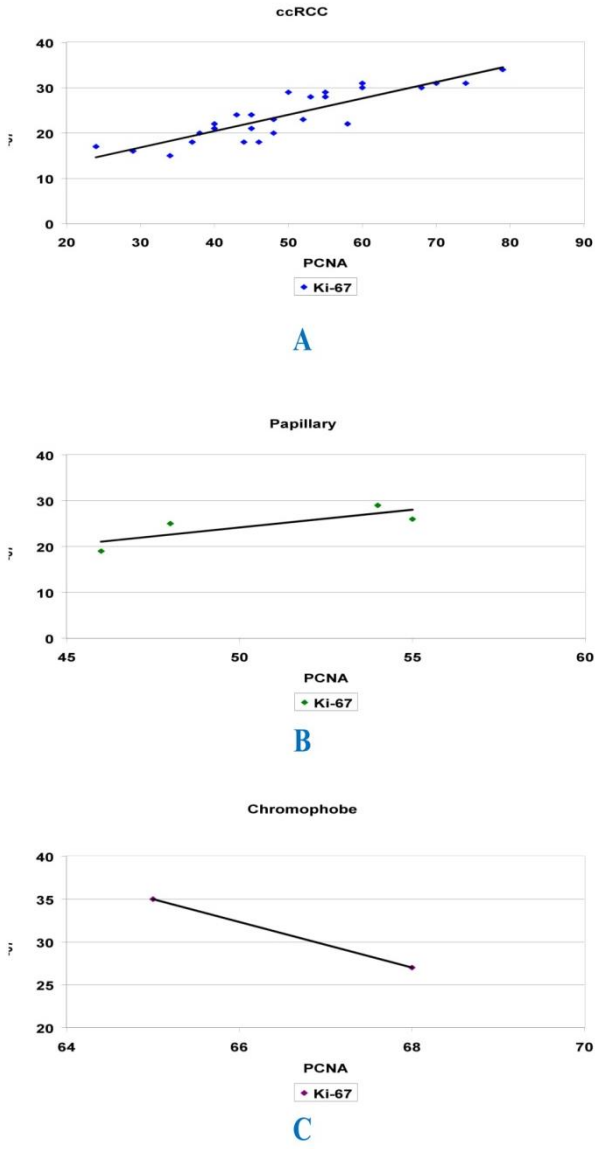

Figure 3: (A) Correlation between PCNA and Ki-67 for CCRCC; (B) correlation between PCNA and Ki-67 for papillary RCC; (C) correlation between PCNA and Ki-67 for chromophobe RCC.

For chromophobe RCC, there was negative correlation between PCNA and Ki-67 (r_1=-0.910; p<0.001). The values of PCNA and Ki-67 significantly negatively correlated. Thus the values of PCNA significantly decreased with the values of Ki-67 (Figure 3C).

Correlation between PCNA & Ki-67 for different stages of RCC: For stage-I, there was positive correlation between these two markers (r_{11}=0.697; p<0.001). The values of PCNA and Ki-67 significantly positively correlated. Thus the values of PCNA significantly increased with the values of Ki-67 (Figure 4A).

For stage II, there was positive correlation between these two markers (r_{11} = 0.725; p<0.001). The values of PCNA and Ki-67 significantly positively correlated. Thus the values of PCNA significantly increased with the values of Ki-67 (Figure 4B).

