Immunohistochemical expression of ERG and P63 in some prostatic lesions
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ABSTRACT
Background: prostate cancer (PCa) ranks second among the most common types of solid tumors and the sixth among the leading causes of cancer deaths in men worldwide. In Egypt, prostatic cancer formed the majority of male genital cancer (60.7%) at the NCI in the last 10 years. Aim of the work: the present study aimed to detect immunohistochemical expression of ERG and p63 (as a basal cell marker) in benign prostatic lesions and prostatic carcinoma and to investigate the potential use of ERG and p63 expression levels to discriminate prostatic carcinoma from other lesions and to investigate the association of immunohistochemical expression of ERG with the other prognostic parameters of prostatic carcinoma such as age, plasma PSA level and pathological Gleason score. Methods: fifty prostate lesions, which were collected from the surgical files of the Pathology Department of Al-Azhar University Hospital and from a private lab. Clinicopathological and histological features were taken from patients files and confirmed by H&E examination. Immunohistochemical study was done by using two markers ERG and P63. Results: expression of ERG was restricted to malignant tissue (Prostatic carcinoma) and was negative in BPH and PIN specimens (P < 0.001) ERG is highly specific but less sensitive marker (40 % of PCa were negative). Expression of ERG revealed inverted significant correlation with Gleason grade and plasma PSA level (P < 0.05). P63 is highly specific and sensitive marker for non-carcinomatous prostatic lesions. Conclusion: ERG showed strong nuclear endothelial expression in all lesions.

Keywords: ERG, P63, PSA, prostate cancer

INTRODUCTION
Prostatic carcinoma is the sixth most common type of neoplasm in the world and the second in prevalence among men (10% of all cases). Statistics worldwide indicated that prostate cancer (PCa) has high prevalence and lethality, with three-quarters of cases among 65-year-oldsters (1). Prostatic intraepithelial neoplasia (PIN) is considered a morphological equivalent of prostate pre-cancer. It develops as a result of proliferative changes of ductal epithelium and acini of the prostate. Many researchers distinguish two forms of PIN: low-grade PIN (Low-grade prostatic intraepithelial neoplasia) and high-grade PIN (high-grade prostatic intraepithelial neoplasia) depending on pronouncement of cytological and structural changes of epithelium lining the prostate (2). The present study was an attempt to determine the expression of ERG and P63 as a (Basal myoepithelial marker) on different carcinomatous and non-carcinomatous prostatic lesions, by immunohistochemical study of both markers upon different Gleason of PCa specimens.

AIMS of the WORK
The aims of this study were:

1. To study the immunohistochemical expression of ERG and p63 (as a basal cell marker) in non-cancerous prostatic lesions and prostatic carcinoma.
2. To investigate the potential use of ERG and p63 expression levels to discriminate prostatic carcinoma from benign lesions.
3. To investigate the association of immunohistochemical expression of ERG with the other prognostic parameters of prostatic carcinoma such as age, plasma PSA level and pathological Gleason score.

MATERIALS and METHODS:
The current study comprised 50 prostate lesions, (17 were benign prostatic hyperplasia, 3 were PIN, while the remaining 30 lesions were prostatic carcinoma), which were collected from the surgical files of the Pathology Department of Al-Azhar University Hospital and from private lab during the period from March 2016 to March 2018. The study was approved by the Ethics Board of Al-Azhar University.

The clinical data were retrieved from the accompanying clinical sheets. Each case was subjected to histologic typing and grading. The
cases were classified according to the WHO histologic classification of prostate tumors and was graded according to Epstein et al. (3) grading system, in which three main grade groups were used to report prostatic carcinoma grading as follow: group I: GS ≤ 6, group II: GS = 7 and group III: GS ≥ 8.

IMMUNOHISTOCHEMICAL STUDY: The paraffin embedded blocks for each case was cut and subjected to:

1. Routine hematoxylin and eosin staining to confirm the original diagnosis and to revise the histological features such as perineural invasion and pathological Gleason score.

