Immunohistochemical expression of β-Catenin in urinary bladder urothelial carcinoma

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Abstract:

Background: Bladder cancer is the ninth common cancer in the world, the third common cancer among men in the Arabic and Western Asian countries. In Egypt, UC has become the most frequent type with its incidence rising from 16% to 65.8%. Tumor grade and stage have been shown to have a strong correlation with tumor recurrence and progression to invasive bladder cancer. B-catenin functions as part of the E-cadherin/β-catenin complex and plays a role in cell-to-cell adhesion and Wnt/β-catenin pathways have been reported to regulate urothelial homeostasis and carcinogenesis. Several studies have assessed the role of β-catenin and malignant transformation, as well as tumor progression.

Objectives: Evaluation of the expression of β-catenin in urothelial bladder carcinoma and its correlation with other clinicopathological parameters of prognostic importance.

Patients and methods: 50 specimens; either transurethral resection (TUR) or cystectomy will be Formalin fixed and paraffin-embedded and tissue sections will be examined histopathologically by routine H&E stain. Immunohistochemical evaluation for expression of β-catenin.

Results: The age range of the 50 studied patients with UC of the urinary bladder was 17-80 years, the mean age was 55.74 years, and the median age was 58 years, with male: female ratio 6:1. The cases were immunostained with β-catenin antibody and revealed that β-catenin was expressed in all cases of invasive UC with variation in its expression as it was strongly expressed in 13/28 (72.2%), moderately expressed in 9/28 (57.1%), mildly expressed in 6/28 (40%) and didn't express in 0/28 (0%) of cases. And was expressed in 19/22 cases of non-invasive UC as it was strongly expressed in 5/22 (27.8%), moderately expressed in 5/22 (35.7%), mildly expressed in 9/22 (60%) of cases and didn't express in 3/22 (100%) of cases. β-catenin showed variation in its expression in high-grade UC as it was strongly expressed in 13/27(72.2%), moderately expressed in 8/27 (57.1%), mildly expressed in 6/27 (40%) and didn't express in 0/27 (0%) of cases. Expression of β-catenin in low-grade UC was; strong in 5/23 (27.8%), moderate in 6/23 (42.9%), mild in 9/23 (60%) and not expressed in 3/23 (100%) of cases.

Conclusions: Expression of β-catenin increases with increasing grades of the tumor and also it showed an increase in expression by the presence of invasion.

Keywords:- B-catenin, Urothelial carcinoma.

Introduction:

Bladder cancer is the ninth common cancer in the world, the third common cancer among men in the Arabic and Western Asian countries(***Hosein et al., 2018).*** The most common histological type of bladder cancer is urothelial carcinoma which constitutes more than 90% of all bladder cancers (***Youness et al., 2018***.) In Egypt, UC has become the most frequent type with its incidence rising from 16% to 65.8%,becoming at present the most common tumor type due to increased
exposure to etiological factors as smoking and pesticides and/or other causative agents ***(Khaled et al., 2013.***Tumour grade and stage have been shown to have a strong correlation with tumor recurrence and progression to invasive bladder **cancer** ***(Pasin et al., 2008).***

β-Catenin is a subunit of the cadherin protein complex; it also acts as an intracellular signal transducer in the Wnt signaling pathway ***(MacDonald et al., 2009***).Which recognized in many different tissues regulating angiogenesis, proliferation, invasion, and metastasis ***(Kastritis et al., 2009)***.

Thus, aberrant activation of the Wnt pathway contributes to the progression of several major human cancers, and inhibition of Wnt effects has major therapeutic potential ***(Tang et al., 2009).***

Several studies have assessed the role of β-catenin and malignant transformation, as well as tumor progression. Some of these studies showed High β-catenin expression in muscle-invasive tumors others proposed an impaired expression with biological aggressiveness, and of being a common feature of high-grade invasive carcinomas, suggesting a role in tumor progression ***(Senol et al., 2015).***

Patients and methods:

studied specimens was 50 cases divided into 24 cases low-grade UC, 26 cases high-grade UC, 29 noninvasive UC and 21 invasive UC, which were obtained from specimens referred to the Pathology Department from cases admitted to the Urology Department, South Valley University Hospitals. Clinical data were obtained from the referral clinical reports. These data included: age, sex, clinical presentations, laboratory tests and the method of obtaining the specimen. The biopsies were obtained by either transurethral resection (TUR)or radical cystectomy.

-Sections were deparafinized then they were rehydrated, then were put in citrate puffer and left to cool at room temperature for about 30 minutes then washed in phosphate buffer.
-We used diluted monoclonal β-catenin antibody in normal goat serum (NGS) and sections were incubated in a humid chamber at room temperature for overnight to block nonspecific interactions.
-Sections were washed in PBS and Biotinylated goat anti polyvalent antibody (HRP Polymar).
-Streptavidin peroxidase was applied .
-The slides were incubated with 1 micron chromogen to 25 micron DAB.
-The slides were counterstained with Mayer's hematoxylin for one minute .

Results:

This study included 50 specimens of UC of the urinary bladder. Their age range was 17-80 years, mean age was 55.74 years, and median age was 58years. Forty three; 43/50 (86%) were males with male: female ratio was 6:1. UC was graded in accordance with the WHO/ISUP grading criteria to:

Table 1.Classification of studied cases

	Invasive UC	Non-invasive UC
High grade UC	24	3
Low grade UC	4	19

β -catenin expression:

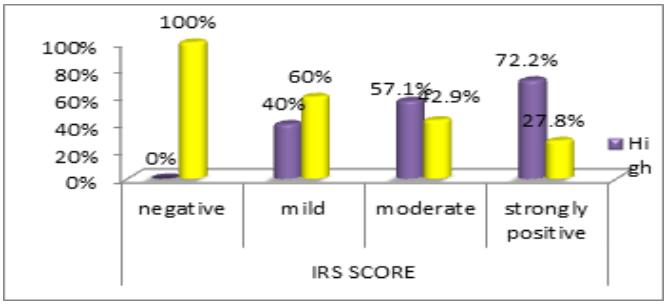

Figure 1.Grade distribution according to IRS score in the studied group.

