EVALUATION OF MINIMAL PROSTATE CANCER IN NEEDLE BIOPSY SPECIMENS USING AMACR (P504S), P63 AND KI67

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ABSTRACT: Background: Prostate cancer is a major health problem throughout the developed world. Immunohistochemistry plays a very important role in the diagnosis of minimal prostatic adenocarcinoma and to exclude one of its benign mimickers**,** but it should always be interpreted in the context of the H&E appearances**.** In some cases of minimal prostate cancer morphologic features do not allow a diagnosis of carcinoma. In such situation, the presence of AMACR and the absence of basal cell marker (p63) confirm the presence of prostate cancer. In addition,Ki-67 a proliferating marker have a strong relationship with Gleason's grading, which has an important relationship with the prognosis of prostate cancer. **The aim:** Assessing the usefulness of immunohistochemical analysis with Alpha-methylacyl-CoA racemase (AMACR) and p63 to confirm the diagnosis of minimal prostate cancer. Also , detection of the prognostic role of ki67 in minimal prostate cancer detection and its association with p63 expression in prostate cancer. **Methods:** 50 needle biopsy specimens, including 30 with small foci of prostatic adenocarcinoma and 20 benign prostate (≤ 1mm or <5% of needle core tissue) were stained immunohistochemically with AMACR, P63 and Ki67 antibodies. **Results:** Of 30 cases of small foci of prostatic adenocarcinoma, 27 (90%) expressed AMACR; all malignant glands were negative for basal cell staining p63 (nuclear stain). All benign glands were recognized easily by basal cell marker (p63) positivity. There was focal positive staining with AMACR in 2 benign cases showing atrophy. A statistical significant correlation was observed between ki67 expression and increased Gleason's grade (p=0.02). Cytoplasmic expression of p63 was high in high grade prostate cancer, and it was associated with higher frequency of ki67 positive cells in prostatic adenocarcinoma. **Conclusions:** Immunostaining with the p504s and p63 could improve the diagnostic performance and helped in avoid carrying out new biopsies in small foci of prostatic carcinoma detection. An important relationship with the prognosis of prostate cancer was noticed through the strong relationship of Ki-67 marker with Gleason's grading. Therefore, we propose that this marker can be applied along with other prostate cancer prognostic factors.

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1.INTRODUCTION

Prostate cancer incidence increased in most high-income countries, the increased detection was due to more frequent digital rectal examination, PSA, incidental diagnosis due to the increasing use of transurethral resection of the prostate (TURP) and developments in diagnostic techniques such as transrectal ultrasound (TRUS) imaging and fine needle biopsies. (Cremers et al. 2010).

The diagnosis of prostatic adenocarcinoma, especially in needle biopsy samples, can occasionally be challenging, either because they only show small foci of prostatic adenocarcinoma, or because of the difficulty in distinguishing prostatic carcinoma from benign mimickers (Hameed and Humphrey, 2006). The difficulty in the diagnosis of prostatic adenocarcinoma is mostly seen with minimal (limited<1mm) carcinoma in needle tissue (Thorson and Humphrey 2000).

Many major and minor histologic features important for the diagnosis of minimal prostatic carcinoma should be assessed specifically at low- and high-power magnification. The first of the major criteria is an infiltrative growth pattern which frequently presents as the presence of small malignant glands between larger, more complex (and often paler), benign glands. This is because the invading glands usually don't elicit a desmoplastic or inflammatory response, which characterizes many other types of invasive carcinoma. The second most common pattern of infiltration was a haphazard or disorderly arrangement of glands, with random dispersion of glands in stroma, without availability of benign glands as a reference point. On occasion, the invasive glands formed a column spanning the width

of the needle core, uncommon patterns of growth are cords of cells, single cells and cribriform glands (Epstein, 1995).

These infiltrative growth patterns are a hallmark of moderately to poorly differentiated; Gleason's score 5 to 6 adenocarcinoma of the prostate and were found in 82% of the minimal carcinoma (Thorson et al., 1998).

It was proposed that well-differentiated, Gleason's score 2 to 4 adenocarcinoma should not be diagnosed in prostate needle biopsy tissue (Epstein, 2000).Since in the vast majority of cases, this represent an under grading of the carcinoma in the whole gland (Thorson et al., 1998).