2. Immunohistochemical staining for ERG and P63 antibodies.

Immunohistochemistry: Avidin–biotin–peroxidase complex immunohistochemical method were performed on formalin-fixed, paraffin-embedded tissue cut at 4 µm and placed on positively charged slides. Slides were deparaffinized in xylene, and rehydrated in graded alcohols. To reduce non-specific background staining, endogenous peroxidase were blocked by 0.3% hydrogen peroxidase in methanol and slides were rehydrated in PBS, pH 7.4. After that incubation with a primary antibody at room temperature were performed. The primary antibodies which used were ERG, (Anti-ERG-MAb, clone 9FY cat. no. CM421C; Biocare Medical, Concord, CA, USA, mouse monoclonal antibody, 1/50 dilution, overnight at room temperature) and P63 (Mouse monoclonal antibody, Clone: 4A4, Dako HIER - Dako Target Retrieval Ph 9, 1/100 dilution, for 40 minutes). Antigen detection were carried out by exposure to a biotinylated universal secondary antibody followed by a streptavidin—peroxidase complex working solution. After another PBS wash, the antigen antibody complex was visualized by staining with diaminobenzidine/hydrogen peroxidase chromogen solution (DAB). The sections were rinsed in tap water, counterstained with hematoxylin, dehydrated in graded alcohols followed by xylene and then mounted in a DPX mounting medium. Positive and negative controls were used for each marker.

POSITIVE CONTROL: As regard ERG antibody, endothelial cells within the prostate tissue were used as internal control, while basal myoepithelial cells of benign prostatic glands was used as a control in the condition of P63 marker.

Staining pattern: the staining pattern of ERG and P63 was nuclear in both of them.

Positive/negative results ERG marker: tumors were considered positive if any tumor cell stained brown and negative if tumor cells did not stain brown.

Percentage score for ERG: the percentage of positive cells (PP) for ERG was scored as the following:

0 = no staining, 1 = positive cells ≤ 30 %, 2 = positive cells 30% - 70%, 3 = positive cells >70% of cells

The intensity score for ERG: the intensity score (IS) was scored on a 3 point scale: 1 = weak, 2 = moderate, 3 = strong

Immunoreactivity score (IRS): IRS for ERG was calculated by multiplying percentage score and intensity score (IRS = PPxIS). Score less than 3 were negative for ERG and score ≥ 3 was positive. Evaluation of ERG positivity was done according to Kim et al. (4).

P63 marker: benign prostatic glands were the positive control. The result was considered positive if the nuclei of myoepithelial cells stained brown, and negative if not stained. The basal myoepithelial cells positivity by P63 was evaluated according to Bachurska et al. (5).

No positive cells: negative staining
Less than 60% of cells: partial staining
More than 60% of cells: diffused staining

Digital images for immunostained slides for both markers were obtained with a digital camera system (Olympus, Tokyo, Japan).

RESULTS: The present study revealed that the group of age 61-70 years were the most affected by PCa, N = 12/30 (40%). The present study included 22 cases (73.3%) of Gleason score ≤ 6, 5 (16.6%) cases of Gleason score = 6 and 3 (10%) cases of Gleason score ≥ 8. As regard plasma PSA level of the studied prostatic carcinoma cases, 7/30 (23.3%) of them showed plasma PSA level ≤ 10 ng/dl and 27/30 (76.7 %) of the studied specimens showed plasma PSA level > 10 ng/dl. ERG immunoreactivity was negative in all the studied benign lesions (BPH and PIN) with strong positive stromal endothelial cells as an internal control (Table 1). Eighteen out of thirty 18/30 (60%) prostatic carcinoma showed positive immunostaining for ERG, while 12/30 (40%) were negative (Figures 1 & 2). The positivity was variable from moderate to strong nuclear expression. Negative cases showed positive surrounding endothelial cells as a positive control, Hence, the present study
revealed a significant difference of ERG expression in prostatic carcinoma versus benign prostatic lesions (P < 0.001) (Table 1). Means of ERG expression were 4.6±0.67, 2.6±0.87 and 2±0.2 in GS ≤ 6, GS = 7 and GS ≥ 8 respectively. The present study revealed inverted significant correlation between ERG expression and Gleason score, (r=0.931, P < 0.01) (Table 1).

Table 1: ERG immunoreactivity in the different studied groups and different Gleason scores

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>No. of Cases</th>
<th>positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign prostatic hyperplasia</td>
<td>17</td>
<td>0</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Prostatic intraepithelial neoplasia (PIN)</td>
<td>3</td>
<td>0</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Prostatic adenocarcinoma</td>
<td>30</td>
<td>18 (60%)</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Gleason group I</td>
<td>22</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>18 (60%)</td>
<td>12 (40%)</td>
</tr>
</tbody>
</table>

Means of ERG IRS were 4.7±1.2 and 3.8±0.64 in cases of PSA level ≤ 10 ng/dl and > 10 ng/dl respectively. The present study revealed inverted significant correlation between serum PSA level and ERG IRS (r= 0.943, P < 0.05). All benign prostatic cases N=20 (BPH, basal cell hyperplasia and PIN) displayed diffsed strong expression for p63 as a brown coloration in the nucleus of basal myoepithelial cells, while all cases of prostatic adenocarcinoma were negative for P63 antibody (Table 2 and figure 2).

