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Concordance of nuclear morphometric analysis with Fuhrman nuclear grade and pathologic stage in conventional renal cell carcinoma

Konvansiyonel böbrek hücreli karsinomda Fuhrman nükleer derece ve patolojik evre ile nükleer morfometrik analizin uyumu

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ABSTRACT

Quantitative image analysis has been applied to renal cancers for diagnostic and prognostic purposes. The aim of this study was to investigate whether there is any correlation between the results of morphometric measurements and Fuhrman nuclear grade, pathologic stage and size of conventional (clear cell) renal cell carcinoma. Morphometric nuclear parameters such as roundness factor, form ellipse, area, length, breadth and perimeter were evaluated in hematoxylin-eosin stained slides of 37 conventional renal cell carcinomas by using computer assisted image analysis system. The relationship between Fuhrman nuclear grade, pathologic stage, tumor size and morphometric results were determined by correlation analysis. Mean nuclear areas correlated with pathologic stages (r:0.413, p=0.05) and with Fuhrman nuclear grade (r:0.588, p=0.01). Mean nuclear length correlated with pathologic stage (r:0.446, p=0.01) and with Fuhrman nuclear grade (r:0.580, p=0.01). Mean nuclear breadth were correlated with pathologic stage (r:0.377, p=0.05) and with Fuhrman nuclear grade (r:0.544, p=0.01) and with tumor size (r:0.366, p=0.05). Mean nuclear perimeter correlated with pathologic stage (r:0.449, p=0.01) and Fuhrman nuclear grade (r:0.593, p=0.01). Mean nuclear roundness factor correlated with pathologic stage (r:0.418, p=0.05) and Fuhrman nuclear grade (r:0.456, p=0.01). In this study, the relative concordance of morphometric results and pathologic stage and Fuhrman nuclear grade, exhibited the importance of nuclear morphometric analysis in the assessment of conventional renal cell carcinoma.

Key words: Conventional renal cell carcinoma, Fuhrman nuclear grade, pathologic stage, nuclear morphometry

ÖZET

Kantitatif görüntü analizi, konvansiyonel böbrek hücreli karsinomda tanısal ve prognostik amaçlarla kullanılmıştır. Bu çalışmanın amacı, konvansiyonel (berrak hücreli) böbrek hücreli karsinomda tümör boyutu, patolojik evre ve Fuhrman nükleer derece ile nükleer morfometrik ölçüm sonuçları arasında bir ilişki olup olmadığını araştırmaktır. Bilgisayar destekli görüntü analiz sistemi kullanılarak, toplam 37 konvansiyonel böbrek hücreli karsinomun hematoksilen- eozin kesitlerinde yuvarlaklık faktörü, elipslik indeksi, alan, uzunluk, genişlik ve perimetreyi içeren nükleer morfometrik parametreler değerlendirilmiştir. Morfometrik sonuçlar ile tümör boyutu, patolojik evre ve Fuhrman nükleer derece arasındaki ilişkiyi saptamak için korelasyon analizi uygulanmıştır. Ortalama nükleer alan patolojik evre (r:0.413, p=0.05) ve Fuhrman nükleer derece (r:0.588, p=0.01) ile; ortalama nükleer uzunluk patolojik evre (r:0.446, p=0.01) ve Fuhrman nükleer derece (r:0.580, p=0.01) ile; ortalama nükleer genişlik patolojik evre (r:0.377, p=0.05), Fuhrman nükleer derece (r:0.544, p=0.01) ve tümör boyutu (r:0.366, p=0.05) ile; ortalama nükleer perimetre patolojik evre (r:0.449, p=0.01) ve Fuhrman nükleer derece (r:0.593, p=0.01) ile; ortalama nükleer yuvarlaklık faktörü patolojik evre (r:0.418, p=0.05) ve Fuhrman nükleer derece (r:0.456, p=0.01) ile ilişkili bulunmuştur. Bu çalışmada, patolojik evre ve Fuhrman nükleer derece ile morfometrik ölçüm sonuçları arasında saptanan ilişki, konvansiyonel böbrek hücreli karsinomda nükleer morfometrik analizin önemini ortaya koymaktadır.

