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RESEARCH ARTICLE

Targeting Potential Tumor Biomarker for Therapeutic Target of Urothelial Carcinoma: B-Catenin, the Promising One Potential Biomarker for Urothelial Carcinoma

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ABSTRACT

Urothelial carcinoma is the malignant invasion of epithelial neoplasms into the urothelial layer. It is a substantial health issue, with bladder carcinoma ranking as the tenth most common type of cancer globally. β-catenin promotes malignant cell proliferation and invasion through many routes. Numerous cancers, such as urothelial carcinoma, disrupt this pathway. This study to examine the correlation between β-catenin expression among different T-stage states of urothelial cancer using the immunohistochemical method. A cross-sectional technique was used in an analytical observational design. The sample consisted of 47 paraffin blocks obtained from radical cystectomies conducted from January 2014 to December 2023. We utilised the immunohistochemical technique to analyse β-catenin expression and quantified it using the H-score. We conducted the T-staging evaluation strictly following the guidelines provided by the WHO and AJCC. The statistical analysis tests are conducted at a significant value with $p<0.05$. Results: The mean age of the patients was 55.6 years, predominantly male (93.6%). Most samples were T2 stage (42.6%) and high-grade tumors (89.4%). β-catenin expression significantly differed between stages ($p = 0$. Conclusions: There is a significant correlation between β-catenin expression and tumor stage, suggesting its potential as a useful prognostic indicator for urothelial cell carcinoma. Additional studies will reveal the processes and therapeutic effects of β-catenin in treating urothelial cancer.

INTRODUCTION

The type of urinary tract cancer most often found is Urothelial Carcinoma (UC). The histology grade at diagnosis has a significant role in determining the prognosis of this disease (Amin, 2022; Heryanto et al., 2017; Izzah & Susilo, 2023). Urothelial carcinoma is characterized by the invasion of epithelial malignant neoplasms in the urothelial layer of the urinary tract (Amin, 2022). This carcinoma represents a significant health problem, with bladder carcinoma being the 10th most common cancer worldwide. Based on the Global Cancer Incidence, Mortality, and Prevalence Data (GLOBOCAN), there were 573,278 cases of bladder carcinoma identified in 2020, accounting for approximately 3% of all cancer types. Approximately 212,536 deaths worldwide in 2020 were attributed to bladder cancer, and it ranks as the 13th most frequent cause of mortality due to cancer. Men have a fourfold higher

occurrence and mortality rate of bladder cancer than women, with rates of incidence of 9.5 over 100,000 men and 2.4 over 100,000 women around the world (Amin, 2022; Halaseh et al., 2022). The bladder cancer incidence rate in Indonesia is 7.33 per 100,000 individuals, with 7,828 newly diagnosed cases and a fatality rate of 0.18 per 1,000 persons (Sung et al., 2021).

The incidence of urothelial carcinoma is rising, primarily due to smoking. Risk factors include genetics, diet, radiation, drug use, infection, and occupational exposure (Amin, 2022). Over 90% of bladder cancers are urothelial carcinoma based on histopathological appearance (Halaseh et al., 2022; Krismaningrum et al., 2022). The T stage is a crucial prognostic indicator for urothelial carcinoma, as the stage of tumor invasion determines it. Stage T1 cancer cells invade the subepithelial connective tissue; stage T2 cancer cells invade the muscularis propria; stage T3 cancer cells invade the perivesical tissue; and stage T4 cancer cells invade the prostate, uterus, seminal vesicles, vagina, pelvic or abdominal wall (Amin, 2022). Current therapies for urothelial carcinoma, such as surgery, radiation, and chemotherapy, have demonstrated encouraging clinical results for individuals in the first phases of urothelial cancer. Five-year survival rates for advanced-stage urothelial carcinoma remain poor, ranging from 5% to 35% (Halaseh et al., 2022).

The cadherin protein complex subunit β-catenin is linked to enhanced tumor cell invasion, differentiation, and proliferation (Liu et al., 2022; Pai et al., 2017). The correlation between β-catenin and T-stage diagnosis in urothelial carcinoma is still not completely recognized. This study used immunohistochemistry techniques to determine if a correlation was found within the T stage of urothelial cancer and the the quantity of β-catenin found. It is considered that the study's results will contribute to promoting the development of targeted therapies that will decrease the mortality rate among people with urothelial cancer.

