Introduction

More than two thousands of people around the world suffer from different types of kidney carcinoma with the highest prevalence in North America and Europe and the lowest in central Africa. Kidney cancer is the third common urologic cancer in Iran following bladder and prostate cancers (Akbari et al., 2005; Basiri et al., 2014). Also, a quarter of the affected patients face locally invasive or metastatic cancer (Jemal et al., 2005). In this regard, one-third of individuals with renal carcinoma undergo resection of localized disease may experience a recurrence. Hence, there is a need for more effective surgical and medical therapies as well as accurate estimating survival of the patients. Besides traditional risk factors for renal cell carcinoma including smoking, central obesity, existing kidney conditions, hepatitis infections, long-term use of non-steroidal anti inflammatory drugs, hypertension, low intake of fruits and vegetables, and family history of kidney cancer (Chow et al., 2000; Chow et al., 2010; Karami et al., 2013; Sanfilippo et al., 2014; Washio et al., 2014), some genetic-based causes and mutations involved in protein over-expression and up-regulation have been recently identified to develop renal cell carcinoma (Moore et al., 2005; Haas et al., 2014). Moreover, the poor prognosis of cancer not only can be predicted by clinical (palpable abdominal mass) (Yap et al., 2013), some paraclinical factors (such as a high level of serum...
Materials and Methods

In a cohort study, patients with kidney cancer referred to Hasheminejad Kidney Center between 2007 and 2009, underwent radical nephrectomy and had pathology reports of clear cell, papillary or chromophobe renal cell carcinoma were included into the study. Other histological types of renal cell carcinoma were excluded from the study. Baseline characteristics regarding demographics, medical history, medications, and pathologic data included pTNM stage, tumor size, Fuhrman nuclear grade and the presence of vascular invasion were collected by reviewing the recorded files. The patients with missed, incomplete or poor quality paraffin blocks were also excluded from the study. Overall ninety one patients met the criteria.

For evaluation of immunohistochemical reactivity, representative areas from each case were selected from paraffin blocks based on hematoxylin and eosin-stained sections. IHC staining was carried out using an anti-p53 monoclonal antibody (NOVOCASTRA clone DO-7, ready to use for immunohistochemistry) and anti-MDM2 antibody (NOVOCASTRA clone 1B10 used at a dilution of 1:200 for immunohistochemistry). Both positive and negative control samples of both P53 and MDM2 parameters were taken in every IHC examination. Breast cancer and well differentiated liposarcoma were used as positive controls and cerebellar tissue and pleomorphic liposarcoma for negative controls of P53 and MDM2 respectively. All IHC slides were examined and scored by two pathologists independently and the mean were assigned. The intensity of nuclear immunostaining of P53 and MDM2 in individual tumor cells was scored on a scale of 0 (no staining), 1 (mild staining), 2 (moderate staining), and 3 (strongest intensity), and the percentage of cells staining at each intensity was scored as 1 (0 to 2 percent), 2 (3 to 10 percent), 3 (11 to 50 percent), and 4 (more than 50 percent). Cases with intensity score of 3 and percentage score of 4 were considered as positive. Other combinations of intensity and percentage scores regarded as negative.

The five-year survival was determined by the patients’ medical files and telephone follow-up.

For analysis of our experience, results were presented as mean±standard deviation (SD) for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Continuous variables were compared using t test, quantitative variables were, on the other hand, compared using chi-square test or Fisher’s exact test. Cancer-specific survival and overall survival were estimated using the Kaplan-Meier method, Multivariate Cox proportional hazards models using a backward stepwise selection method with the likelihood ratio criterion (inclusion/exclusion:P0.05/P.10) were used to determine the independent influence of variables on survival. All statistical tests were 2-sided and were performed at a significance level of 0.05. All analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL).