In a very small minority of cases, there are well differentiated, Gleason's score 2 to 4 carcinoma that are by definition, well circumscribed closely packed, pale, small acini (Epstein, 1995).

The second of the major criteria is absence of basal cells in the atypical glands (Cleary et al., 1983). Nuclear atypia in the form of nuclear enlargement and nucleolar enlargement is the third of the major criteria (Thorson et al., 1998). The major criteria don't include a quantitative threshold
for the number of glands required to establish a diagnosis of malignant neoplasm. Most urologic pathologist believed that 3 glands constituted typical lowest numeric cutoff (Grignon, 1998).

Minor diagnostic criteria are intraluminal wispy blue mucin, pink amorphous secretions, mitotic figures, intraluminal crystalloids, adjacent high-grade PIN, amphophilic cytoplasm and nuclear hyperchromasia. These minor diagnostic attributes are not specific for carcinoma but are useful for prompting in-depth study of the glands harboring these changes, with a view toward assessment of the aforementioned major diagnostic criteria (Algaba et al., 1996).

P63 gene is expressed in the regenerative epithelial compartment of several organs, and shares extensive homology with p53 (Yang et al., 1998). Prostate requires p63 expression for its development and it is expressed like in breast, in myoepithelial cells surrounding normal acinar glands; therefore p63 is routinely used to evaluate the presence of normal basal cells thus distinguishing between benign and malignant glands (Reis-Filho et al., 2003; Hameed et al., 2005).

In adenocarcinoma, p63 tends to be under-expressed (Di Como et al., 2002) and in prostate cancer specifically, negative immunohistochemical staining of p63 is clinically useful tool for identifying benign mimickers (Signoretti et al., 2000). Other studies have identified p63 as important in signatures of advanced disease, with lower expression associated with disease progression and the development of lethal prostate cancer (Bismar et al. 2006; Mucci et al. 2008).

Alpha-methylacyl-CoA racemase (AMACR), formerly known as P504s, is a mitochondrial and peroxisomal enzyme involved in the beta-oxidation of branched fatty acids and bile acid intermediates (Ferdinandusse et al., 2000). AMACR is a marker that selectively labels adenocarcinoma of the prostate and it is proposed as a positive marker in prostatic adenocarcinoma (Molinié et al.,2006).

Several benign mimickers of prostatic adenocarcinoma, including atrophy, atypical adenomatous hyperplasia, crowded benign glands, nephrogenic adenoma and mesonephric hyperplasia can stain negatively with basal cell markers. Although the absence of staining is in most cases usually only focally seen in scattered glands, a negative basal cell marker immune-stain alone does not exclude a diagnosis of benignancy. AMACR expression can also be identified in high-grade PIN, prostatic atrophy, atypical adenomatous hyperplasia and benign prostatic glands, and accordingly a diagnosis of prostatic adenocarcinoma should not be based solely on a positive AMACR immunostain, and basal cell markers should always be run with AMACR. The use of AMACR and basal cell markers can greatly facilitate the distinction between prostatic adenocarcinoma and its benign mimickers, especially when only limited tissue is available for staining (Hameed and Humphrey, 2006).

The Ki67 is a nuclear protein and it is the most widely recognized marker of proliferating cells. The antigen detected by ki67 antibody is localized primarily in nucleoli and is present only in proliferating cells. Its content increases during the S and G2-phases .The antigen appears to be degraded after mitosis, thus it could not be detected in resting (G0) cells (Revelos et al., 2005). Many studies have shown that Ki67 is associated with increased tumor aggressiveness and metastases (Cowen et al., 2002).

We aimed in this study to confirm or rule out small focal prostatic carcinoma in limited biopsy material using AMACR and p63.Also, detection of ki67 as a prognostic role in limited carcinoma and its correlation with p63 expression in prostate cancer.

2.MATERIALS AND METHODS:

2.1.Materials:

2.1.1.Subjects:

Patients (carcinoma group)with age range from from 52 to 81 years (mean = 69.5±8.2)and control group with age range from 49 to 82 years (mean = 65.4±10.1) obtained from the department of pathology, Faculty of Medicine, Zagazig University during the period from 2010 to 2012.
were chosed for this study.

