ABSTRACT

Background and Purpose: Topoisomerase II α (Topo II α) and Her-2/neu are two important targeted therapeutic molecules. The immunohistochemical expression of both of them has not been widely studied in prostatic carcinoma and benign prostatic hyperplasia (BPH). The aim of this study was to evaluate the immunohistochemical expression of Topo II α and Her-2/neu in prostatic carcinoma and BPH and compare the expression patterns of both genes in cases of prostatic carcinoma in relation to Gleason score and hormonal status.

Material and Methods: Paraffin blocks of 30 cases of prostatic carcinoma (categorized by Gleason score and hormonal status) and 5 cases of BPH presented to the Department of Pathology, Faculty of Medicine, Tanta University during the period from 2005 to 2008 were retrieved from the files. The immunohistochemical expression of Topo II α and Her-2/neu antibodies in the above-mentioned diagnostic categories was investigated and compared. The percentage of nuclei staining for Topo II α was semiquantitated; overexpression was defined as ≥5% nuclear staining. Her-2/neu immunoreactivity was scored from 0 to 3+ depending on membrane staining intensity and pattern.

Results: The expression of Topo II α varied significantly among the different studied groups (p<0.001). Topo II α expression increased significantly with increased Gleason score in prostatic carcinoma (p=0.001). Its expression in both moderately and poorly differentiated carcinomas was significantly higher than in BPH (p=0.005 and 0.002 respectively); however the difference between its expression in well-differentiated carcinoma and in BPH was statistically insignificant (p=0.171). Her-2/neu expression was higher in prostatic carcinoma than in BPH, however the difference did not reach the level of statistical significance and the increase in its expression with increased Gleason score was statistically insignificant (p=0.100). There was a significant correlation between Topo II α and Her-2/neu expression (p=0.008, r=0.478). Hormone resistant carcinomas showed higher expression of Topo II α and Her-2/neu than carcinomas with no hormone treatment, however, the differences were statistically insignificant (p=0.594 and 0.667 respectively).

Conclusion: Topo II α expression was significantly higher in poorly differentiated and moderately differentiated prostatic carcinoma compared to BPH. There was a significant increase in Topo II α expression with increased Gleason score. Her-2/neu expression was higher in prostatic carcinoma than in BPH, however the difference did not reach the level of statistical significance and the increase in its expression with increased Gleason score was also statistically insignificant. Topo II α and Her-2/neu were co-expressed significantly. Hormone resistant carcinomas showed higher expression of both markers, however, the differences were statistically insignificant. The latter finding may have important therapeutic implications, however, further large scale studies are required for confirmation.

Key Words: Topo II α – Her-2/neu – Prostatic carcinoma – BPH.

INTRODUCTION

Topoisomerase II-alpha (Topo II α) is an essential cellular enzyme that functions in the segregation of newly replicated chromosome pairs, in chromosome condensation and in altering DNA superhelicity. DNA topoisomerases participate in nearly all biological processes governing DNA and untangle intertwined DNA strands before cell division by transiently breaking and then re-ligating duplex strands of DNA. Chemotherapeutic drugs that target Topo II α, such as etoposide, doxorubicin and mitoxantrone, act by stabilizing a normally transient DNA-topoisomerase II complex, thus increasing the concentration of double-stranded DNA breaks.
This phenomenon triggers mutagenic and cell death pathways [1,2].

An immunohistochemical stain was developed that is capable of detecting Topo II α in formalin-fixed, paraffin-embedded sections of human tissues [3]. Using this immunohistochemical stain, the enzyme has been found to be elevated in neoplasms inherently sensitive to etoposide and other topoisomerase-targeting drugs, such as small-cell lung cancer [4] and Hodgkin’s disease [5]. The enzyme can also serve as a proliferation marker for use in identifying the number of cycling cells in normal tissues [6] and in a number of human cancers [7]; so, it has been reported as a prognostic marker in tumors of several tissues [8-21]. Unfortunately, little information is available about the expression of Topo II α in prostatic carcinoma [22].

Her-2/neu is a receptor tyrosine kinase that belongs to the epidermal growth factor receptor family. Overexpression of Her-2/neu, which is seen in 20%-30% of breast and ovarian cancers, results from gene amplification and is associated with poor prognosis [23-26]. It has also become a therapeutic target in breast cancer with the advent of antibodies generated against its extracellular domain [27]. In breast cancer clinical trials, one such antibody, trastuzumab (Herceptin) has been shown to be effective when co-administered with other chemotherapeutic agents [28,29]. In prostatic carcinoma, the assessment of Her-2/neu overexpression has been more problematic and the results were controversial [30].