Anahtar sözcükler: Konvansiyonel böbrek hücreli karsinom, Fuhrman nükleer derece, patolojik evre, nükleer morfometri

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INTRODUCTION

The prognosis of patients with renal cell carcinoma (RCC) is dependent on mainly pathologic stage and nuclear grade (1,2). Fuhrman system is the most widely used nuclear grading system for RCC. The disadvantage of this grading system is its subjective nature (3). Quantitative image analysis has been applied to renal cancers for diagnostic and prognostic purposes. The potential advantage in histopathology is morphometric measurement is an objective method (4,5). Ouantitative assessment of nuclear morphometry is possible with computer imaging systems, providing a useful and reproducible method of predicting prognosis for many cancers (6-8). In present study we aimed to investigate whether there is any correlation between the results of morphometric measurement and Fuhrman nuclear grade, pathologic stage and size of conventional (clear cell) RCC.

MATERIALS and METHODS

Pathologic Examination: This study included 37 cases, which had been diagnosed as conventional RCC at the Departments of Pathology in Zonguldak Karaelmas University School of Medicine and Lutfi Kirdar Kartal Training and Research Hospital between 2005 and 2007. All patients underwent simple or radical nephrectomy. Clinical features of the cases were derived from hospital records. All histologic sections were fixed in formalin, embedded in paraffin, cut into 5 µm sections, and stained with hematoxylin-eosin (H&E). Slides of all cases were rescored according to Fuhrman nuclear grading system and staged pathologically according to the 1997 TNM criteria by the same pathologist (SB) without having any knowledge about the prior scores and stages. Tumour sizes were also recorded.

Nuclear Morphometry: Morphometric analysis was performed on H&E stained histological sections by the same pathologist. The microscope (Leica, DMLB-100S) was connected to a computerized video camera (Leica, DFC-280) set. After transferring microscopic images to the computer, morphometric parameters were automatically measured by an image analysis program (Leica, QWINPlus v.3.1.0). About 100 nuclei with sharply demarcated contours were included for morphometric analysis in the highest-grade area of tumour on represantative slide from each case. Nuclei that were markedly distorted during preparation and those that were overlapping were not selected for analysis. The nuclear morphometric parameters studied were as follows: nuclear area, nuclear roundness factor, nuclear form ellipse, nuclear length, nuclear breadth and nuclear perimeter. Nuclear roundness factor is given by the equation: "perimeter ^{2} / 4π x area". Nuclear form ellipse is given by the equation: "longest diameter/shortest diameter". These shape descriptors yield a minimal value of 1.00 for a perfect circle and increase as the shape of a contour deviates from circularity. Nuclear area is the area enclosed inside the contour, the perimeter is the contour perimeter, and the length and breadth are the longest and shortest orthogonal projections, respectively. All measurements were made under 400X magnification and expressed in microns. Mean nuclear area (MNA), mean nuclear roundness factor (MNRF), mean nuclear form ellipse (MNFe), mean nuclear length (MNL), mean nuclear breadth (MNB) and mean nuclear perimeter (MNP) were evaluated in a total of 37 conventional RCCs. Statistical analysis of the measurements was performed by using SPSS for Windows, v.11 statistical package. The relationships between Fuhrman nuclear grading score, pathologic stage, tumor size and morphometric results were determined by correlation analysis with Pearson correlation coefficient.

RESULTS

Of the patients, 13 (35.1%) were female, and 24 (64.9%) were male. The mean age of pa-

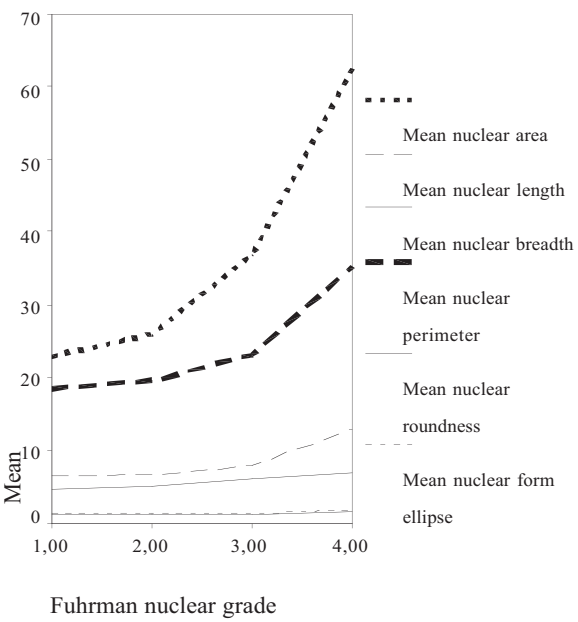
tients (± standard deviation) were 58.65 years± 12.34, ranging from 32 to 79 years. The maximum diameter was ranging from 2 to 14 cm (mean±SD, 6.78±3.14) in all tumors. According to Fuhrman nuclear grading system the distribution of patients were as follows: Grades 1 (n=3; 8.1%), 2 (n=23; 62.2%), 3 (n=10; 27%) and 4 (n=1; 2.7%). The pathological stages were also classified as stages 1 (n=20; 54.1%), 2 (n=13; 35.1%), 3 (n=2; 5.4%), and 4 (n=2; 5.4%). The average number of cancer fields measured was 4.3 for the all tumor slides. The morphometric nuclear parameters of tumors were as follows: MNA: 13.18-62.43 μm^2 (mean±SD, 29.61± 11.64), MNRF: 1.046-1.599 (mean±SD, 1.15± 0.085), MNFe: 1.165-1.945 (mean±SD, 1.37± 0.12), MNL: 4.45-12.85 (mean±SD, 7.18± 1.54), MNB: 3.5-7.75 (mean±SD, 5.28±0.97) and MNP: 13.27-35.22 (mean±SD, 20.86± 4.28). Table 1 summarizes the clinicopathologic features and nuclear morphometric results of 37 patients.

