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Immunohistochemical detection of P53 protein as a prognostic indicator in prostate carcinoma

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ABSTRACT

Mutations of the p53 tumor suppressor gene can result in unregulated cellular growth and have been implicated in numerous malignancies. The aim of our study was to evaluate the prognostic significance of p53 protein immunoreactivity for prostate cancer and to determine whether p53 immunoreactivity correlates with the Gleason tumor grade in primary adenocarcinoma. Prostate fragments were fixed in 10% neutral-buffered-formalin, paraffin-embedded, sectioned and standard Hematoxylin–Eosin stained, then examined using histological grade (Gleason system). P53 expression was studied using immunohistochemistry with monoclonal antibody anti-p53 mutant, 1 : 100 (DO-7) on tissue samples obtained during transurethral resection, in 40 patients with prostate carcinoma: Staining was defined as positive for p53 whenever any specific nuclear staining was detected. We considered tumors to overexpress p53 protein only when strong nuclear staining was present. lmmunoreactivity (IR) was categorized semi-quantitatively from 0 to 10% = negative, 11% to 40% weak, 41% to 70% moderate and 71% to 100% strong. 7 of 40 (17,5%) tumors are strong nuclear staining, 18 of 40 (45,0%) are moderate, 10 of 40 (25,0%) are weak and 5 of 40 (12,5%) are negative. Results were then compared to Gleason score. lmmunohistochemical detection of mutant p53 appears not to be an independent predictor of progression.

Keywords: immunohistochemistry, prostate carcinoma, p53

INTRODUCTION

Prostate cancer is the most common lethal malignancy diagnosed in American men and the second leading cause of male cancer mortality. American Cancer Society 2014 estimated to account for 233000 (27%) new cancer diagnoses and 29.480 (10%) men will die of metastatic disease. On a global scale, the incidence and mortality of prostate cancer varies widely [1]. Black men have the highest cancer incidence and death rates about double those of Asian Americans, who have the lowest rates. Cancer incidence and death rates are higher among black than white men for every site. Factors known to contribute to racial disparities vary by cancer site and include differences in exposure to underlying risk factors [2]. The biological behavior of prostate cancer is unpredictable in individual patients, ranging from slowly growing, non-life-threatening to highly aggressive cancers [3]. The currently most established prognostic factors in prostate cancer are histological grade (Gleason system) and tumor stage [4]. Cellular proliferation and programmed cell death (apoptosis) are associated with tumor growth in general, and prostate cancer growth in particular. Protein expression of the tumor suppressor gene p53 (a regulator of cellular proliferation

and apoptosis) have been proved as useful prognostic indicators in prostate cancer progression [5]. Mutation of the p53 tumor suppressor gene is the most common genetic alteration in malignant human tumors.[6] The abnormal p53 protein produced by the mutant gene is more stable than the wild type protein, tends to accumulate in the cell, and thus can be detected by a immunohisto-chemistry using monoclonal antibodies. In contrast, the wild type protein is labile and its concentration in the nucleus is believed to be below the limits of detection by immunohistochemistry. Any detection of p53 protein immunoreactivity thus can be considered indirect evidence of p53 gene mutation. Immuno-histochemical expression of p53 protein was found to be widespread among human malignancies [7]. In a recent study p53 immunoreactivity was seen in 17% of prostate cancer. A low level of p53 immunoreactivity was not prognostically important. In contrast, a high level p53 immunoreactivity, which was limited to 6% of the cancers, was associated with high histological grade, deoxyribonucleic acid aneuploidy, and high cell proliferation rates, and it defined a small subset of aggressive prostate cancer [8]. The objective of the current study was to evaluate the immuno-histochemical expression of p53 protein by prostate cancer and to examine its utility as a prognostic indicator.

EXPERIMENTAL SECTION

Patients

All patients in this study were diagnosed and treated for adenocarcinoma of the prostate at Division of Urology Adam Malik Hospital Medan between January 1, 2014 and December 31, 2015. A total of 40 patients with a mean age of 67,9 years (range, 49 to 82 years) were included in the study. The tissue samples obtained during transurethral resection of the Prostate, all specimens prior to initiation of any treatment were submitted for this study. Prostate fragments were fixed in 10% neutral buffer formalin, paraffin-embedded, sectioned and standard Hematoxylin–Eosine stained, then examined by light microscopy (Olympus CX 21). Gleason histological grading system was determined by adding the numbers for the two most predominant patterns. The Gleason grading system takes into Low Grade Gleason Score 2-6 (n= 27), and High Grade Gleason Score 7-10 (n=13). (Table 1). A single representative block was selected for immuno-histochemical staining.

Table 1. Gleason Score

Gleason Score	No. of cases
2-6	27 (67,5%)
7 - 10	13 (32,5%)

Immunohistochemistry

Immunohistochemical stains were performed on 4 µm sections of the formalin fixed, paraffin embedded prostate specimens. Sections on poly-L-lysine coated glass slides put into Bond-max Fully Automated IHC instruments and work for 4 hours. Primary antibodies: mouse monoclonal anti p53 antibody (clone DO-7, Novocastra). The anti p53 antibody DO-7 recognizes an epitope on the N-terminus of the p53 protein and reacts with mutant p53 proteins. The working dilution of these antibodies was 1:50 respectively. p53 positive colon adenocarcinoma served as a positive control for p53 immunostaining. All slides were evaluated by two pathologists. We scored p53 as positive if greater than 10% of prostate tumor cells demonstrated nuclear reactivity. 11-40 % weak, 41-70 % moderate and 71-100 % strong.

Statistical Analysis

Statistics were performed using the Fisher's Exact test. The patient population was divided into two groups according to p53 immunostaining and Gleason score.

