

Correlation between the conventional, routine histological grading of transitional cell carcinoma of the urinary bladder and morphometric analysis

Zainab A.J. Al-Obaidi *M.B.,Ch.B., F.I.B.M.S (pathology)
Hassanain A.J Al-Obaidi **M.B.,Ch.B., M.Sc. (Human Anatomy)

Summary:

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Background: Transitional cell carcinoma of the urinary bladder is one of the important malignancies in both sex groups .It is considered as a heterogenous neoplasm with different biological behavior, in which the majority are early non invasive with tendency for recurrence and some may progress to invasive tumor. An important clinicopathological features are ,the tumor stage and histological grade which are used as prognostic parameters of the tumor and play an important role in therapy. Due to the subjectivity of the histological grading , the reproducibility was low . Many studies showed the value of quantitative analysis of the tumor as an important method in determining the recurrence of the tumor and muscular invasion, some other studies showed the value of nuclear measurement as a prognostic tool for bladder carcinoma.

Aim of the study: To evaluate the benefit of nuclear image analysis as an objective method for grading of transitional cell carcinoma of the urinary bladder and compare it with the subjective routine histological grading.

Material and method: Sixty two cases of transitional cell carcinoma of the urinary bladder, histologically diagnosed and graded according to WHO grading system ,were selected . In each case 8-10 HPF were examined &10-20consecutive cells were studied, also we measured the largest 10 nuclei for each case separately by a computerized image analysis system at x400 magnification. Nuclear area and roundness were determined. Statistical analysis was performed using the analysis of variance and Tukey's test (HSD).

Results: There was no statistical difference in the mean value of nuclear roundness between the three grades ($P<0.05$), while there was a statistical difference between grade I&III of their mean nuclear area (MNA) &MNA of the largest 10 nuclei ($P<0.05$). No such difference was found between grade I&II or grade II&III ($P<0.05$).

Conclusion: Morphometric analysis should be based on the selection of special areas and not by random measurement as done on routine histological grading.

Key words: Transitional cell carcinoma of the urinary bladder, image analysis system, nuclear morphometry.

Introduction:

Cancer of the urinary bladder is the fifth most common cancer in men and the second most common urological malignancy in western countries with an incidence rate of 29.8 per 100.000 male per year. ⁽¹⁾ In Iraq transitional cell carcinoma is the main histological type (56%), followed by squamous cell carcinoma (28%) , undifferentiated carcinoma (5.3%) and adenocarcinoma (3%).⁽²⁾ In USA , transitional cell carcinoma constitutes about 90-92% of all primary bladder tumor.⁽³⁾

Transitional cell carcinoma (TCC) of the urinary bladder is a heterogenous neoplasm showing different biological behavior. The majority

of TCC are early non invasive lesions with a tendency for recurrence and some times progress to invasive tumors .⁽⁴⁾

Tumor stage & histological grade are important parameters that determine therapy and prognosis of TCC. ^(4, 5) However, due to the subjectivity of histological grading; its reproducibility is low. ^(6, 7)

The need for an objective and reproducible method for grading has simulated the researchers for the study of quantitative analysis by various techniques for TCC.^(6,8,9,10) Cytomorphometric features of the primary tumor were considered important factors in determining the tumor recurrence.^(7,11,12,13) Other studies showed that the nuclear area(NA) &standard deviation of the nuclear area(NASD) showed increasingly higher values with increase tumor grade, and it raises in those tumors having muscular invasion.⁽¹⁴⁾ Also it was found that image analysis based grading

* College of medicine / Al-mustansiryah University
** Ministry of Health

showed almost identical correlation with mitotic density & expression of P53 protein as a subjective grading parameters, therefore it is of benefit to use this kind of image analysis as a prognostic tool for urinary bladder carcinoma.⁽¹⁵⁾

The use of morphometric measurement of NA has an extended benefit in that it has a great potential as a generic screening tool for malignancy in cytological specimens.⁽¹⁶⁾ In one study the mean NA of the biggest ten cells & mean NA of all cells that are measured in each case showed a positive correlation with the histological grade.⁽¹⁷⁾

In this study using an image analysis system, the nuclear area and roundness were analyzed & were correlated with the histological grade of bladder TCC cases.

Material & Method:

Sixty two cases of TCC of the urinary bladder were included in this study. They were retrieved from the files of the pathological laboratory of Al-Yarmouk Teaching Hospital. Each case was examined and graded according to WHO grading system (grades I-III) (GI,14cases; GII,20cases; GIII,28 cases).⁽¹⁸⁾ Tumors of grade I and II were designated low-grade tumors & those of grade III as high grade tumors. Sections were fixed in formalin, cut at 5µm thick sections from paraffin embedded blocks and stained with hematoxylin and eosin. In each case 8-10 representative high power fields were examined and 10-20 consecutive tumor cells with clear nuclear border were outlined. Overlapping nuclei were excluded from the study. Also we measured the largest 10 nuclei for each case separately. In each case these nuclei were examined at x400 (x40 objective magnification, x10 camera ocular), by the use of an image analysis system run by "global lab image 2" software GLI2 (data translation Inc., USA). The system composed of, personal computer PC with frame grabber (DT3120k-1 data translation Inc., USA) attached to the PC, and a microscope (Olympus BH, Japan) with a video camera (KGB, cc-8603, Taiwan).

