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Nuclear morphometric analysis in gastrointestinal stromal tumors: A preliminary study

Gastrointestinal stromal tümörlerde nükleer morfometrik analiz: Ön çalışma

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Background/aims: *Gastrointestinal stromal tumors are considered a specialized group of mesenchymal neoplasms. In this study, the histomorphologic and immunohistochemical features of gastrointestinal stromal tumors are compared with nuclear morphometric results.* ***Methods:*** *Morphometric nuclear parameters such as mean area, mean roundness factor, mean form ellipse, mean length and mean perimeter were evaluated in hematoxylin and eosin stained slides of 22 gastrointestinal stromal tumors (9 benign and 13 malignant) by using a computer-assisted image analysis system. Morphometric results were compared with tumor behavior and tumor size, the presence of necrosis, mitotic index, and immunohistochemical expressions of p53 and proliferating cell nuclear antigen.* ***Results:*** *We found that tumor necrosis was correlated with mean nuclear roundness factor, mean nuclear form ellipse, mean nuclear length and mean nuclear perimeter ($p<0.05$). Mitotic index was also correlated with mean nuclear roundness factor and mean nuclear form ellipse ($p<0.05$). However, no correlation was found between morphometric features and gastrointestinal stromal tumor behavior, tumor size, or index of proliferating cell nuclear antigen and p53 expressions ($p>0.05$).* ***Conclusions:*** *In this preliminary study, the relative concordance of the morphometric results and general histomorphologic data exhibited the importance of nuclear morphometric analysis in gastrointestinal stromal tumors. Studies including larger series of cases investigating detailed nuclear morphometric analysis of gastrointestinal stromal tumors are needed.*

Key words: Gastrointestinal stromal tumor, histomorphology, nuclear morphometry

Amaç: *Gastrointestinal stromal tümörler, mezenkimal neoplazmlar içinde yer alan özel bir tümör grubudur. Bu çalışmada, gastrointestinal stromal tümörlerin histomorfolojik ve immünhistokimyasal özellikleri, nükleer morfometrik analiz sonuçları ile karşılaştırılmıştır.* ***Yöntem:*** *Dokuzu benign ve 13'ü malign davranış gösteren 22 gastrointestinal stromal tümörün hematoksilen- eosin boyalı kesitlerinde, bilgisayar destekli görüntü analiz programı kullanılarak, ortalama nükleer alan, ortalama nükleer yuvarlaklık faktörü, ortalama nükleer elipslik indeksi, ortalama nükleer uzunluk ve ortalama nükleer perimetre ölçülmüştür. Morfometrik analiz sonuçları, tümörlerin davranış, boyut, mitoz sayısı, nekroz varlığı, immünhistokimyasal olarak p53 ve proliferating cell nükleer antijen ekspresyonları ile karşılaştırılmıştır.* ***Bulgular:*** *Tümör nekrozu ile ortalama nükleer yuvarlaklık faktörü, ortalama nükleer elipslik indeksi, ortalama nükleer uzunluk ve ortalama nükleer perimetre arasında arasında anlamlı ilişki saptanmıştır ($p<0.05$). Ayrıca mitotik indeks ile de ortalama nükleer yuvarlaklık faktörü ve ortalama nükleer elipslik indeksi arasında anlamlı ilişki saptanmıştır ($p<0.05$). Bununla birlikte, nükleer morfometrik ölçüm sonuçları ile tümör davranışı, boyutu, p53 ve proliferating cell nükleer antijen ekspresyonları arasında bir ilişki bulunamamıştır ($p>0.05$).* ***Sonuç:*** *Bu ön çalışmada, gastrointestinal stromal tümörlerin histomorfolojik verileri ile nükleer morfometrik ölçüm sonuçları arasında saptanan anlamlı ilişki, morfometrik analizin değerini ortaya koymaktadır. Gastrointestinal stromal tümörlerde bu yöntem ile elde edilen sonuçların, daha geniş olgu serilerinde araştırılması yerinde olacaktır.*

Anahtar kelimeler: Gastrointestinal stromal tümör, histomorfoloji, nükleer morfometri

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) originate in the interstitial cells of Cajal, and are the most common nonepithelial tumors of the digestive tract. They are characterized by expression of CD117. GISTs arise predominantly in the stomach and small intestine but also occur in the rectum, esophagus, and in a variety of other locations. GIST patients range in age from the teens to the 90s; peak age is around 60 years. The symptoms most often depend on the site of origin of the tumor. These tumors have a wide clinical spectrum varying from benign to malignant behavior. The

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most important and easily applicable criteria for predicting the tumor behavior are tumor size and mitotic activity. Several studies have identified additional prognostic factors, including presence of necrosis, hemorrhage, immunohistochemical markers like proliferating cell nuclear antigen (PCNA), Ki-67, p53, and c-kit mutation (1-9).

