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Quantitative Histopathology in Superficial Bladder Tumors: The Value of Mean Nuclear Size in Progression and Recurrence

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Abstract: The aim of this study was to evaluate the quanititation of the nuclear size of tumor cells in superficial bladder carcinomas in progression and recurrence.

Thirty-four cases diagnosed primarily as superficial bladder carcinoma grade II without muscle invasion (Stage pTa and pT1) between 1986-1993 at the Department of Pathology of Akdeniz University Faculty of Medicine were analysed in three groups:

1- Ten cases without recurrent tumor.

2- Twelve cases with primary and recurrent superficial bladder tumor.

3- Twelve cases with superficial primary tumor and recurrent tumor with muscle invasion.

The mean nuclear diameter of the tumor cells were obtained by using simple light microscopic morphometric analysis. On each slide the diameters of 800 cells were measured and the mean nuclear volume of each was calculated.

In group 1, the mean nuclear volume was 142±9.58 μm^3. In group 2 and 3 the mean nuclear volumes were 207±39.5 μm^3 and 299±36.5 μm^3 , respectively. Our results showed that the primary tumors with high mean nuclear volume had higher rates of recurrences and muscle invasion than the primary tumors with low nuclear volumes and quantitation of nuclear size couldd be beneficial for follow-up of the recurrence and progression of grade II superficial bladder tumors.

Key Words: Morphometry, Bladder, Transitional Cell Carcinoma.

Introduction

In the urologica practise, one of the most important problems is the management of patients with superficial bladder carcinoma (1, 2).

When compared to 30% of patients with tumor invading lamina propria recurrence with tumors merely limited to the mucosa occurs in 4% of patients after transurethral resection (3, 4). Furthermore in superficial bladder carcinoma (Ta, T1) this conservative treatment procedure appears insufficient to prevent the development of the recurrent disease and the progression to high grade carcinoma which occur in 15% to 25% of patients or to muscle invasion (5, 6).

Unfortunately, with their unpredictable course and variable survival rates grade II carcinomas (WHO Classification) constitute a large heterogenous group (1, 7, 9). In the management of transitional cell carcinomas of the urinary bladder, the theropeutic approach depends considerably on the histopathologic grading, but a completely objective grading system is still unavailable (4, 10). As shown by Ooms et al. (10) the consistency and reproducibility of the histologic grading by different pathologists is very low. For these reasons superficial and low grade tumors need more objective grading criteria (4, 9-18).

Recently, several authors have shown that morphometric analysis is a valuable tool for grading urinary bladder carcinomas as in other organ tumors (12,14).

In this study using simple light microscopic morphometric analysis, we compared the mean nuclear volume of primary tumor cells with tumor progression and recurrence in cases diagnosed as grade II, stage pTa-pT1 bladder carcinoma.

Materials & Methods

Selection of cases: In our study we analyzed 34 cases (mean age 59 years, min 24-max 74 years), which all had grade II transitional cell carcinoma staged pathologically as pTa and pT1 between 1986-1993 at the Department

of Pathology of the Akdeniz University Faculty of Medicine.

Acording to the 5 year clinical followw-up the cases were seperated into 3 groups:

Group 1: Ten cases with no subsequent recurrence at least 5 years after the diagnosis of the primary tumor. Group 2: twelve cases with superficial primary tumor (pTa-pT1) and superficial recurrent tumor (pT1). Group 3: twelve cases with superficial primary tumor (pTa-pT1) and recurrent tumor with muscle invasion (pT2-pT3). One case from each group was staged as pTa.

Histological specimens: 4 µm thick H-E stained sections were cut from the original routinely processed, paraffin embedded tissue blocks.

Staging and grading: TNM system was used for the surgical staging, AJCC for the pathological staging and the grading was performed basically according to the WHO system (7, 8).

Morphometric analysis: The morphometric analysis was carried out by a researcher without any knowledge of which patients the tumors came from and the clinical outcome.

With a Nikon Optiphot microscope with a 100X oil immersion lens-the final magnification was 1500-the slides of each case were examined with a 100 µm micrometer inserted into the microscope ocular.

On the slides, the areas of necrosis or primary fibrous tissue were not evaluated.

Ten areas including superficial and basal layers were selected. In each case the diameters of 800 cells were measured.

The nuclear diameters were calculated by the square root of the multiplication of the long and short axis of the cell nuclei by the measurement of eighty cells in each area.

Cells with pyknotic nuclei, degenerated cells or superficial cells near the lumen were not seleted for this study.

