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Original Article

Pathology Section

Histopathological Evaluation and Analysis of Immunohistochemical Markers p53 and CD44S in Renal Cell Carcinoma: A Cross-sectional Study

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ABSTRACT

Introduction: Renal Cell Carcinoma (RCC) is the 9th and 14th most common cancer in men and women, respectively, and among the most lethal urological cancers. Upto 30% of patients experience recurrence within five years postnephrectomy. Therefore, the predictors of disease survival and molecular markers associated with clinical prognostic parameters should be identified.

Aim: To examine the histopathological features of RCC and investigate the association of Tumour protein p53 (p53) and Cluster of Differentiation 44S (CD44S) expression with prognostic factors.

Materials and Methods: The present prospective cross-sectional study was conducted in the Department of Pathology, M.S Ramaiah Medical College and Hospitals, Bengaluru, Karnataka, India, from June 2017 to May 2019. Radical and partial nephrectomy specimens of RCC patients were obtained for the study. In each case age, tumour histologic type, tumour size, Fuhrman nuclear grade, rhabdoid features, necrosis, tumour stage and p53 and CD44S status were evaluated through Immunohistochemistry (IHC). The Chi-square test was used to evaluate differences in CD44S and p53 expression among different groups. Data were entered in Microsoft (MS) excel version 11 and was analysed using International Business Machines Statistical Package for the Social Sciences (IBM SPSS) software version 20.0.

Results: The study included 50 RCC patients with a mean age of 56.64 years and male:female ratio was 2.8:1. CD44S and p53 positivity were noted in 33 (66%) and 27 (54%) patients, respectively. Weak, moderate, and strong CD44S expression were observed in 13 (26%), 8 (16%) and 12 (24%) patients, respectively and those of p53 in 9 (18%), 14 (28%) and 13 (26%) patients, respectively. Patients with higher Fuhrman nuclear grades exhibited stronger CD44S (n=24, 72.7%) and p53 (n=19, 70.3%) expression than did those with lower grades.

Conclusion: The clear cell carcinoma is the most common histologic subtype. Presence of rhabdoid features and necrosis is seen with advanced Tumour, Nodal and Metastasis (TNM) stage. A significant association between p53 and CD44S immunohistochemical expression and higher Fuhrman nuclear grade suggests increased expression of p53 and CD44S and indicates an aggressive clinical course that can be used as a marker of poor prognosis.

Keywords: Fuhrman nuclear grading, Prognosis, Rhabdoid differentiation, Tumour necrosis

INTRODUCTION

The RCC represents approximately 2-3% of all cancers, with the highest incidence occurring in Western countries [1]. During the last two decades, an annual 2% increase in RCC incidence has been noted both worldwide and in Europe, leading to 99,200 new RCC cases and 39,100 kidney cancer-related deaths in the European Union in 2018 [1]. The prognosis of RCC is poor once metastasis begins [2]. Therefore, the predictors of disease survival and molecular markers related to clinical prognostic parameters and staging should be identified. Histopathological parameters, such as stage, tumour size, histologic subtype and nuclear grade, should be examined in tumour nephrectomy specimens [3]. Several studies have focused on individual tumour aggressiveness markers in RCC, and the roles of p53 and CD44S were recently investigated [4].

The p53 tumour-suppressor gene is a critical regulator of cellular proliferation, Deoxyribose Nucleic Acid (DNA) repair, and apoptosis [4,5]. Loss of p53 function is considered a critical event in the evolution of RCC [5]. Findings on the association of p53 expression with RCC prognostic markers, such as nuclear grade, are inconsistent, with some studies finding no association and other demonstrating a strong relationship between them. Thus, p53 expression is a potential marker for determining the prognosis of patients with RCC [5].

The histologic marker CD44 is a receptor for hyaluronic acid and involved in cell motility [6]. CD44 serves as a marker of tumour aggressiveness in numerous malignancies [7]. Expression of the standard isoform of CD44 (CD44S) is associated with poor clinical prognosis in RCC [6,8]. However, some studies did not confirm the prognostic role of CD44S in RCC [7,8]. Thus, the present study examined the histopathologic features of RCC and the association of p53 and CD44S expression with prognostic markers, such as tumour stage and grade.