Different diagnostic and prognostic methods have been investigated due to the varying tumor behavior in morphologically similar lesions which can not be distinguished by conventional methods. In this sense, computer-assisted image analysis systems have become an important approach. Image analysis permits pathologists to obtain quantitative measurements on cytologic and histologic preparations, so that visual impressions can be augmented by quantitative morphometry. It is generally accepted that many nuclear morphometric descriptors (e.g., nuclear area, roundness, ellipticity, perimeter), either alone or in combination, are potentially useful tools for predicting the prognosis of different types of cancers (10-14). To our knowledge, nuclear morphometric studies on GISTs have not been reported in the literature to date. The aim of this study was to assess the characteristics of the cell nuclei of GISTs, and compare the histomorphologic and immunohistochemical features of GISTs by using a computer-assisted image analysis system.

MATERIALS AND METHODS

Pathologic Examination

This study included 22 cases diagnosed as GIST at the Department of Pathology in Hacettepe University Faculty of Medicine from 1999 to 2002. Hematoxylin and eosin (H&E) stained slides were reviewed for each case. Clinical features of the cases were obtained from hospital records. GISTs were diagnosed as clinically malignant when any of the following criteria were met: peripheral invasive growth, lymph node metastasis, metastasis to another organ, recurrence, or death. The following features were evaluated in all cases: primary tumor location, tumor size, presence of necrosis, and number of mitoses in 10 high power fields. For immunohistochemical staining, formalin-fixed paraffinized sections were prepared; after deparaffinization, the resulting sections were stained with labeled streptavidin biotin. The following primary antibodies were used: PCNA (1:1 dilution) (PC10, mouse, DAKO, Denmark) and p53 (1:500 dilution) (D0-7, mouse, Neomarkers, USA). Positive nuclear reactions for p53 were classified by using the following criteria: ≤1%= 0, 2-25%= 1, 26-50%= 2, 51-75%= 3, >76%= 4; and the number of cells with brownish nuclei per 1000 cells were counted to determine the PCNA labeling index (%).

Nuclear Morphometry

Morphometric analysis was performed on H&E stained histological sections. The microscope (Leica, DMLB-100S) was connected to a video camera (Leica, DFC-280) that was also connected to a computer. After transferring microscopic images to the computer, morphometric parameters were automatically measured by an image analysis program (Leica, QWINPlus v.3.1.0) (Figures 1-3). About 50 nuclei with sharply demarcated contour were included for morphometric analysis in each case. The nuclear morphometric parameters studied were as follows: nuclear area, nuclear roundness factor, nuclear form ellipse, nuclear length, and nuclear perimeter. Nuclear roundness factor is determined by the equation: "perimeter ^{2} / 4π x area" and nuclear form ellipse 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. Mean nuclear area (MNA), mean nuclear roundness factor (MNRF), mean nuclear form ellipse (MNFe), mean nuclear length (MNL) and mean nuclear perimeter (MNP) were evaluated in a total of 22 GISTs.

Nuclear morphometric results were compared with tumor behavior and size, presence of necrosis, mitotic index, and immunohistochemical expressions of p53 and PCNA. Statistical analysis

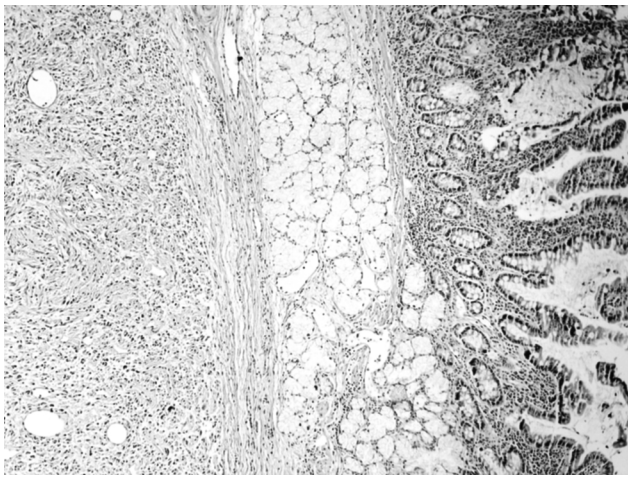

Figure 1. Typical GIST is present in the duodenal mucosa (H&E)