After the measurement of the mean nuclear diameters for each tumor the mean nuclear volume was calculated with the equatin:

V: Volume

r: Mean radius of the cells

$$V = \frac{4\pi \times r^3}{3}$$

Statistics: Student's T test, t test for paired observation and coefficient of correlation tests were applied.

Results

The mean nuclear volume of the tumor cells in the patients with one primary tumor without subsequent recurrence for at least 5 years varied from 108 μm^3 to 215 μm^3 with a mean of 142.5± 9.58 μm^3 with one patient above this value.

In group 2 the mean nuclear volume of tumor cells in the original bladder tumor varied rom 75 μm^3 to 577 μm^3 with a mean of 207±39.5 μm^3.

Eight of the 12 patients had values above 142.5± 9.58 μm^3. In the same group the mean nuclear volume of the recurrent superficial tumors ranged from 101μm^3 to 193 μm^3 with a mean of 152±28.42 μm^3. In the group of patient with recurrence leading to invasion, the mean nuclear volume varied from 94 μm^3 to 496 μm^3 with a mean of 299±3.65 μm^3. Ten of the 12 patients had values above 142.5±9.58 μm^3 .

The mean nuclear volume of the recurrent tumors with muscle invasion was 386±142.66 μm^3 ranging from 155 μm^3 to 666 μm^3.

There was no evident difference between the mean nuclear volumes of group 1 and group 2 patients ($p>0.05$). However the differences between group 1 and group 2 and group 3 were statistically significant ($p<0.05$) (Figure 1).

The primary and recurrent values of group 1 and group 2 were similar with a low variation of distribution. However a higher value and variation were found in group 3. While the coefficient of correlation of the primary and recurrent values of group 2 was r= 0.34 the value of group 3 was r= 0.80 ($p<0.05$).

Discussion

Factors influencing recurrence and progression of superficial bladder cancer have been investigated many times and conflicting results reported in several papers 19,24 The proliferation index of Ki-67 and PCNA, overexpression of p53, bcl-2, c-myc oncogenes and the presence or absence of growth factor receptors on tumor cells were evaluated (19, 20, 21, 23, 24).

However at present we are not able to identify accurately which patient with superficial bladder carcinoma is at increased risk of the invasive disease.

On the other hand, there is increasing evidence demonstrating the value of nuclear morphometry and recently a number of reports considered cell and nuclear size, nuclear areas and cell areas as a marker of tumor behavior (15,18). The detection of tumor progression

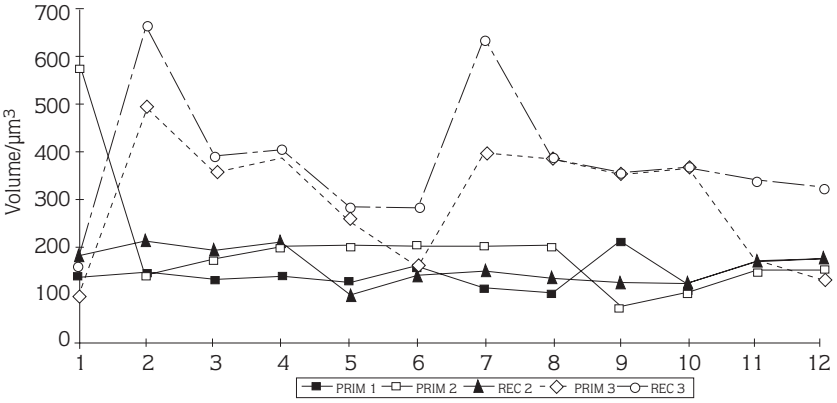

Figure 1. Distribution of mean nuclear volume of primary and recurrent tumors for three groups. Prim: primary tumor, Rec: recurrent tumor. Prim1: Ten patients with only one tumor. Prim 2, Rec 2: Twelve patients with recurrent noninvasive tumor. Prim 3, Rec3: Twelve patients with bladder tumor leading to invasion.

