ORIGINAL ARTICLE

Estrogen Receptor-beta Expression in Different Histopathological Grades of Urothelial Tumours

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ABSTRACT

Aims: To evaluate and compare estrogen receptor-beta expression status between different histopathological grades and invasive and non-invasive urothelial carcinoma.
Methods: A total of 50 cases of primary urothelial carcinoma of different grades and stages, from different ages of both sexes, were taken by convenience sampling in this cross-sectional study. Routine immunohistochemical staining protocols were used to detect ER-β expression in formalin-fixed and paraffin-embedded tissue from trans-urethral resection of urothelial carcinoma. The percentage of cells with positive nuclear stain for ER-β was assessed semi-quantitatively by using immuno-reactive score with IRS > 2 considered as ER-β positive.
Results: The ages of all the patients ranged from 42 to 95 years. These included 37 males and 13 females. According to the interpretation of IRS for ER-β nuclear immunostaining, 27(54%) patients were classified as ER-β positive. Significantly higher proportion of high grade (*p= 0.0006)* and invasive (*p = 0.0001)* cancers showed ER-β expression.
Conclusions: Stronger and higher ER-β positive expression in significantly greater proportion of high grade and invasive carcinomas as compared to low grade and non-invasive tumours.
Key words: Estrogen receptor-beta, urothelial carcinoma, immuno-reactive score

INTRODUCTION

Human urinary bladder cancer is the most prevalent and the eleventh most common malignancy occurring world-wide[1]. Urothelial carcinoma is the predominant and most frequent histological sub-type representing 90% of all the bladder cancers[2]. Highest incidence rates are documented in the countries of Europe, North-America and Northern Africa[3]. In one of the studies conducted in Pakistan, bladder cancer ranked tenth among the cancer cases registered from 1994 to 2004.[4]Urothelial variant of bladder cancer accounts for 86-93% of all the urinary bladder carcinomas, in Pakistan[5].

An elevated incidence of urinary bladder carcinoma has been observed to be influenced by hormonal factors both in females and males[6]. Hormone-dependant susceptibility has been studied in reference with androgen, estrogen and progesterone hormones and is influenced by the status and biology of sex hormone-receptors[7]. Estrogens are steroid hormones influencing growth, differentiation and functioning of many target tissues in humans. Estrogen mediates its physiological effects and biological responses to tumors, both in females and males, through binding to thehormone-receptors[8]. Both interaction and activation aredependant upon the receptor occupancy by the ligand.In the understanding of estrogen signaling, from molecular, biochemical, biological and structural perspectives, substantial advances have been made during the past decade[9]. In 1996, Jan-AkeGustafsson and his research group announced the electrifying discovery of another form of estrogen receptor. After the cloning of this new estrogen receptor, estrogen receptor-beta (ER-β), the former estrogen receptor was designated as estrogen receptor-alpha (ER-α)[10]. ER-β plays an important regulatory role in many human tissues. The expression of ER-β has been observed in normal tissues like breast, testes, gastro-intestinal tract, skeletal muscles, nervous and immune systems,thyroid, ovary, spleen, lungs and urogenital tract[8]. These findings indicate a significant role played by estrogen receptor-beta in elucidating the physiology and also malignant transformation mediated by estrogens[10].

Human urinary bladder transcribes and translates only ER-β sub-type in the lining transitional epithelium, suggesting that urothelial cells respond to estrogen mainly through ER-β[11].

Significant expression of estrogen receptor-beta has been reported in a variety of malignancies including transitional cell carcinoma of bladder,[12] adenocarcinomas of prostate[13] and colon, tumors of esophagus[14], endometrial carcinomas[15], medullary carcinoma of thyroid[16], as well as, rhabdomyo-

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sarcoma and lung cancers. ER-β has been detected in human breast cancers and may also contribute to hormonal sensitivity and resistance. ER-β act both as predictive and prognostic marker for breast carcinoma and anti-estrogen like tamoxifenis an efficient adjuvant therapy for breast[17]. ER-β is a predominant sub-type observed in the cystic neoplasm of pancreas[18] and in certain salivary gland carcinomas[19] and is a paradigm to show the role of ER-β in promoting the development and aggressiveness of these tumors. Only ER-β expression is observed in testicular germ cell tumors especially in endodermal sinus tumors and in teratomas with significantly higher expression in advanced grades. ER-β expression is down-regulated in many tumors like seminomas and embryonal cell carcinoma of testes[13], astrocytic brain tumors and gastric adenocarcinomas[20]. It is also an important regulator of proliferation in ovarian cancers and is variably expressed in different types of ovarian carcinomas[21]. ER-β is a potent regulator of proliferation and invasion in a wide array of tissues[10].