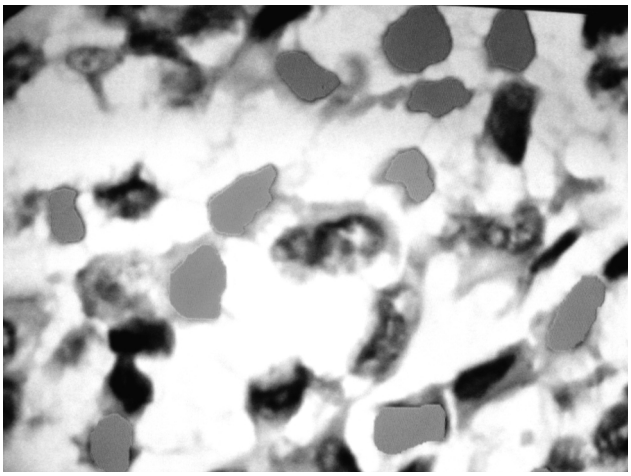

Figure 2. Marked nucleus of tumor cells for morphometric nuclear area measurement (H&E)

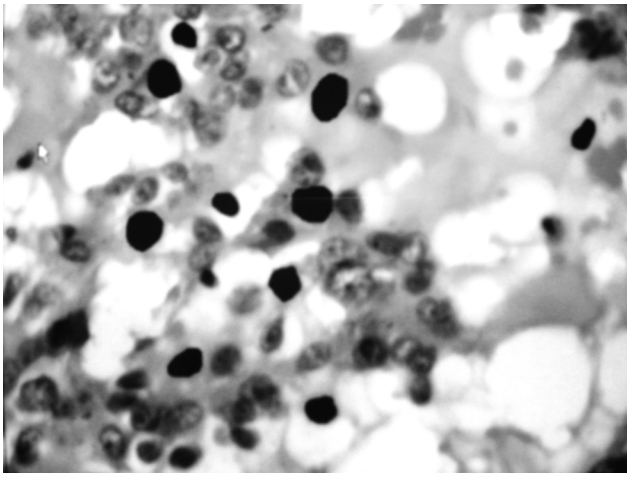

Figure 3. Marked nucleus of tumor cells for morphometric nuclear roundness measurement (H&E)

of the measurements in this study was performed using SPSS for Windows, v.11 statistical package. The relationship between nuclear morphometric parameters and clinicopathological factors was assessed by Mann-Whitney U test; $p< 0.05$ was considered statistically significant.

RESULTS

Twenty-two patients with GISTs were clinically classified as benign (40.9%) and malignant (59.1%). Of those, 11 were male and 11 were female, and the male-to-female ratio for benign and malignant groups were 2/7 and 9/4, respectively. Age range was from 30 to 73 years (mean±SD, 48.8±12.9) for all subjects, 33 to 73 years (mean±SD, 52.5±13.4) in the benign group and 35 to 65 years (mean±SD, 46.1±12.3) in the malignant group. A great majority of patients with known clinical history (20 of 22 patients, 91%) presented with abdominal discomfort and gastrointestinal bleeding. The locations of tumors were as follows: 8 in the jejunum-ileum, 5 in the duodenum, 3 in the anorectal area, 2 in the stomach, 2 in the colon and 2 in the mesentery-omentum. Jejunum-ileum was most common site of the tumors for both benign and malignant groups. The types of primary operation varied from simple local excision to radical resection.

The maximum tumor diameter ranged from 1 to 20 cm (mean±SD, 8.9±5.67) in the subjects overall, from 1 to 18 cm (mean±SD, 7.33±5.74) in the benign group, and from 2 to 20 cm (mean±SD, 10±5.58) in the malignant group. Intratumoral necrosis was present in 13 (59.1%) tumors - in 3/9 of the benign group (33.3%) and in 10/13 of the malignant group (76.9%) (Figure 4). The mitotic index (mitoses per 10 high power fields) ranged from 0 to 27 cm (mean±SD, 5.63±7.39) for all tumors, from 0 to 16 cm (mean±SD, 2.6±5) in the benign group, and from 1 to 27 cm (mean±SD, 7.7±8.2) in the malignant group. The p53 expressions of tumors for scores 0, 1, 2 and 3 were 11, 9, 1 and 1, respectively. There was no score 4 for p53 expression. The p53 scores ranged from 0 to 1 in the benign group and from 0 to 3 in the malignant group. The mean PCNA labeling index (%) of tumors was 13.6 (range: 0.4-75, SD±18.67). The PCNA labeling index (%) ranged from 0.5 to 16.2 (mean±SD, 7.4±5.1) in the benign group and from 0.4 to 75 (mean±SD, 17.9±23.3) in the malignant group.