- ***Criteria of choosing cases***:

Serum PSA level before biopsy ranged 2.3 to 21 ng/ml (mean 8.02 ng/ml), and from 4 to 150 ng/ml (mean 37.02 ng/ml) for the control and carcinoma groups, respectively.

2.1.2.Samples:

A total of 50 prostate needle biopsy specimens, including 30 prostate needle biopsy specimens with small foci (≤ 1 mm or less than 5% of needle core tissue) of prostatic adenocarcinoma and 20 benign prostates

The diagnosis of prostate cancer was established from:

* Examination of multiple levels of H&E-stained sections and was confirmed by absence of basal cell staining (p63) and/or positivity for AMACR (P504S). All radical prostatectomy specimens from cases with a small focus of prostate carcinoma in needle biopsy specimens showed residual prostate cancer. No false-positive cases were found in follow-up radical prostatectomy specimens.

*Morphological evaluation:

Thirteen specimens of prostatic adenocarcinomas were intermediate grade Gleason (5-7) and 17 were high grade Gleason (8-10).

2.2.Methods:

2.2.1.Immunohistochemical Analysis

Immunohistochemical staining was carried out using streptoavidin-biotin immunoperoxidase technique (Dako-cytomation, CA). Three to five micrometer thick sections, cut from formalin fixed paraffin embedded blocks, were deparaffinized in Xylene and rehydrated in graded alcohol. The mounted sections were immersed in ready to use Dako target retrieval solution (PH 6.0), then boiled in this solution in a microwave for 20 min and then washed in phosphate buffer saline (pH 7.3).Then blocking of endogenous peroxidase activity by 6% H_2O_2 in methanol was attained. The slides were then incubated over night using a polyclonal anti-AMACR antibody (1:2000 dilution; DakoCytomation), Ki-67 antibody (clone MIB-1, 1:50 dilution, overnight incubation; DakoCytomation) and incubated with a 1:600 dilution of the 4A4 mouse monoclonal antibody (Lab Vision Corporation, Santa Cruz), which binds to all isoforms of p63. After a buffer rinse, bound antibodies were detected with the DAKO Envision System. Slides were counterstained with hematoxylin, and rinsed again. The slides were allowed to air dry and were cover slipped with permanent mounting media. Negative controls, in which the primary antibodies were replaced by PBS, were carried out for each primary antibody. Squamous cell carcinoma for p63 and human tonsillar tissue for ki-67 were used as positive controls. For AMACR, prostate carcinoma was used as positive internal control.

- *Immunohistochemical Evaluation:*

Evaluation of ki67::

Each slide was evaluated at×40 magnification in order to find areas with maximum positive nuclei. Then these areas were examined at ×400 magnification and the percentage of positive nuclei to total nuclei was calculated. In this study, at least 500 cells were counted and only the cells that were definitely positive were considered. The tumors were divided into five groups regarding the percentage of positive cells. Cases in which the percentage of stained cells was less than or equal to 2% were considered negative. Cases with Ki-67 index of less than or equal to25% were considered 1+, 26-50% as 2+, 51-75% as 3+ and 76-100% as 4+ (Minner et al., 2011).

- *Evaluation of AMACR and P63:*

All glandular tissue that was seen on the whole needle biopsy section was taken into consideration for each case. The percentage of glands (extensiveness) that stained for the immunohistochemical markers (AMACR and P63) was evaluated in a semiquantitative fashion using the following scale: negative, <10%, 10%-50%, 50%-90%, and >90% as previously described (Abrahams et al., 2002; Diaz et al., 2000). The intensity of the P63 was classified as negative, weak, moderate, and strong (Shah et al., 2002). The AMACR staining intensity was graded as negative, weak (weak non-granular cytoplasmic staining), moderate (granular staining with weak or moderate intensity), and strong (granular staining with strong intensity) (Zhou et al., 2004).

2.2.2.Statistical analysis

The results of the study were statistically analyzed using SPSS version15 statistical program. Data were expressed as mean± SD for quantitative variables, numbers and percentage. For categorical variables, student t test was used. For statistical analysis of Gleason's grading Spearman's statistical test was used. P< 0.05 was considered the significant limit.

