Expression of p53 oncoprotein and bcl-2 in renal cell carcinoma

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ABSTRACT

Objective: The aim of the present study was to investigate the significance of p53 and bcl-2 as prognostic factors among others in renal cell carcinoma patients.

Methods: We evaluated the stages, histological grades, tumor diameters, cellular patterns and the presence of mutant p53 protein and bcl-2 overexpression in 57 cases of renal cell carcinoma (RCC). Kaplan-Meier and log-rank tests estimated the survival function of each parameter. The study was carried out in the Department of Pathology and the Department of Urology, Faculty of Medicine, Dicle University Hospital, Diyarbakır, Turkey, in 2003.

Results: The p53 mutation was 35% and bcl-2 overexpression incidence was 89.4% in the RCC cases included in the study. The 5-year disease specific survival rates of mutant p53 positive was 46.6% and p53 negative cases were 83.3%, (p=0.0063). There was no pathological parameter associated in bcl-2, and it has no prognostic significance.

Conclusion: The tumor stage, grade, diameter and p53 mutations affect the survival of RCC cases. The bcl-2 staining did not play any role to estimate patients at high risk of the disease progression.

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The clinical behavior of renal cell carcinoma (RCC) is generally unpredictable, and surgery remains the only effective method of treatment for this tumor. Approximately 50% of patients with RCC have poor prognosis.[1-5] Several factors have been examined with respect to their prognostic abilities in RCC including pathologic grade, stage, tumor size, nuclear morphometry, tumor cytogenetics, histomorphology and tumor vascularity.[6] The growth rate of neoplasm depends on the proliferation and death rates of cancer cells. The p53 has been one of the tumor suppressor genes, which is located on chromosome 17p. Specifically, it has been implicated in controlling a checkpoint during the G1 phase of the cell cycle by monitoring the state of DNA before its entry into the S-phase. Cells with damaged DNA are driven to apoptosis due to a blockage of the G1 phase of the cell cycle. Inactivation of p53, either by mutation or loss of heterozygosity, leads to attenuation of apoptosis and rapid tumor progression.[7,8] Also, the presence of mutated p53 protein in some tumors is associated with advanced disease and poor prognosis. The mutation of p53 has been considered as one of the most common genetic alterations in human cancers and are accumulated in the nucleus, which is detectable immunohistochemically.[9-11] Apoptosis, namely programmed cell death, may be related in part to the death rates of cancer cells as a negative regulating system in the growth of neoplasm.[12] The bcl-2 overexpression inhibit

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apoptosis, which may contribute to the development of tumors and modify their clinical behavior. Multiple studies have since verified an association between bcl-2 overexpression and many carcinomas, including breast, lung, larynx, ovary, bladder, and prostate.[13]

The aim of the present study was to investigate the significance of p53 and bcl-2 as prognostic factors among others in RCC patients.

Methods. The study consisted of 57 selected patients (30 male, 27 female) with RCC treated between 1980 and 2000 by radical nephrectomy. We evaluated the stage, histological grade, tumor diameter, cellular pattern, p53 and bcl-2 positivity with immunohistochemical staining in all cases. The age of the patients ranged from 24-65 years (mean: 47.6 years). The mean follow up period of the patients was 33.1 months (range: 2-65 months). Survival time and disease free period was evaluated by retrospective analysis. The disease free interval was defined as no evidence of recurrence or metastases by examination with imaging procedures such as sonography, abdominal and thoracic computed tomography (CT), or bone scan. After surgery, tissue specimens of RCC were routinely fixed in 3% formalin solution and embedded in paraffin. All cases of RCC were staged pathologically according to the Tumor, Node, Metastasis (TNM) classification (1997) after radical nephrectomy. The Fuhrman system for assessing nuclear grade was used to grade the tumors.[14] The tumors' cellular patterns were classified as clear cell, granular cell, spindle cell and mixed cellular types histologically.

Immunohistochemical staining. Serial sections (5 mm) from formalin-fixed, paraffin embedded tissue blocks were cut and transferred to poly-L-lysine-coated slides, and after the routine deparaffinization, these were placed in a glass Coplin jar containing 10 mM sodium citrate buffer at pH 6. The jar was heated in a microwave oven for 4-5 minutes cycles. After microwaving, the sections were left at room temperature to cool for approximately 15-20 minute then rinsed in phosphate buffered saline (PBS). To reduce the nonspecific staining, the slides were incubated in hydrogen peroxidase for 10-15 minute and rinsed in PBS. The sections were then incubated for 30 minutes at room temperature with primary antibody against p53 and bcl-2 (Neomarkers, United States of America) already prediluted for the method. The slides were rinsed again in PBS, then biotinylated goat anti-mouse solution were dripped and waited for 20 minute. The slides were rinsed in PBS. Streptavidin peroxidase solution were dripped and waited for 20 minutes. After the slides were rinsed a final time in PBS, they were incubated with chromogen 3-amino-9-ethylcarbazole (AEC) and rinsed in pure water. Finally, they were counterstained with Mayer's hematoxylin.