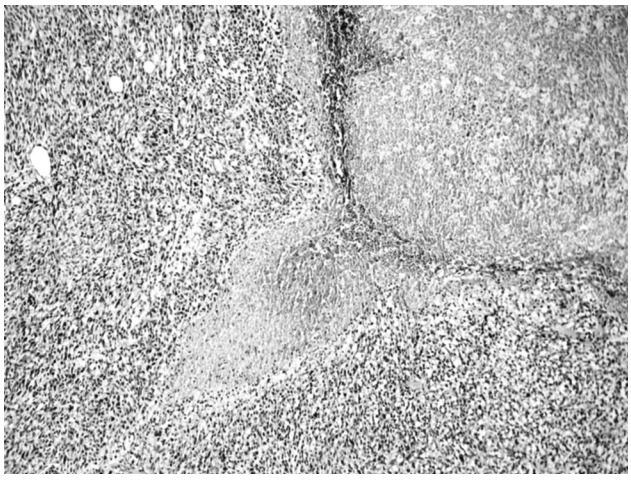

Figure 4. GIST with necrosis in a case (H&E)

Table 1. Clinicopathologic features and nuclear morphometric results of 22 patients with gastrointestinal stromal tumors

	Benign (n: 9)	Malignant (n: 13)
Age		
Range	33-73	30-65
Mean ± SD	52.5 ± 13.4	46.1 ± 12.3
Sex		
Male/Female	2/7	9/4
Site		
Jejunum-ileum	4	4
Mesentery-omentum	1	1
Duodenum	2	3
Colon	-	2
Anorectal	-	3
Stomach	2	-
Tumor size (cm)		
1-5	4	3
6-10	3	5
11-15	1	3
16-20	1	2
Necrosis		
Yes/No	3/6	10/3
Mitotic Index		
Range	0-16	1-27
Mean ± SD	2.6 ± 5	7.7 ± 8.2
p53 score		
Score 0	6	5
Score 1	1	6
Score 2	-	1
Score 3	-	1
Score 4	-	-
PCNA LI (%)		
Range	0.5 - 16.2	0.4 - 75
Mean ± SD	7.4 ± 5.1	17.9 ± 23.3
MNA (μm²)		
Min/max	19.30 - 40.56	20.42 - 42.81
MNRF		
Min/max	1.19 - 2.10	1.16 - 2.74
MNFe		
Min/max	1.58 - 3.56	1.52 - 4.33
MNL (μm)		
Min/max	6.26 - 12.94	6.90 - 12.78
MNP (μm)		
Min/max	17.37 - 31.40	19.29 - 29.53

Tumor size: Size represents the single greatest dimension, Mitotic index: Mitoses per 10 high power fields, Positive nuclear reactions were classified using the following criteria for p53 score: ≤1%= 0, 2-25%=1-26-50%=2, 51-75%=3, >76%= 4, PCNA LI: Proliferating cell nuclear antigen labeling index, MNA: Mean nuclear area, MNRF: Mean nuclear roundness factor, MNFe: Mean nuclear form ellipse, MNL: Mean nuclear length, MNP: Mean nuclear perimeter

The morphometric nuclear parameters of tumors were as follows: MNA ranged from 19.30 to 42.81μm² (mean±SD, 30.46±6.59) for all tumors, and from 19.30 to 40.56μm² (mean±SD, 30.91±7.22) and 20.42 to 42.81 μm² (mean±SD, 30.15±6.40) in benign and malignant groups, respectively. MNRF ranged from 1.16 to 2.74 (mean±SD, 1.65±0.38.) for all tumors, and from 1.19 to 2.10 (mean±SD, 1.74±0.29) and 1.16 to 2.74 (mean±SD, 1.58±0.44) in benign and malignant groups, respectively.