3.RESULTS

3.1.Staining results with AMACR and p63

AMACR expression in malignant glands had much more extensive and intensive staining results than benign glands (P<0.001). Prostatic carcinoma showed a brown cytoplasmic granular staining pattern of AMACR in the malignant glands and cells (Fig 2B, 3C1, 3C2, 4C). Out of 30 cases of small foci of prostatic carcinoma 27 (90%) expressed AMACR (p504s), of 27 cases, AMACR positivity was detected in more than 90% of the malignant glands in 22, 50% to 90% of malignant glands in 3, and 10% to 50% of malignant glands in 2 (Table1). Eighty six percent of

the positive samples with AMACR had moderate to strong staining intensity (Table2). All benign glands adjacent to the malignant glands were recognized by absence or very low level of AMACR expression. There was focal positive staining with AMACR in 2 benign cases showing atrophy. Out of 30 cases of adenocarcinoma,one case showed weak focal p63 nuclear staining.It may represent out-pouching from high-grade PIN or alternatively, flat, high grade PIN (Table1).

Table (1): Extensiveness of AMACR & p63immunohistochemical staining in malignant glands.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
>90%	22	73.3	0	0.0
50-90%	3	10	0	0.0
10-50	2	6.7	1	3.3
<10%	0	0.0	0	0.0
0%	3	10	29	96.6
Total	30	100	30	100

Table (2): Intensity of immunohistochemical staining in malignant glands.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
Negative	3	10	29	96.6
Weak	1	3.3	1	3.3
Moderate	11	36.7	0	0.0
Strong	15	50	0	0.0
Total	30	100	30	100

Benign glands adjacent to cancer were identified in 23 cases. Among these 23 cases, none showed positive staining for AMACR in benign glands. Virtually all cells in the basal layer of the epithelium in normal glands stained strongly for p63. The staining was confined exclusively to the nuclei of basal epithelial cells. No staining was observed in the secretary epithelial cells or in the stroma (Table3, 4) (Fig 1B, 1C).

Table (3):Immunohistochemical staining extensiveness of benign glands in carcinoma plus control groups.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
>90%	0	0.0	39	90.7
50-90%	0	0.0	3	7
10-50	2	4.6	1	2.3
<10%	0	0.0	0	0.0
0%	41	95.4	0	0.0
Total	43	100	43	100

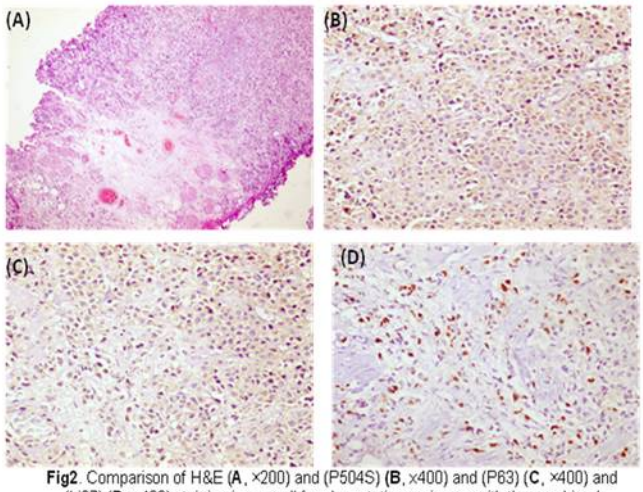

Fig2. Comparison of H&E (**A**, ×200) and (P504S) (**B**, x400) and (P63) (**C**, ×400) and (ki67) (**D**, ×400) staining in a small focal prostatic carcinoma with the combined Gleason's grade 10.**B**, tumor cells show a brown cytoplasmic granular staining (α-methylacyl coenzyme A racemase/P504S). **C** tumor cells show p63 cytoplasmic stain. **D** tumor cells show dark brown nuclear stain of ki67

Cytoplasmic staining for p63 was observed in tumor cells, which is a rare
expression pattern for p63 protein which is normally absent in prostate adenocarcinoma and that usually exhibits strong nuclear staining in basal cells of benign prostate gland. We observed higher levels of cytoplasmic p63 expresison in high grade prostatic adenocarcinoma, also higher levels of cytoplasmic p63 were associated with higher frequency of Ki-67 positive cells. But it was statistically un significant (P = 0.076) (Fig 2C, 3B).

