

BIOCHEMICAL AND CELLULAR ARCHIVES

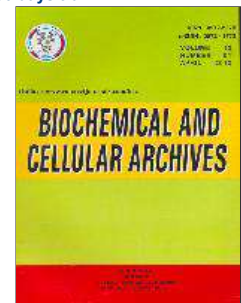
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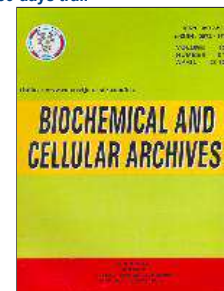
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Name	G. TRIPATHI
Dept./Organisation	Dept Zoology
Address	J N V University, Jodhpur-342 001, India
Email	drgst@rediffmail.com
Name	G. ARCHUNAN
Dept./Organisation	Dept Animal Science
Address	Bharathidasan University, Tiruchirappalli-620024, India
Email	garchu56@rediffmail.com
Name	RAED H. OGAILI
Dept./Organisation	Dept Maxillofacial Surgery
Address	College of Dentistry, Kerbala University, Iraq
Email	raedogaili@gmail.com
Name	VELAZHAHAN RETHINASAMY
Dept./Organisation	Dept Crop Sci.
Address	Sultan Qaboos University, Al-Khod, Muscat 123, Sultanate of Oman.
Name	MD. ABDULLAH-AL-MAMUN
Dept./Organisation	Dept. Fish Health Manag.
Address	Sylhet Ag. University, Sylhet - 3100, Bangladesh
Email	mamunff@gmail.com
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Email	dr.hassan198366@yahoo.com
Name	NIHAD KHALAWA TEKTOOK
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Email	drnihadkhalawe@gmail.com
Name	P. PADMANABHAN
Address	Cognitive Neuroimaging Centre, Nanyang Technological Univ., Singapore -636921
Email	ppadmanabhan@ntu.edu.sg
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Dept./Organisation	Faculty of Sci. & Technol.
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Email	envirokrishna@gmail.com
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CYCLIN D1, p53, AND Ki-67, THEIR ROLES IN UROTHELIAL CARCINOMA OF BLADDER IN DR. SOETOMO GENERAL ACADEMIC HOSPITAL, SURABAYA, INDONESIA DURING 2010-2019

Anny Setijo Rahaju^{1,2,3,4}, Arifa Mustika^{5*}, Etty Hary Kusumastuti^{2,4}, Priangga Adi Wiratama^{2,4}, Sutrisno², Lukman Hakim^{4,6} and Doddy M. Soebadi^{4,6}

¹Medical Doctoral Program Student, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

²Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

³Universitas Airlangga Hospital, Universitas Airlangga, Surabaya, Indonesia.

⁴Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

⁵Division Pharmacology and Therapy, Department of Anatomy, Histology and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁶Department of Urology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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ABSTRACT : Bladder cancer is a malignancy of the genitourinary system, which is ninth cancer globally with 430,000 new cases estimated in 2012. More than 50% of all bladder cancer incidents globally. Urothelial cell carcinoma is that commonest histological type. There are no good therapeutic options to date. Cancer occurs through various pathways, through disruption of tumor suppressor genes that inhibit apoptosis, the cell cycle continues so that cell proliferation occurs continuously and uncontrollably. Apoptosis can be seen from the p53 protein expressed by tumor cells as a mechanism for cell suicide, to remove cells that are not functioning. This p53 expression can assess tumor size and assess tumor cell proliferation, including urothelial bladder cancer cells. A nuclear protein called Ki-67 represents cell proliferation that highly active tumors. Ki-67 overexpression is related with worse oncologic outcomes in urothelial tumors. An increased level of cyclin D1 expression identified by immunohistochemistry is related to good progression and survival in bladder cancer. The analytical observation was conducted on 53 Urothelial Carcinoma of bladder patients who underwent cystectomy in Dr. Soetomo General Academic Hospital during 2010-2019. Immunohistochemistry on paraffin blocks was performed using p53, Ki-67, and cyclin D1 antibodies in the pathology laboratory. Expression of p53, Ki-67 and cyclin D1 were semiquantitative measured then statistically analyzed. In the Spearman correlation test, the grade of bladder urothelial cell carcinoma correlated strongly with p53 expression. There was also a correlation between Ki-67 and T stage of bladder urothelial cell carcinoma. Still, p53 and Ki-67 are promising biomarkers for urothelial tumors.

