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The relation between gleason score, and nuclear size and shape factors in prostatic adenocarcinoma*

Aim: The aim of this study was to assess the size and shape characteristics of prostatic adenocarcinoma cell nuclei using a computer-assisted analysis system, and to compare the results with the Gleason score.

Materials and Methods: Morphometric nuclear parameters, such as roundness factor, form ellipse, area, length, and perimeter, were evaluated based on specimen slides of 130 prostatic adenocarcinoma cases (77% needle biopsies and 23% prostatectomy specimens) using a computerized image analysis system. Correlation analysis between Gleason score and morphometric results was performed.

Results: The Gleason score was correlated with mean nuclear area (r = 0.516, P = 0.01), mean nuclear length (r = 0.298, P = 0.01), and mean nuclear perimeter (r = 0.303, P = 0.01) for all specimens. In the needle biopsy group the Gleason score was correlated with mean nuclear area (r = 0.522, P = 0.01), mean nuclear length (r = 0.398, P = 0.01), and mean nuclear perimeter (r = 0.432, P = 0.01), whereas in the prostatectomy group the Gleason score was correlated only with mean nuclear area (σ = 0.619, P = 0.01) and mean nuclear roundness factor (σ = −0.425, P = 0.05).

Conclusions: Nuclear size and shape factors, especially mean nuclear area, were concordant with the Gleason score. Nuclear size and shape assessment may aid in the evaluation of the pathological status of prostatic adenocarcinoma.

Key Words: Gleason score, nuclear size, nuclear shape, prostatic adenocarcinoma

Prostat adenokarsinomunda gleason skor ile nükleer boyut ve şekil faktörleri arasındaki ilişki

Amaç: Bu çalışmada, bilgisayar destekli görüntü analiz sistemi kullanılarak prostatik adenokarsinom hücre nükleuslarının boyut ve şekil özellikleri değerlendirilmiş, sonuçlar olguların Gleason skorları ile karşılaştırılmıştır.

Yöntem ve Gereç: Görüntü analiz sistemi kullanılarak, 130 (% 77'si iğne biyopsi ve % 23'ü prostatektomi materyali) prostat adenokarsinomunun histolojik kesitlerinde yuvarlaklık faktörü, elipslik indeksi, alan, uzunluk ve perimetreyi içeren nükleer morfometrik parametreler değerlendirilmiştir. Morfometrik sonuçlar ile Gleason skorları arasındaki ilişki korelasyon analizi ile saptanmıştır.

Bulgular: Tüm materyaller arasında Gleason skor ile ortalama nükleer alan (r = 0,516, P = 0,01), ortalama nükleer uzunluk (r = 0,298, P = 0,01) ve ortalama nükleer perimeter (r = 0,303, P = 0,01) arasında ilişki saptanmıştır. Organ iğne biyopsi grubunda ise Gleason skor ile ortalama nükleer alan (r = 0,522, P = 0,01), ortalama nükleer uzunluk (r = 0,398, P = 0,01) ve ortalama nükleer perimeter (r = 0,432, P = 0,01) ilişkili bulunmuştur. Prostatektomi grubunda Gleason skor ile ortalama nükleer alan (σ = 0,619, P = 0,01) ve ortalama yuvarlaklık faktörü (σ = −0,425, P = 0,05) arasında ilişki tespit edilmiştir.

Sonuç: Nükleer boyut ve şekil faktörleri, özellikle ortalama nükleer alan Gleason skor ile ilişkili bulunmuştur. Nükleer boyut ve şekil ölçümü, prostatik adenokarsinomun patolojik durumunun değerlendirilmesine katkı sağlayabilir.