Table (4):Immunohistochemical staining intensity of benign glands in carcinoma plus control groups.

	AMACR		P63 (nuclear stain)	
	NO.	%	NO.	%
Negative	40	93.1	0	0.0
Weak	2	4.6	0	0.0
Moderate	1	2.3	2	4.6
Strong	0	0.0	41	95.4
Total	43	100	43	100

Table (5): Frequency of the Ki-67 labeling index in relation to differentiation and Gleason's grade

Ki-67 labeling index	Gleason grade				Sum
	Poorly differentiated tumors		Moderately differentiated tumors		
	NO.	%	NO.	%	
<2	1	5.9	8	61.9	9
<25	8	47.1	4	30.8	12
26-50	3	17.6	1	7.7	4
51-75	2	11.8	0	0.0	2
76-100	3	17.6	0	0.0	3
Total	17	100	13	100	30

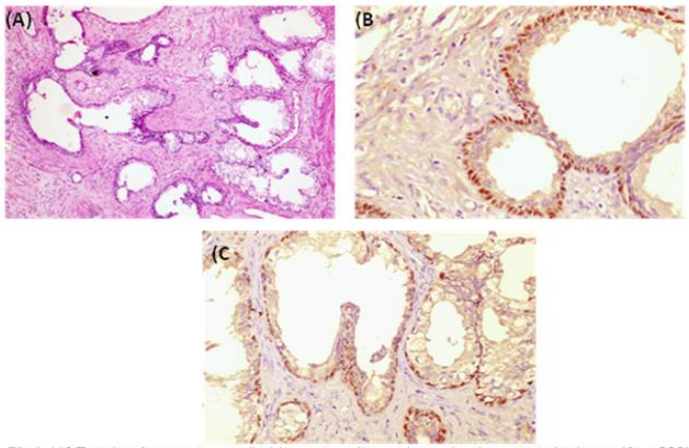

Fig1. H&E stain of prostate needle biopsy specimen shows benign prostatic tissue (**A**, ×200). Immune expression of **p63** in benign glands, positive expression is shown as brown circumferential nuclear **p63** stain in benign glands (immunoperoxidase stain). (**B, C,** ×400**).**

3.2.Ki-67 immunoreactivity results:

In studied specimens of benign prostatic tissue, only (1/20) specimen (5%) was positive for Ki-67 immunostaining. It has been shown that Ki-67 is significantly up-regulated in prostate cancer (P = 0.023) as compared with benign prostatic lesions (Fig 3D).In poorly differentiated carcinoma, one case (5.9%) was negative, whereas 8 cases (47.1%) were +1, 3 case (17.6%) +2, two cases (4.8%) 3+, and three cases (17.6%) 4+ (Table 5). Of 13 cases of moderately differentiated tumors eight cases (61.5%) were negative, while 4 cases (30.8%) were indexed as 1+ and one case (7.7%) as 2+. Consequently a statistical significant correlation was observed between Ki-67 positivity and increased Gleason's grade (P = 0.02) (Table 5).