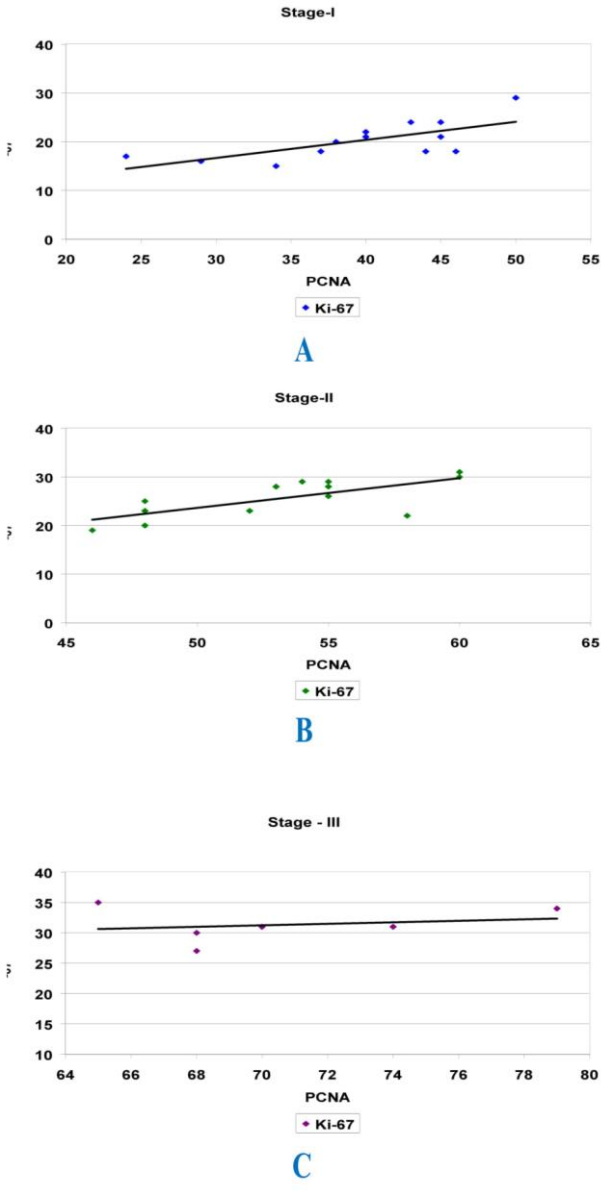

Figure 4: (A) Correlation between PCNA and Ki-67 for stage I RCC; (B) correlation between PCNA and Ki-67 for stage II RCC; (C) correlation between PCNA and Ki-67 for stage III RCC.

For stage III, there was positive correlation between these two markers (r_4 = 0.215; p=0.681). The values of PCNA and Ki-67 positively correlated but it was not significant (Figure 4C).

DISCUSSION

Thirty two Radical Nephrectomy samples were evaluated during study period. As written in the literature RCC is more prevalent in the 6th to 7th decade of life, this study

also shows 65.6% of patients were in 6th decade of life or older.[3] As stated before male are predominantly affected by RCC, this study also shows male predominance i.e. 75%.[3]

Tobacco is a well-known established risk factor for RCC. In a study it is found that 20 to 30% of total RCC were associated with tobacco abuse.[19] But this study revealed that 78.1% of patients were using it in some form. As revealed in various studies, this research also shows clear cell carcinoma as the most common histopathological subtype in RCC. This is 81.2% in this study followed by papillary RCC 12.5%.[4]

To understand the association of Ki-67 with potential for proliferation of cells of RCC, expression of PCNA was measured. 4.54% of the normal tissues cells had nuclear PCNA expression and mainly tubular epithelial cells were discovered as proliferating cells. ccRCC samples had increased proliferation (49.8%) which was statistically significant ($p<0.05$). Other subtypes also had increased proliferation (50.7%-66.5%) all through the lesions when compared to normal tissue. Stage I had significantly increased proliferation (39.6%) whereas there was steady increase in proliferative index in stage II and stage III which were 53.2% and 70.6% respectively. It indicates the clinical importance of this marker with evolution of the cancer. Previous studies have also documented stage wise increment in expression of nuclear PCNA in RCC indicating the importance of this marker in prognosis of the disease.[20,21]

Few previous studies have documented that labelling of Ki-67 antigen has got correlation with proliferation of tumour cells as well as clinical outcome in wide spectrum of malignancies. Expression of Ki-67 has been found to be ranging from <1% to 30% in RCC, as per previous studies.[22,23]

This study also corroborates with the finding of previous studies. Normal tissues had 1.75% of cells showing Ki-67 expression. There were significantly ($p<0.05$) increased expression in ccRCC samples (23.96%) and other different subtypes (24.75%-31%). Significantly increased expression was noticed in cases of stage I (20.23%) followed by steady increase in cases of stage II (25.62%) as well as stage III (31.33%), indicating the clinical importance of this marker with tumour progression.

In few of the new studies Ki-67 labelling has been compared with tumour grade and there was positive correlation between these two variables.[24]

In 1993, de Riese et a1, upon examination of the prognostic significance of Ki-67 antigen expression in a series of 58 node-negative RCCs, concluded that, Ki-67 staining had positive correlation with tumour grade as well as tumour recurrence.[25] Survival analysis marked Ki-67 antigen labelling as a good predictor of outcome independent of both histologic grade and tumour stage on multivariate testing.[25]

When the authors compared the expression of PCNA with Ki-67 in RCC patients, they found that the expression of PCNA, in the current study was consistently higher than that of Ki-67. This can be clarified by the findings of previous studies which give an explanation that "low levels of PCNA are present in actively proliferating cells, with accentuation of levels being observed during the S-phase of the cell cycle" and PCNA has a longer half life than Ki-67.[26]

However the authors were able to find that expressions of both these molecular markers are significantly raised with increment in stage of the disease and expression varies in different histological types. These findings certainly have some significance in prognostication of the disease. Nevertheless larger studies are required for reinforcing their findings.

CONCLUSION

Findings of this study illustrated that expression profiles of Ki-67 and PCNA have significant association with RCC irrespective of different sub-types. This may have implication probably in diagnosis as well as disease prognosis. PCNA and Ki-67 expression is increased in RCC when compared with normal tissues. Their expression increases with stage of RCC. PCNA expression is positively correlated with another marker Ki-67 in different stages and histopathological groups of RCC except chromophobe RCC where it is negatively correlated. They can be potential therapeutic targets in future. However, our findings were acquired in small population of RCC patients and additional structured studies are required in large RCC population in this regard.

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