MATERIALS AND METHODS

Research designs

This research used an analytical observational design within a cross-sectional study. The procedure took place at the Anatomical Pathology Laboratory, Dr. Soetomo General Hospital in Surabaya. This study protocol received ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Hospital in Surabaya (No 0856/KEPK/XII/2023).

Samples

Samples were paraffin blocks that met the following criteria: (1) Urothelial carcinoma tumor tissues obtained during radical cystectomies (RC); (2) The T stage of the sample can be determined based on histopathological examination; (3) The paraffin block still has tumor cells representative enough for immunohistochemical examination purposes. Paraffin blocks that were missing, broken, or could not be cut were excluded. Frozen section specimens were excluded. Samples were obtained from RC procedures conducted at Dr. Soetomo General Hospital between January 2014 and December 2023. A purposive sampling method was employed to obtain the samples. To calculate the sample size, the Lemeshow formula was employed. The total samples obtained were 47 samples, consisting of T1 (6 cases), T2 (20 cases), T3 (8 cases), and T4 (13 cases).

T-Staging of Tumor

Staging of the tumor has been established using the World Health Organization (WHO) Classification of Tumors of the Urothelial Tract 2022 and the American Joint Committee on Cancer (AJCC) classifications, which evaluates the extent of tumor invasion into the surrounding tissues. The T-stages were defined as follows: T1 tumors invade the subepithelial connective tissue, T2 tumors invade muscularis propria, T3 tumors invade perivesical tissue, and T4 to prostate, uterus, vagina, pelvic or abdominal wall (Amin, 2022). Two anatomical pathologists determined the staging using

an Olympus CX31 binocular light microscope (Olympus Optical Co. Ltd, Japan) at 20X, 40X, 100X and 400X magnifications.

β-catenin expression in urothelial carcinoma

β-catenin expression was assessed by immunohistochemical examination with an antibody monoclonal that selectively targets β-catenin (E-5): sc-7963 (diluted 1:200, Santa Cruz Biotechnology, USA). The manufacturer's specified procedure was used to perform the work. β-katenin was positively expressed in the cytoplasm and membrane (Ciurea et al., 2013). β-catenin expression was quantified using the histochemical score (H-score). The H-score system was calculated based on the proportion and intensity of stained cells (H-Score = (% of cells with weak stainingx1) + (% of cells with moderate stainingx2) + (% of cells with strong stainingx3)). A classification of the H-score results was made into four tiers as follows: (1) 0: Negative (0-50); (2) 1: Weak (51-100); (3) 2: moderate (101-200); (4) 3: strong (201-300) (Jeon et al., 2021; Ren et al., 2020; Ruengwanichayakun, 2021). Two anatomic pathologists used an Olympus CX31 binocular light microscope (Olympus Optical Co. Ltd., Japan) with 400X objective magnifications to score the samples. H-scores from these two pathologists will be averaged into final H-scores.

Data collection and Statistical Analysis

Data collection included the following: Analysis of the clinicopathological characteristics of the paraffin block samples (patient age and gender, T-stage, invasion status, and histopathological grade) and β-catenin expression. Analysis of β-catenin expression variations in different T stages (T1, T2, T3, and T4) of urothelial cancer was performed using the Kruskal-Wallis test. A statistical significance difference level of $p < 0.05$ was applied. This study used the Spearman correlation test to examine the correlation between β-catenin expression and different T-stages of urothelial carcinoma. A statistical level of significance of $p < 0.05$ was used. Statistics were analyzed using EZR software from Japan's Jichi Medical University Saitama Medical Center.

RESULTS

A total of 47 radical cystectomy samples from bladder urothelial carcinoma patients which fit the inclusion criteria were analyzed for this study.

Clinicopathological Characteristics of Samples

The clinicopathological characteristics of the samples are shown in Table 1. Patient mean age was 55.6 years, with the youngest aged 33 and the oldest aged 70. Almost all patients were male (93.6%). Most samples were at the T2 stage, 42.6%, followed by the T4 stage, 27.6%. Over 87.2% of the samples had muscle invasion. Most of the samples were high-grade tumours (89.4%).