Several lines of evidence have linked estrogen to bladder carcinogenesis. Estrogen acts as a mitogenic agent on hormone-sensitive tissues and has proliferative effect on transitional epithelial cells of urinary bladder[12]. ER-β expression is found to be related with the initiation and progression of bladder urothelial carcinogenesis[22]. Urothelial carcinoma predominantly expresses ER-β[12] and shows biologically significant and divergent immuno-reactivity for ER-β[23]. Estrogen receptor-alpha is rarely found in malignant urothelialtumor of human urinary bladder[12]. Additional efforts are needed to determine the ER-β mediated regulation of neoplastic cell growth and survival and utility of hormonal or anti-hormonal therapy as an adjuvant tool in malignancies previously unconsidered for ER-β expression[17].

The clinical significance of bladder cancers depends on their histological grade and differentiation and, essentially on the depth of invasion of the lesion.[11]Currently the tools used for therapeutic evaluation of urothelial carcinoma are stage and grade of the tumor. However, the biological behaviour of urothelial carcinoma cannot be adequately predicted by histopathological criteria alone and improved methods of detection and treatment have had a significant influence on disease outcome[24].

Estrogen receptor-beta has been more oftenly observed in higher grades of urothelial carcinom.[12]Positive correlation of estrogen receptor status with aggressive features of urothelial carcinoma has been described[24]. The ER-β expression is significantly higher in the urothelial carcinomas with higher grade and deep-muscle invasion as compared to the tumors with lower grade and without invasion of muscularis-propria.[11] Superficial and non-invasive transitional cell carcinomas of the bladder show less degree of ER expression[25]. The ER-β expression is more wide-spread and is significantly elevated in high grade urothelialcarcinomas[12]. The duration of survival without progressing to advanced disease is more in the urothelial carcinomas with lower expression of ER-β as compared to the tumors with higher ER-β expression[26].

MATERIALS AND METHODS

This cross-sectional study was planned and conducted at Lahore General Hospital and Post-Graduate Medical Institute, Lahore. A total of 50 samples were collected by convenience sampling. Male and female patients of all age groups with both invasive and non-invasive urothelial carcinoma of all grades were included. Patients on hormone-therapy, chemo-therapy or radio-therapy and with recurrent and malignant tumors other than primary urothelial carcinoma were excluded. The history and relevant data was taken and recorded in a proforma, after obtaining an informed concent. The specimens were collected from trans-urethrally resected samples of urinary bladder tumor (TUR-BT) and were preserved in 10% formalin solution. After routine automatic processing of the specimen, the prepared sections were stained with hematoxylin and eosin stain and estrogen receptor-beta immunostain. The tumor grading and staging of the hematoxylin and eosin stained sections was based on World Health Organisation(W.H.O.) histological classification of tumors of urinary tract. The sections were also immunostained for estrogen receptor-beta using ER-β polyclonal primary antibody against human estrogen receptor-beta proteins (Innovex biosciences US) per the manufacturer's instructions. The staining results were evaluated semi-quantatively by calculating an immuno-reactive score (IRS). The data was statistically analysed by using Fisher's exact test and the results were considered statistically significant at a $p \leq 0.05$.

RESULTS

During the study period, 50 samples of trans-urethrally resected specimens of urinary bladder tumors, diagnosed as urothelial carcinoma on histological sections, were obtained. The pathological grading revealed 29 (58%) patients of high grade urothelial carcinoma and 21 patients (42%) were graded as low grade urothelial cancer. According to the criteria of invasion of muscularispropria of urinary bladder by urothelial carcinoma, frequency of non-

invasive tumours was observed in 31 cases (62%) as compared to 19 cases (38%). There was no significant correlation between different grades and invasive and non-invasive urothelial carcinoma. Out of 21(42%) low grade carcinomas, 15(71.43%) were non-invasive whereas, 16 out of 29(55.2%) high grade tumourswere non-invasive (Table 1).

The distribution of the calculated IRS is shown in figure 1. According to the interpretation of IRS for ER-β nuclear immunostaining, 27 patients (54%) were classified as ER-β positive and 23 cases (46%) were observed to be ER-β negative. The data of ER-β detection in urothelial carcinoma, by using the criteria of immuno-reactive score (IRS) for nuclear staining, are shown in figures (2-5).

The distribution of ER-β staining intensity in low and high grades of urothelial carcinoma showed a majority of rank 1 tumours i.e., 12 out of 15(80%) were low grade tumours (Fig. 2). Similarly, 16 out of 18(88.9%) rank 2 tumours and 9 out of 11(81.1%) rank 3 tumours were high grade carcinomas (Fig. 2).