Results

Overall, ninety one patients were assessed. The mean age of patients was 56.7±12.3 years (ranged 28 to 86 years) and 58.2% were male. The mean size of lesions was also 8.5±5.4 cm ranged 2.5 to 18cm. left-sided lesions were observed in 50.5% and right-sided in 49.5%. Regarding type of lesions, 69.2% were clear cell, 19.8% were chromophobe, and 11% were papillary. 15.1% were categorized as grade 1, 52% as grade 2, 27.0% as grade 3, and 6.2% as grade 4. With regard to organ involvement, renal sinus was involved in 71.3%, perirenal fat involvement in 23%, renal pelvis involvement in 7%, renal vein involvement in 9%, vena cava involvement in 2.2% and adrenal gland involvement in 3.2%. Necrosis was noted in 51% of cases. Also, vascular and neural
invasions were observed in 23% and 3.3% respectively. Lymph node involvement was noted in 8%. Recurrence was revealed in 1.1%, while metastasis in 5-7 years follow up was occurred in 17.6%.

Regarding the intensity of immunostaining for anti-p53 monoclonal antibody, the score was 0 in 82.4%, 1 in 7.7%, 2 in 5.5%, and 3 in 4.4%. In this regard, the percentage of cells staining at intensity was scored as 1 in 78.0%, 2 in 17.6%, 3 in 3.3%, and 4 in 1.1%. Thus, 1.1% of all samples were revealed to be positive for P53. Regarding intensity of immunostaining for anti-MDM2 antibody, the score was 0 in 31.9%, 1 in 17.6%, 2 in 19.8%, and 3 in 30.8%. Also, the percentage of cells staining at intensity was scored as 1 in 33.0%, 2 in 12.1%, 3 in 29.7%, and 4 in 25.3%. Thus, 20.8% of all samples were revealed to be positive for MDM2. Regarding risk factors, 14.3% were smoker and 12.1% were hypertensive. Regarding stage of disease, 18.6% had stage I, 5.4% had stage II, 69.2% had stage III, and 6.5% had stage IV.

The patients were all followed for 5 years survival. In this regard, 5-year mortality was 30.5% and thus 5-year survival was thus 5-year survival was 85.3%. There was no difference in 5-year survival between men and women (86.4% versus 83.9%, p=0.834). The mean age of non-survivors was significantly higher than survivors (60.9±13.7 years versus 55.0±11.4 years, p=0.039). Also, 5-year survival in clear type was Also, 5-year survival in clear type was 82%, in chromophobe type was 100%, and in papillary type was 77.8% (p=0.014). 5-year survival rate was adversely associated with tumor grade (grade 1: 90%, grade 2: 88.9%, grade 3: 76.5%, grade 4: 66.7%, p<0.001) and stage stage (stage I: 96.7%, stage II: 90.9%, stage III: 68.2%, stage IV: 0%, p=0.001). The higher intensity of immunostaining for anti-P53 antibody was

![Figure 1. Positive Controls for p53 and MDM2](image)

**Figure 1. Positive Controls for p53 and MDM2**  
(A) p53 Nuclear Positivity in Invasive Ductal Carcinoma of Breast Cancer with Original Magnification ×20, (B) MDM2 Nuclear Positivity in Well Differentiated Liposarcoma with Original Magnification ×20

![Figure 2. Positive Nuclear Staining of p53 and MDM2 in Renal Cell Carcinoma.](image)

**Figure 2. Positive Nuclear Staining of p53 and MDM2 in Renal Cell Carcinoma.** (A) p53 Positive Immunoreaction with Original Magnification ×20, (B) MDM2 Positive Immunoreaction with Original Magnification ×20

![Figure 3. Negative Nuclear Staining of p53 and MDM2 in Renal Cell Carcinoma.](image)

**Figure 3. Negative Nuclear Staining of p53 and MDM2 in Renal Cell Carcinoma.** (A) p53 Negative Immunoreaction with Original Magnification ×20, (B) MDM2 Negative Immunoreaction with Original Magnification ×20

