Original Article

Procaspase- 3 Status in Benign Prostatic Hyperplasia and Carcinoma (A Correlative Retrospective Study)

Alaa A. Qader* **MBChB, MSc**
Nabeel W. Rasheed ** **MBChB, MSc**

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Summary:

Background: Benign prostatic hyperplasia and prostatic adenocarcinoma are two of the most common pathologic mass lesions. Both are encountered mainly in elderly males. The caspases family is a group of at least 15 known proteases that serve as initiator & effector molecules of the apoptosis pathway. Caspase-3, in particular, is thought to play a pathogenetic role in both prostatic hyperplasia and carcinoma. Finasteride is a medication that has routinely been given to patients with hyperplasia and carcinoma; its prostate size-reducing effect is thought to be mediated through caspases.

Patients and methods: fifty patients with prostatic mass lesions were included in this study (20 with hyperplasia & 30 with adenocarcinoma); all were on finasteride treatment. The carcinoma cases were graded according to Gleason scoring system. All cases were analyzed for procaspase-3 strength of staining.

Results: benign hyperplasia & well-differentiated carcinomas show high expression of procaspase-3, in contrast loss of expression of this marker was noted in moderately & poorly differentiated carcinomas.

Conclusion: there is a strong statistical correlation between caspase-3 expression and the degree of tumor differentiation. This may allow the utilization of this marker as a potential prognostic factor, especially in limited biopsy samples.

Key words: prostatic hyperplasia, prostatic adenocarcinoma, procaspase-3, Gleason grade

Introduction:

Benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma (PAC) are two of the most common lesions in aging men. The incidences of both are age-related.1 the progression of the normal gland to BPH seems to rely on the action of androgens, mainly dihydrotestosterone (DHT).2 the latter results from the conversion of testosterone through 5α-reductase enzyme in both stroma & epithelial cells. The drug finasteride inhibits 5α- reductase activity & thus its administration is associated with up to 90% decrease in DHT level within the gland, which eventuates in 20% to 30% reduction of lesion size.3,4 Evidences suggest that finasteride treatment, through testosterone ablation, provokes the apoptotic process, which is a caspase-dependent and restricted to epithelial cells. The therapeutic significance of manipulating apoptosis in the treatment of prostate cancer emerges from evidences suggesting that like normal prostate epithelial cells; cancer cells maintain sensitivity to androgens & undergo apoptosis in response to androgen withdrawal.5 Caspase–3 (and 6) seems to play a key mediating role in this programmed cell death (apoptosis). Gleason score is the most commonly used system for grading prostatic adenocarcinoma.6 the system describes a score between 2 and 10; these figures reflect the least aggressive and the most aggressive cancers respectively. It is based on the degree of glandular differentiation and the growth pattern of tumor in relation to the stroma as evaluated on low power examination.

* *Dept. of Pathology, Al-Anbar Faculty of Medicine*
** *Dept. of Pathology, Baghdad Faculty of Medicine*

Patients and Methods:

Between August 2006 and through January 2007, fifty patients with prostatic mass lesions were studied; these were represented by specimens of formalin-fixed, paraffin-embedded blocks. The sources of the cases were the laboratories of Specialized Surgeries & Medical City Teaching Hospitals. All the fifty patients were on finasteride treatment for at least a month prior to the biopsy procedures. The specimens comprised a combination of TURP (15 cases), total prostatectomy (8 cases) and core needle biopsies (7 cases). The cases were selected to represent BPH (20 cases) and PAC (30 cases). The carcinoma cases were chosen to represent well-differentiated, moderately-differentiated, & poorly-differentiated adenocarcinomas (10 cases each). Through the application of the Gleason scoring system of grading, the well-differentiated carcinoma cases showed a score of 2 to 4, the moderately differentiated a score of 5 to 7, whereas the remaining poorly differentiated cases had scores of 8 to 10. Sections (4 microns-thick) were also stained immunohistochemically with reagents for the detection of caspase-3 (Bioten-alkaline-phosphatase

complexed antibodies) (chemicon–international cat. No. APR050, APR150, APR500 USA & Canada), and according to the instructions given in the leaflets of the kit. Both positive & negative controls were also applied to confirm the specificity of the primary antibody. The immunostaining patterns of procaspase-3 protein were in the form of essentially cytoplasmic red dots. For the evaluation of caspase -3 expressions, tumors were divided according to the following scheme

None = no staining

Weak = < 15% of tumor cells were positively stained.