In figure 3, the grading of ER-β staining done according to different grades of transitional cell carcinoma displayed largest number of grade 1 tumours i.e., 13 out of 16(81.2%) were low grade cancers. Whereas, 9 out of 10(90%) grade 2 and 11 out of 1(84.6%) grade 3 tumours were high grade carcinomas having more than 10% cells with ER-β nuclear positivity (Figure 3). The grade 4 of staining was seen only in high grade carcinomas and no reactivity of this highest staining grade was observed in the low grade tumours (Fig. 3).

The classification of ER-β staining intensity in invasive and non-invasive urothelial carcinomas showed 9 out of 11(81.1%) tumours with rank 3 intensity were invasive carcinomas while, 12 out of 15 neoplasms belonging to the rank 1, (80%) were non-invasive cancers (Fig. 4).

The percentage of ER-β stained nuclei graded according to invasive and non-invasive urothelial cancers exhibited most of the carcinomas 13 out of 16 cases (81.2%) of staining grade 1 were non-invasive. None of the non-invasive tumours had more than 80% cells with positive ER-β nuclear staining, i.e., grade 4 staining pattern (Fig. 5).

The comparison of ER-β expression status in low and high grade tumour was highly significant (p=0.0006). ER-β staining was positive in 5(23.81%) low grade carcinomas while 22(75.86%) high grade cancers revealed ER-β positivity (Table 2). Similarly, a comparison between ER-β expression in invasive and non-invasive urothelial carcinoma revealed 17(89.47%) invasive tumours positive for ER-β, whereas, 21(67.74%) non-invasive cancers were ER-β negative (Table 6). The difference was statistically significant (p=0.0001).

Fig 1: Frequency of Immuno-reactive scores (IRS) for ER-β nuclear staining in urothelial carcinoma.

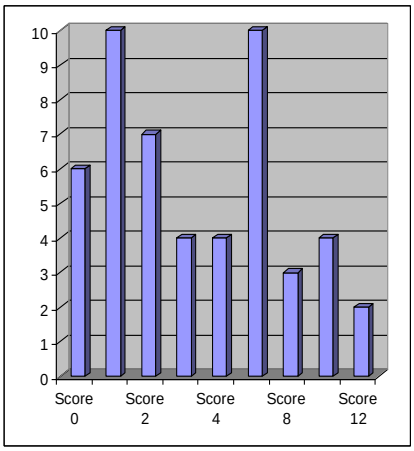

Fig. 2: Distribution of ER-β staining intensity in different grades of urothelial carcinoma.

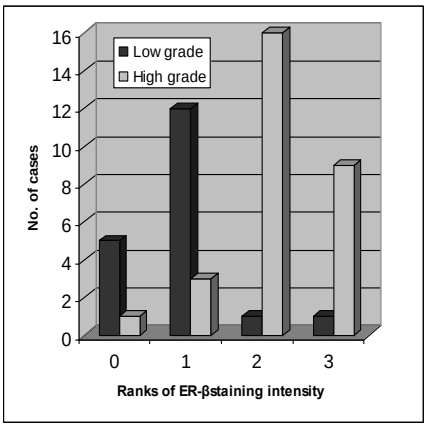

Fig. 3 :Distribution of ER-β staining grades in low and high grades of urothelial carcinoma.

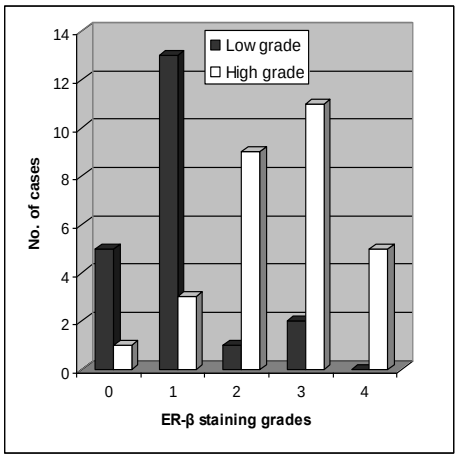

Fig. 4: ER-β staining intensity in invasive and non- invasive urothelial carcinoma

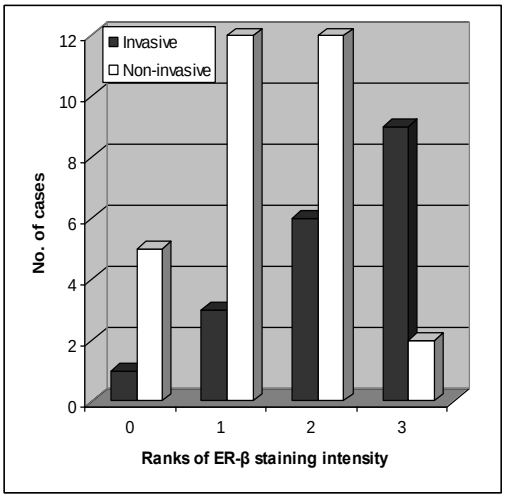

Fig. 5: Distribution of ER-β staining grades in invasive and non- invasive urothelial carcinoma.

