A Comparison Of The KI67 Proliferation Index And CD117 & Cox-2 Expressions In Renal Carcinomas

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ABSTRACT

The present study has three main aims; a) to investigate the relationship between the tumor grade (TG), type (TT) and size (TS) on the renal carcinoma cancer (RCC) types, and demographic features of the patients such as gender and age, b) to find out the correlation between KI67 proliferation index and COX-2 and CD117 on the RCC, and c) to find out whether the immunohistochemical (IHC) reagents can determine the prognosis of some renal tumor diseases.

In this research, 50 cases with RCC in Dicle University Hospital (30 clear cell type, 8 multi-lobular type, 5 sarcomatoid type, 4 papillary type, 3 chromophobe type), and 1 with radical nephrectomy were investigated retrospectively.

The TG was carried out using Fuhrman grading system (FGS) and IHC examination was carried out on KI67, COX2, and CD117.

The results of the present study did not indicate that gender had any effects on tumor parameters, but CD117 and age had an effect on CD117 and COX2. The results indicated strong correlations between tumor parameters such as TT and TG and CD117, KI67, TG and TT and CD117, and COX2 and KI67.

Keywords: CD117, COX2, KI67, Renal Carcinomas

INTRODUCTION

The most frequent histological subtype among the renal cell carcinoma (RCC) has been found as clear cell type (Alpers & Fogo, 2008). There have been numerous studies on the prognoses of clear cell RCC (CL-RCC), multi-lobular cystic RCC (M-RCC), sarcomatoid RCC(S-RCC), papillary RCC(P-RCC), and chromophobe RCC(CHR-RCC). However, very few of these studies investigated the effects of multi-factors on tumors. The present study will contribute to the literature with its specific focus on the relationships between tumor type (TT) and size (TS), nuclear grade of tumor (TG), and various IHC markers by exploring KI67, CD117, COX2 expressions, and their relations with other prognostic In this research, 50 cases with RCC in Dicle University Hospital (30 clear cell type, 8 multi-lobular type, 5 sarcomatoid type, 4 papillary type, 3 chromophobe type), and 1 with radical nephrectomy were investigated retrospectively.

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MATERIAL AND METHOD

Immunohistochemistry

To increase the reaction of primary antibodies (P-Ak), antigen (Ag) retrieval was made by heating the sections in 0.01 M citrate buffer (Ph6.0). They were gradually deep-frozen for four times (5 mins each) in the microwave (800watt). They were incubated with P-Ak(7,0ml); Mouse monoclonal KI67 (KI67Ab4: Cat#RB-1510-R7), COX2: (Cat#RB-9072-R7), CD117 (Cat#RB-9038-R7), Using Value P-Ak Enhancer as a coenzyme, the sections were kept at room temperature for 20 mins. Then, the sections were exposed to Value HRP Polymer without light for 30 mins, soaked in AEC (3-Amino-9-Ethylcarbazole) single solution for 10 mins and washed with distilled water (P-Ak, Ultra Vision LP value Detection System HRP Polymer & AEC Chromogen; Thermo Scientific, USA). Nuclear adversary staining was carried out by using Mayer's Hematoxylin for 2 mins. They were dried at room temperature and kept in Aqueous mounting (*Lab Vision Ultra Mount*). The cases were examined under a light microscope with control blocks.

ANALYSIS AND RESULTS

Immunohistochemical Evaluation

The cases were scored differently during the analysis. The unstained cases with CD117 and COX2 tumor areas had no points but those were stained with light focal, diffuse cytoplasmic or membranous stain had 1 point.

The staining of the cases with KI67 was carried out under the light microscope (with 40x10 magnifying) on 10 microscopic areas by counting 1000 tumor cells according to the following scores:

1. 1% and fewer cells
2. %1- 10 cells
3. %11- 50 cells
4. %51- 100 cells

Statistical Analysis

Using SPSS 15.0 for Windows, descriptive analysis was used to describe the basic features of the data, inferential statistics (Mann-Whitney U test) to see the effects of gender on other variables, and a cross-tabulation and correlation analysis were to explore the relations between TT, TS, TG, and KI67, COX2, and CD117 expressions.

Clinicopathologic Findings

The patients' clinicopathologic characteristics are listed in Table 1. There were 26 male and 24 female patients. The age of the patients ranged from 26 to 81, and the average age was 57 (57,92+13,47). The largest TS was 13 cm, and the smallest TS was 2 cm, and the average was 7 (7,12+2,670) cm. Out of 50 patients, 30 of them had CL-RCC, 8 patients M-RCC, 5 patients S-RCC, 4 patients P-RCC, and 3 patients CHR-RCC. Nuclear grading of the tumors was carried out by using the FGS. Seven of the patients (16%) were at TG-1, 29 (58%) at TG-2, 9 (18%) were at TG-3, and 5 (10%) at TG-4. The average grade was 2 (2,24 + 0,822 median 2).