MATERIALS AND METHODS

The present prospective cross-sectional study was conducted on partial and radical nephrectomy specimens of RCC, received in the Department of Pathology, M.S Ramaiah Medical College and Hospitals, Bengaluru, Karnataka, India, for routine histopathological evaluation from the Departments of Urology and Surgical Oncology, M.S Ramaiah Medical College and Hospitals, Bengaluru, India, from June 2017 to May 2019. Ethical permission was obtained from Institutional Ethical Committee (IEC no. SS-1/EC/009/2017).

Inclusion criteria: All nephrectomy specimens from patients of age ≥18 years with RCC were included in the study.

Exclusion criteria: Cases, where there was extensive tumour necrosis without sufficient viable tumour cells for accurate

evaluation of the immunohistochemical results and patients who had not given consent were excluded from the study.

Study Procedure

The specimens were received in 10% formalin. In every case, the standard protocol for surgical grossing of nephrectomy specimens was followed. After a detailed gross specimen examination, multiple representative tissue bits were taken from the tumour, surgical margins and all the lymph nodes. The latter were processed as per standard protocol and paraffin embedded tissue blocks were cut and stained by Haematoxylin and Eosin (H&E). The H&E stained slides were studied for the tumour histology, grade, lymphovascular invasion, lymph node metastasis and other features like rhabdoid differentiation and tumour necrosis. The tumour was staged according to American Joint Committee on Cancer (AJCC) (8th edition-2017) cancer staging system and Fuhrman nuclear grading system was used for grading the tumour [9].

Processing for immunohistochemistry: Immunohistochemical detection of p53 and CD44S proteins was done on 4 µm thick sections, cut from a paraffin block of tumour tissue. For each case, two sections, one for p53 and other for CD44S was taken. The technique for IHC using "Super Sensitive Link Label HRP detection system" includes antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibodies against p53 (clone DO7, Biogenex) and CD44S (clone DF1485, Biogenex) proteins, linking with rabbit antimouse secondary antibody (Biogenex), enzyme labelling with streptavidin-horseradish peroxidase, developing chromogen with Deaminobenzidine (DAB) and counterstaining with haematoxylin. Positive (p53: breast carcinoma, CD44S: lymph node) and negative controls were run with each batch of slides. Staining was defined as positive for p53 protein, whenever any specific nuclear staining was detected. In each case, the percentage of positive staining tumour cells (the number of positive tumour cells over the total number of tumour cells) was evaluated. A semi-quantitative assessment of staining was done as follows:

- Strong positive (3+): 91-100% of tumour cells showing p53 positivity.
- Moderate positive (2+): 11-90% of tumour cells showing p53 positivity.
- Weak positive (1+): upto 10% of tumour cells showing p53 positivity.
- Negative (0): No p53 immunoreactivity detectable.

For statistical analysis only samples scored as 3+ and 2+ were considered as positive. Samples scored as 1+ and 0 were considered negative [5]. Staining was defined as positive for CD44S protein, whenever any specific cytoplasmic and/or membranous staining is detected in more than 5% of the tumour cells. In each case, the percentage of positive staining tumour cells was evaluated. Immunostaining of less than 5% of tumour cells was considered negative [10].

The CD44S immunostaining was scored into four grades based on staining intensity:

- Strong positive (3+): more than 75% of tumour cells showing CD44S positivity.
- Medium positive (2+): 25-75% of tumour cells showing CD44S positivity.
- Weak positive (1+): 5-24% of tumour cells showing CD44S positivity.
- Negative (0): less than 5% of tumour cells showing CD44S positivity [10].