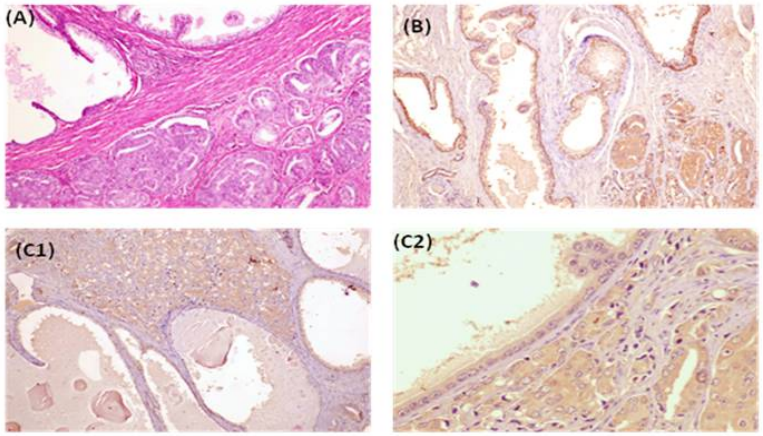

Fig3. Cluster of adenocarcinoma glands with the combined Gleason's grade8 invading between benign glands H&E (**A**, ×200). **P63**staining in a small focal prostatic carcinoma, the tumor glands show brown p63 cytoplasmic staining, whereas adjacent benign glands show dark brown nuclear staining of basal cells (**B**, ×200).**AMACR** expression in benign and malignant glands (immunoperoxidase stain, **C1** ×200) Brown color shows strong positive reaction in malignant glands whereas benign glands among malignant glands show complete negativity. (**C2** ×400) shows strong positive reaction in malignant glands and also in adjacent high grade PIN

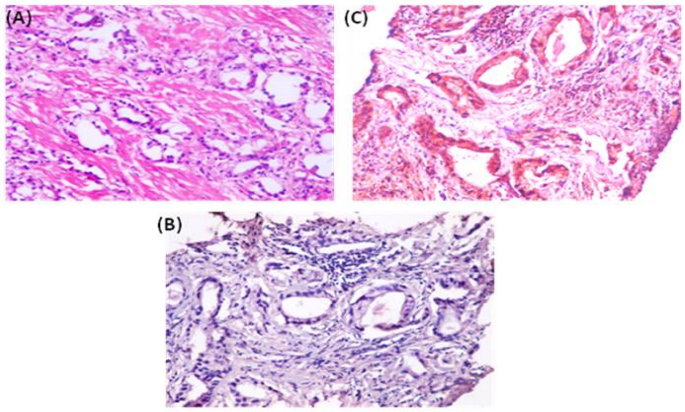

Fig4: A-Minimal carcinoma in prostatic smooth muscle stroma, without adjacent benign glands as reference points to assess infiltration, with the combined Gleason's grade6 (H&Ex200). **(B**, x200) **p63** immunostaining shows no reactivity in malignant glands. (**C**, x200) **AMACR** immunostaining shows a strong cytoplasmic reaction.

4.DISCUSSION

Prostate needle biopsy is the preferred method for diagnosing of early prostate cancer. It has low morbidity and provides specific information on the grade and extent of the tumor.

However, histologic confirmation of prostate carcinoma sometimes remains challenging in biopsy samples. The difficulty with needle biopsy stems not only from the small amount of tissue available for histological examination, but also from the fact that biopsies often identify only a few malignant glands (small focus carcinomas) or several histological benign mimics of cancer (Molinié et al. 2006). Diagnostic difficulty in indeterminate cases concerns 1.5%–9% of prostate biopsy (Iczkowski et al. 2002).

AMACR is used as a confirmatory stain for prostate cancer in conjunction with morphology and a basal cell–specific marker. Using AMACR as a positive marker alone might be misleading because weak expression of AMACR might be seen in benign glands and expression of AMACR is seen in high grade prostatic intraepithelial neoplasia (PIN) (Jiang et al. 2001) and atypical adenomatous hyperplasia (AAH) (Yang et al. 2002). Therefore, using AMACR as a positive marker along with basal cell-specific negative marker (p63) will enhance the diagnostic accuracy in minimal prostate cancer and reduce the chance of misdiagnosis (Srigley, 2004; Epstein, 2004).

In our study, only 3 cases of carcinoma group (10%) showed negative immune reaction with AMACR, and 83.3% of malignant glands showed >50% staining. Eighty six percent of the positive samples with AMACR had moderate to strong staining intensity. We also detected focal positive immune-stain with AMACR in two benign cases showing atrophy. This is due to the fact that the reverse transcriptase-polymerase chain reaction and quantitative IHC have shown that benign prostate epithelium also expresses AMACR mRNA and protein, respectively, but at very low level (Rubin et al. 2002).We consider only circumferential, diffuse or apical, granular, cytoplasmic staining of luminal cells that can be identified at x100 magnification as positive, while the adjacent benign prostatic glands should not show more than weak, partial (non-circumferential) staining as described in study done by Hameed and Humphrey (2006).