Table 1. Clinicopathologic features and nuclear morphometric results of 37 patients with conventional renal cell carcinomas.

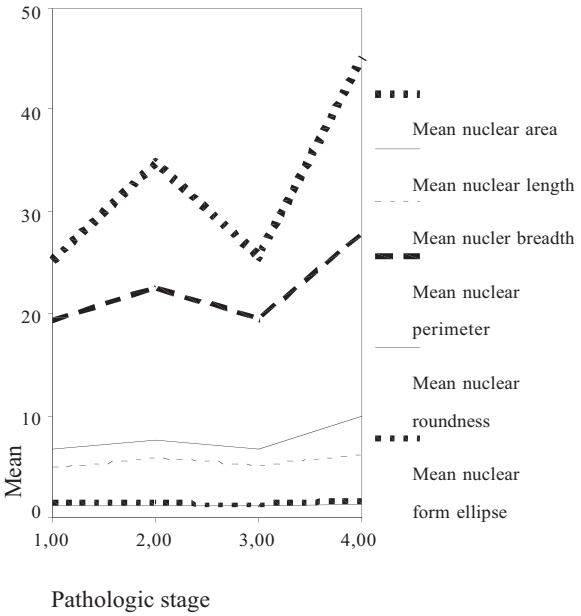
Clinicopathologic Features	Cases (n:37)
Age *(Mean±SD)*	32-79 years (58.65±12.34)
Sex *(Male/Female)*	24/13
Tumor size (cm)	
2.0-4.0	11
4.1-6.0	6
6.1-8.0	9
8.1-10.0	7
10.1-12.0	1
12.1-14.0	3
Fuhrman Nuclear Grade	
1	20
2	13
3	2
4	2
Pathologic Stage	
1	3
2	23
3	10
4	1
MNA (μm^2)-*Min/max*	13.18-62.43
MNRF-*Min/max*	1.046-1.599
MNFe-*Min/max*	1.165-1.945
MNL (μm)-*Min/max*	4.45-12.85
MNB (μm)-*Min/max*	3.5-7.75
MNP (μm)-*Min/max*	13.27-35.22

Tumor size: Size represents the single greatest dimension, MNA: Mean nuclear area, MNRF: Mean nuclear roundness factor, MNFe: Mean nuclear form ellipse, MNL: Mean nuclear length, MNB: Mean nuclear breadth, MNP: Mean nuclear perimeter.