The Topo II α gene is located adjacent to Her-2/neu on chromosome 17q. Co-amplification of Her-2/neu and Topo II α has been described in breast and bladder carcinomas [31,32]. In prostatic carcinoma, the expression patterns of both genes have not been widely compared in a single study; it is not clearly known if tumors that overexpress Topo II α co-express Her-2/neu [33].

In the light of this, the current work aimed at studying the immunohistochemical expression of Topo II α and Her-2/neu in prostatic carcinoma and benign prostatic hyperplasia (BPH) and comparing the expression patterns of both genes in cases of prostatic carcinoma in relation to the Gleason score and the hormonal status.

MATERIAL AND METHODS

Case selection and review:

Available archival paraffin blocks of thirty cases of prostatic carcinoma and five cases of BPH presented to the Department of Pathology, Faculty of Medicine, Tanta University during the period from 2005 to 2008 were retrieved from the files. Cases consisted of prostatectomy specimens and transurethral resections. Case selection was based on the availability of sufficient material for immunohistochemical staining. Histopathological reports were reviewed for all carcinoma cases to ensure the adequacy of diagnosis and grading. All prostatic carcinomas had been graded based on the Gleason score [34].

The cases were classified into four groups: BPH "n=5"; well-differentiated prostatic carcinoma of Gleason score 6 or lower "n=8"; moderately differentiated prostatic carcinoma of Gleason score 7 "n=10" and poorly differentiated prostatic carcinoma of Gleason scores 8-10 "n=12". All the poorly differentiated carcinoma cases received no hormonal treatment prior to surgery except for 4 cases, that were unsuitable for curative resection and were treated with hormonal therapy and palliative transurethral resection of the prostate, however, they showed increasing PSA and thus underwent transurethral resection of the prostate for reevaluation. These 4 cases were categorized as hormone resistant carcinomas [22].

Immunohistochemistry:

For immunohistochemistry, 3µm sections were deparaffinized in xylene for 30 minutes and rehydrated with graded alcohol series. Sections were then subjected to antigen retrieval by boiling the tissue sections in 10mM citrate buffer, pH 6.0 (Lab Vision catalog # AP-9003), for 10 minutes followed by cooling at room temperature for 20 minutes and rinsing with phosphate buffered saline (PBS) for one minute. Endogenous peroxidase was blocked by immersion of the sections in 3% hydrogen peroxide solution for 10 minutes, then washing them in PBS. Immunohistochemical staining was performed using the UltraVision Detection Kit (TP-015-HD, Lab Vision, USA) according to the manufacturer’s protocol. Sections were incubated for 10 minutes with Ultra V block to prevent non-specific background staining, fol-
followed by rinsing the sections with PBS. Afterwards, an overnight incubation was done in a humidity chamber with monoclonal primary antibody against Topo II α (Catalog # MS-1819-R7 “Ready to Use”, Lab Vision, USA) and Her-2/neu (clone SPM495; Catalog # MS-10463-P0, Lab Vision, USA, at 1:100 dilution), followed by washing in PBS. Sections were then covered with 4-5 drops of UltraVision biotinylated goat anti-polyvalent secondary antibody, incubated at room temperature for 10 minutes, then washed in PBS, followed by incubation with streptavidin peroxidase solution for 10 minutes at room temperature, then rinsing with PBS. Sections were then covered for 15 minutes by adding one drop of 3-3'-diamino-benzidine-tetra-hydrochloride (DAB) chromogen mixed with 2ml of DAB substrate. Finally, sections were counterstained with Mayer’s haematoxylin, dehydrated in alcohol and mounted in di-n-butyl-phthalate-polystyrene-xylene (DPX).

To test for immunohistochemical positivity, external controls previously positive for the antigen of interest were used. Positive (3+) ductal carcinoma of the breast was used for Her-2/neu and normal tonsil was used for Topo II α. Negative controls were prepared by omission of the primary antibody.

Immunohistochemical evaluation and scoring:

Topo II α expression was semi-quantitated, by cell count. Topo II α index is equal to the percentage of tumor cell nuclei staining positively for the enzyme. To determine this number, at least 500 tumor cells were counted in the areas of highest staining [21]. Topo II α overexpression was defined as ≥5% positive nuclear staining [33].