Anahtar Sözcükler: Gleason skor, nükleer boyut, nükleer şekil, prostatik adenokarsinom

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Introduction

The Gleason grading system is widely used for grading prostatic adenocarcinoma. This system is based entirely on the histological pattern of carcinoma cells in hematoxylin and eosin (H&E)-stained prostatic tissue sections (1-3). Problems with Gleason grading include interobserver and intraobserver variation, and an imprecise predictive value (4,5). Morphologic changes in nuclei are characteristic of cancer cells. In particular, changes in nuclear size, shape, and the nuclear-to-cytoplasmic ratio are common features of cancer (6). These nuclear alterations can be translated into quantifiable features with digital image analysis and a process known as quantitative nuclear morphometry. Image analysis permits pathologists to obtain quantitative measurements of cytological and histological preparations, as visual impressions can be augmented by quantitative morphometry. This procedure also facilitates exact measurement of cell size, shape, and organization, which is not possible with other methods (7). Nuclear size and shape variation are important prognostic indicators in breast carcinoma, renal cell carcinoma, and colorectal adenocarcinoma (8-10). In an effort to develop an objective method for grading and predicting the prognosis of prostate cancer, many investigators have used morphometric characteristics, such as nuclear area, roundness, ellipticity, length, perimeter, and volume (7,11-14). In the present study we investigated the relationship between Gleason score and nuclear morphometric features in 130 prostatic adenocarcinomas, based on needle biopsies and prostatectomy specimens, and evaluated the diagnostic effects of these characteristics.

Materials and Methods

Pathological Examination

This study included 130 cases that were diagnosed with prostatic adenocarcinoma at the Karaelmas University, Faculty of Medicine, Department of Pathology between 2001 and 2006. One hundred of the samples studied (77%) were prostate needle biopsies and 30 (23%) were radical prostatectomy specimens. Clinical features of the cases were obtained from hospital records. All histological samples were fixed in formalin, embedded in paraffin, cut into 5-μm sections, and stained with H&E. All specimen slides were rescored according to the Gleason system by one pathologist (S.B.) that was blinded to the previous scores.

Nuclear Morphometry

Morphometric analysis was performed with H&E-stained histological sections by one pathologist (S.B.). A microscope (Leica, DMLB-100S) was connected to a video camera (Leica, DFC-280) and computer. After transferring microscopic images to the computer, morphometric parameters were automatically measured by an image analysis program (Leica, QWINPlus v.3.1.0). About 150 nuclei with sharply demarcated contours were included in the morphometric analysis of each case. Nuclei that were markedly distorted during preparation and those that were significantly overlapped were excluded from analysis. The nuclear morphometric parameters studied were as follows: mean nuclear area (MNA), mean nuclear perimeter (MNP), mean nuclear length (MNL), mean nuclear roundness factor (MNRF), and mean nuclear form ellipse (MNFe). Nuclear roundness factor was given by the equation: $perimeter^2/4\pi \times$ area. Nuclear form ellipse was given by the equation: longest diameter/shortest diameter. These shape descriptors yielded a minimal value of 1.00 for a perfect circle and increased as the shape of a contour deviated from circularity. Nuclear area was the area enclosed inside the contour, perimeter was the contour perimeter, and length was the longest orthogonal projection. All measurements were made with the 400x objective and were expressed in micrometers. In all, 130 prostatic adenocarcinoma cases were evaluated for MNA, MNP, MNL, MNRF, and MNFe, and the results were compared with the Gleason score.

Statistical Analysis

Statistical analysis of the measurements was performed using SPSS v.11 for Windows. The association between Gleason score and the morphometric variables was analyzed using Pearson's and Spearman's correlation coefficients. Before beginning the study, a priori power analysis

was performed to determine the necessary sample size for the prostatectomy group. A priori power analysis indicated that 30 patients would be sufficient to detect a moderate correlation with a power greater than 75% at a level of significance of P < 0.05.