Table 2: P63 immunoreactivity in different study groups

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>No. of Cases</th>
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<td>0</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>20 (40%)</td>
<td>30 (60%)</td>
</tr>
</tbody>
</table>
Figure 1: BPH showing negative ERG expression in benign hyperplastic prostatic glands with positive endothelial cells as an internal control (ERG x400)

Figure 2: showing diffused strong positive expression in the prostatic carcinoma (ERGx400)
DISCUSSION:
In the present study, the age range of the PCa patients was 60-95 years with a high prevalence in 60-70y, group of age, N = 12/30 (40%). The current study was in accordance with results of Kelly et al. (6) who reported that 31 (51.7%) of the prostatic carcinoma patients were older than 50 and younger than 69 years old. There was predominance of cases in Gleason score GS ≤ 6, representing 22 (73.3%) this is in agreement with results of Navaei et al. (7) who found that 33% of cases were in GS=6. Also, Kuroda (8) noted that the Gleason score 9/13 (69%) adenocarcinomas was 3+3=6. The present study showed that 27/30 (76.7%) of the studied PCa specimens showed plasma PSA level > 10 ng/dl. This result is in agreement with those of Baig et al. (9), they decided marked elevation in serum PSA levels (>10ng/ml) in vast number of examined prostatic carcinoma specimens. This finding is in contrast with results of Çalışkan et al. (10) and Brooks et al. (11) who found that means of the plasma PSA levels were 8.03 ± 5.21 ng/ml and 8.7 ± 8.8 ng/ml respectively. This discrepancy may be due to difference in numbers of the studied cases. In the present study, there was inverted relationship between ERG expression and Gleason score, (r=0.931, P < 0.01). The current result is in agreement with results of Kelly et al. (6); Navaei et al. (7); and Lee et al. (12). All of these studies displayed higher ERG expression in low grade Gleason score. This result is in contrast to results of Hoogland et al. (13) who displayed no correlation between ERG expression and Gleason score. This discrepancy may be due to the difference of genetic factors of patients. Eighteen out of thirty 18/30 (60%) of PCa specimens showed positive immunostaining for ERG, while 12/30 (40%) were negative for ERG, while it was not expressed in any benign prostatic lesion. This agrees with results of Bachurska et al. (5) and
Hoogland et al. (13) who reported negative ERG expression in the benign prostatic glands. Also, there was inverted significant relationship between ERG expression and serum PSA level. These results are in line with those of Brooks et al. (11) and Song et al. (14). However, Abdel-Hady et al. (15) decided no important correlation between ERG expression and serum PSA level. All the studied BPH and PIN biopsies were positive for P63 which highlighted the basal cells around the glands. This result is in concordance with those of Baig et al. (9) and Oksana et al. (16), they revealed positive expression of p63 in non-cancerous lesions. Brustmann (17) reported that all cases with benign and all cases signed out as carcinomas were completely negative for P63. All biopsies of PCa were completely negative for P63, this result was expected, because myoepithelial cells play an active role in tumor suppression by secreting protease inhibitors, down regulating matrix metalloproteinases and producing tumor suppressive proteins such as p63, maspin, Wilms tumor 1 and laminin. These data support the absence of myoepithelial cells, resulting in the transition from preinvasive to invasive cancer (17). The present study demonstrated strong nuclear endothelial cells expression. This result is in agreement with results of Navaei et al. (7) who showed that vascular endothelial cells were strongly positive for ERG expression, hence they were used as the internal positive control. Kohashi et al. (19) reported that ERG was immunoexpressed in the vascular endothelial tumors, plastic extramedullary myeloid tumors and tumors with ERG-involved translocation, such as prostate carcinoma or Ewing sarcoma. ERG plays a role in endothelial cell migration and has been linked to angiogenesis (20). It plays a role in capillary morphogenesis which is an important step of the angiogenic cascade (21).

Conclusion:

1) Expression of ERG was restricted to malignant tissue (Prostatic carcinoma) and was negative in BPH and PIN specimens. ERG is highly specific but less sensitive marker (40% of PCa were negative).

2) Expression of ERG revealed inverted significant correlation with Gleason grade and plasma PSA level (P < 0.05).

3) ERG showed strong nuclear endothelial expression in all lesions.

4) P63 is highly specific and sensitive marker for benign prostatic lesions.

REFERENCES:


8. Kuroda N (2014): Application of combined immunohistochemical panel of AMACR(P504S)/p63 cocktail, cytokeratin 5 and D2-40 to atypical glands in prostatic needle biopsy. The