MNFe ranged from 1.52 to 4.33 (mean±SD, 2.50±0.78) for all tumors, and from 1.58 to 3.56 (mean±SD, 2.77±0.64) and 1.52 to 4.33 (mean±SD, 2.32±0.84) in benign and malignant groups, respectively. MNL ranged from 6.26 to 12.94 μm (mean±SD, 9.81±1.96) for all tumors, and from 6.26 to 12.94 μm (mean±SD, 10.59±2.08) and 6.90 to 12.78 μm (mean±SD, 9.27±1.75) in benign and malignant groups, respectively. MNP ranged from 17.37 to 31.40 μm (mean±SD, 24.78±3.77) for all tumors, and from 17.37 to 31.40 μm (mean±SD, 26.02±4.30) and 19.29 to 29.53 μm (mean±SD, 23.93±3.26) in benign and malignant groups, respectively. Table 1 summarizes the clinicopathologic features and nuclear morphometric results.

No correlation was found between morphometric features and GIST behavior, tumor size, and index of PCNA and p53 expressions ($p>0.05$). However, we found that tumor necrosis was correlated with MNRF, MNFe, MNL and MNP ($p<0.05$), but not with MNA ($p>0.05$). Mitotic index was also correlated with MNRF and MNFe ($p<0.05$), but no correlation was found between mitotic index and MNA, MNL and MNP ($p>0.05$).

DISCUSSION

GISTs comprise the great majority of primary mesenchymal tumors of the digestive tract and their clinicopathologic profile is not completely understood. GISTs arise most commonly in the stomach followed by the small intestine and then the colon, rectum, or esophagus. Occasionally, GISTs originate outside the intestinal tract (1-4). In our series, small intestinal GISTs comprised the largest group at presentation. The most useful clinicopathologic prognostic parameters in GISTs are tumor stage, size, histologic type, degree of necrosis, cellularity, nuclear pleomorphism, and mitotic activity. Among those, the most consistent histopathologic features to predict aggressiveness are tumor size and number of mitoses. A high Ki-67 index and high expression of p53, PCNA, Bcl-2, and vascular endothelial growth factor are frequently associated with poor prognosis (1-9). Recurrence or metastasis is occasionally observed in lesions

diagnosed histopathologically as benign GISTs. There has been debate on the histopathological criteria for assessing the malignant potential of GISTs. Thus, new histological malignancy classifications for GISTs were recommended (4).

The histopathological and morphological aspects of neoplasms are important in diagnostic procedures. Computer-assisted quantitative image analysis is a method of assessing computerized images of histologic preparations. The ability to study nuclear shape (nuclear morphometry) quantitatively has been made possible by advances in computer imaging technology. Nuclear morphometric descriptors, either alone or in combination, are potentially useful tools for predicting the prognosis of different types of cancers (10-14). Increases in nuclear size are detected more frequently in carcinomas than in benign neoplasms. Nuclear size is an important prognostic indicator in oral squamous cell carcinoma, breast carcinoma, renal cell carcinoma and prostatic carcinoma (10-19). Shape alterations are hallmarks of malignancy, and nuclear shape is a component of many histologic grading systems. Nuclear shape has been found to be significant in delineating the metastatic potential of colorectal adenocarcinoma (20, 21). Irregularity in shape occurs more frequently in ovarian carcinomas than in borderline tumors (22). Thus, several studies have demonstrated the clinical usefulness of nuclear morphometric parameters as prognostic factors, compared with the conventional grading systems for malignancies of various organs. This method also allows for exact measurement of cell and tissue size, shape, organization and quantification that no other methods can. Computer-assisted image analysis system allows reproducible analysis of large numbers of specimens with an accuracy and sensitivity exceeding 99% (23). To our knowledge, nuclear morphometric studies on GISTs have not been reported in the literature to date. We suggest that computer-assisted image analysis in GISTs is as valuable as in other malignancies.

In this preliminary study, we found that tumor necrosis was correlated with MNRF, MNFe, MNL, and MNP in GISTs ($p<0.05$). Mitotic index was also correlated with MNRF and MNFe ($p<0.05$), but no correlation was found between morphometric features and GIST behavior, tumor size, and index of PCNA and p53 expressions ($p>0.05$). The relative concordance of the results and general histomorphologic data has demonstrated the importance of nuclear morphometric analysis of GISTs. The major limitation of our study is the small sample size. Studies including larger series of cases with specific histologic subtypes investigating detailed nuclear morphometric analysis of GISTs with longer periods of observation are required in order to demonstrate the association between clinical outcome and morphometric parameters.

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