Results

Patient age ranged from 46 to 94 years (mean: 67.23 ± 8.26 years). Distribution of Gleason scores was as follows: 4 in 1 case (0.7%), 5 in 11 cases (8.4%), 6 in 42 cases (32.3%), 7 in 58 cases (44.6%), 8 in 9 cases (6.9%), 9 in 8 cases (6.1%), and 10 in 1 case (0.7%). Morphometric nuclear parameters were as follows: MNA ranged from 12.14 to 51.93 μm^2 (mean: 22.43 ± 7.43 μm^2), MNP ranged from 13.32 to 28.48 μm (mean: 18.73 ± 3.15 μm), MNL ranged from 4.64 to 10.27 μm (mean: 6.59 ± 1.07 μm), MNRF ranged from 1.09 to 1.38 (mean: 1.20 ± 0.06), and MNFe ranged from 1.27 to 1.8 (mean: 1.50 ± 0.11). Mean number of cancer fields measured was 4.2 for entire study population, 4.1 for the needle biopsy group, and 4.6 for the prostatectomy group.

The Table summarizes the clinicopathological features and nuclear morphometric results of the needle biopsy and prostatectomy groups.

There were no correlations between age, and Gleason score and nuclear morphometric parameters. Among the specimens, Gleason score was significantly correlated with MNA (r = 0.516, P = 0.01), MNL (r = 0.298, P = 0.01), and MNP (r = 0.303, P = 0.01), whereas it was not correlated with MNRF or MNFe (Figure 1). In the needle biopsy group Gleason score was significantly correlated with MNA (r = 0.522, P = 0.01), MNL (r = 0.398, P = 0.01), and MNP (r = 0.432, P = 0.01), and negatively correlated with MNFe (r = −0.213, P = 0.05). The correlation between Gleason score and MNRF was statistically insignificant (Figure 2). In the prostatectomy group Gleason score was positively correlated with MNA (σ = 0.619, P = 0.01) and negatively correlated with MNRF (σ = −0.425, P = 0.05). The correlations between Gleason score, and MNP, MNL, and MNFe were not statistically significant in the prostatectomy group (Figure 3).

Table. Clinicopathological and nuclear morphometric features of the needle biopsy and prostatectomy groups.

	Needle Biopsy Group (n = 100)	Prostatectomy Group (n = 30)
Age		
Range (mean ± SD)	46-94 (68.42 ± 8.42)	53-78 (63.3 ± 6.34)
MNA (μm^2)		
Range (mean ± SD)	12.14-49.51 (22.06 ± 7.13)	13.87-51.93 (23.66 ± 8.36)
MNP (μm)		
Rang (mean ± SD)	13.32-28.45 (18.43 ± 3.07)	15.76-28.48 (19.77 ± 3.25)
MNL (μm)		
Range (mean ± SD)	4.64-10.07 (6.54 ± 1.07)	5.41-10.27 (6.75 ± 1.06)
MNRF		
Range (mean ± SD)	1.10-1.38 (1.21 ± 0.05)	1.09-1.31 (1.18 ± 0.06)
MNFe		
Range (mean ± SD)	1.10-1.38 (1.21 ± 0.05)	1.09-1.31 (1.18 ± 0.06)
Gleason Score: 4	n = 1 (1%)	-
Gleason Score: 5	n = 7 (7%)	n = 4 (13.3%)
Gleason Score: 6	n = 32 (32%)	n = 10 (33.3%)
Gleason Score: 7	n = 44 (44%)	n = 14 (46.7%)
Gleason Score: 8	n = 9 (9%)	-
Gleason Score: 9	n = 6 (6%)	n = 2 (6.7%)
Gleason Score: 10	n = 1 (1%)	-

