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NUCLEOLAR MORPHOMETRY IN PROSTATE CANCER

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Abstract: *Prominent nucleoli are an important diagnostic feature of prostate cancer. Various attributes of nucleoli are useful markers for diagnosis and prognosis. The aim of the present study was to analyse morphometric nucleolar parameters and to compare the results to histologic tumour grading (Gleason grading system).*

Keywords: *nucleoli, morphometry, prognosis, prostate cancer.*

1. Introduction

Cancer cells are notably distinguished from noncancer cells by alterations in the nucleolar structure. In particular, changes in nucleolar number, size, and shape are common features in cancer [2, 9]. Most of these nucleolar features can be translated into quantifiable measures by digital image analysis, a method that utilizes the ability of a microscope to capture nuclei in a digital form for analysis, process known as quantitative nuclear morphometry [4, 7]. Nucleolar morphometry has been used as a tool to predict progression of different types of cancer, thus supplementing diagnostic and prognostic information. Quantitative nucleolar morphometry has been shown to predict metastasis and biochemical recurrence of prostate cancer [1, 3, 8].

The aim of the present study was to analyse some of these morphometric nucleolar parameters and to compare the results to histologic tumour grading, according to the Gleason grading system.

2. Materials and Methods

For this study 35 cases of prostatic carcinoma were selected, collected during 2006-2007 out of the archive of the Department of Pathology, District Hospital of Brasov. Samples were obtained from formalin-fixed and paraffin-embedded pathology specimens prepared from transurethral resection of the prostate. 5µm random sections were cut onto microscope slides and stained with conventional haematoxylin & eosin stain. All slides were graded using the Gleason system (grade and score).

According to the Gleason three-grade system tumours were classified as well-differentiated (corresponding to combined Gleason grades 2 to 4), moderately-differentiated (corresponding to combined Gleason grades 5 to 7), and poorly differentiated (corresponding to combined Gleason grades 8 to 10).

Morphometric estimation of nucleolar features (number, area, perimeter, diameter) was carried out using an original stereologic software, created by a group led by Professor Olinici C.D. from the Department of Pathology, University of

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Medicine and Pharmacy, and Professor Ing. Vaida M.F. from the Department of Communications, Technical University of Cluj-Napoca. All measurements were performed using an Olympus microscope equipped with a Sony CCD video camera and with an x100 oil-immersion lens at a final magnification of x1000.

A mean of 30 fields of vision were examined in each case. Averages of 100 nuclei were sampled per case, 50 from each of the two main Gleason's grade.

A comparison between morphometric nuleolar values in tumour area with differrent Gleason grade and score was performed.

Mean ± SD was calculated by Statistica for Windows (StatSoft Inc) package. Comparison between means was performed using the Student's *t*-test; $p<0.05$ was considered significant.

3. Results

The mean number of nucleoli/nuclei increased significant in parallels with Gleason grade (p=0.021) (Table 1)

Table 1

Mean nucleolar number in fields with different Gleason grades

Gleason grade	Mean ± SD
1	1.44 ± 0.2
2	1.86 ± 0.27
3	2.4 ± 0.4
4	2.87 ± 0.31
5	2.94 ± 0.56

The mean number of nucleoli/nuclei increased significant in parallels with Gleason score (p = 0.01) from 1.47 ± 0.12 in tumours with Gleason score 2 to 3,31 in those with Gleason score 10.

The mean number nucleoli/nuclei also increased significant (p = 0.026) from well-differentiated (combined Gleason grade or score 2-4) to poor-differentiated (combined Gleason grade or score 8-10) adenocarcinomas (Figure 1).

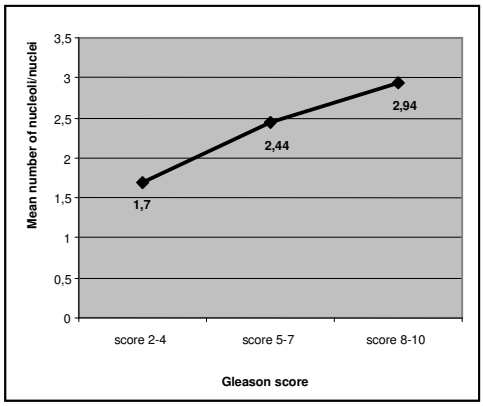

Fig. 1. *Mean nucleoli number/nucleus vs. tumours differentiation (Gleason score).*

Statistical analysis of nucleoli distribution (Pearson's correlation co-efficiency) showed a significant correlation between the percentage of nucleolated nuclei and Gleason grade (p = 0.09). The increase in the percentage of bi- and trinucleolated nuclei was accompanied by a decrease of the uninucleolated nuclei (Figure 2).