Key words : Urothelial carcinoma, cyclin D1, p53, Ki-67.

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INTRODUCTION

Bladder cancer is a malignancy of the genitourinary system (Siegel *et al*, 2018), which is ninth cancer globally, with an estimated 430,000 new incidents based on the accumulated data in 2012. More than 50% of all bladder cancer incidents globally. Urothelial cell carcinoma is that commonest histological type. Men dominate 75% of all bladder cancer cases (Antoni *et al*, 2017; Maffezzini *et al*, 2010). Bladder cancer mortality is about 17,000 deaths

each year (Antoni *et al*, 2017; Katz *et al*, 2017; Siegel *et al*, 2018). Multifactorial etiology proposed in bladder cancer, for example: environmental factors, genetic factors and smoking. Urothelial cell carcinoma is that commonest histological type. There are no good therapeutic options to date (Qiao, 2010). Cancer occurs through various pathways, namely, through disruption of tumor suppressor genes that inhibit apoptosis. The cell cycle continues so that cell proliferation occurs

*Corresponding author: Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. e-mail arifa-m@fk.unair.ac.id

continuously and uncontrollably. Apoptosis can be seen from the p53 protein expressed by tumor cells as a mechanism for cell suicide, to remove cells that are not useful. This p53 expression can assess tumor size and assess tumor cell proliferation, including urothelial bladder cancer cells (Ansori *et al*, 2020; Fadholly *et al*, 2020a; Fadholly *et al*, 2020b; Kharsima *et al*, 2020).

Tumor cell proliferation can also be seen through the activity of Cyclin D1, which inhibits the cell cycle so that decreased Cyclin D1 expression will cause the cell cycle to continue and cause the tumor to increase in size and invasion of the surrounding tissue (Ansori *et al*, 2020; Fadholly *et al*, 2020a; Fadholly *et al*, 2020b; Kharsima *et al*, 2020). In addition, the rate of tumor cell proliferation can also be assessed from Ki-67 protein expression, which is a recognized proliferative index for various tumors, including bladder cancer. These reasons are the thoughts in this study so that it can be developed for the selection of anticancer derived from herbs or medicines in Indonesia.

MATERIALS AND METHODS

This research used analytical observation method. Samples are consisted of 53 urothelial carcinoma of bladder patients slides, who conducting cystectomy in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia during 2010-2019. This study had been approved by the Dr. Soetomo General Academic Hospital. Immunohistochemistry was performed using Ki-67, p53, and cyclin D1 antibodies in Laboratory of Anatomical Pathology Department and Dr. Soetomo General Academic Hospital. The slides were observed used binocular and CX31 Olympus Microscope. Expression of immunohistochemistry was semiquantitative measured and statistically analyzed to find the association among Ki-67, p53 and cyclin D1 with grade and T stage.

RESULTS AND DISCUSSION

In the Spearman correlation test, the grade of bladder urothelial cell carcinoma correlated strongly with p53 expression, but there is no association of grade with Ki-67 and cyclin D1 expression (Table 1).

We did the Kruskal-Wallis difference test. There was a significant difference at any stage for p53. The p53 difference is between T1 and T3, also between T3 and T4 (Mann-Whitney test: p-value = 0.023 and 0.011). There was also a correlation between Ki-67 and T stage bladder urothelial cell carcinoma.

In Dr. Soetomo General Academic Hospital, there was 403 bladder urothelial cell carcinoma patients in 10 years from 2010-2019. Fifty-three patients have sampled

Table 1 : Correlation and Difference Test of IHC expression on any grade.