Table 1 Clinopathological characteristics of samples

Characteristics	n	%
Age (years)		
31-40	4	8.51
41-50	9	19.1
51-60	20	42.5
61-70	14	29.8
Gender		
Male	44	93.6
Female	3	6.4
Tumor stage		

T1	6	12.8
T2	20	42.6
T3	8	17.0
T4	13	27.6
Muscle invasion		
Yes	41	87.2
No	6	12.8
Tumor Grade		
Low	5	10.6
High	42	89.4

Correlation Between β-catenin Expression and Tumor Stage

β-Catenin expression measured by histochemical (H-score) is shown in Figure 1.

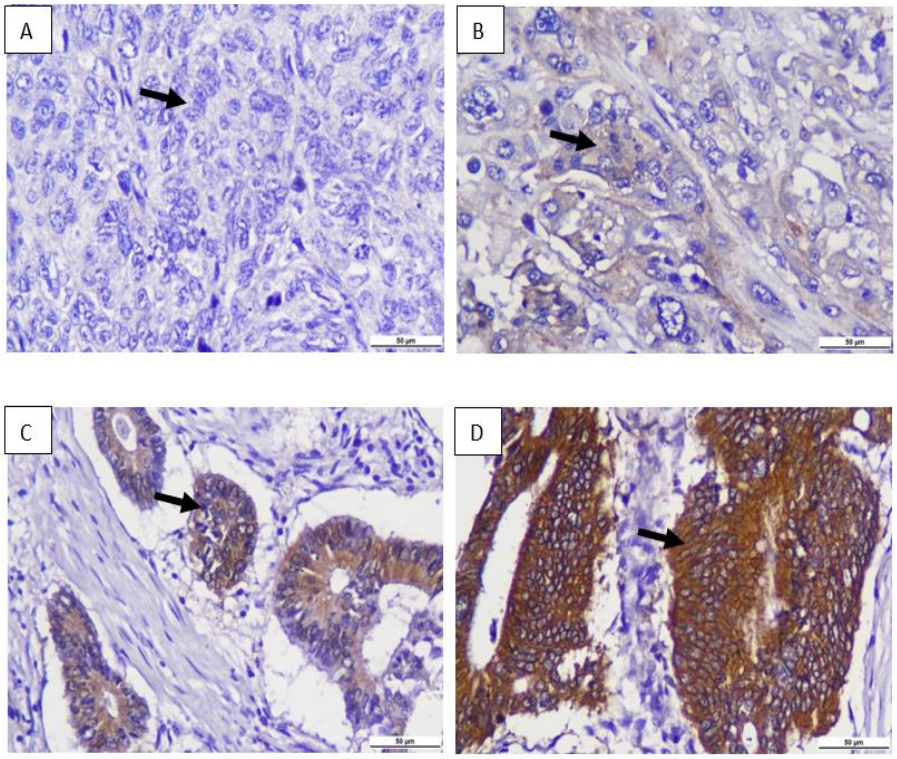
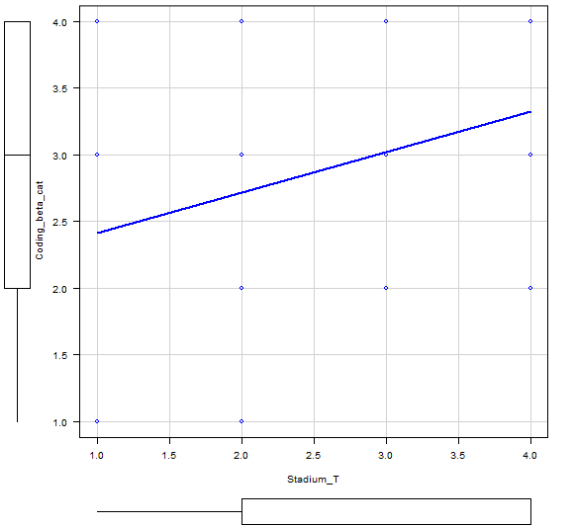


Fig. 1. Expression of β-catenin in urothelial carcinoma tumour cell's cytoplasm and membrane (black arrow). (A) Negative staining x400. (B) Weakly positive staining x400. (C) Moderately positive staining x400. (D) Strongly positive staining x400.