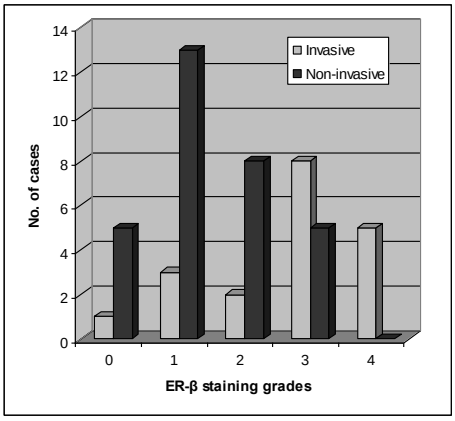

Table 1: Correlation between different histological grades and invasiveness of urothelial carcinoma

Histological grades	Urothelial Carcinoma	
	Invasive	Non Invasive
Low	6(28.6%)	15(71.4%)
High	13(44.8%)	16(55.2%)

p =0.4 (Not significant)

Table 2: Comparison of ERr-β expression in different grades of urothelial carcinoma

Histological grades	ER-expression	
	Invasive	Non Invasive
Low	05	16
High	22	07

p = 0.0006

Table 3: Expression of ER-β in invasive and non-invasive urothelial carcinoma

Urolithial carcinoma	ER-β Expression	
	Positive	Negative
Invasive	17	02
Non-Invasive	10	21

p=0.0001

DISCUSSION

Urothelial carcinoma is a major cause of mobidity and mortality throughout the world. It is one of the most common malignancies in Pakistan. Of all the newly diagnosed urothelial carcinomas, about 70% are low grade tumours and approximately 10 to 20% have tendency to progress to the aggressive malignancy. The frequency of different grades of urothelial carcinoma as observed in our study are, in contrast, with the international data. Majority of the studied cases belonged to the high grade i.e., 58% patients were diagnosed as high grade carcinoma, in this study. This discrimination may be due to delayed presentation of our patients.

Different studies reported that, at the initial diagnosis, around 50% to 70% of urothelial carcinomas are non-invasive. The current study showed similar findings with 62% non-invasive tumours and these findings same to the observations of the above mentioned studies.

In a few studies, the data regarding the association between different grades and invasiveness of urothelial carcinoma has shown that about 70% low grade tumours are non-invasive. Although our study revealed statistically non-significant results regarding the correlation between low and high grade and invasiveness of urothelial carcinoma, the 71.4% low grade cancers in this study were non-invasive. These findings are in accordance with the observations of above mentioned studies.

The ER-β expression is observed in urothelial carcinoma, with 54% positive cases in our study. These results are similar to the study reports carried out internationally.Several studies reported a positive correlation between higher grade of the transitional cell carcinoma and stronger ER-β expression status both in terms of staining intensity and percentage of cells with positive nuclear staining. The present study revealed most of the low grade tumors exhibiting staining intensity of rank 1 i.e., 80% cases of rank 1 were low grade neoplasms, whereas, majority of the high grade carcinomas belonged to the staining intensity ranks 2 and 3. This study also showed low grade tumors mostly displaying less than 10% cells with positive nuclear staining while high grade cancers belonged to the higher staining grades i.e., 2 and 3, and the staining grade 4 was found only in the high grade carcinomas. These findings are similar to the observations of the above mentioned studies. In addition many studies also indicated ER-β expression in higher number of high grade urothelial carcinomas. These observations support the findings of this study. The comparison of ER-β expression in low and high grades has been highly significantly

established in the present study revealing 75.86% high grade cancers with ER-β positivity while 69.56% ER-β negative carcinomas were of low grade phenotype.

The comparison of staining intensity and percentage of cells with ER-β nuclear expression between invasive and non-invasive urothelial cancers were studied in some studies. These studies observed invasive carcinomas with stronger staining intensity and higher staining grade as compared to the non-invasive tumors. The current study also indicated 80% staining intensity rank 1 in non-invasive tumors while more stronger and higher ranks 2 and 3 of colour intensity were seen in the invasive carcinomas. Similarly, in this study, 81.25% staining grade 1 cases were non-invasive while most of the invasive carcinomas revealed higher staining grades with none of the non-invasive tumors had the highest grade 4 staining.

ER-β positivity appears to be a biomarker related to a more aggressive clinical outcome and a correlation with advanced features of urothelial carcinoma has been established. This tudy can provide new clues for the elucidation of the estrogen dependant mechanisms of urothelial carcinoma of urinary bladder and the clinical and therapeutic benefits for the patients.

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