RESULTS AND DISCUSSION

Positive p53 staining (Figure 1) was seen in 35 of the 40 primary prostate cancers (85%) examined. Low Gleason Score (2-6) in 25 (62,5%), High Gleason Score (7-10) in 10 (25%) (Table 2). Weak positive in 10, moderate in 18 and strong in 7. Staining was observed in the patients with Low Grade Gleason Score are 7 in weak, 14 in moderate,

and 4 in strong. In patient with High Grade Gleason Score are 3 in weak, 4 in moderate, and 3 in strong. There was no statistically approved relationship between increased levels of apoptosis regulator proteins (p53) and Gleason score, p value = 0,469 (Table 3).

Table 2. P53 positivity compared to Gleason score

Gleason Score	No. of cases	P53 positivity
2 - 6	27 (67,5%)	25 (62,5%)
7 - 10	13 (32,5%)	10 (25%)

Table 3. Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.[illegible]	1	.[illegible]		
Continuity Correction[b]	.016	1	.898		
Likelihood Ratio	.[illegible]	1	.[illegible]		
Fisher's Exact Test				1.000	.499
Linear-by-Linear Association	.[illegible]				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.63.
b. Computed only for a 2x2 table

Several studies have sought parameters to predict the outcome for patients with prostate cancer. Certain his-tological Gleason score, tumor volume; and tumor stage are the best prognosticators of tumor progression. Recent studies have focused on genes related to tumor behavior as efficient predictors of patient outcome. Gilvan Neiva Fonseca at all, showed p53 expression in 30% of the tumors, which was directly related to tumor stage. In an earlier study, this group was able to find p53 expression in 31% of 118 prostate carcinomas and could prove the association of p53 with tumor stage [11]. Cheng at all reported nuclear p53 accumulation was detected in 50 of 55 prostate carcinomas (91%), respectively. Increased p53 nuclear accumulation correlated with cellular proliferative activity [12].

Navone showed p53 expression in 4.4% of localized prostate tumors and in 45% of bone metastasis [13]. The positive relationship of p53 to tumor grade was very significant in another series, in which p53 was expressed in only 7.5% of well-differentiated prostatic carcinomas and in 80% of poorly differentiated tumors [14]. Quinn et al. defined p53 expression as tumors presenting clusters of 12 positive cells within a 200-power magnification field and showed p53 expression in 52% of the cases [15].

P53 over expression has been investigated independently in a large number of different malignancies for their potential value as a prognostic marker. Mutation of the p53 tumor suppressor gene is a common genetic alteration in malignant human tu-mors and can be inferred from the immunohisto-chemical detection of the accumulated mutant gene .Mutation of the p53 tumor suppressor gene is a common genetic alteration in malignant human tumors and can be immunohistochemical detected [16].

The role of p53 in human prostate adenocarcinoma is still unclear and remains controversial. While a number of groups demonstrated a high p53 mutation and/or protein accumulation rate in prostate cancer others reported rare mutations. Such frequency differences of the p53 in prostate cancer among various groups could partially be due to the geographic or demographic factors as well as methods used for detecting p53 abnormalities. Bookstein R et al. reported that 23% of stage III or IV tumors and 4% of stage 0–II tumors had abnormal nuclear p53 accumulation and that 20–25% of advanced cancers, but none of early prostate cancer had mutations of the p53 gene [17]. However, two studies suggested that p53 abnormalities may be an early event in prostate cancer progression [18].

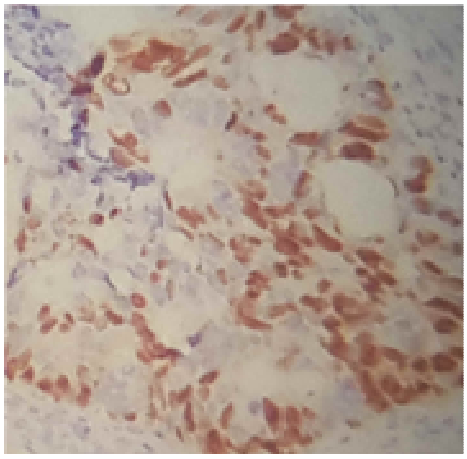

Figure 1.Immunohistochemical staining of p53, original magnification 100x

Shurbaji et al, examined the expression of p53 in 109 prostate cancers. They concluded that mutation of p53 might be involved in the development of some prostate cancers. Patients whose prostate cancers showed p53 immunoreactivity had significantly worse prognosis than patients with p53-negative cancers [19].

Prendergast et al, studied 18 patients with locally recurrent prostate carcinoma after radiotherapy (RT) and found that 72% had p53 nuclear immunoreactivity; while all 5 patients with available pre-RT biopsies had p53 immunoreactivity [20].

Cheng et al examined p53 abnormalities using immunohistochemistry in lymph node-positive prostate cancer. They found that a significant proportion of primary tumors (52%) and matched lymph node metastases (58%) showed nuclear accumulation of the p53 protein [21]. However, other studies examining the expression of p53 in 55 prostate cancers concluded that it was not a useful prognostic indicator, and also found no association between p53 expression and patient outcome [22].

Wahda M. Al- Nuaimy at all, reported P53 expression was detected in 15 cases of prostate carcinoma (29%) and relationship was found between p53 expression and tumor differentiation. P53 had higher expression in poorly differentiated adenocarcinoma of prostate (48.2%) [23].

In our study p53 positivity are 85%, strong are 7, but unfortunately distribution of weak, moderate and strong positive staining are not correlate with Gleason score. We found no statistically relationship between increased levels of apoptosis regulator proteins (p53) and Gleason score.

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