![Figure 4. Association of Intensity of Immunostaining for Anti-p53 Antibody with Survival](image)

**Figure 4. Association of Intensity of Immunostaining for Anti-p53 Antibody with Survival**

<table>
<thead>
<tr>
<th>Intensity of p53</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>Survival</td>
<td></td>
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<tr>
<td>5-years</td>
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<td>80.10%</td>
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<td>60%</td>
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<td>88.30%</td>
<td>71.40%</td>
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<tr>
<td>7-years</td>
<td>59.70%</td>
<td>71.40%</td>
<td>0%</td>
<td>0%</td>
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![Figure 5. ROC Curve for p53 Showing Limited Role for Predicting Long-term Survival](image)

**Figure 5. ROC Curve for p53 Showing Limited Role for Predicting Long-term Survival**
associated with lower 5-year survival (87.1% for score 0, 80.1% for score 1, 75.6% for score 2, and 60.0% for score 3, \( p=0.008 \)) (Figure 4) (Table 1), but no difference was found in 5-year survival between different scores for immunostaining for anti-MDM2 antibody (78.3% for score 0, 76.9% for score 1, 100% for score 2, and 87% for score 3, \( p=0.545 \)) (Figure 5) (Table 2). According to the Cox proportional hazard analysis, positive P53 marker was only predictor for patients’ 5-year survival that the presence of positive p53 increased the risk for long-term mortality up to 2.8 times (HR=2.798, 95%CI: 1.176-6.660, \( P=0.020 \)). However, the presence of MDM2 could not predict long-term mortality. In this regard, analysis by the ROC curve (figure 6) showed a limited role for predicting long-term survival by confirming p53 positivity (AUC=0.610, 95%CI: 0.471-.750, \( P=0.106 \)).

Discussion

The present study first aimed to determine the frequency of positive anti-P53 and also anti-MDM2 antibodies in patients who underwent radical nephrectomy leading a considerably low positivity of anti-P53 in 1.1%, but a positivity of anti-MDM2 in one-fifth of study subjects. However, only 1.1% of all subjects had positivity for both markers. Second, we showed that both indices could not predict long-term mortality and thus long-term survival in the patients. In this regard, analyzing area under the ROC curve was not resulted in a proper cutoff value of both P53 and MDM2 for predicting 5-year survival in the patients. On the other hand, the present scoring system for both biomarkers regarding intensity and percentage of the cells staining seems to be not useful for assessing long-term mortality in our population. In this regard, we did not achieve homogeneity of findings in Cox modeling and ROC curve analysis.

Reviewing the literature reveals contradictory results. In a study (Hejnold et al., 2014), about 6.6% of patients were P53+/MDM2+ that was higher compared to our results as 1.1%. In another study (Noon et al., 2012), the presence of P53 but not its mutation was associated with reduced survival. Also, MDM2 expression was related to reduce survival. Other study (Polanski, 2010) showed that the concurrent expression of both p53 and MDM2 was associated with considerably decreasing survival. In another research (Uchida et al., 2002) the expression of P53 and MDM2 was showed in 13.4% and 1.8% that was contrary to our finding. In that study, the expression of both markers could predict poor outcome in the patients. In other study (Haitel et al., 2000) study, simultaneous expression of both P53 and MDM2 was related to tumor progression. There is also a similar (Hashimoto et al., 2000) that indicated that P53 could not predict disease outcome and thus only type of infiltration was predictor for poor outcome. Other research (Moch et al., 2013) also indicated positive P53 in 16% and positive MDM2 in 30% of subjects with a strong association between the two biomarkers and also with poor prognosis of the disease. In total, it seems that both P53 and MDM2 markers in Iranian population may not be able to predict long-term survival in patients who underwent radial nephrectomy that can be affected by genetic and racial differences. However, this claim should be assessed in further studies.
term survival.

Acknowledgements

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References