Variables	Frequency			p-value	r
	Low Grade n=7	High Grade n=46	Total		
p53					
Score 0	6	19	25	0.031*	0.299*
Score 1	1	22	23		
Score 2	0	4	4		
Score 3	0	1	1		
Ki-67					
Score 0	0	4	4	0.431	0.109
Score 1	6	25	31		
Score 2	1	13	14		
Score 3	0	4	4		
Cyclin D1					
Score 0	1	5	6	0.545	0.084
Score 1	4	21	25		
Score 2	1	13	14		
Score 3	1	7	8		

*Significant at $\alpha < 0.05$

for bladder urothelial cell cancer with an age range between 34 to 81 years, 60.43 years in mean, and predominantly male (92.46%). According to previous literature, in the US, bladder cancer is rarely diagnosed at less than 40 years. The mean age is 73 years and four times more common in men (Flaig *et al*, 2020; Saginala *et al*, 2020).

This study's sample was dominated by high grade, stage T4 and invasive urothelial bladder carcinoma. This profile is due to all samples was obtained from cystectomy. Cystectomy is a standard procedure for muscularis invasion of bladder cancer (MIBC). This data is consistent with research in several regions in Indonesia (Umbas *et al*, 2015), but shows contradictory results to various other areas such as Western countries, China, Japan, and Korea, which most cases found are non muscularis invasion of bladder cancer (NMIBC) (Cumberbatch *et al*, 2018; Kakehi *et al*, 2010; Kim *et al*, 2016; Pang *et al*, 2016). This difference is due to the lack of knowledge and understanding of bladder cancer and people's tendency to get treatment with traditional medicine first at the beginning of the diagnosis. Another difference also states in the literature that more than 50% of novel identified cases are NMIBC. Mostly, exophytic, grows papillary and no invasion beyond the Ta, T1, or CIS. Bladder urothelial tumor cells are often fragile and bleeding. A residual history of the disease with symptoms

Table 2 : Correlation and difference test of IHC expression on any stage.

Variable	Frequency					p value	r
	T1 n=9	T2 n=14	T3 n=14	T4 n=16	Total		
p53							
Score 0	6	6	3	10	25	0.040*	0.017
Score 1	3	6	8	6	23		
Score 2	0	1	3	0	4		
Score 3	0	1	0	0	1		
Ki-67							
Score 0	2	1	1	0	4	0.080	0.279*
Score 1	5	11	5	10	31		
Score 2	2	2	6	4	14		
Score 3	0	0	2	2	4		
Cyclin D1							
Score 0	2	2	1	1	6	0.457	0.180
Score 1	4	6	9	6	25		
Score 2	3	3	2	6	14		
Score 3	0	3	2	3	8		

*Significant at $\alpha < 0.05$

similar to the initial stage or further will cause the patient to come for medical help (Flaig *et al*, 2020; Raspollini *et al*, 2020).

Tumor grade is also one of the determinants of patient prognosis. This study found more high-grade cases than low-grade. This data is consistent with other studies in Indonesia (Umbas *et al*, 2015). Still, it is different from several studies in Japan, China, and Korea, which show a balanced proportion between high grade and low grade (Kakehi *et al*, 2010; Kim *et al*, 2016; Pang *et al*, 2016). The chi-square test results showed no significant differences in grade, T stage, invasion status, cyclin D1 expression, Ki-67 expression, and p53 expression in males and females, proving that urothelial carcinoma bladder is not influenced by sex as in the study in Chennai. That study showed no correlation between p53 expression and patient gender and age (Hammed *et al*, 2020; Hegazy *et al*, 2015). Another study in China presents no significant correlation between Ki-67 and age neither sex (Tian *et al*, 2016).

p53 expression correlates to grade in bladder urothelial carcinoma in this study. This result contradicts the literature suggesting that p53 is not associated with tumor grade (Cumberbatch *et al*, 2018; Ibrahim *et al*, 2009). However, it represents some literature that states that p53 overexpression and its intensity correlate well with tumor grade. In bladder carcinoma, p53 is biologically

aggressive and discovered more often at high-grade than low-grade or moderate ones, and p53 leads pathogenesis of carcinoma related to tumor grade (Mahdi, 2020).

The existence of p53 gene mutation can be proven by immunohistochemistry. Tumors that show the p53 mutation are of higher grade and stage and show a tendency to recur. p53 mutation/change is an important biological event in bladder tumorigenesis, and the detection of these changes can set necessary entities about the history of the disease. It can provide useful information in making therapeutic decisions (Esrig *et al*, 1993).