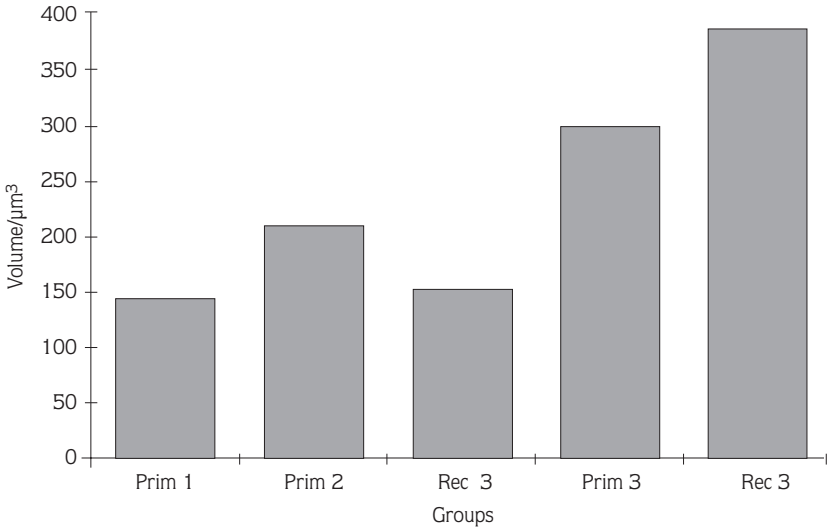

Figure 2. The mean nuclear volume of tumor cells in three groups of patients. Prim: Primary tumor, Rec: Recurrent tumor. Prim 1: Group 1, Prim 2, Rec 2: Group 2, Prim 3, Rec 3: Group 3.

and recurrence morphometric parameters has also been applied in the field of bladder carcinoma, but the results remain controversial (4, 10, 25-29).

Colombel et al (26) reported that morphonuclear parameters correlated well with tumor progression and recurrence in bladder carcinoma. Borland pointed out that nuclear morphometry could be useful in the detection of recurrence in deeply invasive tumors (26) . In contrast Vale did not observe any predictive no additional prognostic information in superficial bladder carcinoma (27).

In our study, in group 3 patients there was a 55 μm^3 increase in the mean nuclear volume of recurrent tumor values compared with the primary tumor (Figure 2). For the primary tumors of this group, the mean nuclear volume of the primary tumor cells was significantly larger than the other two groups and this value was the greatest in their reccurences.

In our cases, while 10 of the 19 primary tumors with a mean nuclear volume above the 142.5±9.58 μm^3 distribution value had subsequent musle invasion.

This finding supported the concept that the mean nuclear volume of the primary superficial grade II tumor cells in the great majority of cases ending with recurrent muscle invasion was larger than the cases with no recurrence or superficial recurrence. Although there are numerical variations when compared with the study by Nielsen et al (4, 10), our results had similar mean nuclear volume relations.

Our results showed that in the group of patients with no recurrence the mean nuclear volume of the primary tumor was significantly lower than the primary tumors of the patients who had recurrence. However, this difference was not statistically significant.

In group 2 patients there was an 86.2 μm^3 decrease in the mean nuclear volume of the recurrent tumor values compared with the primary tumors. In other words there was an inverse correlation betwween the primary and recurrent tumors of this group. The difference between the mean nuclear volume of the recurrent tumor cells of group 2 and primary tumor cells in group 1 was also very low (9.5±18.84 µm). The decrease in the mean nuclear volume of the recurrent tumor cells in superficially recurrent tumor compared with their primary and the close proximity of this value to the mean nuclear volume of the tumor cells of the group 1 remains to be explained. Perhaps the metabolic status of the nucleus of the tumor cells with no recurrence or superficially recurrence potential could be similar. However we could not agree with the above findings and think that this subject needs further investigation concerning molecular basis. To our knowledge, there is no report concerning this finding at present. The mean nuclear volume of the primary tumor of group 2 patients was slightly larger than the primary tumor with no recurrence, but lower than the primary tumor of the group 3 patients ending up with muscle invasion and this difference was statistically significant ($p<0.05$).

It is obvious that all tumors diagnosed as grade II superficial bladder carcinomas by using histopatologic criteria, form a large heterogeneous group with different behavior during their follow-up. While some of these tumors do not recur others recur and also progress.

The aim of our study was to evaluate the accuracy of the mean nuclear volume of the primary tumor cells in grade II superficial bladder carcinomas (pTa-pT1) in the progression and recurrence during the follow-up.

Our results showed that the mean nuclear volume of the primary tumor cells could be valuable in the detection of invasive behavior of tumor cells and their recurrence.

In our study we obtained the mean nuclear volume of the tumor cells after the measurement of the mean nuclear diameters with an ocular micrometer. This method is more time-consuming and semi biased when compared with highly sensitive image analysis systems and stereology, but is more objective for detecting recurrence than simple morphological grading (4,13,15).

In addition the methods mentioned above are expensive and require technical equipments not easily available everywhere. However we do not suggest that our method can be used instead of computerized image analysis and the other systems, but can be a supportive method to simple morphological grading.

As a conclusion, the mean nuclear volume of tumor cells is a useful determinant for predicting the future behavior of grade II, pTa-pT1 transitional cell carcinomas of the bladder, even with our simple method.

In the future, a standart for measurement will be carried out and a better comparaison of bladeler tumor materials will be possible.

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