MNA: Mean nuclear area; MNP: mean nuclear perimeter; MNL: mean nuclear length; MNRF: mean nuclear roundness factor; MNFe: mean nuclear form ellipse.
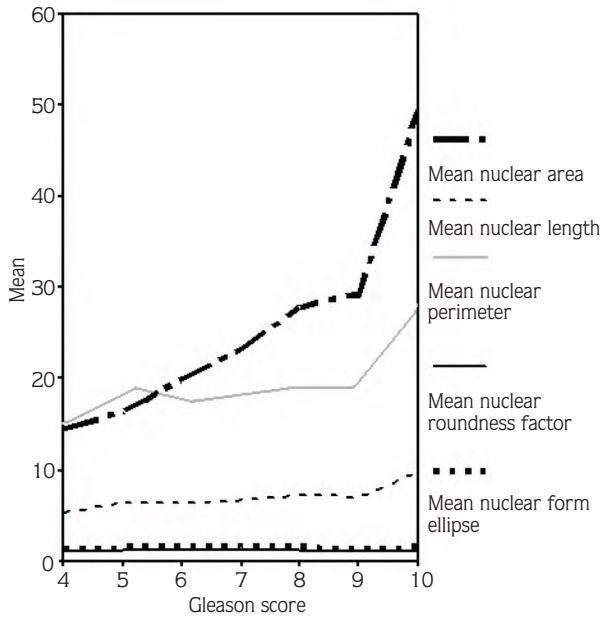
Figure 1. Relationships between Gleason score and nuclear morphometric parameters in the total study population.
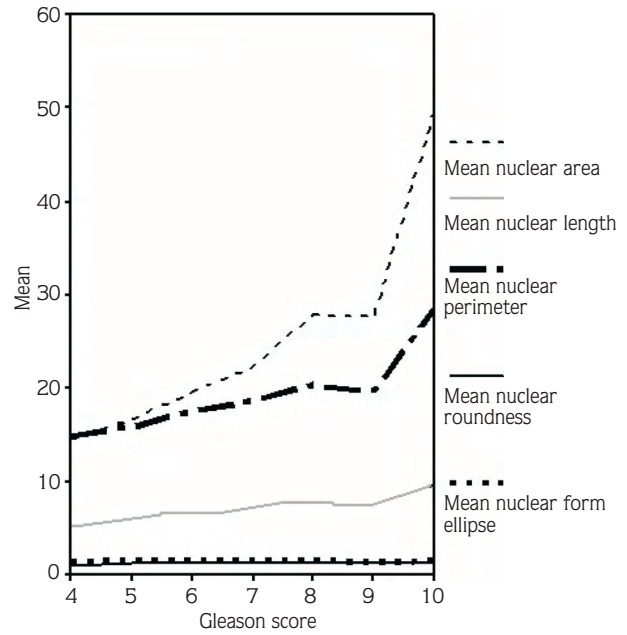

Figure 2. Relationships between Gleason score and nuclear morphometric parameters in the needle biopsy group.

Discussion

The Gleason grading system is widely used to determine the degree of malignancy of prostatic cancer and to predict the prognosis (1-3,15); however, it is difficult to reproduce identical results among pathologists because of the subjective nature of the system (4,5,16). In recent years many methods have been studied in an effort to overcome the subjectivity of the conventional histologic grading system, one of which is quantitative measurement of the pathological features of tumor cells. The potential advantages of morphometric analysis in histopathology are its objectiveness, accuracy, and efficiency (17,18). There are many different mathematically derived nuclear morphometric descriptors of H&E-stained tissue sections that can be quantitatively calculated using image analysis systems. The initial results of nuclear morphometry reported in 1982 by Diamond et al. showed that nuclear roundness accurately identified 27 patients with stage B1 or B2 prostate cancer that were treated surgically (19). Similarly, Partin et al., Mohler et al., Hurwitz et al., and Martinez-Jabaloyas et al. reported a very good correlation between prognosis, and the nuclear roundness factor and nuclear form
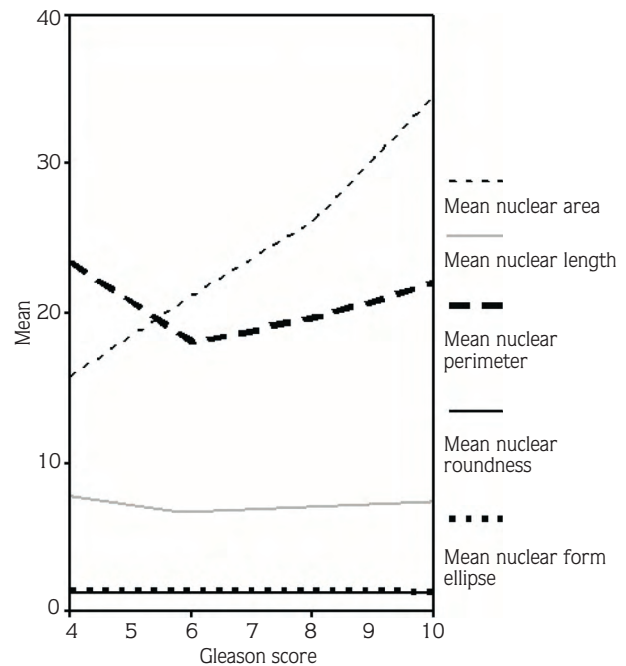