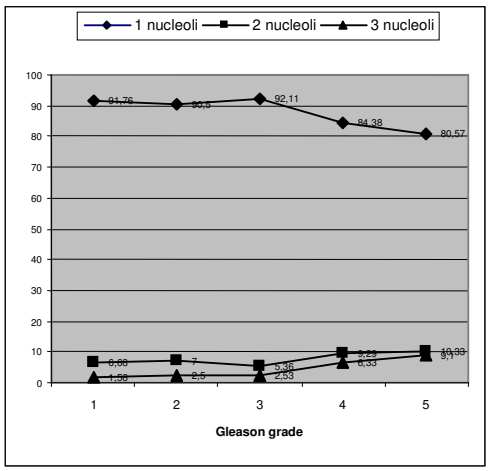

Fig. 2. *Percentage of nucleolated nuclei, bi- and trinuleolated nucleui vs. Gleason grade*

The values of morphometrical parameters studied in the individual nucleoli (area, perimeter, diameters) increased in Gleason grade 2 tumours as compared with grade 1 tumours. In tumour higher grades there was, however, a progressive decrease of these values (Figure 3 and 4).

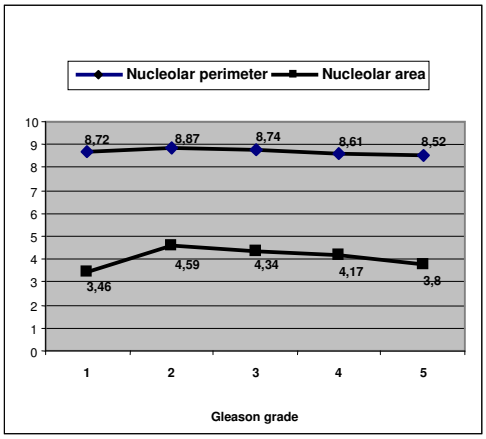

Fig. 3. *Nucleolar area and perimeter vs. Gleason grade.*

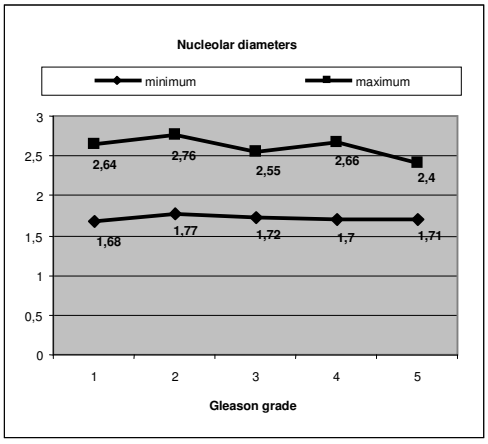

Fig. 4. *Nucleolar diameters vs. Gleason grade.*

4. Discussion

Morphologic changes in the structure of cells, primarily the nuclei, are characteristic features of cancer cells. In recent years, quantification of these nuclear features has been used to assess progression of different cancers [5, 6]. However, the mechanisms underlying these nuclear alterations are not clear.

Recently, studies have shown that alterations in nuclear structure can predict progression and metastasis in prostate cancer [8].

Nucleolar morphometry can predict progression as well as biochemical recurrence in prostate carcinomas. The quantitative nucleolar grade includes features such as size and shape [7].

Changes in nucleolar number, area, perimeter, and diameter are frequent events in prostate cancer cells. Whether any are associated with a more aggressive cancer phenotype has not been shown until now.

This study showed that Gleason score was positively correlated with prominent nucleoli, which are often quoted as being essential for the diagnosis of prostate cancer.

4. Conclusions

Our findings indicate that morphometric changes in nucleolar number, area, perimeter, and diameter should be added to the list of histological features that are helpful in the diagnosis of prostate cancer on transurethral resection. However, measurements should be done from several cell groups in each sample.

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