This study found that there was no relation between Ki-67 expression and grade in bladder urothelial cell carcinoma. Previous research has associated Ki-67 overexpression with adverse clinicopathological parameters. Low Ki-67 predicts independently bladder tumor recurrence. Ki-67 also be related with bad prognosis in post radical nephroureterectomy primary urothelial carcinoma patients (Wu *et al*, 2015). Ki-67 expression was related with bad comprehensive survival and decreased tumor stage in MIBC patients and was highly related with PD-L1 expression. PD-L1 and Ki-67 immunophenotyping will assist in therapy choices (Rubino *et al*, 2020).

This research finds that Cyclin D1 does not correlate with urothelial bladder cancer grade. Other literature

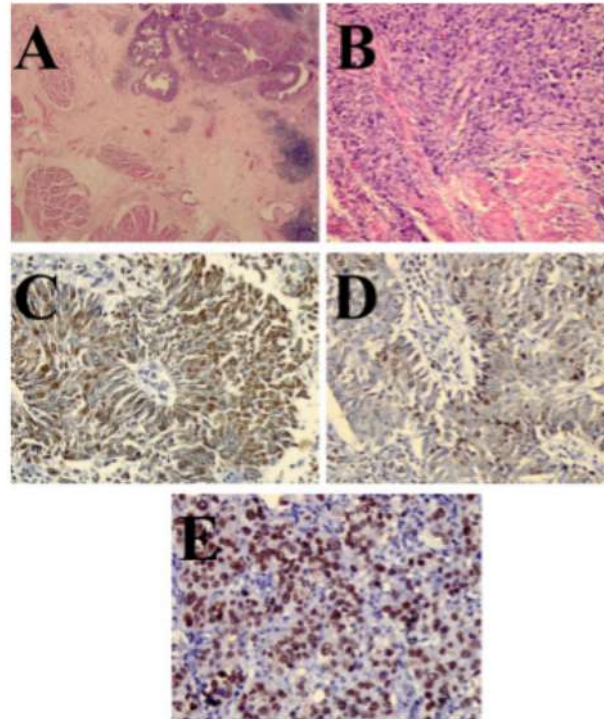


Fig. 1 : A. Non muscularis invasion of bladder cancer, 40 HPF; B. Muscularis invasion of bladder cancer, high grade, 200 HPF; C. Cyclin D1 expression, 400 HPF; D. Ki-67 expression, 400 HPF and E. p53 expression, 400 HPF.

presents a different result: patient prognosis correlates to cyclin D1 expression. Low cyclin D1 expression is related with high-grade, advanced stage and tumor metastasis to lymph nodes and p53 overexpression (Albasri *et al*, 2019). However, cyclin D1 is an oncogenic gene that contributes to cell cycle dysfunction. Its overexpression is found in many human malignancies. Cyclin D1 can control the normal cell cycle and affect the epithelial proliferation pathway. Cyclin D1 has a vital role in regulating the G1-S phase of the cell cycle by forming complexes with different cyclin kinases (Shan *et al*, 2015).

A significantly elevated of Cyclin D1 expression was in tumors with invasive phenotype in bladder tumors than the non-invasive ones. Cyclin D1 expression in the nucleus is absent in the majority of invasive malignancies and nodal metastases. In the muscular-invasive group, there is a significant correlation reported between Cyclin D1 and various parameters, such as bladder tumor histological type, grade, stage, *Muscularis propria* invasion, vascular invasion, and lymph node invasion (Albasri *et al*, 2019). Other literature also states that an increased level of expression of cyclin D1 presented by immunohistochemistry is related with survival and good progression in bladder cancer (Ren *et al*, 2014).

