

Correlation Between Morphometric Analysis and Routine Histological Grading of Transitional Cell Carcinoma of The Urinary Bladder

Dr. Fadhil Husam Ahmed,¹ Dr. Jalal Alwan Mejbel,² Dr. Mohammed Ubaid Hamza,³ Dr. Nour Taqi Khudair,⁴

^{1,2,3,4} Al-Farahidi University, College of Medical Techniques, Baghdad, IRAQ.

Corresponding author: fadil.hussam@alfarahidiuc.edu.iq

jalalafandimejbel@gmail.com,

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Abstract

Urinary bladder transitional cell carcinoma is serious cancer that affects both men and women. It is a heterogeneous neoplasm with varying biological behavior, with most cases being early non-invasive with a proclivity for recurrence and some cases progressing to invasive tumors. The tumor stage and histological grade, which are employed as predictive indicators for the tumor and play a critical role in therapy, are key for clinicopathological aspects. The repeatability was low because of the subjectivity of histological grading. Many studies have shown the importance of quantitative tumor analysis in assessing tumor recurrence and muscular invasion, while others have shown the utility of nuclear measurement as a prognostic tool for bladder cancer. This study compares the benefits of nuclear image analysis as a method for evaluating transitional cell carcinoma of the urinary bladder to subjective standard histological grading.

keywords: analysis system of image, image J, morphometry of nucleus, roundness of nuclei, the urinary bladder transitional cell carcinoma.

Introduction

With a rate of 29.7 per 100.000 males per year, cancer of the urinary bladder is the fifth most predominant cancer in men and the second most normal urological danger in Western nations ⁽¹⁾. Transitional cell carcinoma (55%) is the most widely recognized histological sort in Iraq, trailed by squamous cell carcinoma (27%), undifferentiated carcinoma (5.4%), and adenocarcinoma (5.4%)^(2,3). Transitional cell carcinoma accounts for 91-92% of all primary bladder tumors in the United States ⁽⁴⁾. Urinary bladder transitional cell carcinoma (TCC) is a heterogeneous tumor with a variety of biological characteristics. Most TCC are non-intrusive early lesions with a proclivity for repeat and the possibility to develop into obtrusive tumors^(5, 6). Tumor stage and histological grade are critical factors in TCC treatment and prognosis ⁽⁶⁻⁸⁾. However, because histological grading is subjective, its repeatability which limited ^(9, 10). The need for a grading approach that is aim and repeatable has prompted scholars to investigate quantitative analysis using various methodologies for TCC ⁽⁹⁻¹²⁾. Cytomorphometric characteristics of the initial tumor were thought to be important in predicting tumor recurrence. Different examinations tracked down that the nuclear area (NA) and standard deviation of the nuclear are increased with increasing tumor grade, and that it increased much more in tumors with muscle invasion ⁽¹³⁻¹⁸⁾. They also discovered that image analysis-based grading had a nearly same connection with mitotic density and P53 protein expression as subjective grading criteria, suggesting that this type of image analysis could be useful as a predictive tool for urinary bladder cancer ⁽¹⁹⁻²²⁾. Using morphometric NA measurement has the added benefit of having a lot of potential as a general evaluating strategy for malignancy in cytological material (10) In one investigation, they found the mean NA of the ten biggest cells and the mean NA of all cells analyzed for each case to have a positive connection with the histological grade ⁽²³⁾. Using an image analysis system, the NA and roundness of bladder TCC cases which examined and linked with the histological grade. The study did not include nuclei that overlapped. We also counted the largest ten nuclei in each sample. Each case on its own.

Materials and methods

In this investigation, there were 70 cases with TCC of the urinary bladder. We took them from Alkindi Teaching Hospital's pathology laboratory files. Each case is to investigate and evaluated using the WHO grading system (grades I-III) (GI, 18 cases; GII, 22 cases; GIII, 30 instances) (24). We classified tumors with a grade of I or II as low-grade tumors, while those with which we classified a grade of III as high-grade tumors. They fixed sections in neutral buffer formalin 10%, cut from paraffin embedded blocks at 5 um thick sections, and stained with hematoxylin and eosin (H&E). In each example, 7-10 high-resolution fields which examined, and 11-16 tumor cells with a distinct nuclear border which explained. The study did not include nuclei that overlapped. In addition, for each case, we measured the largest eleven nuclei independently. They studied these nuclei at x400 (x40 objective lens, x10 camera ocular) using an image analysis system controlled by the "Image J®" software in each case (program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin). The system comprises from PC, proway™ microscope and a microscopic camera (Olympus, EP50). They captured the photos from the sections in JPGE format at a resolution of 1280X720 pixels. They evaluated the following morphometric characteristics:

Area (a) and roundness (r) of the nucleus = $4\pi a/p^2$ (a= area, p= perimeter). Before each measurement, they calibrated the system using a micrometer slide. We entered the data into a Microsoft® excel work sheet and expressed in micrometers; We statistically assessed the variations in morphometric measurement between the three grades of bladder cancer investigated using analysis of variance (IBM SPSS statistics Data Editor®) and Tukey's HSD test.

Result

The findings are shown in the tables below (1-4). The mean values of nuclear roundness didn't contrast significantly among the three grades (I, II, and III) using analysis of variance, although the mean values of nuclear area organized (<P0.05). The mean nuclear area differed significantly between grades I and III, but not between grades II and III (Tukey's test: <P0.05).

Table 1: Descriptive nuclear parameters expressed in mean± s.d.

Parameter	Grade I	Grade II	Grade III
Nuclear Roundness	0.71±0.09	0.72±0.09	0.70±0.07
Nuclear Area	36.12±9.54	38.59±12.49	40.31±15.09

Table 2: One-Way Analysis of Variance for nuclear roundness.

Variance source	Freedom degree	Summation of Squares	Mean of Squares	F
Among groups	2.09	1130.89	565.48	3.18
Within groups	454.15	80901.10	178.25	
Total	82031.85	456.10		

Table 3: One-Way Analysis of Variance for nuclear area.

Variance source	Freedom degree	Summation of Squares	Mean of Squares	F
Among groups	10.54	2.18	5.27	0.91
Within groups	2656.37	454.09	5.89	
Total	2666.91	456.27		

Table 4: shows the mean s.d. of nuclear area and the nuclear area of the largest 11 nuclei in various histological grades.

Parameter	Grade I	Grade II	Grade III
Nuclear Area of the 11 largest nuclei	45.19±8.48	49.44±13.56	53.26±17.46
Nuclear Area	36.21±9.53	38.68±12.48	40.43±15.10

Discussion:

The most frequent histological type of bladder cancer is transitional cell carcinoma (TCC), which affects people all over the world. In TCC, tumor grade and stage are essential clinico-pathological features, and treatment options are determined by these factors ⁽¹⁰⁾, making them difficult to repeat. This prompted researchers to seek a more objective system of grading, and because of advances in technology, they could do it. Advances in photography and software have aided in the refining of our current morphometric system. The cytomorphometric characteristics of the initial tumor were found to be an essential factor in predicting tumor recurrence ^(5, 10-12). Other studies have found that the NA and standard deviation of the NA rise with increasing tumor grade, and that it increases in cancers with muscle invasion ⁽¹³⁾. Using morphometric NA measurement has a wide range of applications in the screening of cytological materials for malignant alterations. The goal of this study was to see if nuclear morphometric features (nuclear area and roundness) studied by random (and not selected area) measurement through the use of an image analysis system were compatible with subjective routine histological grading on routine histological sections of TCC of the urinary bladder with different grades (I-III). The mean value of NA increased with increasing tumor grade in this study. (Table 1, 4) Nuclear roundness did not differ between the three categories (See Table 1 and 2). There was a statistically significant difference in mean NA and mean NA of the largest 11 nuclei between grades I and III (larger values in higher grades), but no such difference found between grades I&II, II&III. The fact could explain this that, in standard histological grading, the pathologist determines the final tumor grade based on the cytomorphological aspects of random cell evaluation rather than targeted cell selection. We then examined these cells using an image analysis method, which revealed no significant differences between grades I and II and grades II and III. Because cancers of grade I&II (low-grade tumors) that normally treated conservatively, whereas those of grade III require more violent treatment, it is critical to distinguish between grades II and III using morphometric analysis of certain tumor cell regions, namely:

- Deep-seated cells (cells near the stroma).
- Cells on the surface (near the lumen).
- Cells with a big nucleus (regardless of the location).

Because this was not done during the standard random cell evaluation, the objective morphometric analysis could not distinguish between these classes after it appraised them. As a result, when dealing with TCC of the urinary bladder, it should use morphometric analysis with caution, especially when attempting to differentiate between grades II and III.

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