Her-2/neu immunohistochemical positivity was indicated by the presence of circumferential membranous staining. The membrane staining intensity and pattern were considered for scoring according to the Food and Drug Administration approved criteria: 0, no staining or membrane staining observed in less than 10% of tumor cells; 1+, partial membrane staining in more than 10% of tumor cells; 2+, circumferential weak to moderate staining observed in more than 10% of tumor cells and 3+, strong circumferential membrane staining observed in more than 10% of tumor cells. Areas that were poorly preserved, crushed, cauterized, folded, or retracted were specifically avoided. Scores of 2+ and 3+ were considered positive [35].

Statistical analysis:

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) software for Windows, Version 13.0. The following tests were done: Kruskal-Wallis test for comparing marker expression between more than two different groups; Mann-Whitney test for comparing marker expression between two groups; Spearman Rank Correlation Coefficient to assess if there was a correlation between Topo II α and Her-2/neu expression in the studied cases; Chi-square and Fisher’s exact tests for comparing qualitative data. A probability value (p) of less than 0.05 was considered statistically significant.

RESULTS

Topo II α status in prostatic carcinoma and BPH (Tables 1,2):

Evaluation of Topo II α expression in the studied cases of prostatic carcinoma revealed that 25 cases (83.3%) were Topo II α positive (Figs. 1,2), while 5 cases (16.7%) were negative. Based on the cutoff point of ≥5% nuclear positivity, 11 tumors (36.7%) showed Topo II α overexpression (Fig. 2). All these carcinomas were poorly differentiated of Gleason score ≥8 except for 3 moderately differentiated carcinomas of Gleason score 7. Neither well–differentiated carcinomas nor BPH cases showed Topo II α overexpression. There was a significant difference in Topo II α expression among the different studied groups (p<0.001). Topo II α expression was significantly higher in moderately differentiated and poorly differentiated carcinoma cases compared to BPH (p=0.005 and 0.002 respectively), while, the difference between its expression in well-differentiated carcinoma and in BPH was statistically insignificant (p=0.171).

Within the spectrum of prostatic carcinoma, there was a statistically significant increase in Topo II α expression with increased Gleason score (p=0.001).

Her-2/neu status in prostatic carcinoma and BPH (Tables 3,4):

Interpretation of Her-2/neu immunohistochemistry was possible for all the studied cases. Within prostatic carcinoma cases, 7 tu-
mors (23.3%) showed 3+ immunoreactivity, 15 tumors (50%) showed 2+ immunoreactivity, and 8 tumors (26.7%) showed 1+ immunoreactivity but no tumors were devoid of immunoreaction. Considering that Her-2/neu scores of 2+ and 3+ are positive, 11/12 (91.7%) poorly differentiated carcinomas of Gleason score ≥8 were positive (Fig. 3), 7/10 (70%) moderately differentiated carcinomas of Gleason score 7 were positive (Fig. 4) and 4/8 (50%) well-differentiated carcinomas of Gleason score ≤6 were positive (Fig. 5). Within the BPH cases, 2 cases (40%) showed 2+ staining (Fig. 6), 2 cases (40%) showed 1+ staining and the remaining case (20%) showed 0 staining.

Fig. (7) shows the distribution of Her-2/neu scores among the different studied groups.
Her-2/neu expression was higher in prostatic carcinoma than in BPH, however, the difference did not reach the level of statistical significance ($p=0.084$). Within the spectrum of prostatic carcinoma, although there was an increase in Her-2/neu expression with increased Gleason score, yet the difference was also statistically insignificant ($p=0.100$).

Correlation between Topo II $\alpha$ and Her-2/neu expression in prostatic carcinoma (Fig. 8):

There was a statistically significant positive correlation between Topo II $\alpha$ and Her-2/neu expression ($r=0.478$, $p=0.008$). Evaluation of Topo II $\alpha$ overexpression together with Her-2/neu immunopositivity was available within the poorly and moderately differentiated carcinoma groups. Within the group of poorly differentiated carcinoma of Gleason score $\geq 8$, 7 cases (58.3%) showed both Topo II $\alpha$ overexpression and Her-2/neu positivity, one case (8.3%) showed Topo II $\alpha$ overexpression alone and 4 cases (33.3%) showed Her-2/neu positivity alone. In the moderately differentiated carcinoma group of Gleason score 7, 2 cases (20%) showed both Topo II $\alpha$ overexpression and Her-2/neu positivity, one case (10%) showed Topo II $\alpha$ overexpression alone and 5 cases (50%) showed Her-2/neu positivity alone.