Evaluation of the immunohistochemical staining. A tumor tissue section was examined and scored by 2 researchers who had no prior knowledge of the clinical and pathologic data. All tissues were scored by overall tissue expression on the nuclear intensity of p53 and cytoplasmic intensity of bcl-2 as follows: 0, no staining; 1+, 1-25% staining; 2+, 26-50% staining; 3+, 51-75% staining; 4+, 76-100% staining.

Statistical analysis. The Statistical Package for Social Sciences version 10 for Windows was used for the statistical analysis. Kaplan-Meier and log-rank tests estimated the survival function of each parameter.

Results. Histopathological staging according to the TNM classification was pT1 in ten of 57 cases, pT2 in 29 cases, pT3 in 14 cases and pT4 in 4 cases. Of the 57 patients, the histologic grade of the tumor was 12 for G1, 24 G2, 16 G3, and 5 G4 RCC according to the Fuhrman grading system. Twelve of the cases (21%) had tumor diameters under 7 cm and 45 (79.9%) over 7 cm. Histological examination of the tumors demonstrated 44 (77.1%) clear cell, 8 (14%) granular cell, 2 (3.5%) spindle cell tumors and 3 (5.2%) mixed types of cellular pattern. During the follow up interval 13 out of the 57 patients revealed tumor recurrence (local relapse or metastases). Six of these 13 patients died of renal cancer. Two patients died of other causes without tumor relapse. Of the 57 tumors, 31 were situated at right and 26 left side. The main characteristics of the patients included in this study are shown in **Table 1**.

Stages, survival, expression of p53 and bcl-2. The 5-year disease specific survival rates of pT1 -2 cases were 73.6%, and T3-T4 cases 48.2%, (p=0.0472). The incidence of p53 mutations and bcl-2 overexpression are shown in **Table 1.**

Effects of p53 and bcl-2 on survival at each stage. The disease specific survival rates of mutant p53 negative cases in stages pT1-2 were 85.3% and pT3-4 were 77.7%. The survival rates of p53 positive cases in stages pT1-2 were 50.3% while pT3-4 were 40.0%, (p=0.0261). However, bcl-2 positivity was not associated with any stage.

Tumor grade, survival, expression of p53 and bcl-2. The 5-year disease specific survival rates of grade 1-2 were 86.4% and grade 3-4 cases were 45.2%, (p=0.0057). The incidence of p53 mutations and bcl-2 overexpression are shown in **Table 1**.

Effects of p53 and bcl-2 on survival at each tumor grade. The disease specific survival rates of mutant p53 negative cases in grade 1-2 were 83.6% and grade 3-4 were 68.9%, (p=0.0247). The survival

Table 1 - Patients characteristics

Characteristics	Cases		p53 + cases		Bcl-2 + cases	
	n	(%)	n	(%)	n	(%)
Stage						
T1	10	(17.5)	2	(20)	9	(90)
T2	29	(50.8)	10	(34.4)	26	(89.6)
T3	14	(24.5)	5	(35.7)	13	(92.8)
T4	4	(7)	3	(75)	3	(75)
Grade						
1	12	(21)	1	(8.4)	11	(91.7)
2	24	(42.1)	6	(25)	21	(87.5)
3	16	(28)	9	(56.2)	15	(93.7)
4	5	(8.7)	4	(80)	4	(80)
Cell Type						
Clear cell	44	(77.1)	11	(25)	40	(90.9)
Granular cell	8	(14)	5	(62.5)	7	(87.5)
Spindle cell	2	(3.5)	2	(100)	1	(50)
Mixed cell	3	(5.2)	2	(66.7)	3	(100)
Diameter						
≤7 cm	12	(21)	2	(16.7)	11	(91.7)
>7 cm	45	(79.9)	18	(40)	40	(88.9)

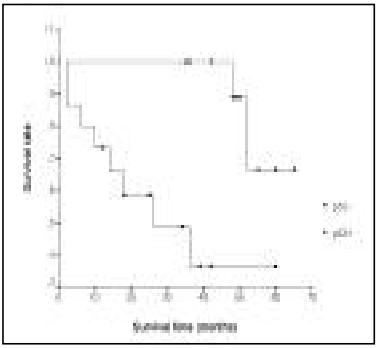

Figure 1 - The 5-year disease specific survival rates of mutant p53 positive and p53 negative cases.

rates of p53 positive cases in the same grade were 73.8 and 52.6%, (p=0.0133). However, bcl-2 positivity was not associated with any grade.

