Original Article

P53 in Renal Cell Carcinoma: a biomarker for Disease Progression

Manal A. Habib MBChB, MSc, PhD*
Mazin J. Ibrahim MBChB, MSc, PhD*
Saad D. Farhan MBChB, FICM**

Summary:

Background: P53 is an important tumor marker in many malignancies, The P53 gene is a tumor suppressor gene that plays a key role in the regulation of the cell cycle. When DNA damage occurs, the level of P53 protein increases, causing cell cycle arrest and repair of DNA. Mutations in the P53 gene result in the production of an abnormal protein product, allowing cells with damaged DNA to continue through the cell cycle.

Material and methods: The nuclear expression of p53 protein was determined by immunohistochemical analysis in renal cell carcinoma specimens from 20 patients and was correlated with age, sex, stage, and grade.

Results: p53 overexpression was observed in different stages of renal cell carcinoma, most in T2 (P=0.56) and in different grades of RCC, most in grade II, with significant correlation (P =0.026).

Conclusions: In renal cell carcinoma, significant correlations were observed between p53 protein expression and tumour grade.

Keywords: P53, RCC (Renal Cell Carcinoma), grade, stage.

Fac Med Baghdad 2011; Vol. 53, No. 1
Received July. 2010
Accepted Jan. 2010

Introduction:

Renal cell carcinoma is the most frequent renal neoplasm (90-95%); it constitutes 3% of malignancies among adults. It is more common in men than women: the male-to-female ratio is 1.6:1. Its incidence has been decreasing over the last decade (1). There are at least five subtypes of RCC currently recognized and the most common form is clear cell cancer, which accounts for roughly 70% to 80% of cases. (2). the unpredictable outcome of RCC following diagnosis is a major obstacle to the effective management of this disease. The tumor ; it is a key regulator in preventing cancer formation, plays a vital role in cell cycling, growth, DNA repair, cell cycle arrest, and apoptosis (3) . The importance of p53 inactivation in RCC has been the subject of conflicting observations (4,5,6). The studies that describe a low frequency for p53 detection by immunohistochemistry are fairly limited in both number and scale.

Therefore the aim of this work was to shed light on the role of p53 as a possible prognostic indicator in RCC.

Materials and Methods:

A total of twenty paraffin embedded blocks of renal cell carcinoma were collected. Those archieved paraffin embedded blocks with their histopathological reports were taken from histopathological laboratories of The Baghdad teaching hospitals and The Hospital of Specialized surgeries from March 2009 to January 2010.

Two slides were prepared from each block in pathology department of Baghdad College of medicine, one for Hematoxyllin and Eosin staining and were reexamined by a histopathologist to confirm the diagnosis. The other was prepared for immunohistochemical staining of P53 protein which was carried out in the histopathological laboratories of The Hospital of Specialized surgeries.

Immunohistochemistry: In this study we used the Dako Cytomation LSAB+System-HRP immunostaining kit that utilizes a refind avidin biotin technique in which a biotinylated secondary antibody reacts with several peroxidase conjugated streptavidine molecules, that is why the kit offers an enhanced signal generation system for increasing staining intensity. The steps of immunostaining were followed as indicated in manufacturer protocol of the kit. The fixed paraffin embedded tumor tissues were placed inside a hot air oven at 65°C overnight then dipped in xyelene and ethanol (100%, 95% & 70%). After deparaffinization and rehydration, slides were incubated in 3% H_2O_2 for 5 minutes. The slides were placed in 10 mmol/L Tris buffer, 1 mmol/L, EDTA at pH 9.0 and underwent epitope retrieval for 10 min at 680 W in a microwave oven. Primary antibody (Mouse monoclonal antibody anti-human p53 (1:25) (Dakocytomation) was added. Slides were incubated at room temperature for 30 minutes followed by sequential incubations with biotinylated linker antibody and peroxidase labeled streptavidin. Staining was completed after incubation with substrate-chromagen (DAB) solution resulting in a brown to black precipitate. Slides then were counterstained with haematoxylin, dipped in ammonia water then mounted and coversliped. Sections treated without primary antibodies served as negative control. Twenty normal kidneys tissues were used and considered as a negative control and known positive controls were run in parallel to give reliable results. P53 nuclear staining only was rated positive. The

*Dept of pathology, college of medicine, university of Baghdad
**Dept of surgery, college of medicine, university of Baghdad

results were given percentage scores, based on the number of positive cells. A scale of 0-3 was used to score relative findings with 0 corresponding to no detectable reaction and 1, 2, 3 equivalent to low, intermediate, and high intensity of reaction respectively. Positive cells were counted in ten different fields taken randomly for each sample and the average of positive cells of the ten fields was determined assigning cases to one of the three following categories(7) .

