Original Article

Relationship between grade and MDM2 oncoprotein overexpression in transitional cell carcinoma of the urinary bladder

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ABSTRACT

Objective: Transitional cell carcinoma (TCC) of the urinary bladder is the second common cancer of the genitourinary tract. Several parameters such as clinical and pathological parameters, molecular factors, and etc play a role in determination of prognosis and type of treatment. In this research study, the relationship between grade and MDM2 oncoprotein overexpression in TCC of bladder was evaluated.

Material and Methods: This cross-sectional study was carried out on 75 paraffin-embedded tissue blocks (deposited in Pathology Department) from patients with TCC of urinary bladder from 2002 to 2005. In this respect, 3 μm sections were taken from each block. In one of the sections, grade of tumor was determined according to WHO/ISUP criteria using hematoxylin-eosin staining method. In another section, percent of MDM2 positive cells were determined by microscopic observation of 100 cells in each section using immunohistochemical technique and specific antibody.

Results: Mean of MDM2 positive cells was %9.08 ± 4.7. In addition, mean of MDM2 positive cells in low malignant potential tissue (grade I), low grade tissue (grade II), and high grade tissue (grade III) was %3.6 ± 2.2, %8.2 ±3.5, and %13.1 ± 3.4 respectively. Meanwhile, a significant relationship was found out between grade and overexpression of MDM2 oncoprotein ($p = 0.012$).

Conclusion: Overexpression of MDM2 oncoprotein exhibits a direct relationship with grade of TCC of the urinary bladder. The obtained results were consistent with the most previous studies. Therefore, through performing studies with a greater follow-up and entailing a larger population of patients, overexpression of MDM2 oncoprotein could be used as a prognostic parameter in TCC of urinary bladder.

Key words: Transitional Cell Carcinoma of the urinary bladder (TCC), Grade, MDM2 oncoprotein

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Introduction

Transitional cell carcinoma (TCC) of the urinary bladder is the fifth common cancer in Iran and the second common cancer of the genitourinary tract after considering prostate cancer (1,2). In case of correct and early diagnosis of this condition, there is a higher lifespan for patients. Several factors are involved in determination of prognosis and selection of type of treatment. In this regard, one of the known and independent factors is tissue tumor grade (1). In addition, other factors are tumor growth, patient lifespan, and invasiveness of the condition. These factors may be not sufficient in some patients for final determination of prognosis and other parameters are required. In this respect, MDM2 oncoprotein overexpression can be considered for patients tumors. This nucleus-located oncoprotein has been associated with some kinds of human cancers (3-6). Therefore, rate of expression of this oncoprotein was determined in TCC of the urinary tract and its relationship with tumor grade was evaluated in the present study.

Material and Methods

The cross-section strategy of this study was conducted on patients with TCC of the urinary bladder whom referred to Mostafa Khomeini hospital during the years 2002-2005. For this purpose, 75 available tissue blocks were evaluated. For this purpose, 4 µm sections were prepared from paraffin-embedded blocks and stained using Hematoxylin-Eosin method. Then, tumor grade was determined on the basis of WHO/ ISUP criteria. Another section from each block was used for immunohistochemical evaluation of MDM2 oncoprotein. For this purpose, the blocks were deparaffinized and processed as follows: 1) the samples were placed in oven at 50-60 °C for 30 min, 2) the samples were rinsed in 100% xylol, ethanol 100%, 85%, 75%, and distilled water, 3) rinsed in PBS (10%) solution, 4) 10 min exposure to H2O2 and ethanol at a ratio of 1:9 for 10 min, 5) rinsed in PBS (10%), 6) placed in EDTA buffered solution (pH = 8) for 10 min at 120 °C at a pressure of 0.5 atmosphere, 6) rinsed in PBS (10%), 7) stained using Histostain Plus board spectrum (DAB) which itself included the following steps: 1) blocking serum was added to slides for 10 min and then dried, 2) addition of MDM2-specific primary antibody at a dilution of 1/200 for 30-60 min at room temperature, 3) rinse in PBS (10%), 4) addition of Broad spectrum antibody for 10 min, 4) rinsed in PBS (10%), 5) addition of conjugated solution (HRP-streptoavidin) for 10 min, 6) rinsed in PBS (10%), 7) addition of 100 µl of DAB, 8) rinsed in PBS (10%), 9) dehydrated in distilled water, alcohols 75%, 85%, and 100%, xylol, 10) coverslipped and coded. The slides were evaluated under light microscope with maximum magnification. The number of positive cells/ 100 counted cells was determined. Data were statistically analyzed using SPSS software.

Results

Out of the patients, 49 (65.3%) and 25 (34.7%) were male and female cases respectively. The age range of patients was 39-80 years with a mean age of 56.34 ± 9. Out of the samples, 14 (18.7%), 35 (46.7%), and 26 (34.7%) cases were low malignant potential (grade I), low grade (grade II), and high grade (grade III) respectively. Meanwhile, percent of positive specimens were 96% (73 samples). In addition, mean of MDM2 positive cells in low malignant potential tissue (grade I), low grade tissue (grade II), and high grade tissue (grade III) was %3.6 ± 2.2, %8.2 ±3.5, and %13.1 ± 3.4 respectively. A linear relationship equal to 0.72 was obtained between tissue grade and overexpression of MDM2 using Spearman correlation analysis. Furthermore, X^2 analysis also showed a significant association between these factors.

Discussion

In this research study, the relationship between tissue grade and MDM2 oncoprotein overexpression was evaluated in transitional cell carcinoma of the urinary bladder. The results of previous study by Tuan et al on 80 samples of TCC of the bladder showed that increased number of

P53 and MDM2 positive cells is closely associated with tissue grade and tumor relapse using the same immunohistochemical technique (7), which is consistent with obtained results in this study. In another study by Scholett et al on 71 samples, PCR and mRNA quantification techniques were used regarding MDM2 oncoprotein and their results are consistent with the present study. In this respect, it was found out that patients with a higher level of MDM2 mRNA have a more malignant and more invasive tumor. In addition, these researchers determined the related risk factors (8). Furthermore, Korkolopaulou et al found out that increased tissue grade and tumor stage are not correlated with expression of MDM2 protein at a non-significant level. The experimental procedure of their study was the same as in the present study and a total of 106 samples were evaluated. In addition, they reported that MDM2 overexpression could not play a role in prognosis determination (9). On the other hand, Uchida et al (2002) through evaluation of 119 samples of TCC of the bladder found out that in contrast to our obtained results, there is no significant relationship between MDM2 overexpression and the related clinical and pathological findings (10). Although they used a larger number of samples, but their findings were contrary to other studies, which may be attributed to differences in staining kits and other factors . In addition, Hashimoto et al performed an immunohistochemical study on 74 samples of TCC of ureter and pelvis and found out that MDM2 overexpression and simultaneous evaluation of P53 are not applicable for determination of prognosis and tumor growth. These results can not be compared to the obtained results of this study due to different areas of involvement (11).

Conclusion

Since previous and the current study have shown a relationship between tissue grade and overexpression of MDM2 oncoprotein, therefore, this protein is very critical in prognostic determination of TCC of the urinary bladder. Finally, it is recommended to perform more complete researches with a longer follow up and using a larger number of patients.

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