Tumor diameter, survival, expression of p53 and bcl-2. The 5-year disease specific survival rates of tumors ≤7 cm were 79.5% and for >7 cm were 43.6%, (p=0.0237). The incidence of p53 mutations and bcl-2 overexpression are shown in **Table 1.**

Effects of p53 and bcl-2 on survival at tumor diameter. The disease specific survival rates of mutant p53 negative cases in tumors ≤7 cm were 84.1% and >7 cm were 75.3%, (p=0.0376). While the disease specific survival rates of mutant p53 positive cases in tumors ≤7 cm were 68.5% cm and >7 cm were 43.9%, (p=0.0275). However, bcl-2 positivity was not associated with any tumor diameter.

Cellular patterns, survival, expression of p53 and bcl-2. The 5-year disease specific survival rates of clear cell was 79.5%, granular cell 57.2%, mixed cell tumor was 50.6% and spindle cell tumors was 0%, (p=0.0152). The p53 mutations were observed in clear cell by 25%, 62.5% in granular cell, 100% in spindle and 66.7% in mixed cell tumors. Statistical significance was not found in survival rates of all cellular patterns for mutant p53 (p=0.173) and bcl-2 (p= 0.256).

Expression of p53, bcl-2 and survival. The p53 mutations were demonstrated immunohistochemically in 35% (n=20) of 57 RCC cases. The proportion of positively marked tumor cells ranged from 5-85%. The 5-year disease specific survival rates of mutant p53 positive was 46.6% and for p53 negative cases was 83.3%, (p=0.0063) (**Figure 1**), but we did not find any association with age, gender, side of disease and cellular pattern. Also, bcl-2 was expressed in 51 of 57 (89.4%) in the cytoplasm and nuclei of cancer cells and bcl-2 positivity was not associated with any pathological parameter, and had no prognostic significance (p=0.347).

Discussion. Renal cell carcinoma is one of the unpredictable neoplasms with a poor prognosis. Identification of patients with a particularly high tumor progression risk level is of great interest from an oncological point of view. In this connection, tumor related prognostic factors include several gross, microscopic and molecular findings.[15] In our study, we investigated the prognostic importance of the p53, bcl-2 proteins and some other prognostic factors in RCC tumors. The p53 gene, located on the short arm of chromosome 17, encodes nuclear phosphoprotein. It is one of the major factors controlling cell proliferation, suppressing both growth and transformation of cells.[16] Loss of this tumor suppressor function can actively promote proliferation. The protein product of the normal gene has a very short half-life, which makes its detection difficult; the product of the mutant gene is more stable and easily detectable immunohistochemically.[17] Alterations in p53 expression were found in human renal cancers in approximately 0-85% of the cases, but controversial results exist on their prognostic significance in the literature.[12,18,19] Vasavada et al[19] reported an infrequent p53 expression in RCC samples of 0% while and Sejima et al[12] reported the sample by .5%.

