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PROGNOSTIC SIGNIFICANCE OF MIB-1 (Ki-67) EXPRESSION IN PROSTATE CANCER AND MATCHED LYMPH NODE METASTASES

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Abstract: *MIB-1 is a marker expressed only in proliferating cells. The aim of this study was to evaluate MIB-1 (Ki-67) labeling index in primary prostate cancer and matched nodal metastases for its prognostic value. Forty radical prostatectomy specimens from 2008 to 2009 were randomly selected for this study. Tumor blocks were immunostained using the monoclonal antibody MIB-1 (Dakorp. Denmark). MIB-1 expression was evaulated as percentage of positive nuclei of all tumor cells. A mean of 1000 cells were analyzed in each case. There were highly significant differences between primary tumors with negative metastases versus primary tumors with lymph node metastases (p=0.0001), and primary tumors with lymph node metastases versus lymph node metastases (p=0.004). Our data indicate that assessment of cell proliferation using MIB-1 as proliferation marker has predictive value in prostate cancer patients with lymph node metastases.*

Key words: *prostate cancer; MIB-1; metastases; prognosis.*

1. Introduction

MIB-1 is a monoclonal antibody that reacts with cells undergoing DNA synthesis by binding to the Ki-67 antigen, a marker known to be expressed only in proliferating cells. By measuring the amount of tumor cells expressing MIB-1, an estimate of DNA synthesis can be determined. Studies suggest that MIB-1 analysis of paraffin-embedded tissue specimens may provide useful prognostic information in various tumor types [4, 11]. The aim of this study was to evaluate the prognostic value of the immuno-histochemical expression o MIB-1 (Ki-67) antigen in specimens of primary prostate cancer and matched nodal metastases.

2. Materials and Methods

In this retrospective study, 40 radical prostatectomy specimens diagnosed from 2008 to 2009 at the Department of Urology, Clinical County Hospital of Braşov, were randomly selected.

Representative blocks of the primary cancer and paired lymph node metastases were selected from each patient for immunostaining. Staining was performed on 3-µm, formalin-fixed, paraffin-embedded sections using the avidin-biotin complex technique. Primary monoclonal antibodies were used for evaluation of MIB-1 labeling index (Ki-67; Dakorp Denmark). Diaminobenzidine was used as

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the chromogen, and hematoxylin-eosin was used as the counterstain. MIB-1 expression was evaulated as percentage of positive nuclei related to all tumor cells. A mean of 1000 cells were analyzed in each case.

In the present study, prostate specimens were classified as group I+II (all prostate cancers), group I (primary prostate cancer with negative lymph node metastases), group II (primary prostate cancers with positive lymph node metastases), and group II-LN (matched lymph node metastases).

Data were analyzed by Statistica for Windows 4.3 software. The Pearson correlation was used to assess the relationship between two continuous variables. Mean differences in microvessel counts were compared with the use of *t* tests (the calculated p-value below 0.05 was considered statistical significant)

3. Results

The mean patient age was 66 years (range, 47–79).

MIB-1 positive nuclei were brown coloured, due to diaminobenzidine, and most of them were part of the proliferative tumor section. The cytoplasm of these showed no MIB-1 positivity.

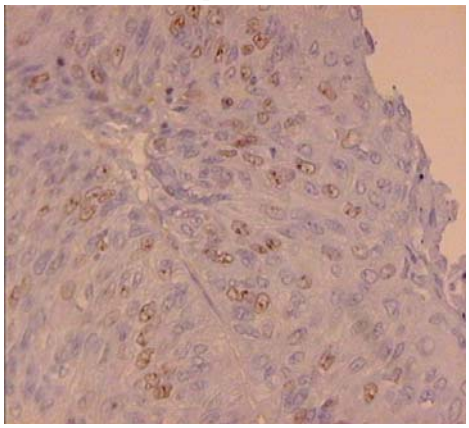

Fig. 1. *MIB-1 expression in an intermediate differentiated prostate cancer (20x).*

Figure 1 shows a representative example of immunostaining for MIB-1.

MIB-1 labeling was positive in most of the prostatic tumors analyzed. Four of the 40 primary tumors (1%), and one of the 20 lymph node metastases (0.5%) were MIB-1 negative. Therefore, only positive tumors were included in this study.

As determined with MIB-1 monoclonal antibodies, table 1 shows the mean, standard deviation of imunohistochemical labeling (expressed as percentage of positive tumor nuclei), and the number of cases from each studied group.

Table 1

Mean, standard deviation (SD) and number of MIB-1 pozitive cases in the different groups

Group	MIB-1		No. cases
	Mean	SD	
I+II	38.34806	13.43596	36
I	29.96288	7.483421	17
II	45.85058	13.24469	19
II-LN	40.96379	10.72450	19

The Student *t* test for independent samples and for dependent samples showed highly significant differences between primary tumors with negative metastases (group I) and primary tumors with lymph node metastases (group II). Tumors of the first group had a very low proliferative capacity, as compared with group II - MIB-1 (I) = 29,9629 +/-7,4834, almost one third lower than the mean labeling index than the index of group II - MIB-1 (II) = 45,8506 +/-13,2447. The *t* test showed no significant differences between lymph node metastases (group II-LN) vs matched primary tumors (group II), although *p* was close to the significant one (p = 0,062388), showing therefore aa easy lowering of the proliferative index MIB-1 in metastases vs. primary tumors (Table 2). Despite lack of significant differences between metastases and cooresponding

primary tumors, we analyzed the possible correlation between the two groups using the Pearson correlation index r(X,Y). The resulted index, r = +0,617722, showed a direct (r positiv), intense (r close to 1), and very high significant differences (p = 0,004828) between group II and II-LN (Table 3).

Table 2
t test between group I vs II and II vs II-LN

MIB-1	Group	
	I	II
Mean	29.9629	45.8506
SD	7.4834	10.72450
t		13.2447
p		0.000115
No.	17	19
MIB-1	Group	
	II	II-LN
Mean	45.85058	40.96379
SD	13.24469	10.72450
t		3.238735
p		0.004828
No.	19	19

Table 3
Correlation matrices: Pearson moment product r(X,Y)

MIB-1	Group	
	II	II-LN
Mean	45.85058	40.96379
SD	13.24469	10.72450
r(X,Y)		+0.617722
t		3.238735
p		0.004828
No.		19

4. Discussion

Ki-67 is expressed in all stages of the cell cycle except G_0 phase, making it a valuable measurement for cell proliferation [6]. The prognostic value of Ki-67 has been reported in various tumors, including cancers of breast, soft tissue, lung, cervix, prostate, and brain [3], [8-9], [14].

Our data indicate that cell proliferation in nodal metastasis is closely associated with the biological behavior of prostate cancer. Within the group of carcinomas, Ki-67 indices in patients with metastatic disease were significantly higher than in those without metastasis. However, the results suggested that high Ki-67 index could define a group of patients with poor prognosis.

Some of initial studies showed that MIB-1 is an independent predictor of outcome [1-2], [10], [12-13], and the most significant determinant of metastasis in prostate cancer [5], [12]. Only a few studies showed that reduced MIB-1 expression is not a predictor of poor outcome in prostate cancer [7], [16]. A MIB-1 overexpression revealed an increased risk of cancer progression in two studies [1], [15].

5. Conclusion

Evaluation of MIB-1 expression has the advantage to be highly reproducible. Our data indicate that assessment of cell proliferation in nodal metastasis using MIB-1 as proliferation marker has predictive value in prostate cancer patients with lymph node metastases.

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