Statistical analysis using Spearman's test showed a moderate correlation (p=0.036) of expression β-catenin with different T-stages. The findings are shown in Figure 2.




```
        Spearman's rank correlation rho

data:  Dataset$Coding_beta_cat and Dataset$Stadium_T
S = 12001, p-value = 0.03637
alternative hypothesis: true rho is not equal to 0
sample estimates:
      rho
0.3061293
```

```
> cat(gettextRcmdr( "Spearman's rank correlation coefficient"), signif(res$estimate, digits=3), gettextRcmdr(
+   "p.value"), " = ", signif(res$p.value, digits=3), "
+ ")
```

```
Spearman's rank correlation coefficient 0.306 p.value  =  0.0364
```

Fig. 2 Statistical analysis of the correlation between β-catenin expression and different T-stages of urothelial carcinoma using Spearman's test.

This study employed the Kruskal-Wallis test to examine the fluctuations in β-catenin expression throughout different T stages (T1, T2, T3, and T4) of urothelial carcinoma. Statistical analysis significantly differed between β-catenin expression and various T stages (p= 0.0492). The results are shown in Table 2.

Table 2 β-catenin expression across different T-stages

β-Catenin Expression (H-score)	**Stage**								**Total (n)**	***p* value**
	T1		T2		T3		T4			
	n	%	n	%	n	%	n	%		
0	1	16.7	6	30	0	0	0	0	7	
1	0	0	5	25	2	25	3	23.1	10	
2	3	50	6	30	2	25	4	30.8	15	0.0492
3	2	33.3	3	15	4	50	6	46.1	15	
Total (n)	6	100	20	100	8	100	13	100	47	

0: negative; 1: weak; 2: moderate; 3: strong

DISCUSSION

Clinicopathological characteristics of patients

This study's patients had a median age of 55.63 years, with the majority (42.55%) aged 51–60 years and the fewest (8.51%) aged 31–40. These findings obtained the same results as the reported results by Saginala *et al*, Ng *et al* and, Mustafa A, Safriadi F, revealed that 90% of newly diagnosed bladder cancer cases in the US are discovered in people aged 55 years and above (Ng, 2022; Saginala et al., 2020). However, WHO findings show that most diagnoses occur after the seventh decade (Amin, 2022). However, the link between older age groups and long-term exposure to risk factors is consistent in this literature, which highlights smoking and hazardous chemicals as significant contributors to carcinogenic mutations in bladder cells (Krismaningrum et al., 2022; Ng, 2022; Saginala et al., 2020).

Age also impacts treatment outcomes, with older patients often facing comorbidities, affecting treatment tolerance and survival rates. The five-year overall rate of survival for patients with bladder carcinoma in the United States is 77%. Survival varies significantly by tumor stage, with metastatic cases having less than a 5% five-year survival rate (Saginala et al., 2020).

This study also found that male patients outnumbered females greatly. WHO attributed this to gender differences in tobacco use and higher occupational exposure to carcinogens (Amin, 2022; Halaseh et al., 2022; Ng, 2022). This study's finding of a higher male-to-female ratio reflects these global trends. Smoking generates reactive oxygen species (ROS) that damage DNA and promote cancer progression via TGF-β activation, which increases the risk and aggressivity of bladder cancer (Ng, 2022; Wu et al., 2019).

This study found that the predominant pathological stage was T2. Given the cancer's invasive nature at this stage, the higher number of T2 cases can be attributed to radical cystectomy being the primary treatment for T2 urothelial carcinoma. For cases of T1 urothelial carcinoma, the recommended treatment is Transurethral Resection of Bladder Tumor (TURBT), followed by intravesical therapy or strict surveillance. Thus, we rarely get samples of cystectomy T1. Radical cystectomy is advised in cases of more aggressive disease or when initial therapy fails (Amin, 2022).