Figure 3. Relationships between Gleason score and nuclear morphometric parameters in the prostatectomy group.

ellipse (18,20,21,23); however, Blom et al. did not observe any correlation between prognosis and these nuclear shape descriptors (22). In the present study Gleason score was negatively correlated with MNFe in the needle biopsy group. We also observed that Gleason score was negatively correlated with MNRF in the prostatectomy group; however, Gleason score was not correlated with MNRF or MNFe in the total study population. It has been reported that nuclei in benign prostate hyperplasias have less nuclear area and greater mean nuclear form ellipse than nuclei in prostate adenocarcinomas. In addition, it has been shown that mean nuclear area has prognostic value and correlates with Gleason score (23-25). Published data suggest that mean nuclear area greater than 28 µm² and mean nuclear diameter greater than 5 µm predict malignancy. In the present study MNA and MNL ranged from 12.14 to 51.93 µm² (mean: 22.43) and 4.64 to 10.27 µm (mean: 6.59), respectively. In contrast to previous studies, we detected lower MNA values (26.27). Possible explanations for these discrepant results are compression artifacts during needle biopsy and artifacts due to delayed fixation of prostatectomy specimens.

Our results demonstrate that Gleason score was significantly correlated only with MNA in both the needle biopsy and prostatectomy groups, and in the total study population; however, MNRF showed significant concordance with Gleason score only in the prostatectomy group. Needle biopsy is a routine diagnostic procedure for patients with prostate cancer. It is expected to provide useful pathologic information that can aid in treatment decisions and in predicting cancer progression. Therefore, the correlation between MNA and Gleason score, particularly in the needle biopsy and prostatectomy specimens observed in the present study, indicates that MNA may provide additional assistance in evaluating the pathologic status and prognosis of the disease, and contribute to preoperative treatment.

Nuclear morphometric characteristics provided improved prediction of outcome when compared with standard predictive tools. Moreover, recent data indicate that nuclear morphometry, with or without clinical and histopathologic parameters, may be beneficial in predicting recurrence and prognosis of prostatic adenocarcinoma (14,28-30).

Technical considerations limiting the use of nuclear morphometry are mainly associated with cost and reproducibility. Mohler et al. measured the accuracy and reproducibility of such a system for nuclear morphometry measurements, and reported a reproducibility and accuracy of greater than 95% when studying standard microscopic shapes (17,18).

Quantitative assessment of nuclear morphometry is possible with computer imaging systems, providing a useful and reproducible method of predicting the prognosis of many cancers. Nuclear morphometric parameters have been compared with conventional grading systems for malignancy of various organs (8-10). Many studies have demonstrated that a computer-based nuclear morphometry system can add to the prognostic information provided by the Gleason scoring system (21,23,24,31). Additionally, the World Health Organization recommended the use of nuclear morphometry in prostate cancer (32). In the present study nuclear size and shape factors were concordant with Gleason score in both the needle biopsy and prostatectomy groups. Nuclear size and shape assessment, especially MNA, may support the evaluation of the pathological status of prostatic adenocarcinoma.

To summarize, nuclear morphometry may be used as a diagnostic tool to supplement the Gleason grade in prostatic adenocarcinoma. Considering its exact and objective measurement of cell size and shape, morphometric analysis might help improve our understanding of the diagnostic and prognostic features of prostatic adenocarcinoma.

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