Table (1): Topo II $\alpha$ immunoreactivity in the different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Median</th>
<th>Kruskal-wallis test</th>
<th>$X^2$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPH &quot;n=5&quot;</td>
<td>0-0.4</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-differentiated prostatic carcinoma &quot;n=8&quot;</td>
<td>0-3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately differentiated prostatic carcinoma &quot;n=10&quot;</td>
<td>0-6</td>
<td>3.5</td>
<td>19.048</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated prostatic carcinoma &quot;n=12&quot;</td>
<td>0-20</td>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant.
* One case
* 2 cases with the same values of expression
* 3 cases with the same values of expression
* 4 cases with the same values of expression

Table (2): Correlation between the Topo II $\alpha$ index and Gleason score in the studied prostatic carcinoma cases.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range</th>
<th>Median</th>
<th>Kruskal-wallis test</th>
<th>$X^2$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason score (≤6) &quot;n=8&quot;</td>
<td>0-3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason score (7) &quot;n=10&quot;</td>
<td>0-6</td>
<td>3.5</td>
<td>13.726</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Gleason score (8-10) &quot;n=12&quot;</td>
<td>0-20</td>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant.
Groups showing different letters show a statistically significant difference.

There was a significant positive correlation between Topo II $\alpha$ and Her-2/neu expression within the studied carcinoma cases ($p=0.008$, $r=0.478$). The positivity of both markers was overlapping in 7 poorly differentiated carcinoma cases and 2 moderately differentiated carcinoma cases.

Her-2/neu expression was higher in prostatic carcinoma than in BPH, however, the difference did not reach the level of statistical significance ($p=0.084$). Within the spectrum of prostatic carcinoma, although there was an increase in Her-2/neu expression with increased Gleason score, yet the difference was also statistically insignificant ($p=0.100$).
Correlation of Topo II α and Her-2/neu expression with the hormonal status (Table 5):

On analyzing the group of poorly differentiated carcinomas of Gleason score ≥8 according to the hormonal status, 3/4 (75%) of the hormone resistant tumors showed Topo II α overexpression, while 5/8 (62.5%) of those with no hormonal treatment showed overexpression, however, the difference between both groups was statistically insignificant (p=0.594). Also, on evaluating Her-2/neu expression in these cases, all the hormone resistant cases (100%) were positive, while 7/8 (87.5%) of those with no hormonal treatment showed Her-2/neu positivity, however, the difference was also statistically insignificant (p=0.667).

### DISCUSSION

Antitumor therapy that targets tumor specific molecular functions or surface antigens has shown an increasing interest in scientific research since early 1980s. The goal of such therapy is to improve specificity and decrease morbidity compared to cytotoxic chemotherapy [33]. DNA Topo II α is emerging as an important molecular target for many anticancer drugs [36]. Many experimental studies have clearly demonstrated that cellular sensitivity to Topo II α active agents is dependent on high levels of the enzyme [37]. Also, Trastuzumab (herceptin) was one of the first targeted therapies to show a survival benefit in patients with breast cancers that expressed Her-2/neu [38,39]. Recent studies have shown an additional survival benefit in Her-2/neu positive breast cancers that show co-amplification of Topo II α and Her-2/neu genes when treated with Topo II α inhibitor therapy [32]. The possibility of enhanced sensitivity to Topo II α inhibitors places new significance on the presence of Topo II α and Her-2/neu co-expression in other tumors [33].

This work evaluated the immunohistochemical expression of Topo II α and Her-2/neu in prostatic carcinoma and BPH and compared the expression patterns of both genes in cases of prostatic carcinoma in relation to the Gleason score and the hormonal status.

Little information is available on the expression of Topo II α in prostatic carcinoma or, in particular, in identifying whether its expression is increased in comparison with BPH. This study investigated 5 cases of BPH, which showed
little expression of Topo II α (range 0-0.4), which was lower than that found by Willman & Holden [21] and Hughes et al. [22], who identified higher expression of Topo II α in BPH (range 0.2-1.0 and 0-3.3 respectively). These differences might be attributed to a number of factors including the type and extent of fixation and tissue processing, the different antibodies used and the sensitivity of the techniques used for detection.