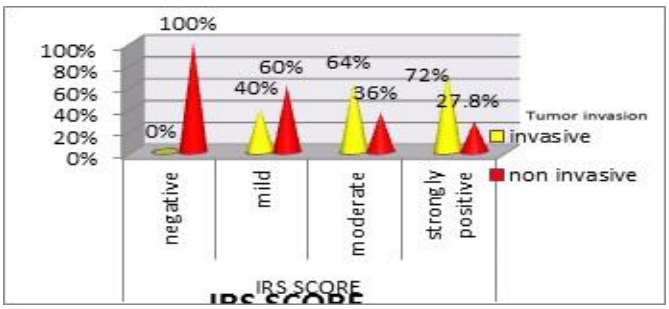

Figure 2.Tumor invasion according to IRS score in the studied group

There was significant correlation between B-catenin expression and tumour grade, P value = (0.038). There was increase in its expression with increasing grade **(Graph 1)**. There was also significant correlation between B-catenin expression and invasion, P value = (0.031). There was increase in its expression with invasion **(Graph 2)**

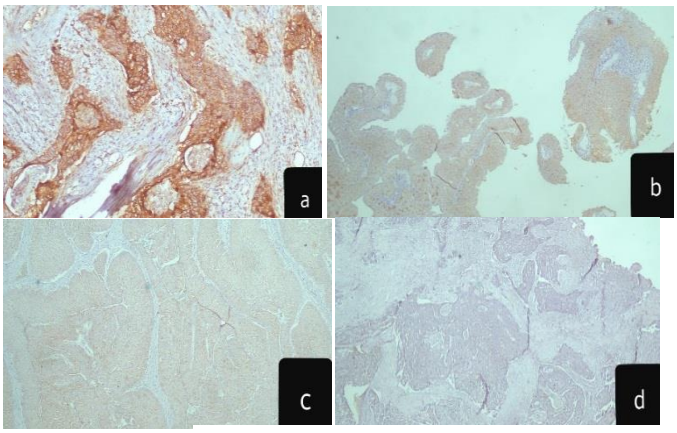

Figure 3. a-Strong B-catenin expression in invasive, high-grade UC. b- Moderate B-catenin expression in invasive low-Grade UC. c- Weak B-catenin expression in non-invasive, low-Grade UC d- negative B-catenin expression in low-Grade UC.

Discussion:

In this study we examined fifty specimens of UC; 27 cases of them were high grade and 23 cases were low grade. β-catenin showed positive expression in 27/27

(2015) who found that High β-catenin expressions and positive expression of p53 were significantly associated with high tumor grade (P = 0.007 and P = 0.032, respectively). Similar data were reported by Fatma A. and colleagues who found that statistically significant correlation between high β-catenin score and high tumor grade (P = 0.001), lymph node metastasis (P = 0.014), advanced stage (P = 0.001), vascular invasion (P = 0.02), perinural invasion (P = 0.001), high mitotic index (P = 0.001), and high MVD (P = 0.003). ***Fatma et al., (2014)*** and ***Niharika et al., (2019)*** found that pS9GSK-3β and β–Catenin proteins were observed to be aberrantly co-expressed in 67.9% (19/28) muscle invasive tumors (P = 0.01), 78.6% (22/28) high grade tumors, 60.7% (17/28) recurrent tumors (P = 0.02).

The fifty studied cases included 28 cases which were diagnosed as invasive UC and 22 cases which were diagnosed as non-invasive UC, B-catenin was expressed in 28/28 (100%) of invasive UC and it was expressed in all cases of non-invasive UC 19/22 with a statistically significant relation (p value= 0.031).

Our results are similar to other studies, which tried to elucidate the role of B-catenin in urothelial carcinogenesis. ***Xiaojing et al., (2018)*** showed in a study on highly-invasive and noninvasive UBC that Wnt7a overexpression was strongly associated with staining of β-catenin in a cohort of UBC patients (P = 0.0463). In addition, we stained the same section with Wnt7a and β-catenin by immunofluorescence. We confirmed that there was less Wnt7a staining and strong membrane β-catenin staining in the urothelium at

the earlier invasive stage, whereas there were stronger cytosolic Wnt7a staining and more staining of β-catenin in the invasive UBC section. Overall, these data supported the notion that Wnt7a overexpression is associated with the activation of the Wnt/β-catenin pathway in UBC samples. ***Serkan et al., (2015)*** found that High β-catenin expression and positive expression of survivin and p53 were significantly associated with a high T stage (≥ T2; P = 0.006, P = 0.003, and P = 0.002, respectively).

Our results are different from other studies which have investigated the role of β-catenin in urothelial neoplasms, and proposed that reduced membranous expression with poorer prognosis.

A study performed on 24 radical cystectomy specimens of high-grade urothelial carcinoma by ***Yi et al., (2013)*** showed that 15 (67.5%), eight (33.3%), and nine (37.5%) cancer cases showed reduced membranous expression , ectopic cytoplasmic expression, and ectopic nuclear expression and Coexisting benign urothelium exhibited β-catenin reactivity mainly with a membranous staining pattern. Deep invasion (≥pT2) was strongly associated with reduced membranous β-catenin compared with non-muscle-invasive tumors (P=0.0474).

Conclusion:

Expression of B-catenin increases with increasing grades of the tumor and also it showed an increase in expression by the presence of invasion.

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