Moderate = 15-50% tumor cells were positively stained.

Strong = >50% tumor cells were positively stained.

Seven cases of the carcinoma group were excluded from the analysis because they were represented by small fragments of core needle biopsies, and the diagnosis of carcinoma was made primarily on the presence of perineural invasion; there were no enough carcinomatous elements present in the sections to make Gleason grading feasible.

All the statistical analyses were done through the SPSS program (version-10) and Excel application.

Results:

All the patients with BPH (20 cases) & all patients with well-differentiated PAC (8 cases with Gleason score 2-4) showed diffuse strong staining (fig. 2 and 3). In comparison, only 2 of 9 (22.2%) of the patients with moderately differentiated PAC (of Gleason score 5-7) showed similar intense staining; the remaining 7 cases (78.8%) showed generally focal staining of either moderate or weak strength (Fig. 4). In contrast, four of six patients (66.7%) with poorly differentiated PAC (Gleason score 8-10) showed no staining at all, while two of six (33.3%) stained weakly (Fig. 5). These results were statistically highly significant (p=<0.01) as shown in the table 1 and figure 1.

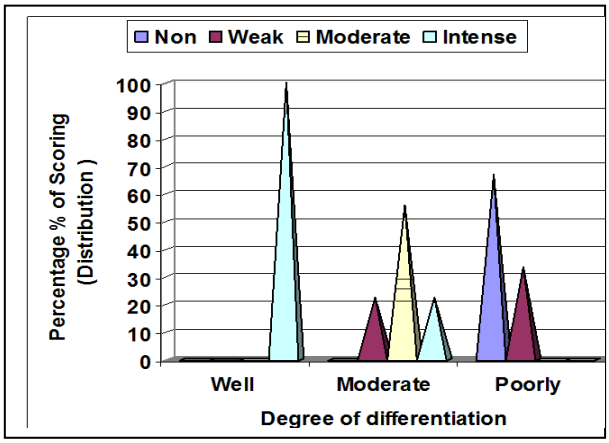

Fig. 1 A Bar chart showing frequency of procaspase staining strength distribution according to intensity among various grades of prostatic adenocarcinoma

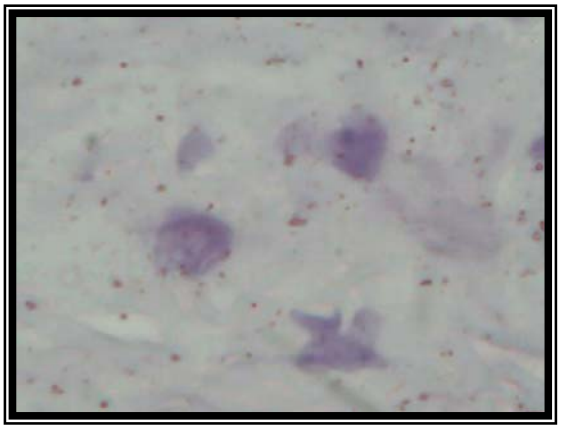

Fig. 2 Benign prostatic hyperplasia, a field showing intense positive reaction with Caspase-3 immunostain (seen as small red granules) (1000 X with oil)