The images from the sections were obtained at 800X600 pixels resolution in BMP format. The digitalized images of the nuclear profile were outlined on the monitor screen using a computer mouse. The morphometric features assessed included:

Nuclear area (a) and nuclear roundness ($r = 4a/p^2$ (a=area and p=perimeter)). The system was calibrated with a micrometer slide before each measurement. The data was transferred to a Microsoft® excel work sheet and were expressed in terms of micrometers, the differences in terms of morphometric measurement between the three grades of bladder carcinoma studied were statistically tested using analysis of variance, and Tukey's HSD test.

Results:

Results are presented in tables (1 to 4).

The mean values of nuclear roundness did not vary between the three grades (I, II and III) by using analysis of variance ($P < 0.05$), in contrast, the mean values of nuclear area varied significantly between the three grades ($P < 0.05$).

On the other hand, the mean nuclear area showed a significant difference between grade I and III, while no such difference appeared between grade I and II or grade II and III (Tukey's test: $P < 0.05$).

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

Parameter	Grade I	Grade II	Grade III
Nuclear Roundness	0.70±0.08	0.71±0.08	0.69±0.06
Nuclear Area	36.11±9.53	38.58±12.48	40.33±15.00

Table 2 One way ANOVA for nuclear roundness.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F
Among groups	10.52	2.00	5.26	0.90
Within groups	2656.30	454.00	5.85	
total	2666.82	456.00		

$F=3.02$

Table 3 One way ANOVA for nuclear area.

Source of variance	Degree of freedom	Sum of squares	Mean squares	F
Among groups	2.00	1130.83	565.42	3.17
Within groups	454.00	80901.00	178.20	
Total	82031.83	456.00		

$F=3.02$

Table 4 The mean± s.d values of nuclear area and the nuclear area of the largest 10 nuclei in different histological grade.

Parameter	Grade I	Grade II	Grade III
Nuclear Area of the 10 largest nuclei	45.15±8.43	49.34±13.56	53.16±17.36
Nuclear Area	36.11±9.53	38.58±12.48	40.33±15.00

Discussion:

Bladder cancer is a worldwide disease in which the most common histological type encountered is transitional cell carcinoma (TCC).

Tumor grade and stage are important clinicopathological features in TCC and the choice of therapy depends on these variables.⁽¹⁹⁾ Many histological grading systems were applied to grade TCC of the urinary bladder, but all have a subjective nature which render their reproducibility low. This had stimulate the researchers to find a more objective method for grading and due to the developing technology, imaging and software advances, they have contributed to the refinement of our current morphometric system. It was reported that cytomorphometric features of the primary tumor were important factor in determining the tumor recurrence.^(7,11,12,13) Other studies showed that the nuclear area (NA) & standard deviation of the nuclear area (NASD) showed an increasingly higher values with increase tumor grade, and it raises in those tumors having muscular invasion.⁽¹⁴⁾ The use of morphometric measurement of the nuclear area has an extended benefit in screening the cytological materials for detection of malignant changes.

The present study was performed on the routine histological sections of TCC of the urinary bladder with different grades (I-III) & the aim was to check whether nuclear morphometric features (nuclear area and roundness) which were studied by random (and not selected area) measurement through the use of an image analysis system were compatible with the subjective routine histological grading.

Here, there was an increase in the mean value of nuclear area with increase tumor grade. (Table 1, 4)

On the other hand, nuclear roundness showed no difference between the three grades. (Table 1, 2)

Statistically speaking, there was a significant difference in the value of mean nuclear area and the mean nuclear area of the largest 10 nuclei between grade I and III (higher values observed in higher

grade), but no such difference was observed between grade I&II, II&III.

This might be explained that in the routine histological grading, the pathologist uses the cytomorphological features of random cell examination, and not specific cell selection, to reach the final tumor grade. These cells were further studied by image analysis system that showed no statistical difference between grade I&II and between grade II&III. Since the tumor grade I&II (low grade tumors) usually treated conservatively, and those with grade III needs a more aggressive therapy, so it is important to separate grade II and III by morphometric analysis of specific selected areas of the tumor cells namely:

- Deep cells (cells near the stroma).
- Superficial cells (near the lumen).
- Cells with large nuclei (regardless the location).

which was not done during the usual routine random cell examination which make it unable to discrimination between these grades after they were assessed by the objective morphometric analysis.

This concludes that using morphometric analysis should be treated with caution in dealing with TCC of the urinary bladder especially when trying to discriminate between grade II and III.

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