1.Low: 1-25%.
2.Intermediate: 26-50%.
3.High: >50%.

Results:

In this study a total of twenty cases of RCC were included in addition to other twenty normal kidneys and known positive controls. Nine (45 %) were males and 11 (55%) were females. Their ages ranged between 30 and 76 years. The correlation between sex of the patients with the stage and grade of RCC were shown in tables 1 and 2 respectively with no significant findings.P53 expression was positive in nine patients (45%); its expression was seen as brown to black immunostaining of the nuclei of the tumor cells (figure2) with no detectable immunostaining in the normal renal epithelium adjacent to the tumor areas in the same sections and in the tissues of normal kidneys as negative controls.

P53 expression revealed a significant correlation with the grade of RCC (p=0.026) (table3). When P53 expression scores were correlated with different criteria, high score was seen mainly among RCC cases with stage T2 with borderline P value (P=0.056). All the details of the correlations of P53 expression and P53 scores with different available criteria were shown in tables 3 and 4 respectively.

Discussion:

In the present study, 50-60% of the RCC cases were of grade II according to the RCC grading system which is similar to the studies around the world, (8, 9, and 10). Studying the average age of patients at different levels of grading, SPSS statistical method showed no significant relationship between age and tumor grading .No relationship between patient gender and other variables was observed as it would be expected. Similar results were reported by Sakineh Amouian (11). Our study also showed that p53 was expressed in 45% of RCC cases with significant correlation with grade. A slightly lower percentages reported by another study conducted on large sample (n = 97) when p53 found to be up-regulated, typically indicative of p53 mutation, in roughly 36% of RCC cases (5). A larger (n = 246) and more recent study obtained similar results with 29.5% of RCC's being p53 positive using 20% positive cells as a cutoff value (6). In both these studies, p53 was a significant prognostic indicator. Several relatively small studies have suggested that p53 mutation, inferred from immunohistochemical detection in clinical samples, is relatively rare (25%, n = 36; 16%, n = 31; and 2%, n = 53; refs. 3–5).

The important challenge now becomes to use this information to develop appropriate diagnostics and therapeutics to manage this notoriously difficult disease. In Conclusion, using immunohistochemical staining of mutated P53 marker is an easy and reliable marker that could be applied on formalin fixed tissue for better assessment of the biologic behavior of RCC and probably predicting patients' outcome. In addition, the assessment of this marker will help to reduce inter-observer variability which is a frequent issue. Two important recent articles strongly suggest that loss of p53 function is a critical event in the evolution of renal cell carcinoma (5,6), similarly in this study only the malignant cells show P53 expression while dysplastic cells in the negative control cases did not show P53 expression . It is better to use combination of percentage score "number of positive cells /100 cells and intensity score "the strength of staining "and this reflects the amount of protein present in a cell .

Table.1 : correlation of the sex of the patients with the stage of RCC

		Tumor Stage			
		T1	T2	T3	Total
Sex	Male	3 (15%)	4 (20%)	2 (10%)	9 (45%)
	Female	4 (20%)	6 (30%)	1 (5%)	11 (55%)
	Total	7 (35%)	10 (50%)	3 (15%)	20 (100%)

Table.2: correlation of sex of the patients with grade of RCC

		Tumor Grade		
		Grade II	Grade III	Total
Sex	Male	8 (40%)	1 (5%)	9 (45%)
	Female	8 (40%)	3 (15%)	11 (55%)
	Total	16 (80%)	4 (20%)	20 (100%)

Table.3: P53 expression in relation to different criteria in RCC

criteria	Mutated P53 expression		P value
	Positive	Negative	
Age <50 > 50 total	7 (35%) 2 (10%) 9 (45%)	4(20%) 7(35%) 11 (55%)	.092
Sex Male Female Total	3 (15%) 6(30%) 9(45%)	6(30%) 5(25%) 11(55%)	0.406
Grade II III Total	5(25%) 4 (20%) 9(45%)	11(55%) 0(0%) 11(55%)	.026 *
Stage T1 T2 T3 Total	2 (10%) 6 (30%) 1 (5%) 9(45%)	5(25%) 4(20%) 2(10%) 11(55%)	.485

Pearson Chi-Square
* Significant correlation (p value < 0.05%)