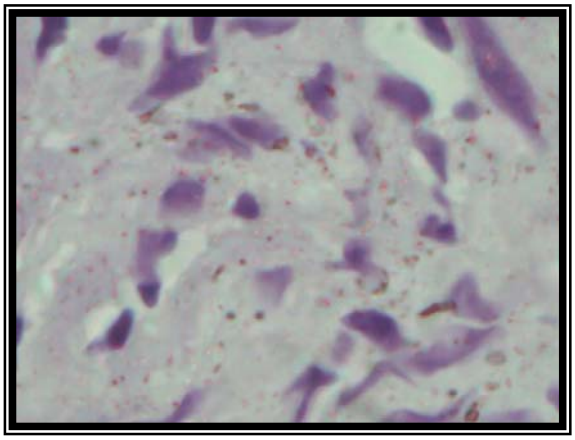

Fig. 3 Well- differentiated prostatic adenocarcinoma, a field showing intense positive reaction with Caspase-3 immunostain (seen as small red granules) (1000 X with oil)

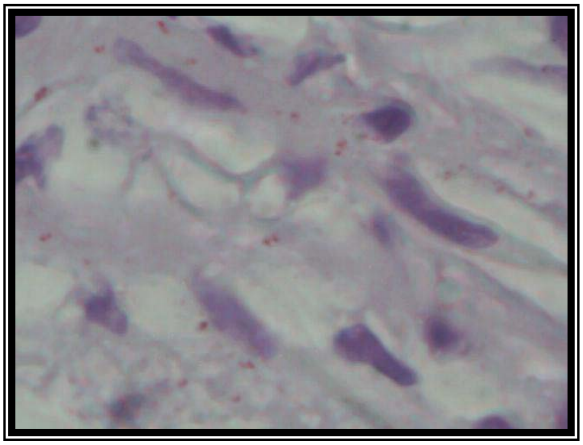

Fig.4 Moderately- differentiated prostatic adenocarcinoma stained with Caspase-3, a field showing generally focal staining by red granules of either moderate or weak strength (1000 X with oil)

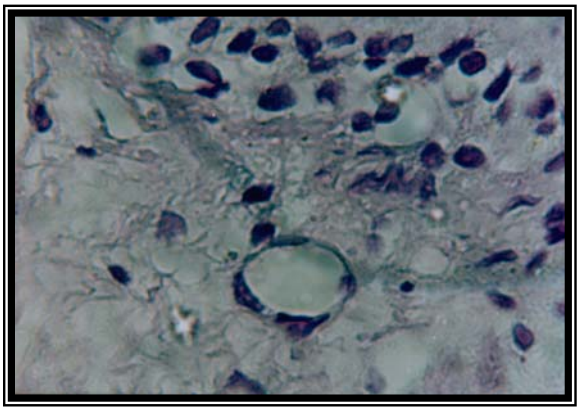

Fig.5 poorly differentiated prostatic adenocarcinoma stained with Caspase-3; there is absence of red granules (1000 X with oil)

Discussion:

Apoptosis is a regulated process of autodigestion of cells. The chief effectors of the apoptotic pathway are the caspase family of proteases including caspase 3. Activation of the caspase cascade has been correlated with the onset of apoptosis. This has been the case in normal prostate as well as in both BPH & PAC. Activation of the caspase family occurs in response to diverse mechanisms, including androgen ablation (e.g. using the drug finasteride).3, 7, 8 in this study, we demonstrated a diminished detection of caspase -3 proteins in human prostate cancer compared with the BPH. We studied retrospectively a selection of 50 patients suffering from BPH & PAC. All of them were on finasteride treatment for at least one month prior to the biopsy procedure. Twenty of such patients were suffering from BPH and the remaining 30 were having PAC in which the Gleason's score was assigned. We found that there was a reduced expression of caspae-3 in moderately & poorly differentiated PAC, as compared with well-differentiated PAC & BPH. These findings were analogous to those in two other studies; Kadkol et al 8 who found a similar correlation between Caspase-3 expressions & intermediate & high grade prostate cancer by in situ hybridization, and O'Neill et al 9 who studied six cases of BPH, 6 cases of intermediate-grade adenocarcinoma & 10 cases of high-grade adenocarcinoma for Caspase-3 expression. The latter marker was over-expressed in BPH. However, significant loss of expression was observed in the epithelial cells of intermediate-grade adenocarcinoma, & rare staining was noted in cells of high-grade adenocarcinoma. It should be mentioned, however, that Steiner et al 10, found no correlation between the Gleason's score & caspase-3 expression. Finasteride was shown to trigger apoptosis in cells affected by BPH in the first week of treatment; the peak of apoptosis was observed 6-8 days after the commencement of treatment. Finasteride is known to be effective in reducing prostate size but by only of 20-30% of its initial volume; presumably because it primarily affects the epithelial cell component.11 Finasteride treatment induces the activation of caspase-3 during the apoptotic cell death process in BPH tissues. However, in our study we could not validate this for lack of controls i.e. those with BPH who had not received finastride treatment, since giving such a drug has become a routine treatment modality.