Vasavada et al[19] detected low level of mutant p53 for they investigated only large (mean 6.2 cm), clinically localized T2 tumors and not sarcomatoid RCC. Moch et al[20] using immunohistochemical methods, reported 16% rate of p53 expression in RCC, but no difference was noted in either survival or clinical stage of the RCC patients. However, Shiina et al[21] reported that the positive rate for the p53 protein was 40.3% and that p53 positivity and lymph node metastasis were independent significant factors for predicting survival. In our study, the incidence of p53 mutations was 35%. This result reflects that p53 mutations are not common in RCC, although prognostically significant. Uhlman et al[22] reported that p53 overexpression was noted in 49 (28%) of 175 RCC samples studied and was associated with high tumor grade and stage, but not with cell type or histologic pattern. Tumor stage, grade and p53 overexpression represent a useful combination of prognostic markers for patients with RCC.[23] In our study, p53 overexpression demonstrated association with grade, stage and tumor diameter, but we did not find any association with age, gender, side of disease and cellular pattern. In addition, the 5-year survival rate was 83.3% for patients with non-staining tumors versus 46.6% for patients with p53 positive tumors (p=0.0063). These data demonstrate that p53 positivity is a useful prognostic factor in patients with RCC. Reiter et al[24] demonstrated that 33% of tumors had evidence of mutant p53 expression and most of these tumors were from higher stage and grade. Reiter et al[24] also suggested that mutations of the p53 locus are involved in the tumor progression of RCC and in the development of a higher grade of malignancy or more advanced tumor stage. Immunohistochemical overexpression of p53 seems to be accompanied with metastatic progression of the disease and poor survival of patients with RCC.[25,26] On the other hand, Kuczyk et al[26] could not confirm this finding in multivariate analysis and there was no significant correlation between p53 gene, alteration and tumor stage or malignancy grade. Hofmockel et al[18] using immunohistochemical methods reported no difference, either in survival or in clinical stage of RCC patients concerning p53 expression. Girgin et al[27] reported that p53 mutations were not observed in grade 1 and 2 cases, but was frequently encountered in grade 3 (31%) and grade 4 (50%) cases. As a result of these findings, they reported that as the nuclear grade increases the incidence of p53 mutations also increases.[27] The tumor stage and grade were found to be an important prognostic parameters of RCC and they increases the incidence of p53 mutations, which is similar to the present study. Kanamaru et al[28] and Amin et al[29] reported that the presence of sarcomatoid change was a bad prognostic factor in RCC cases. Higher levels of p53 expression have been demonstrated in those tumors that have undergone sarcomatoid transformation or in higher stage tumors.[27,30] Two cases with sarcomatoid change lived with a mean of 10.3 months and RCC with sarcomatoid change had a significantly poor prognosis in the present study. Also, Girgin et al[27] reported that p53 mutations were more common in cases with spindle and mixed cellular morphology. In this study, all RCC having spindle and 2 mixed cellular morphology had p53 immunoreactivity but it is impossible to get favorable result due to the low number of cases. Some investigators demonstrated that just tumor stage and grade had the most important prognostic factors in RCC cases.[2,3,15,19] Further studies implicated the tumor grade as an important prognostic factor.[1,31] The results of our study demonstrated that both tumor stage (p=0.0472) and tumor grade (p=0.0057) were important prognostic factors in the follow up of RCC cases. Guinan et al[5] demonstrated that tumor size was related to stage and survival. Larger tumors were generally associated with a higher stage as well as poorer survival, and they concluded that, for stages II, III and IV, tumor size may contribute additional prognostic information on patient survival. Similarly, the present study has concluded that tumors over 7 cm had a poorer prognosis compared to tumor size under 7 cm. The p53 mutation rate was higher in cases with tumor diameters over 7 cm. The bcl-2 gene product regulates programmed cell death, and a number of studies have suggested that bcl-2 is involved in the selection and maintenance of long living cells and in rescuing them from apoptotic cell death.[32] In connection with the immunoreactivity of bcl-2 in cancer cells, Sejima et al[12] have shown the immunoreactivity of bcl-2 has been detected mainly in the cytoplasm and nuclei of cancer cells and has expressed in 64% of all cases. Also, they reported that, although, bcl-2 positivity was not associated with any pathological parameters, the prognosis in bcl-2 positive cases was somewhat associated with a poor prognosis compared with bcl-2-negative cases. Whereas, reported that bcl-2 was expressed in 20% of tumors and was expressed commonly in well differentiated small tumors, while the expression was reduced in tumors that exhibited features related to high malignancy.[14] Huang et al[13] reported that 70% of RCC samples showed bcl-2 overexpression but no correlation was noted with tumor grade and stage. However, infrequent expression of bcl-2 staining in RCC was reported by Hofmockel et al, [18] with only 2 of 31 cases showing positive bcl-2 staining of tumor cells. Vasavada et al[19] demonstrated a high level of bcl-2 staining in clear cell adenocarcinomas, tubulopapillary and renal oncocytomas. This

expression pattern correlated well with higher tumor grade, but alone as a predictor of adverse outcome, did not achieve a statistically significant level.[19] These contradictions in results are may be due to technical factors. In the present series, bcl-2 overexpression was recognized in 51 (89.47%) of 57 cases of RCC but was not statistically significant.

The present study demonstrated that tumor stage, grade, diameter and p53 mutations affect the survival of RCC cases. The cellular pattern had no prognostic significance in RCC cases, excluding the spindle cell variant. The bcl-2 staining did not play any role to estimate patients at high risk of the disease progression.

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