Fuhrman nuclear grading for clear cell carcinoma and papillary RCC:

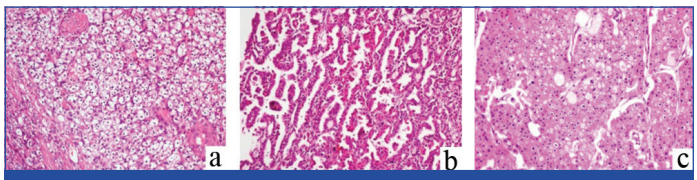
- Grade 1 (G1): Nucleoli are absent or inconspicuous and basophilic at 400X magnification.
- Grade 2: Nucleoli are conspicuous and eosinophilic at 400X magnification and visible but not prominent at 100X magnification.
- Grade 3: Nucleoli are conspicuous and eosinophilic at 100X magnification.
- Grade 4: There is extreme nuclear pleomorphism, multinucleate giant cells, and/or rhabdoid and/or sarcomatoid differentiation [9].

STATISTICAL ANALYSIS

Data were entered in MS excel version 11 and descriptive statistics were employed using IBM SPSS software version 20.0 to express quantitative parameters such as age, duration of the disease etc., and were summarised in terms of percentage with 95% confidence interval. Differences in the proportion of expression between different grades, types, etc. were tested for statistical significance by Chi-square test significance/Fisher's-exact test. Differences in mean values were tested by appropriate student's t-test/Mann-Whitney U test. The p-value <0.05 was considered statistically significant.

RESULTS

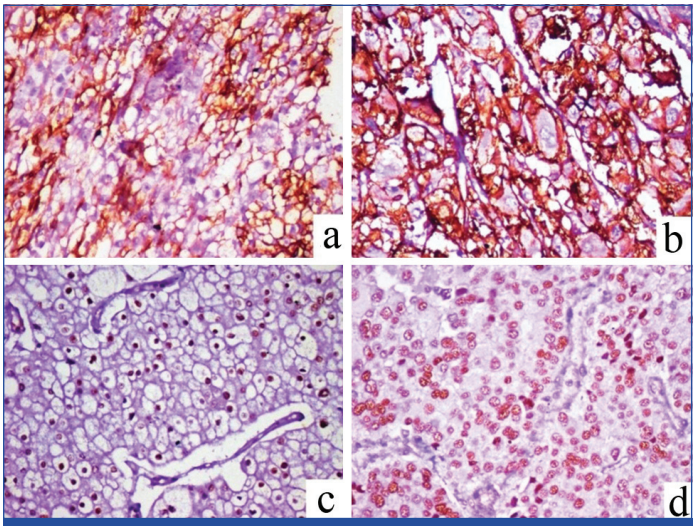
A total of 50 RCC specimens were examined. The mean age of the patient specimens included was 56.64 years with a range of 26-75 years. Men (n=37, 74%) were more susceptible to RCC than women (n=13, 26%); male:female ratio was 2.8:1. The most common RCC site was the upper pole of the kidney (n=15, 30%), followed by the entire kidney (n=13, 26%) and lower pole (n=7, 14%). The most common tumour size was >7 cm (n=21, 42%), followed by >4 cm, but ≤7 cm (n=17, 34%) and ≤4 cm (n=12, 24%). The most common histologic type was clear cell RCC (n=43, 86%), followed by papillary RCC (n=5, 10%) and chromophobe RCC (n=2, 4%) [Table/Fig-1a-c]. The majority (n=20, 40%) of RCCs had G3 Fuhrman nuclear grade, followed by G2 (n=12, 24%) and G1 and G4 (both being n=8, 16%, respectively). Rhabdoid features and necrosis were noted in 3 (6%) cases and 16 (32%) cases, respectively.



[Table/Fig-1]: (a) Clear cell RCC-microphotograph shows tumour composed of nests and islands composed of polygonal cells (G1) (H&E, 10X); (b) Papillary RCC-microphotograph showing tumour cells arranged in small tubules and papillary pattern (G1) (H&E, 10X); (c) Chromophobe RCC-microphotograph showing sheets of tumour cells separated by thin fibrovascular stroma (H&E, 10X).