Conclusion derived from this study as well as of similar ones include

1. Providing a rationale for the contribution of deregulated caspase cascade in prostatic tumorogenesis. As would be expected, loss of expression of the key caspases would confer a growth advantage through inhibition of apoptosis in malignant prostate cells.
2. The present findings may have a high clinical relevance through the identification of a potentially significant values of caspases, not only as markers of disease progression, but also as therapeutic targets for effective activation of the apoptotic process in advanced prostate cancer.

Table1: Frequency distribution of procaspase staining according to its strength among various grades of prostatic adenocarcinoma

Degree of differentiation		Scoring (Distribution)				Total	P value
		None	Weak	Moderate	Strong		
Well	N	0	0	0	8	8	
	%	0	0	0	100	100	

Moderate	N	0	2	5	2	9	< 0.01 HS
	%	0	22.2	55.6	22.2	100	
Poorly	N	4	2	0	0	6	
	%	66.7	33.3	0	0	100	
Total	N	4	4	5	10	23*	
	%	17.39	17.39	21.73	43.47	100	

* Seven cases of the carcinoma group were excluded from analysis in as much as they could not be assessed according to Gleason score being represented by small fragments of needle biopsy cores; the diagnosis of carcinoma was made primarily on the presence of perineural invasion

References:

1. Landis SH, Murray T, Bolden S, Wingo PA: cancer statistics, 1999, CA cancer J Clin 1999; 49:8.

2. Change SM, Chung L.W.K. Interaction between prostatic fibroblast and epithelial cells in culture: role of androgen, Endocrinology 1989; 125: 2719.

3. Thompson IM, Good man PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG et al. The influence of finasteride on the development of prostate cancer, N Engl J Med 2003; 349: 215-24.

4. Shapiro E, Lepor H. Pathophysiology of clinical benign prostatic hyperplasia. Urological Clinics North America 1995; 22: 285-290.

5. Xue Y, Van der lak KJ, Smedts F, et al: neuroendocrine cells during human prostate development: Does neuroendocrine cell density remain constant during fatal as well as postnatal life? Prostate 2000; 42; 116-123.

6. American Joint Committee on cancer: Cancer staging manual. 5th edition. Lippincott. Raven, 1999.

7. Rosette J. dela, G.Alivizatos, S.Madersbacher et al: Giude lines on benign prostatic hyperplasia: European association for urology giude line 2006 edition.

8. Kadkol SS, Brody JR, Epstein JI et al. Noval nuclear phosphoprotein PP32 is highly expressed in intermediate- and high- grade prostate cancer. Prostate 1998; 34:231-237.[Pub Med Citation].

9. O'Neill AJ, Boran SA, O'Keane C. Et al. Caspase-3 expression in benign prostatic hyperplasia and prostate carcinoma.prostate 2001;47:138-188.[Pub Med Citation].

10. Steiner M. S. Barrack ER. Transforming growth factor beta 1 over production in human prostate cancer: effect of growth in vivo and vitro. Mol Cell Endocrinol 1992;6: 15-25.

11. Cunha G.R. The role of androgens and the epithelio-mesenchymal interactions involved in prostatic morphogenesis in embryonic mice. A rat Rec 1990;175:87-96.