Cytochrome P450 aromatase expression in men with urinary bladder carcinoma

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ABSTRACT:

Objective: The goal of this work is to study the alterations in the Cytochrome P450 aromatase, and its role in pathogenicity of bladder cancer in biopsies of men who are initially diagnosed with this disease.

Methods: Immunohistochemistry staining (IHCS) was applied on biopsies taken from 42 men with bladder cancer, and other 38 benign considered as a control group to detect Cytochrome P450 aromatase.

Results: The results revealed a highly significant (P<0.05) level of Cytochrome P450 aromatase in patients in comparison with control group.

Conclusions: It was concluded from the results of the present study that Cytochrome P450 aromatase was good prognostic biomarker has important role in pathogenesis and progression of urinary bladder cancer in men, and these results significantly correlate with the early stages of disease.

Recommendations: It may be recommended using of this biomarker inhibitor may be considered as a target for anticancer therapy.

Key words: aromatase, bladder cancer, Iraqi patients.

INTRODUCTION:

Bladder cancer is one of the most common malignancies with total of 110,500 new cases diagnosed per year in western Europe, and incidence being four times higher in men than in women [1]. 90% of bladder cancer are urothelial carcinoma previously named transitional cell carcinoma, and the majority are papillary low-grade, non muscle invasive that recur in up to 80% of cases. In contrast, 10-20% of tumors are muscle invasive at diagnosis, and 50% of patients die from metastatic disease [2,3]. The most common symptom of bladder cancer is intermittent hematuria (80-85% of patients). Other symptoms include increased frequency, urgency and dysuria (15-20% of patients) [4]. Bladder cancer is staged on the degree of tumor invasion into the bladder wall, stages Ta and T1 are grouped as superficial bladder cancers, while stages T2, T3, and T4 as invasive tumors. The grading of bladder cancer consist of well-differentiated (Grade 1), moderately differentiated (Grade 2), and poorly differentiated (Grade 3) tumors, grading is important for establishing prognosis, as Grade 3 tumors are the most aggressive and the most likely to become invasive [5].

Although cystoscopy remains a fundamental investigative tool in the detection and surveillance of bladder cancer [6]. Considerable research effort continues to be
Directed towards the identification of markers that predict the aggressive potential of superficial bladder tumors. Such could lead to more effective surveillance protocols and permit more aggressive treatment of those patients with tumors most likely to progress to invasive or metastatic disease [7].

Estrogens produced as result of tumoral aromatization has been recently shown to play important roles in proliferation of many types of human cell carcinomas, therefore many studies have been focused on evaluation aromatase expression in tumor cells [8].

The current work designed to detect aromatase content in the bladder cancer lesions, and established the possible role in pathogenicity of bladder cancer, also in trying to manage the result in future diagnosis and prevention or treatment of this disease.

AIMS OF STUDY:
1-Detection of aromatase content in the bladder cancer lesions, and established the possible roles of this marker in pathogenicity of bladder cancer.
2-Also in trying to manage the results of this study in future diagnosis for bladder carcinoma and prevention or treatment this disease.

PATIENTS AND METHODS:
The current study included 42 men with urinary bladder carcinoma and 38 healthy men adjusted as control, excluded those with cardiovascular disease, renal failure, liver disease, diabetes mellitus, arthritis. The patients were classified into four stages and three grades, the stages are Ta, T1, T2, T3, and the grades are I, II, III [WHO, 2010] also into four age subgroups (40-49Y), (50-59Y), (60-69Y), (70-80Y), and two subgroups smokers and non-smokers, and other two subgroups according to size of tumor whether less or more than 3cm.

Aromatase assessment:
The Novolink Polymer Detection Systems (Envision technique) method based on a polymer backbone to which multiple antibodies and enzyme molecules are conjugated. This allows the entire immunohistochemical staining procedure, from primary antibody to enzyme, to be accomplished in a single step. The Novolink Polymer Detection Systems using commercial kit from Novocastra, Newcastle, UK, RE7150-K.

Results:
The results of immunohistochemical staining showed the significant elevation of cytochrome P450 aromatase expression in the urinary bladder malignant lesions in comparison with those of benign. The percent of scoring of the enzyme expression elevated with the progression of the stage and the grade of tumor, and the more significant expression (p< 0.05) was in T3 (75%) in comparison with Ta (47%), T1 (57%), T2 (67%), also in G3 (70%) in comparison with G1 (52%), G2 (40%). The results indicated a significant elevation of aromatase expression in all ages of patients and group of (70-79Y) showed strong aromatase expression and the higher percentage (82%) (p<0.05) in comparison with other groups (40-49Y) (50%), (50-59Y) (57%), (60-69Y) (61%). Result of smokers patients have showed...
more significant expression (63%) (p< 0.05) in comparison with non-smokers patients (10%). Depending on the size of tumor the results showed a significant expression of aromatase (45%)(p< 0.05) in group of tumor size > 3cm , in comparison with others of tumor size ≤ 3cm (24%) (Table 1, Fig.2,3,4).