Jiang et al.,(2002) found that strong immune reaction for AMACR was consistently present in 94.5% of cases of prostate carcinoma and high-grade prostatic intraepithelial neoplasia. However, contrary to prostate carcinoma no expression was detected in most of the cases of benign hyperplasia. In another study, 71% of cancer cases showed positive immune-staining with AMACR, but variable intensities and percentages of cells were present. About 71%–100% of prostatic adenocarcinoma stained with AMACR (Yang et al., 2002; Jiang et al., 2002; Beach et al. 2002; Boran et al. 2011). In contrast to prostatic adenocarcinoma, about 0%–21% of benign prostatic glands stains for AMACR (Beach et al. 2002;Hameed et al., 2005; Boran et al. 2011).

Some variants of prostatic adenocarcinoma, including the atrophic, foamy gland and pseudohyperplastic variants can be AMACR negative. The sensitivity of AMACR in detecting these variants was found to be 70% (Farinola and Epstein 2004), 68% (Zhou et al., 2003) and 77% (Zhou et al., 2003) respectively. Accordingly, one should not render a diagnosis of benignancy based solely on a negative AMACR immune-stain (Hameed and Humphrey 2006).

Invasive prostatic adenocarcinoma lacks basal cells so using IHC to confirm their diffuse absence

in suspicious foci is a very useful diagnostic tool. Several studies showed that p63 is selectively expressed by basal cells in normal prostate gland. (Signoretti et al., 2000; parsons et al., 2001;Weinstein et al., 2002; Davis et al., 2002; Molinié et al., 2006) found that there was persistent basal cell staining with p63 in 1%–100% of atrophic and benign lesions, and a total absence of basal cells after p63 staining in prostate cancer. In another study, 97% of prostate tumors were completely negative for p63, and only a small percentage of p63 positive cells were detected (Signoretti et al., 2000).

Only complete absence of basal cell staining in all of the glands in a particular focus of concern, will support a diagnosis of carcinoma, as almost all of the mimkers of prostatic carcinoma can, at least focally in some glands, be negative with basal cell marker immune-stain (p63). In our study, there was only one subject with malignant glands that had positive p63 reaction in outer basal cells. It may represent out pouching from high grade PIN or flat, high grade PIN. All benign glands in our study showed strong p63 nuclear stain in outer basal cell layer, but no stain was observed in the secretory epithelial cells or in the stroma.

The expression of high levels of p63 exclusively in epithelial basal cells and the complete lack of prostate development in p63 null mice indicates that p63 expression is involved in the control of prostate growth and differentiation. Moreover, it strongly emphasizes the hypothesis that the basal cells represent prostate stem cells. Loss of p63 expression in secretory epithilum appears related to the process of differentiation as basal cells progress into the overlying epithelium and develops into secretory cell (parsons et al., 2001).

When small atypical glands identified by routine H&E staining are negative for basal cell markers and positive for AMACR/P504s, a malignant diagnosis is established. The main value of AMACR immune-staining is that it appears to provide additional diagnostic value beyond that of a negative basal cell marker immune-stain (Browne et al., 2004). Also there might be other explanations for a negative basal cell immunostaining, including the type of marker used as well as the fixative and antigen-retrieval methods used for the specimen (Varma et al., 1999).

Differences in p63 expression are associated with cancer progression or a poor prognosis for several cancer sites, including over-expression in the ovaries and oral squamous cell carcinoma (Lo Muzio et al., 2005;Marchini et al., 2008), down-regulated expression in the upper urinary tract and prostate (Zigeuner et al., 2004;Bismar et al.,2006;Mucci et al.,2008) and aberrant cytoplasmic expression in lung adenocarcinoma (Narahashi et al., 2006).

In our study, there was a predominantly cytoplasmic staining for p63 positive tumor cells, which is a rare expression pattern for p63 protein which is normally absent in prostate adenocarcinoma and that usually exhibits strong nuclear staining in basal cells of benign prostate gland. This expression was high in high grade prostate cancer, also higher levels of cytoplasmic p63 were associated with higher frequency of ki67 positive cells in prostatic adenocarcinoma, but it was statistically insignificant ($p=0.076$) this in contrast to Parsons et al.,(2001) who found that the majority of prostate adenocarcinomas do not express p63 except some tumor cells in high grade prostatic carcinomas representing less than 1% of cells in those carcinoma specimens showed very weak nuclear staining and there was a strong correlation between the expression of p63 in these tumor cells and Gleason grade ($p<0.0001$).

