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Original Article

Evaluation of Neuroendocrine and Proliferative Markers in Prostatic Adenocarcinomas

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Abstract

Background: Certain marker studies have practical importance in the biology of prostate cancer. The purpose of this study was to determine whether the quantification of certain neuroendocrine and proliferative markers obtained during transurethral resection or prostatectomy, would help in the prognostic evaluation of prostatic adenocarcinomas.

Methods: The present study was performed on samples obtained from two groups of patients with acinar type prostatic adenocarcinoma. Each group comprised 21 patients with Gleason scores ≥7 (high-grade) and Gleason scores ≤6 (low-grade). Tumors with their surrounding benign tissues were stained with Ki_{67} and chromogranin A (ChA), and their cell proliferation and neuroendocrine differentiation were examined.

Results: The mean number of neuroendocrine cells (ChA positive cells) in high grade tumors was 21% and that of low grade was less than one percent (P<0.001). Whereas, the mean proliferative index determined by Ki_{67} positive cells was 49% in high grade tumors as compared to less than 4% in low grade tumors (*P*<0.001). No significant difference was found between the mean percentages of chA cells in the non-tumoral tissues of high grade (2.7%) and low grade (1.9%). The mean proliferative index in the non-tumoral tissues of high grade (2.8%) was significantly higher (*P*<0.001) than of low grade tumors (1.4%).

Conclusion: The usage of proliferative index seems to be an acceptable diagnostic index for the determination of tumor grading.
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Keywords ● Prostate cancer ● proliferative index ● chromogranin A ● Ki_{67}

Introduction

Prostate cancer has become the most commonly diagnosed cancer,[1] and the second cause of death in man.[2] Carcinoma of the prostate is a heterogeneous disease with a wide range of biologic activity. Several variables have been evaluated in efforts to predict the biological behavior of prostatic adenocarcinoma. The variables of histological grade and clinical stage are considered as the most useful prognostic parameters.[3] Another factor, neuroendocrine differentiation, as detected by immunohistochemical staining, has also been shown to be present in many cases of benign prostate tissues,[4,5] as well as prostate cancer, but conflicting results have been obtained when trying to correlate these markers

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with their clinical outcomes.[6,7] Some studies indicated that neuroendocrine positivity increases the risk or tumor progression,[8,9] whereas, other studies do not support these results.[10,11]

The proliferation marker Ki_{67} antigen is another prognostic factor. Ki_{67} is present in cycling cell nuclei and has increasing expression as the cell cycle proceeds to the mitotic phase. Some studies have shown a relatively poorer prognosis in tumors with increased proliferation as detected by the Ki_{67} antibody, particularly in breast cancers.[12,13] Few studies also have specifically examined Ki_{67} in the prostate cancers.[14,15]

Certain marker studies may be important in the understanding of biology of prostate cancer. Therefore, in this study we evaluated and quantified neuroendocrine differentiation as detected by chA positive cells and the Ki_{67} proliferative index staining in a series of low and high grade prostatic adenocarcinomas obtained during transurethral resection (TUR) and radical prostatectomy.

Material and Methods

This descriptive-analytical study was carried out in Alzahra and Kashani Hospitals affiliated with Isfahan university of Medical Sciences, Isfahan, Iran. Two groups of patients with acinar type prostatic adenocarcinoma, identified using TUR, were selected from their pathology records and prostatectomy specimens. Each group comprised 21 cases of high-grade (Gleason scores of ≥7) and low-grade (Gleason scores of ≤6) tumors. Adequate tumor mass and surrounding benign tissues for immunostaining were available in paraffin blocks.

Immunohistochemical staining

Avidin-biotin complex technique was performed on formalin-fixed paraffin embedded sections. All cases and appropriate controls were assessed with 1:100 dilution of chA,[16,17] and 1:150 dilution of Ki_{67} (DAKO, Denmark),[18,19] as incubated for 30 min. Each slide was scanned using ×400 to determine the areas of most numerous positive cells. Each specimen was stained with both Ki_{67} and ChA. The areas of tumors with surrounding benign prostatic epithelium were then identified and specifically examined using χ400, for both benign and malignant epithelium. The percentage of positive cells for each marker was recorded by counting 1000 consecutive cells. Areas of transitional epithelium and epithelium containing prostatic intraepithelial neoplasia were excluded from the counts while disregarding any background or stromal staining. Prostatic epithelial cells showing definite cytoplasmic ChA staining were counted as positive and designated as neuroendocrine cells. Prostatic epithelial cells showing definite nuclear staining with Ki_{67} was counted as positive and was referred to as positive proliferative index.