A significant association was noted between advanced TNM stage (III and IV) cancers and presence of rhabdoid features (p-value=0.059) and tumour necrosis (p-value=0.025). Renal vein invasion, lymphovascular invasion, perinephric fat invasion, capsular involvement, and lymph node metastasis were noted in 4 (8%), 8 (16%), 7 (14%), 3 (6%), and 5 (10%) cases, respectively. Most of the cases had T1 stage (n=27, 54%), followed by T3 stage (n=13, 26%), T2 stage (n=9, 18%) and T4 stage (n=1, 2%). Stage-I (n=27, 54%) was the most common presentation, followed by Stage-III (n=15, 30%), Stage-II (n=7, 14%), and Stage-IV (n=1, 2%). Overall, 66% of RCC specimens exhibited CD44S positivity with 34%, 26%, and 24% displaying weak, moderately intense, and strong positivity, respectively [Table/Fig-2a-d,3]. Compared with non conventional RCCs, conventional RCCs were associated with stronger CD44S expression; however, this association was not significant (p-value=0.223) [Table/Fig-4]. Those with higher Fuhrman (G3 and G4) nuclear grades exhibited stronger CD44S

expression, than did those with lower (G1 and G2) nuclear grades; this association was significant (p-value=0.014) [Table/Fig-4]. Advanced stage tumours showed stronger CD44S expression than did early stage tumours. However, this association was not significant (p-value=0.679) [Table/Fig-4]. No significant association was observed between CD44S expression and age (p-value=0.537), pathological T stage (p-value=0.642), and lymph node status (p-value=0.486) [Table/Fig-4].



[Table/Fig-2]: Positive membranous and/or cytoplasmic staining of CD44S (2+) (a) and (3+) (b) in clear cell RCC by immunohistochemistry (IHC, 200X). Positive nuclear staining of p53 (2+) (c) and (3+) (d) in clear cell RCC by immunohistochemistry (IHC, 200X).

Intensity of CD44S	n (%)	Intensity of p53	n (%)
0 (Negative)	17 (34)	0 (Negative)	14 (28)
1+ (Weak)	13 (26)	1+ (Negative)	9 (18)
2+ (Moderate)	8 (16)	2+ (Positive)	14 (28)
3+ (Strong)	12 (24)	3+ (Positive)	13 (26)
Total	50 (100)	Total	50 (100)

[Table/Fig-3]: Intensity of CD44S and p53 in renal cell carcinoma.

Characterstic	CD44S positive n (%)	CD44S negative n (%)	Total n (%)	p-value
Histologic type				
Clear cell RCC	27 (81.8)	16 (94.1)	43 (86.0)	0.223
Papillary RCC	5 (15.2)	0	5 (10.0)	
Chromophobe RCC	1 (3.0)	1 (5.9)	2 (4.0)	
Nuclear grade				
G1	2 (6.1)	6 (35.3)	8 (16.0)	**0.014**
G2	6 (18.2)	6 (35.3)	12 (24.0)	
G3	17 (51.5)	3 (17.6)	20 (40.0)	
G4	7 (21.2)	1 (5.9)	8 (16.0)	
T stage				
T1	16 (48.5)	11 (64.7)	27 (54.0)	0.642
T2	7 (21.2)	2 (11.8)	9 (18.0)	
T3	9 (27.3)	4 (23.5)	13 (26.0)	
T4	1 (3.0)	0	1 (2.0)	
N stage				
N0	29 (87.9)	16 (94.1)	45 (90.0)	0.486
N1	4 (12.1)	1 (5.9)	5 (10.0)	
TNM				
Stage-I	16 (48.5)	11 (64.7)	27 (54.0)	0.679
Stage-II	5 (15.2)	2 (11.8)	7 (14.0)	
Stage-III	11 (33.3)	4 (23.5)	15 (30.0)	
Stage-IV	1 (3.0)	0	1 (2.0)	

[Table/Fig-4]: CD44S expression with respect to histologic type, Fuhrman nuclear grade, pathological T stage, lymph node status and TNM stage.
The p-value in bold font indicates staistically significant values

Overall, 54% cases showed p53 immunopositivity {moderate (2+) and strong (3+) staining} [Table/Fig-2,3]. The gene p53 expression was more common in non conventional tumour subtypes. However, this association was not significant (p-value=0.384) [Table/Fig-5]. Those with higher Fuhrman nuclear grades exhibited significantly stronger p53 expression than did those with lower nuclear grades (p-value=0.006) [Table/Fig-5]. No significant association was observed between p53 expression and age (p-value=0.707), pathological T stage (p-value=0.087), lymph node status (p-value=0.108), and tumour stage (p-value=0.133) [Table/Fig-5]. Compared with p53 negative cases, fewer p53 positive cases exhibited CD44S positivity (70% vs 63%). However, this relationship was not significant (p-value=0.623).