Table 4: P53 scores in relation to different criteria in RCC

criteria	Mutated P53 scores			P value
	Score2	Score3	Score4	
Sex Male Female Total	1 1 2 (10%)	2 3 5 (25%)	0 2 2 (10%)	.762
Grade II III Total	2 0 2(10%)	2 3 5 (25%)	1 1 2 (10%)	.683
Stage T1 T2 T3 Total	2 0 0 2 (10%)	0 4 1 5 (25%)	0 2 0 2(10%)	.056*

Pearson Chi-Square

* Borderline correlation (p value =0.05%)

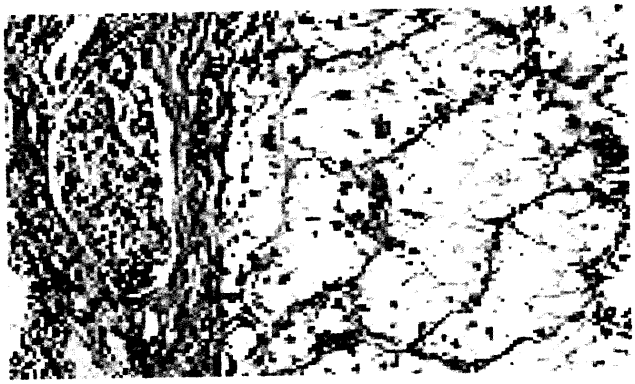

Figure (1): H & E staining of RCC (X400).

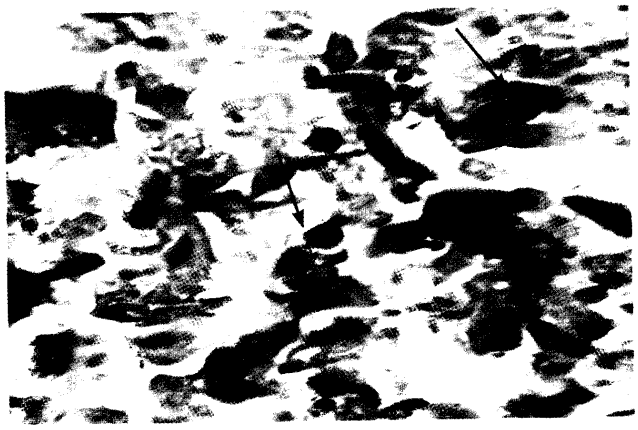

Figure 2: Positive reaction after IHC staining with marker P53 (brown nuclei in tumors cells) (x400)

References:

1- Kashyap MK, Kumar A, Emelianenko N , et al. Biochemical and molecular markers in renal cell carcinoma: an update and future prospects. Biomarkers10(4):258-94 ,2005 .

2- Martel CL, Lara PN: Renal cell carcinoma regarding current status and future directions. Crit Rev Oncol Hematol 45:177–90, 2003.

3- May P, May E : Twenty years of p53 research in structural and functional aspects of the p53 protein. Oncogene 18: 7621-7636 , 1999.

4-. Gurova KV, Hill JE, Razorenova OV, Chumakov PM, Gudkov AV. p53 pathway in renal cell carcinoma is repressed by a dominant mechanism. Cancer Res 64:1951–8, 2004.

5-. Haitel A,Wiener HG, Baethge U,Marberger M, Susani M. mdm2 expression as a prognostic indicator in clear cell renal cell carcinoma: comparison with p53 overexpression and clinicopathological parameters. Clin Cancer Res 6:1840–4, 2000.

6- Zigeuner R, Ratschek M, Rehak P, et al : Value of p53 as a prognostic marker in histologic subtypes of renal cell carcinoma: a systematic analysis of primary and metastatic tumor tissue. Urology 63:651–5, 2004.

7- - Blancato J, Singh B, Liao DJ., Dickson RB. Correlation of amplification and overexpression of the c-myc oncogen in high-grade breast cancer, FISH, in situ hybridization &Immunohistochemical analysis.British Journal Cancer 2004;90:1612-1619.

8--SONG Wei, HUO Shi-wei, LÜ Jia-ju,et al : Expression of p53 isoforms in renal cell carcinoma Chin Med J 122(8):921-926, 2009.

9- Francois C : Classification strategies for the grading of renal cell carcinomas, based on nuclear morphometry and densitometry. J Pathol 183(2):141-50 , 1997 Oct.

10 -Helmy W, Selmy G, Ossman A , et al : Nuclear Morphometry and KI67 proliferative marker in Renul clear cell carcinoma Nat. Cancer Inst. 15: 107-112, 2003.

11- Sakineh Amouian : Expression of P53 and Ki67 Proteins in Renal Cell Carcinoma and Its Relationship with Nuclear Grade . Iranian Journal of Pathology 1: 25-29, 2008.