Statistical analyses

The percentages of ChA and proliferative index markers were considered as dependent variables, whereas Gleason scores and tissue types were regarded as independent variables. Data were analyzed using Student's *t* test and $P<0.05$ was denoted as statistically significant.

Results

The percentages of positive cells for ChA and Ki_{67} markers are presented in Table 1. The mean percentages of neuroendocrine cells (ChA positive cells) and proliferative index (Ki_{67} positive cells) of high-grade and low-grade tumors were significantly different from each other ($P<0.001$). However, no significant differences were found between the mean percentages of ChA positive cells and proliferative index (Ki_{67} positive cells) of high-grade and low-grade of non-tumoral tissues.

Table 1: The mean percentage values of cells stained with chA and Ki_{67} in tumoral (T) and non-tumoral (NT) tissues of high- and low-grade prostatic carcinomas

Tissue	Marker	High-grade (%)		Low-grade (%)	
		Mean±SD	Range	Mean±SD	Range
T	ChA	20.7±24.9*	0-80	0.8±1.1	0-5
	Ki_{67}	48.9±25.5*	20-97	3.3±2.0	0.5-7
NT	ChA	2.7±2.3	0-7	1.9±2.4	0-10
	Ki_{67}	2.8±1.6*	0.3-7	1.4±0.9	0.3-3

*values of high-grade are significantly different from that of low-grade at $p<0.001$

Discussion

There are evidences for neuroendocrine differentiation in many cases of benign and malignant prostatic epithelium.[9] However, the prognostic significance of neuroendocrine differentiation in prostatic malignancy is controversial and there is no definite consensus about their utility.[20] The results of recent studies with chA markers suggest that neuroendocrine differentiation, as reflected by the increased tissue expression or blood concentration of this neuroendocrine secretory product is associated

with poor prognosis and tumor progression.[21-25]

Efforts to define the extent of neuroendocrine differentiation have used different methods of quantification or grading.[4,5,7,10,26] Cohen et al. quantified the neuroendocrine cells in a series of prostatic biopsies obtained from 10 randomly selected high-power fields.[4] Others have atempted to quantify the frequency of neuroendocrine cells by using different stains to maximize the calculation for the area of highest activity of neuroendocrine markers.[9] In the present study the objectivity of the quantification was maximized by using the percentages (based on 1000 cell counts) calculated for the area of highest activity of neuroendocrine marker.

Nearly all of the presented cases of high-grade prostate cancers showed minimally few positive neuroendocrine cells. In addition, there were obvious differences in the amount of neuroendocrine cells between high-grade and low-grade tumors, similar to that of Speights et al.[27] These findings indicate that neuroendocrine products may promote proliferation and confer antiapoptotic capabilities on non-neuroendocrine cells in close proximity to neuroendocrine cells.[27] In this study the results of observations made on the premalignant benign prostate epithelial cells are compared with those of adenocarcinoma and revealed a prominent expression of chA in premalignant benign prostate epithelial cells than adenocarcinoma in low-grade tumors as stated by Sion-Vardy et al.[28]

The results of the present study indicated that the proliferative index measured by Ki_{67} was much higher in high-grade than low-grade tumors. Ki_{67} is a nuclear antigen that is present in all cycling human cells and it is a marker for active cell proliferation. Immuno-histochemical staining of Ki_{67} provides an index that estimates the growth fraction of a population of cells. Ki_{67} nuclear staining has been related to biological aggressiveness, tumor cell growth and the prognosis of several cancers, including breast cancers,[12,13] malignant lymphomas,[29] and prostatic carcinomas.[14,26,30]

Conclusion

Although the proliferative index and the extent of neuroendocrine differentiation were markedly increased in high-grade prostatic cancers as compared to low-grade tumors, the relationship of these findings with their subsequent invasive behaviors are still uncertain and needs further investigation. According to the results obtained here, labeling indices of Ki_{67} is well correlated with tumor grading in prostatic carcinoma and provides additional prognostic indication of biological aggressiveness. Therefore, the use of proliferative index may be suggested as an acceptable and alternative tumor grading.

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