Characteristic	p53 positive n (%)	p53 negative n (%)	Total n (%)	p-value
Histologic type				
Clear cell RCC	22 (81.5)	21 (91.3)	43 (86.0)	0.384
Papillary RCC	3 (11.1)	2 (8.7)	5 (10.0)	
Chromophobe RCC	2 (7.4)	0	2 (4.0)	
Nuclear grade				
G1	5 (18.5)	3 (13.0)	8 (16.0)	**0.006**
G2	1 (3.7)	11 (47.8)	12 (24.0)	
G3	13 (48.1)	7 (30.4)	20 (40.0)	
G4	6 (22.2)	2 (8.7)	8 (16.0)	
T stage				
T1	18 (66.7)	9 (39.1)	27 (54.0)	0.087
T2	2 (7.4)	7 (30.4)	9 (18.0)	
T3	6 (22.2)	7 (30.4)	13 (26.0)	
T4	1 (3.7)	0	1 (2.0)	
N stage				
N0	26 (96.3)	19 (82.6)	45 (90.0)	0.108
N1	1 (3.7)	4 (17.4)	5 (10.0)	
TNM				
Stage-I	18 (66.7)	9 (39.1)	27 (54.0)	0.133
Stage-II	2 (7.4)	5 (21.7)	7 (14.0)	
Stage-III	6 (22.2)	9 (39.1)	15 (30.0)	
Stage-IV	1 (3.7)	0	1 (2.0)	

[Table/Fig-5]: The gene p53 expression with respect to histologic type, Fuhrman nuclear grade, pathological T stage, lymph node status and TNM stage.
The p-value in bold font indicates staistically significant values

DISCUSSION

Histopathologic features of RCC: Clear cell RCC is the most common histological type of RCC, which is similar to the present study results [11]. The Fuhrman nuclear grading system is a validated indicator of RCC prognosis. In the present study, the majority of the cases had nuclear grade G3 (40%). However, studies done by Latif F et al., Kankuri M et al., and Rioux-Leclercq N et al., have most commonly observed nuclear grade G2 in 63.3%, 49% and 42% cases, respectively [3,12,13]. This disparity partly reflects the late presentation of cases in the set-up and can be partly attributed to sample size. Rhabdoid differentiation and necrosis in RCC are independent indicators of aggressive behaviour in RCC [14,15]. Authors observed rhabdoid differentiation and tumour necrosis in 6% and 32% patients, respectively. A significant association was noted between these two pathological features and advanced tumour stage (p-value <0.05 and 0.025 for rhabdoid differentiation and necrosis, respectively). Przybycin CG et al., reported the association between rhabdoid differentiation and advanced tumour stage (p-value <0.001) and Pichler M et al., found

a significant association between necrosis and advanced tumour stage (p-value <0.001) [14,16].

CD44S immunoexpression in RCC: The present study examined the role of the CD44S marker in RCC prognosis by performing immunohistochemical analysis. CD44S is a surface transmembrane glycoprotein that was initially identified on lymphocytes. CD44S participates in lymphocyte homing through interaction with hyaluronic acid on endothelial venules. It participates in normal and tumoural cell-cell and cell-extracellular matrix interactions. Hyaluronic acid overexpression is correlated with metastatic potential in certain malignancies [17]. CD44S expression was noted in 66% cases in the present study, which is comparable to that reported by da Costa WH et al., (57.6%) [7]. However, the prevalence of CD44S expression in RCC considerably varied from one study to another, ranging from 27.4-57.6% [Table/Fig-6] [4,6,7,10,17-21]. Of 50 cases in the present study, 34%, 26%, 16%, and 24% exhibited negative (0; <5% positive cells), weakly positive (1+; 5-24% positive cells), moderately positive (2+; 25-73% positive cells), and strongly positive (3+; >75% positive cells) CD44S expression, respectively [Table/Fig-3]. Lucin K et al., evaluated 173 RCC cases and found that 22.5%, 9.2%, and 8.7% of the cases exhibited 1+, 2+ and 3+ intensities of staining, respectively [19]. Daniel L et al., reported that of 95 cases, 72.6%, 16.8%, 8.4%, and 2.1% exhibited grade 0, 1+, 2+ and 3+ staining, respectively [10].