Immunostaining Analysis

Following results were obtained from the immunostaining analysis:

1. Out of 30 patients with CL-RCC 16had1%, 5 had 1-10%, 7 had 10-50%, 2 had 50-100 cells nuclear stained with KI67.
2. Out of 8 patients with M-RCC 4 had 1-10%, 2 had 0-50%, and 2 had 50-100% cells nuclear stained with KI67.
3. Out of 4 patients with P-RCC, 1 had 1%, 1 had 10-50%, and 2 had 50-100% cells nuclear stained with KI67.

4. Out of 5 patients with S-RCC, 3 had 1%, and 2 had 50-100% cells nuclear stained with KI67.
5. All 3 patients with CHR-RCC were below 1% nuclear stained with KI67.

Out of 30 patients with CL-RCC 17 had, 8 patients with M-RCC 6 had, 5 patients with S-RCC 2 had, 4 patients with P-RCC 1 had and 3 patients with CHR-RCC 1had CD117 staining.

On the other hand, out of the 30 patients with CL-RCC 18 had, 8 patients with M-RCC 3 had, 5 patients with S-RCC 1 had, and 4 patients with P-RCC 3 were examined with COX2 staining. However, 3 patients with CHR-RCC were not found to have COX2 staining.

Statistical Analysis

Inferential statistics were used to see the gender differences regarding TT, TS, and TG measurements. The results indicated that there were no differences between the female and male patients in relation to their TT, TS and TG measurements. However, gender was found to have effects on CD117. Males received high scores than females with CD117 measurements (see Table 1).

Table 1. Mann-Whitney U test results for the effects of Gender on TS, TT, TG

	TS	TT	TG	CD117	KI67	COX2
Mann-Whitney U	240,000	277,000	285,000	238,000	248,000	312,000
Wilcoxon W	540,000	577,000	585,000	538,000	548,000	663,000
Z	-1522	-770	-588	-1664	-1321	000
Asymp. Sig. (2-tailed)	128	441	557	096	187	1000

a. Grouping Variable: gender

A Cross Tabulation is a method used to analyze the relationship between multiple variables. In the present study, the relationships among TS, TT, TG, and CD117, KI67, COX expressions were examined using cross tabulation analysis and correlational analysis. The results of the Chi-square test indicated that there are relationships only between KI67 and tumor measurements such as TS and TT (see Table 2).

Table 2. Chi-Square Tests results for the relationship between TS, TT, TG, and CD117, KI67, COX2 expressions

	CD117	KI67	COX2
TS	p= 0.906	**p= 0.035**	p=0.295
TT	p= 0.438	**p= 0.033**	p=0.112
TG	p= 0.435	p= 0.364	p=0.554

The results of the correlational analysis indicated that their significant relationships between two tumor parameters TT and TG, TS, TG and IHC reagents, and finally between KI67 and COX2 (see Table 3).

Table 3. Correlational analysis results for age, TS, TT, TG, and CD117, KI67, COX2 expressions.

			TS	TT	TG	CD117	KI67	COX2	age
Spearman's rho	TS	Correlation Coefficient	1.000	.159	.316*	-.014	-.024	-.176	-.247
		Sig. (2-tailed)		.272	.026	**.925**	**.868**	.220	.084
		N	50	50	50	50	50	50	50
	TT	Correlation Coefficient	.159	1.000	-.002	-.131	.155	-.243	-.197
		Sig. (2-tailed)	.272		**.989**	.365	.282	.088	.170
		N	50	50	50	50	50	50	50
	TG	Correlation Coefficient	.316*	-.002	1.000	-.042	-.122	-.120	-.126
		Sig. (2-tailed)	.026	**.989**	.	**.772**	.398	.408	.382
		N	50	50	50	50	50	50	50
	CD117	Correlation Coefficient	-.014	-.131	-.042	1.000	-.164	.201	-.003
		Sig. (2-tailed)	**.925**	.365	**.772**		.255	.162	**.985**
		N	50	50	50	50	50	50	50
	KI67	Correlation Coefficient	-.024	.155	-.122	-.164	1.000	-.078	.142
		Sig. (2-tailed)	**.868**	.282	.398	.255		**.590**	.326
		N	50	50	50	50	50	50	50
	COX2	Correlation Coefficient	-.176	-.243	-.120	.201	-.078	1.000	.017
		Sig. (2-tailed)	.220	.088	.408	.162	**.590**		**.909**
		N	50	50	50	50	50	50	50
	age	Correlation Coefficient	-.247	-.197	-.126	-.003	.142	.017	1.000
		Sig. (2-tailed)	.084	.170	.382	**.985**	.326	**.909**	
		N	50	50	50	50	50	50	50

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Relationships between TT, TS, TG, Gender, and Age

The results of the present study did not indicate any relationship between gender and the tumor parameters but CD117. Therefore, gender and tumor measurements can be considered as independent prognostic parameters in tumors. This could be due to sex hormones of patients.

The results of the Spearman test indicated that age had an effect on CD117 and COX2. CD117 measurements were found to be high with the patients between 45 and 65 years old, and COX2 were high with those between 50 and 70 years old.