Dhillon et al.,(2009)found that significant association between cytoplasmic expression of p63 in prostate tumor tissue at the time of diagnosis and fatal prostate cancer and higher levels of cytoplasmic p63 were also significantly associated with increased proliferative activity (ki67) ($p=0.0026$). This difference in the significance of the result may be due to small number of our cases but Dhillon et al.,(2009) study were conducted on 298 men.

Our findings are in contrast with two studies done by Bismar et al.; (2006) and Mucci et al., (2008) that reported an inverse association between p63 expression (as part of a genetic signature) and prostate cancer progression. Bismar et al., (2006) generated 12-gene signature for aggressive prostate cancer that included p63 based on its under-expression in metastatic cancer compared to benign tissue and localized disease.

The nuclear localization of p63 is essential for its role as a transcription factor. Similar to p53, alterations in nuclear-cytoplasmic shuttling may lead to cellular mislocalization, which disrupts regulation of cell cycle checkpoints and apoptosis, contributing to the initiation or progression of cancer (Hood and Silver 2000;Fabbro and Henderson 2003). In patients with lung cancer, inflammatory breast carcinoma and colorectal carcinoma, the cytoplasmic sequestration of p53 is associated with metastasis and poor long-term survival (Moll et al., 1996). The localization shift may arise from disruptions in the nuclear transport pathway (Hood and Silver 2000), such as those mediated by the murine double minute-2 gene (Mdm2) where laboratory data show that p63-induced apoptosis is reduced when Mdm2 exports two isoforms of p63 (TAp63α and TAp63γ) from the nucleus to inhibit their transcription and pro-apoptotic activity (Kadakia et al., 2001). In our study, higher levels of

cytoplasmic p63 are associated with increased proliferation, this is in agreement with Dhillon et al.,(2009) who stated that, the mis-localization and imbalance in p63 isoforms may alter p63 stability and function and thereby disrupt cell cycle arrest and apoptosis, which may have prognostic significance for cytoplasmic sequestration of p63 and the progression of prostate cancer.

The management of patients with prostate cancer depends on an accurate assessment of the biological potential of the tumor. Unfortunately, the current examination techniques are mostly inadequate for correct clinical staging, and assessment of the tumor grade may vary due to subjectivity of the observer. Therefore, the search for additional prognosticators of the cancer behavior is the subject of intense ongoing investigation. Ki-67 is one of the most reliable markers of cell proliferation. Ki-67 was up-regulated in prostate cancer and PIN and was associated with Gleason grades. High ki-67 expression was a predictor of poor prognosis after radical prostatectomy (Nikoleishvili et al., 2008).

In this current study, the Ki67 was significantly up-regulated in prostate cancer ($P<0.023$) as compared with benign prostatic tissue. This finding is in agreement with Nikoleishvili et al., (2008) who found that this marker was highly expressed in prostate cancer as compared with BPH ($P=0.019$).

Ki-67 marker was positive in 21 out of 30 tumors in current study (70%). Forty nine percent of poorly differentiated tumors and 38.5% of the moderately differentiated tumors were positive for Ki-67 in this study. This is in agreement with Nilsson et al.,(1988) who showed a significant correlation between positive cases of Ki-67 and tumor cell differentiation. The present study also showed a statistical significant correlation between Ki-67 marker and increased Gleason's grading with increased number of stained cells ($P=0.02$) and this is in consistent with Madani et al., (2011).

In conclusion, immunohistochemical analysis with an AMACR (P504s) and p63 provides a simple and easy assay that can be used as a routine test, which overcomes the problems of studying limited carcinoma in prostate needle biopsies and increase its diagnostic accuracy. The diagnosis of these small foci of prostate cancer in needle biopsy specimens is one of the major diagnostic challenges in surgical pathology. Ki-67 marker was shown to have a strong relationship with Gleason's grading, which has an important relationship with the prognosis of prostate cancer. Cytoplasmic expression of p63 was high in high grade prostate cancer. Also it was associated with higher frequency of ki67 positive cells in prostatic adenocarcinoma. The mislocalized cytoplasmic expression of p63 was associated with higher proliferative activity, and may suggest an oncogenic role in prostate cancer progression.

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