Studies	Sample size (N)	CD44S expression (%)	Prognostic value of CD44S	p-value
Present study	50	66	CD44S expression strongly correlated with Fuhrman nuclear grade	0.014
Ma C et al., 2016, (Japan) [6]	103	35.9	No statistical significance was seen between CD44 positive cells and the clinical course	0.91
Noroozinia F et al., 2014, (Iran) [4]	66	46.9	No significant correlation was found between CD44S expression and tumour subtype	0.6
da Costa WH et al., 2012, (Brazil) [7]	99	57.6	CD44S expression was associated with clinical stage and Fuhrman nuclear grade	0.02, 0.02
Tawfik OW et al., 2007, (USA) [17]	62	32.2	CD44S did not show significant correlation with pathologic stage	0.94
Kabiri M et al., 2006, (Iran) [18]	46	32.6	CD44 was a significant marker of prognosis in univariate and multivariate analyses	0.039
Lucin K et al., 2004, (Croatia) [19]	173	40.5	CD44S expression strongly correlated with higher nuclear grade, tumour stage	<0.001, 0.023
Zolota V et al., 2002, (Greece) [20]	67	35	CD44 expression was strongly correlated with higher nuclear grade, stage tumours	<0.001, <0.001
Daniel L et al., 2001, (France) [10]	95	27.4	CD44S expression was strongly correlated with tumour size, grade, TNM staging	0.006, 0.0001, 0.008
Paradis V et al., 1999, (France) [21]	66	48	CD44 expression was significantly associated with tumour size, grade and stage	0.002, 0.02, 0.05

[Table/Fig-6]: Comparison of CD44S and its prognostic value in various studies [4,6,7,10,17-21].

Immunoexpression of p53 in RCC: The present study examined the role of p53 in cancer prognosis through immunohistochemical analysis. p53 plays a crucial role in cell proliferation, apoptosis, genomic stability and angiogenesis. Loss of its normal function may lead to uncontrolled cell proliferation and neoplastic progression. The p53 protein binds to DNA and stimulates the expression of p21 protein, which complexes with cell division stimulating protein and stops the cell division process [22]. Mutant p53 protein cannot be effectively bound to DNA, and p21 protein cannot act as the stop signal for cell division, which results in tumourigenesis [22]. Altered p53 expression is observed in various neoplasms including RCC. p53 overexpression is an indicator of poor prognosis and advanced clinicopathological features [12,22-24]. In the present study, p53 expression was found in 54% cases [Table/Fig-3]. Similar to CD44S, the frequency of p53 expression in RCC considerably varies from one study to another. These conflicting data are partly due to different populations, scoring systems, statistical analyses, immunohistochemical techniques, and result evaluation methods. Although immunohistochemistry has been used in most studies, the results have been interpreted using diverse protocol variables and scores, which cause difficulty in comparing study findings [Table/Fig-7] [4,5,12,20,23-29]. In the present study, 28%, 18%, 28%, and 26% cases displayed negative, weakly positive, moderately positive, and strongly positive p53 expression, respectively [Table/Fig-3]. Hodorova I et al., observed negative, weakly positive, moderately positive, and strongly positive, p53 expression in 66.6%, 19%, 9.5%, and 4.8% of 42 cases, respectively [5].