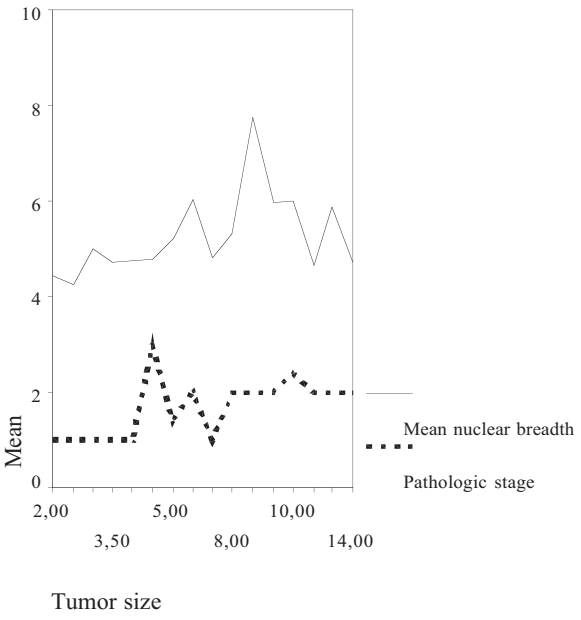
MNAs moderately correlated with pathologic stages (r:0.413, p=0.05) and highly correlated with Fuhrman nuclear grade (r:0.588, p=0.01). MNLs moderately correlated with pathologic stage (r:0.446, p=0.01) and highly correlated with Fuhrman nuclear grade (r:0.580, p=0.01). MNB were moderately correlated with pathologic stage (r:0.377, p=0.05) and highly correlated with Fuhrman nuclear grade (r:0.544, p=0.01) and moderately correlated with tumor size (r:0.366, p=0.05). MNP moderately correlated with pathologic stage (r:0.449, p=0.01) and highly correlated with Fuhrman nuclear grade (r:0.593, p=0.01). MNRF moderately correlated with pathologic stage (r:0.418, p=0.05) and with Fuhrman nuclear grade (r:0.456, p=0.01) (Graphic 1-3). The correlation between MNFe and Fuhrman nuclear grade, pathologic stage, and tumor size was not statistically significant. Tumor size moderately correlated with pathologic stage (r:0.479, p=0.05), Fuhrman nuclear grade was not statistically significant.



Graphic 1. Relationship between Fuhrman nuclear grade and nuclear morphometric parameters in conventional renal cell carcinoma.



Graphic 2. Relationship between pathological stage and morphometric nuclear parameters in conventional renal cell carcinoma.



Graphic 3. Relationship between tumor size, mean nuclear breadth and pathologic stage in conventional renal cell carcinoma.

DISCUSSION

Renal cell carcinoma accounts for 90% of all primary malignant renal tumors in adults (1). Currently, staging and nuclear grading for RCC are considered to be the most important predictor of survival (1,2). Several studies have identified additional prognostic factors, such as tumor size, cell type, histologic subtype, DNA ploidy, proliferation markers, angiogenesis, nuclear morphometry and combination of these parameters (4,9-13). Fuhrman scheme is the most widely used nuclear grading system for RCC grading. This nuclear grading system is based on morphonuclear features including the nuclear size, shape, and the prominence of nucleoli (14). Despite the Fuhrman nuclear grading system is widely accepted, it has some limitations such as its subjective nature and low reproducibility (3,15).

The histopathological and morphological aspects of neoplasms are important as diagnostic and prognostic factors. Shape alterations are hallmarks of malignancy, and nuclear shape is a component of many histologic grading systems. Since visual impressions can be augmented by quantitative morphometry, image analysis permits pathologists to obtain quantitative measurements on tissue sections. Nuclear morphometry was firstly introduced by Diamond et al. in 1982 and later standardized by Mohler et al (16-18). Nuclear morphometric parameters have been compared with the conventional grading systems for malignancies of various organs in the literature (6-8). Quantitative image analysis has been applied to renal cancers for diagnostic and prognostic purposes. Several authors have tried to introduce objective measures for nuclear grading (4,19,20). In this study, we found that MNA, MNL, MNB, MNP, MNRF correlated with pathologic stage and Fuhrman nuclear grade. This correlation might not be surprising, because these nuclear morphometric parameters are quantitative features of nuclear enlargement in malignant cells (5,7,13). On the other hand moderate-high correlation may reflect subjectivity of Fuhrman nuclear grading system as they were not very highly correlated. With respect to the tumor size we found only moderate correla-

tion with MNB. Our findings were in line with previous studies that reported higher grade conventional RCCs with larger nuclei and also a good correlation between nuclear morphometric parameters, nuclear grade and stage in conventional RCC (5,20). Also many studies, nuclear morphometric parameters have been suggested to be of importance in prognostic prediction among patients with conventional RCC (4,5,11,13,20,21). Major limitation of our study is its small sample size. Studies including larger series of cases investigating detailed nuclear morphometric analysis of RCC with longer periods of observation are required in order to demonstrate the association between clinical outcome and morphometric parameters. Considering exact measurements of cell and tissue size, shape, organization and higher sensitivity obtained, morphometric analysis may help better understanding of diagnostic and prognostic features of conventional RCC. In addition, nuclear morphometric analysis may help to improve objectivity of nuclear grading system in conventional RCC.

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