Numerous studies have investigated the relationships between various parameters in tumors, in particular, renal tumors. Murphy et al. (2005) considered TS over 4 cm as a malign prognostic factor in the study. In the present study, a relationship was found between TS and KI67. Tumor size seemed to have strong effects on KI67 expressions.

TG has been examined in the prognosis in renal tumors. Lang et al. (2005), identified the tumor TG-1-2 as low stage and the TG-3-4 as high stage. Rioux-Leclercq et al. (2007) and Al-Aynati et al. (2003) stated that TG was an influential factor in prognosis.

Various renal carcinomas were graded using FGS in the present study. The results of the present study revealed that there was a relationship between TG and TT, and CD117. The relationship seemed to be significant only in the second grade of renal tumors.

The Role of KI67, CD117, and COX2 in the Prognosis of Different Tumors

Ki67 is found in the G1, S, G2 and M phases in the nucleus of all split cells, in other words in proliferous tumor cells. Gerdes et al. (1984) used KI67 as a monoclonal anti-core for nuclear antigen proliferation in the Non-Hodgkin lymphoma cells. In a different study, the researchers found the effects of KI67 expression in rectal cancer and the sensitivity of KI67's to chemoradiotherapy could influence KI67 results.

Wang et al. (2017) found that KI67/MIB-I expression correlated with poor prognosis in RCC on some patients. Similarly, Miyata et al. (2003), found that COX2; TS, grade and mmp-2 expression were meaningful dependent, and KI67 and TS were interdependent parameters.

In the present study, however, KI67 was found to have a relationship with TS. This finding could emerge due to different types of tumors observed in the research. The KI67 values were found high in big size tumors, and this could be a sign of an increase in the cell proliferation and poor prognosis in the RCC.

CD117 has a role in cell signal transmission (Besmer, Murphy & George, 1986). Ahmed & Youssif (2009), obtained different results regarding different subtypes of the renal tumors. The CD117 expression might be determining CHR-RCC and oncocytomas. Huo et al. (2005), Liu et al. (2007), Pan et al. (2004) and Lin et al. (2004) found that CD117 expression of P-RCC and CHR-RCC was more meaningful than the CD117 expression of CL-RCC.

In the present study, CD117 was found to have a strong relationship with TS and TG. The results did not reveal any relationship between CD117 and other variables. This could show that CD117 can be effective on the prognosis of some renal tumor diseases.

COX enzyme exists in two isoforms COX1 and COX2 (16). COX1 is expressed in hemostasis providing cell. COX2 is pro-inflammatory and inducted by mitogens, tumor parameters, cytokinesis, and growth factors. Non-steroidal anti-inflammatory medicines (NSAID) were found to inhibit carcinogenesis in human (Chen, 2004). This anti-neoplastic effect occurs when NSAIDs suppress prostaglandins biosynthesis that is mediated by COX.

COX2 inhibitors increased vascularity of tumor, decreased metastases and increased apoptosis (Grosch, 2006). In their studies, Dirim et al. (2007) found that COX2 played an important role in RCC and angiogenesis. Tabriz et al. (2016) found that the COX2 expression correlated with the histological subtype of the RCC

In the present study, a meaningful relation was found between COX2 and KI67. The results suggested that KI67–COX2 can be used to detect renal tumors in RCC, and KI67 and COX2 expressions may have prognostic significance in CL-RCC and P-RCC.

SUGGESTIONS AND IMPLICATIONS

Taking the cardiovascular side effects into account, COX2 inhibitors can be used in the treatment of CL-RCC and P-RCC (Grosch, 2006). In addition, tyrosine kinase inhibitors can be tried in the treatment of CL-RCC and M-RCC (Baker, 2006).

However, the results also suggested that COX2 expression may not exist in some of the tumors due to the differences in tumor structures. Further research is called to detect COX2 levels in the parenchyma.

In the study, it was found that the KI67 involvement rate was over 1% in M-RCC and P-RCC. This could also be a determining sign for these tumors. Further studies need to focus on this topic.

Previous studies into the S-RCC conducted in different contexts revealed different results originating from the differences in tumor structure. The three IHC determinants were administrated in the study with CHR-RCC. More studies should be conducted on the relationship between them.

LIMITATIONS

The sample size is an important feature of any empirical study in which the goal is to make inferences about a population from a sample. The present study has a limitation with the sample size. The study was conducted in a city on the east of Turkey where the whole population was not very big. The negative effects of this limitation were taken into consideration and tried to be eliminated by using nonparametric tests to analyze the data.

Previous studies indicated that the reasons of renal cell carcinomas are dietary habits (Hemminki et al. 2002) family history, chromosomal abnormalities, high body mass index, hypertension, smoking, frequent urinary infections (Murai & Oya, 2004; Pischon et al. 2006; Hunt et al. 2005).

The researchers reported that the reasons of the disease as mentioned above were not observed with their patients due to the location of the research. The patients live in the rural areas of the city, eat healthy and organic food, and they do not expose their bodies to any chemicals. This could be another reason of the sample size of the present study and needs further investigation.

AUTHOR BIOGARPHIES

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NOTES