Studies	Sample size (N)	p53 expression (%)	Prognostic value of p53	p-value
Present study	50	54	p53 expression strongly correlated with Fuhrman nuclear grade	0.006
Noroozinia F et al., 2014, (Iran) [4]	64	20.3	Study showed correlation between P53 expression and non conventional type of RCC	<0.001
Hodorova I et al., 2012, (Slovak Republic) [5]	42	14.28	p53 expression was not correlated with tumour subtype and nuclear grade	0.063, 0.17
Mombini H et al., 2006, (Iran) [25]	67	45.4	p53 overexpression was more frequent in in non conventional subtypes and higher grade tumours	<0.001, 0.001
Kankuri M et al., 2006, (Finland) [12]	117	12.82	No association was observed between p53 expression and tumour grade	0.105
Uzunlar AK et al., 2004, (Turkey) [23]	57	35	p53 strongly correlated with stage, grade and tumour diameter	0.0472, 0.0057, 0.0237
Zigeuner R et al., 2004, (Austria) [26]	184	22.8	A statistically significant difference in p53 expression was seen among non conventional subtypes	<0.0001
Zolota V et al., 2002, (Greece) [20]	67	13.5	p53 expression strongly correlated with high nuclear grade and stage	<0.01
Girgin C et al., 2001, (Turkey) [27]	50	20	Study showed positive correlation between p53 expression and tumour grade, stage, disease related death	0.0007, 0.0034, 0.0012
Ljungberg B et al., 2000, (Germany) [24]	90	19	p53 immunoreactivity was strongly associated with higher tumour stage and grade	0.016, 0.020
Haitel A et al., 1999, (Austria) [28]	92	35.9	p53 overexpression is strongly associated with high tumour grade	0.0008
Uhlman DL et al., 1994, (USA) [29]	175	28	Increased p53 expression is seen in higher tumour grades and stages	0.02, 0.02

[Table/Fig-7]: Comparison of p53 expression and its prognostic value in various studies [4,5,12,20,23-29].

Association of CD44S and p53 expression with age group: No significant association was noted between CD44S expression and age group in the present study (p-value=0.537); this finding agrees with that of a previous Japanese study by Noroozinia F et al., (p-value=0.07) and Ma C et al., (p-value=0.47) [4,6]. Akin to

the finding reported by Noroozinia F et al., Kankuri M et al., and Zigeuner R et al., the authors noted no association between p53 expression and age group (p-value=0.707) [4,12,26].

Association of CD44S and p53 expression with histopathologic type: In the present study, CD44S positivity was 62.79%, 100%, and 50% in clear cell, papillary, and chromophobe RCC, respectively. A significant correlation was not observed between CD44S and histopathologic type (p-value=0.223). Similarly, Noroozinia F et al., observed no significant correlation between CD44S expression and tumour subtype: 17/30 (26.6%) were conventional and 13/30 (20.3%) were non conventional (p-value=0.6) [4]. In contrast to the present study, Zolota V et al., observed a significant association between CD44S expression in clear cell and sarcomatoid carcinoma compared with other subtypes, such as papillary and chromophobe carcinomas (p-value <0.01) [20]. In the present study, p53 positivity was 51.16%, 60%, and 100% in clear cell, papillary, and chromophobe RCC, respectively [Table/Fig-5]. Although p53 overexpression was more frequent in non conventional tumour subtypes, a significant association between its immunoexpression and histopathologic type was not observed (p-value=0.384). Increased p53 overexpression is observed in non conventional tumour subtypes [4,25,26]. Zigeuner R et al., detected p53 overexpression in 70%, 27.3%, and 11.9% of papillary, chromophobe, and conventional subtypes of RCC, respectively [26]. The difference in p53 overexpression might reflect the presence of alternative tumourigenesis pathways in different subtypes, possibly related to subtype-specific genetic changes [26]. Gelb AB et al., observed that p53 expression was more common and stronger in chromophobe and chromophil RCC than in clear cell RCC [30]. Uhlman DL et al., reported that p53 staining was not associated with any histologic pattern [29].