No significant correlation of p53 and T stage in this study. The TP53 gene is “the guardian of the genome”, that is a tumor gene processor that often mutates. Inactivation of the TP53 gene results in decreased DNA repair function, and genomic instability occurs. TP53 gene plays a vital point when going inactive in progression of tumor, metastasis and bladder cancer aggressivity phenotypes. This gene predicts poor prognosis. The type of mutation and its frequency vary with different types of tumors and depend on the tumor type, stage, and etiology. Mostly all TP53 mutations cause damage to protein function. Due to p53 protein mutation has a longer half-life than normal p53 protein, the TP53 gene might be found both in the gene level and protein level. Immunohistochemistry (IHC) can detect it as a surrogate marker for mutation. Immunohistochemically, markers of TP53 protein expression can be considered to reflect point mutations of the TP53 gene in tumors, although they are not always identical to TP53 mutations (Al-Kashwan *et al*, 2012). Another study on p53 stated that no changes in mortality between positive and negative tumors expressing p53, but overexpression of p53 was associated with advanced grade and stage (Reddy *et al*, 2017).

Ki-67 expression correlates to T Stage in this study urothelial bladder carcinoma. These results are consistent

with previous bladder urothelial cell carcinoma research presenting Ki-67 as an independent marker of tumor progression at the pTa and pT1 stages. Ki-67, as nuclear protein, involves in transcription and specific marker of cellular proliferation. Expression of Ki-67 is strong in proliferating cancer cells fractions and the presence of Ki-67 positive tumor cells indicates a poor prognosis for survival and recurrence. One study analyzing NMIBC found that a combination of p53 and Ki-67 had a predictive value in its recurrence. There is a predictive role of the NMIBC postoperative recurrence risk by using p53 and Ki-67 expression. p53 has prognostic value in predicting the advanced progression of pT1 urothelial bladder cancer (Ziaraan *et al*, 2020).

A meta-analysis study indicated that elevated Ki-67 immunoreactivity correlates significantly to worsening clinical outcomes in bladder urothelial cell carcinoma (Luo *et al*, 2016). Low Ki-67 expression could be an independent predictor of urothelial bladder cancer tumor recurrence, while in primary upper tract bladder urothelial cell cancer patients after radical nephroureterectomy, its overexpression was associated with adverse clinicopathological parameters and poor prognosis (Wu *et al*, 2015).

However, Ki-67 has not been confirmed as a poor prognostic marker in NMIBC patients because of the variation of threshold and the varied immunochemical procedure, making difficulties in direct comparisons. A consensus of experts has found that Ki-67, as well as p53 can predict recurrence and progression of bladder urothelial cell cancer, but the inconsistency of available data suggests that these markers are unreliable. This meta-analysis was performed to improve our understanding of the prognostic significance of Ki-67 in NMIBC patients. Another meta-analysis supports the predictive value of Ki-67 in NMIBC patients (Ko *et al*, 2017). Evaluation of Ki-67, expression and intensity of surviving nuclei and p53 nuclear staining may also add information that allows more precise NMIBC recurrence risk after TURBT (Stec *et al*, 2020). Other literature strengthens the opinion that the combined use of immunomarkers p53 and Ki-67 can provide additional prognostic information along with histological assessment and stage of bladder carcinoma (Thakur *et al*, 2017).

Cyclin D1 regulates the cell cycle and promotes cell growth and proliferation. It is a proto-oncogene located on chromosome 11q13, which modulates an essential step in the cell cycle controlling development. Data contradicting the significance of Cyclin D1 have been reported in urinary bladder tumors, resulting in a controversial prognostic role for the cyclin D1 protein.

Bladder urothelial cell cancer is a complex process that progressively disrupts the normal mechanisms that control epithelial proliferation, inflammation and differentiation (Rubino *et al*, 2020).

The results showed that there was no relation between Cyclin D1 expression and stage T in urothelial bladder cancer. These results are not in accordance with Amer *et al*, who stated that the Cyclin D1 expression was associated with prognostics in urothelial bladder cancer, low Cyclin D1 expression related with poor differentiation, advanced stage, metastasis of tumor lymph nodes and high p53 expression. The cell cycle regulations are the important event for tumor development. Cyclin D1 controls normal cell cycle development and influences epithelial proliferation pathways. Cyclin D1 has an important role in cell cycle regulation and signaling of proliferative targets in the development of G1-S phases; through the formation of complexes with different cyclin kinases.

CONCLUSION

In summary, this study showed a significant correlation between Ki-67 and T stage, but no correlation between Ki-67 and grade. A significant correlation between p53 and grade is found, but no correlation between p53 and T stage. Both grade and T stage do not correlate to cyclin D1.

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