In addition, our study showed a statistically significant higher expression of Topo II α in poorly and moderately differentiated prostatic carcinoma compared to BPH (p=0.002, 0.005 respectively). Also, there was a statistically significant increase in the expression of Topo II α with increasing Gleason score (p=0.001) and this was consistent with published data [21,22,33].

This work showed that Her-2/neu positivity was detected in some BPH cases and in all grades of prostatic carcinoma. Prostatic carcinoma cases showed higher expression of Her-2/neu than BPH cases, However, the difference did not reach the level of statistical significance (p=0.084). Also, there was an increase in Her-2/neu expression with increased Gleason score, however, the difference was statistically insignificant (p=0.100). These results were compatible with the published data [30,40-42]. Gu et al. suggested that the difference in Her-2/neu positivity between prostatic carcinoma and prostatic hyperplasia may indicate that Her-2/neu is involved in growth stimulation and probably contributes to the oncogenic transformation of prostatic cells [40]. However, in contrast to these results, Ibrahim et al., reported that Her-2/neu expression was significantly higher in BPH than in prostatic carcinoma [43].

In fact, the correlation and role of Her-2/neu overexpression in the initiation and progression of prostatic carcinoma remains uncertain since conflicting results from several studies exist. In particular, while Wane et al., concluded that Her-2/neu is overexpressed in both benign and malignant glands of human prostate [44], MacCann et al. failed to detect any immunoreactivity to Her-2/neu in prostatic carcinomas [45]. In contrast, Zhau et al., reported that positive staining was detected in 80% of prostatic carcinomas with absence of positivity both in normal prostatic tissue and benign prostatic hyperplasia [46], while only 16% of prostatic carcinomas overexpressed Her-2/neu by image analysis assisted quantitative immunocytochemistry in a study made by Ross et al. [47]. Michael et al., suggested that the variability of immunohistochemical staining results for Her-2/neu among different laboratories may be due to the variability of the antibodies used in the detection of Her-2/neu oncoprotein [48]. Moreover, Gu et al., added that differences in the immunohistochemical staining techniques and classifications of the degrees of staining as well as normal variations in tumor populations may be responsible for these conflicting results [40].

In the present study, there was a significant positive correlation between Topo II α and Her-2/neu expression in the studied prostatic carcinoma cases (r=0.478, p=0.008). Besides, they were found to be overexpressed in overlapping but distinct subgroups of patients. These results were in accordance with the work of Murphy et al., who recommended therapies directed against Topo II α and Her-2/neu in patients with such co-expression [33]. In breast cancer, Topo II α amplification occurs in a subset of Her-2/neu positive tumors and is associated with increased sensitivity to Topo II α inhibitor chemotherapy as stated by Di Leo et al. [32].

This study included only 4 cases that were hormone resistant among the poorly differentiated carcinomas of Gleason score 8-10. They showed higher Topo II α and Her-2/neu expression compared to prostatic carcinomas with no hormone treatment, however, the differences were statistically insignificant (p=0.594 and 0.667 respectively) and this may be attributed to the small sample size. However, our results were in accordance with Hughes et al., who reported that the importance of this finding would be in providing clinicopathological correlation for the fact that this clinical subgroup of hormone-resistant prostatic carcinomas has shown the best response to the drug etoposide that targets the Topo II α gene [22]. Also, our results agree with the studies which suggest that Her-2/neu is commonly overexpressed in tumors following the development of resistance to hormonal therapy [49,50]. Signoretti et al. and Edward’s et al., suggested the use of Her-2/neu targeted therapies in treating patients following the development of hormone resistance [30,51]. Signoretti et al. and Di Lorenzo et al., added
that the combination of androgen ablation and Her-2/neu targeting could be effective in androgen-dependent tumors in an attempt to prevent the development of androgen-independent, hormone-refractory disease. However, they recommended further studies with increased statistical power and increased numbers of hormone-resistant prostatic carcinoma cases for more conclusive data [30,52].

In conclusion, this study showed that Topo II α expression was significantly higher in poorly differentiated and moderately differentiated prostatic carcinoma compared to BPH. There was a significant increase in Topo II α expression with increased Gleason score. Her-2/neu expression was higher in prostatic carcinoma than in BPH, however the difference did not reach the level of statistical significance, and the increase in its expression with increased Gleason score was also statistically insignificant. Topo II α and Her-2/neu were co-expressed significantly. Hormone resistant carcinomas showed higher expression of both markers, however, the differences were statistically insignificant. The latter finding may have important therapeutic implications, however, further large scale studies are required for confirmation.

REFERENCES