Association of CD44S and p53 with nuclear grade: In the current study, specimens with nuclear grades 3 and 4 exhibited significantly stronger CD44S and p53 expression than did those with nuclear grades 1 and 2 (p-value=0.014 and 0.006 for CD44S and p53, respectively). CD44S expression increased with histological grade, indicating its role in tumour differentiation, as postulated by other studies [7,10,19-21]. Tumours with high Fuhrman grades have a more aggressive phenotype and are associated with higher likelihood of local invasion and distant metastasis [7]. Therefore, the metabolism of adhesion molecules, such as CD44S, can be increased in such tumours, causing the spread of tumour cells [7]. As nuclear grade increases, the incidence of p53 mutations was observed to increase with its overexpression [29].

Association of CD44S and p53 with pathological T stage: In the present study, CD44S positivity was noted in 59.25% of T1 cases, 77.77% of T2 cases, 69.23% of T3 cases, and 100% of T4 cases, and no significant correlation was noted between CD44S expression and pathological T stage [Table/Fig-4]. Lucin K et al., observed decreased CD44S expression in tumours confined within the kidney compared with pT3/T4 tumours [19]. Paradis V et al., observed higher CD44S expression in T3 than in T1-T2 RCC [21]. In the present study, p53 was positive in 66.66% of T1 cases, 22.22% of T2 cases, 46.15% of T3 cases, and 100% of T4 cases; no significant correlation between p53 expression and pathological T stage was noted [Table/Fig-5]. Similarly, an Austrian study showed no significant difference in p53 overexpression in primary tumours with respect to pathological stage [26]. However, Uzunlar AK et al., observed a significant association between p53 positivity and increasing pathological stage (p-value=0.0472). In their study, 20% of T1, 34.4% of T2, 35.7% of T3, and 75% of T4 tumours displayed p53 expression, and they concluded that p53 overexpression is an adverse prognostic indicator [23].

Association of CD44S and p53 expression with lymph node status: In the present study, there was no significant association between CD44S and p53 expression and lymph node involvement (p-value=0.486 and 0.108 for CD44S and p53, respectively; [Table/Fig-4,5]. However, da Costa WH et al., observed that increased CD44S expression was associated with higher pN stage (p-value=0.003) and Haitel A et al., found a significantly higher percentage of p53 positive tumours in patients with lymph node metastases (p-value=0.0066) [7,28]. In the latter study, p53 was expressed in 39.80% (41/103) of lymph node positive cases and 30.86% (25/81) lymph node negative cases [28].

Association of CD44S and p53 expression with TNM stage: In the present study, CD44S positivity was noted in 59.25%, 71.42%, 73.33%, and 100% of stage-I, II III, and IV cases, respectively, and p53 positivity in 66.66%, 28.57%, 40%, and 100% of stage-I, II, III, and IV cases, respectively [Tables/Fig-4,5]. No significant correlation was observed between CD44S and p53 expression and tumour stage. Zolota V et al., demonstrated that CD44S and p53 expression were more common in advanced-stage tumours (p-value <0.01) than early-stage tumours [20]. Similar to the present study, a Japanese study examining 103 RCC cases did not find a significant correlation between CD44S expression and tumour stage [6]. These differences in results may be due to the inclusion of fewer tumour samples and the use of antibodies of diverse origin, that may have differences in specificity. In addition, the use of different techniques may contribute to variability {i.e., immunohistochemistry vs Reverse Transcription-Polymerase Chain Reaction (RT-PCR)}.

Limitation(s)

Additional studies including larger and varied sample sizes are warranted to determine the role of CD44S and p53 in predicting the survival of patients with RCC.

CONCLUSION(S)

The present study provides information on histopathological and prognostic parameters in RCC. Clear cell carcinoma is the most predominant histologic subtype. Presence of rhabdoid differentiation and tumour necrosis is seen with advanced stage tumours. An increased CD44S and p53 immunohistochemical expression was seen in high Fuhrman nuclear grade RCC. Conventional RCC exhibited greater CD44S expression compared with non conventional RCC. Advanced stage tumours showed higher CD44S expression. However, these two associations were not significant. Compared with conventional RCC, non conventional RCC is associated with greater p53 expression.

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