The study also found a predominance of high-grade urothelial carcinoma, consistent with the literature indicating that high-grade invasive urothelial carcinoma is more frequently observed than low-grade carcinoma (Amin, 2022). Apollo et al. reported that over 70% of urothelial carcinomas manifest as non-invasive, low-grade (Ta/PT1/CIS), genetically associated with FGFR3 mutations, and exhibiting a substantial likelihood of recurrence but limited advancement to high-grade invasive malignancy. The remaining 30% of tumors are categorized as high-grade, flat, and genetically linked to changes in the TP53 gene. These cancers advance from severe dysplasia or carcinoma in situ (CIS) to invasive stages (pT2-4) and are linked to treatment resistance and a poor prognosis (Apollo et al., 2019).

β-catenin Expression in Bladder Urothelial Carcinoma

This study found differences and correlations between β-catenin expression and various T stages in urothelial carcinoma. β-Catenin is an essential part of the Wnt signalling pathway and acts as an intracellular signal transmitter. It is critical to maintain tissue homeostasis and regulate the activities of angiogenesis, proliferation, invasion, and metastasis. Under normal conditions, β-catenin preserves epithelial tissue integrity and controls extracellular gene transcription. However, abnormal β-catenin expression can induce oncogenic signalling, promoting tumor initiation, progression, survival, and recurrence (Kristiani et al., 2024).

β-catenin is pivotal in the EMT (Epithelial-Mesenchymal Transition), moving from a junction component in epithelial cells to an active nuclear complex, activating genes, including oncogenes and

tumor suppressors. Reduced cadherin expression is essential for cell separation during EMT and tumour cell invasion. At the plasma membrane, E-cadherin sequesters β-catenin, thereby inhibiting β-catenin and TCF-LEF mediated transcription (Ciurea et al., 2013). Increased β-catenin expression in urothelial carcinoma is related to cancer progression and survival. Increased Wnt/ β-catenin signalling pathway activity in urothelial cancer leads to nuclear and cytoplasmic β-catenin accumulation. It interacts in transcription factor interaction with TCF/LEF to activate genes which promote tumor invasion and metastasis (Garg & Maurya, 2019). Huang *et al*. found a strong correlation with β-catenin and overexpression of Wnt7a in patients with urothelial carcinoma (Huang et al., 2018). Senol *et al*. reported significant associations between high β-catenin expression, positive surviving, p53 expression, and higher T stages (Senol et al., 2015). Advanced T stage, high tumor grade, lymph node metastases, advanced stage, invasion of vascular, invasion of perineural, and high microvessel density strongly correlate with increased β-catenin expression (Sherkawy et al., 2021). Shen *et al*. also found that β-catenin expression increases with cancer stage progression, especially in stages 1 and 2, indicating its role in carcinoma progression and invasion (Ciurea et al., 2013).

The present findings expand upon prior studies that emphasize the significance of both canonical and non-canonical Wnt signaling pathways. Canonical signaling necessitates the existence of β-catenin, but non-canonical pathways do not for this purpose. The initiation of canonical signaling occurs when the DVL protein binds to frizzled receptors and LRP5/6, forming the FZD-LRP5/6 complex. In the absence of Wnt signaling, the degradation of β-catenin is induced by phosphorylation by GSK-3β and casein kinase-1. The activation of Wnt disrupts the destruction complex, enabling the entry of β-catenin into the nucleus to stimulate the transcription gene associated with cell proliferation and invasion, including MMP-9, c-Myc, and cyclin D1, which are linked to urothelial carcinoma progression and metastasis (Guntarno et al., 2021; Hambalie et al., 2021).

This study is the inaugural research conducted in Indonesia. Furthermore, research is required to explore the potential biomarker β-catenin in cases of Urothelial Carcinoma in Indonesia.

This study had several constraints. Firstly, it must be noted that this research was done as a single-centre study. As a result, the sample size was smaller than that of other research. Secondly, because RC treatments are commonly carried out on more aggressive tumors, tumor samples are scarce below stage T2. This scarcity restricts the amount of data available for statistical analysis.

CONCLUSION

This study found notable variations in β-catenin expression throughout different stages. Considering the positive correlation between this marker and tumour progression, it is advisable to consider it promising for targeted therapy in treating urothelial carcinoma. In addition, further research is needed to examine the implication of β-catenin in the urothelial carcinoma grading.

AUTHOR CONTRIBUTION

Each author actively contributed to the paper's data analysis, drafting, and revision and willingly accepted responsibility for all aspects of this work.

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