Table (1) Associations between immunohistochemical expression of cytoplasmic aromatase and clinicopathological parameters in urinary bladder cancer patients.

<table>
<thead>
<tr>
<th></th>
<th>All cases</th>
<th>Number 42</th>
<th>Neg/weak %</th>
<th>Moderate %</th>
<th>Strong %</th>
<th>P value</th>
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<td>P Ta</td>
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<td>P (50-59 Y)</td>
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<td>63</td>
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<td>P Tumor size ≤ 3cm</td>
<td>33</td>
<td>54</td>
<td>18</td>
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<td>P Tumor size &gt; 3cm</td>
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<td>33</td>
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<td>45</td>
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( Figure 1)(5x)cross histological section of normal human placenta stained with Aromatase monoclonal antibody used as control, showing normal aromatase content.
Figure (2) cross histological section of malignant urinary bladder cells : (250x) brown color refers to moderate epithelial aromatase expression in patient GI,Ta.

Figure (3) cross histological section of malignant urinary bladder cells : (250x) brown color refers to strong epithelial aromatase expression in patient GIII,T3.
Figure (4) cross histological section of malignant urinary bladder cells : (a)10x stromal aromatase expression in patient GII,T2. : (b)40x negative aromatase staining in normal cells.

Discussion:

The current study revealed highly expression percentages of aromatase in all stages and grades of urinary bladder cancer cells (Table 1). The aromatase enzyme catalyzes the final step of steroids conversion to estrogen and it is localized in the endoplasmic reticulum of estrogen producing cells [9]. It has been reported that targeted disruption of gene CYP19 (a single gene that encoded aromatase in mice and human) or inhibition of its product effectively eliminates estrogen biosynthesis [10]. Many reports have been described the elevated expression of cellular localization aromatase transcript and protein in endometriotic tissue [11-16]. Several studies have explained that both protein and mRNA level of aromatase was more significant in cancer than transformed tissues [17,18]. Other studies proved overexpression
of aromatase in many types of cancer lead to using of aromatase inhibitors such as anastrozol, letrozole which are a non steroidal, competitive inhibitors for endogenous aromatase enzyme as therapeutic in breast cancer, endometrial cancer, and uterine fibroids [19,20-24]. The study of Weinberg and Colleagues [25] showed proliferation of aromatase-dependent cell of lung carcinoma in mice xenograft model. Similar localization of aromatase has also been reported in many types of cancer such as colon, gastric, oral squamous cell carcinoma, and prostate cancer [26-28]. Epidemiological studies have indicated a higher incidence of testicular cancers after estrogen exposure and higher expression of aromatase from Sertoli cells tumors [29-30]. These studies indicated highly increased of estrogen level in older men compared with younger men and has been hypothesized to be causative factor in many types of cancer [31].

The present study revealed aromatase increased with larger tumor size leading to conclude its role in progression and recurrence of urinary bladder cancer and may this agree with previous studies which indicated increased growth of the cancer cells of prostate gland induced by the targeted disruption of the aromatase gene in mice and hyperplasia of the entire organ [32].

Aromatase protein expression has been localized in the epithelial and stromal cells of the urinary bladder lesions, and the epithelial cells may be the major source of aromatase expression (Figure 2, 3, 4.a) as normally found in other estrogen producing cells such as placenta tissue (Figure 1) and may have the ability to increase the progression of cancer. This result may agree with study of Lin [33] which indicated the overexpression of aromatase leads to increased proliferation in the urothelial layer.

CONCLUSIONS:

The present study may the first report of aromatase expression in human urinary bladder cancer in men after malignant transformation, indicating that estrogens in situ after aromatization could act as autocrine factors promoting neoplastic growth, and the aromatase absence in normal urinary bladder cells (Figure 4.b) may support this conclusion and may lead to use aromatase inhibitors in the medical management to treat or prevent progression of urinary bladder cancer and invasion in men.

RECOMMENDATIONS:

1-Advanced study focusing on genetic DNA analysis of biomarker genes of bladder carcinoma patients to study the relationship between these biomarkers proteins and the gene amplification.

2-It may be recommended that using this biomarker to predict and diagnosis the bladder cancer.

3-It may be recommended using of this biomarker inhibitor, may be considered as a target for anticancer therapy.

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the diagnosis of the urinary enzyme in Iraqi patients.

Summary:
Objective: The objective of this study was to detect the enzyme in the bladder of male patients with bladder outlet obstruction.

Methodology: The study was conducted from September to August 2012 on a sample of 82 patients with bladder outlet obstruction and 63 healthy individuals. The study was performed using a group of 25 patients with bladder outlet obstruction.

Results: The results showed a significant increase in the enzyme level in patients with bladder outlet obstruction compared to healthy individuals.

Conclusions: The results of this study showed that the enzyme was increased in patients with bladder outlet obstruction, which is consistent with previous studies.

Recommendations: Further studies are recommended to determine the mechanism of this increase in the enzyme level.

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