



UROSCIENCE

ORIGINAL ARTICLE

# The expression of p63 in bladder cancer vs. chronic bilharzial bladder

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## ABBREVIATIONS

SCC, squamous cell carcinoma;  
CIS, carcinoma *in situ*;  
TCC, transitional cell carcinoma

**Abstract Objective:** To investigate the immunohistochemical expression of p63 in bladder cancer and the variation of expression in relation to histological type, grade and stage of the tumour, and whether bilharziasis (endemic in Egypt) has an effect on its expression, in an attempt to better understand the tumour behaviour and the possibility of using p63 as a prognostic marker.

**Patients and methods:** In a prospective study, biopsies were taken from the bladders of 70 patients, who were divided into three groups; group A comprised 10 with a normal urothelium, group B comprised 20 with chronic cystitis (bilharzial and non-bilharzial) and group C contained 40 with bladder cancer. The biopsies were examined for the expression of p63, using immunohistochemical techniques.

**Results:** The mean (SD) ages of groups A, B and C were 45.2 (9.5), 50.5 (11.7) and 60.5 (9.9) years, respectively. There was a statistically significant decrease in the expression and immunoreactivity in group C ( $P < 0.05$ ), and a significant decrease with advancing tumour stage and grade ( $P < 0.01$ ). In cases of squamous cell

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carcinoma there was a statistically significant lower immunoreactivity than in transitional cell carcinoma ( $P < 0.05$ ). There was a tendency for a statistically significant decrease in the immunoreactivity in bilharzial cystitis ( $P < 0.05$ ), but in the malignant group, bilharziasis had no apparent effect on the pattern of expression.

**Conclusion:** p63 might be a helpful biomarker and adjunct in predicting the biological behaviour and progression of tumours. Further studies are recommended to elucidate more clearly its role as a prognostic indicator and its utility as a tumour marker.

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## Introduction

In Egypt, bladder cancer is intimately related to the parasitic blood fluke *Schistosoma*, which has been endemic in Egypt at least since 1900 BCE, and is endemic in 74 countries throughout the world [1,2].

Carcinoma of the bladder is the foremost oncological problem in Egypt, constituting 30.3% of all cancers, 40.6% of male cancers and 14.3% of female cancers, as recorded at the National Cancer Institute [3]. Worldwide it is an important health problem, with 386,300 newly diagnosed cases and 150,200 deaths in the year 2008 [4].

Various methods are currently used for detecting bladder cancer, of which the most recent is the use of tumour markers as a complement to the tumour grade and stage, to reflect the potential behaviour of the tumour and the possibility of its progression or recurrence [5].

The gene *p63* is a homologue of the p53 tumour-suppressor gene located at 3q27–3q29 [4]. It is expressed selectively in the basal cells of stratified epithelium, including the urothelium [6]. It is suggested to play a critical role in the normal development and maintenance of the human urothelium [7].

Several studies have assessed the role of p63 in malignant transformation, as well as tumour progression. Some of these studies showed a downregulation in muscle-invasive tumours, and others proposed an impaired expression with biological aggressiveness, and of being a common feature of high-grade invasive carcinomas, suggesting a role in tumour progression and biochemical differentiation [8,9]. Considering the urothelium as a unit acting in the same manner throughout the urinary tract, similar studies on the upper urinary tract transitional cell carcinoma (TCC) showed a significant decrease in immunoreactivity with advancing tumour stage, and an association with a poor prognosis [10]. By contrast, other studies showed that the designation of *p63* as an oncogene or a tumour-suppressor gene might be difficult, because its isoforms might have opposing functions [11].

Thus we evaluated the immunohistochemical expression of p63 in bladder cancer and the variation of expression in relation to histological type, grade and stage of the tumour, and whether bilharziasis has an effect on its expression.

## Patients and methods

The study included 70 patients admitted to the Urology department of Theodor Bilharz Research Institute, Egypt. The patients were prospectively enrolled in the study and divided into three groups; a control group (A) of 10 patients who underwent cystoscopy for any urological disease other than cystitis or bladder tumour; a cystitis group (B) of 20 patients with chronic cystitis (bilharzial and non-bilharzial); and a malignancy group (C) of 40 patients with bladder cancer. According to the results of the biopsy, group B patients were retrospectively divided into bilharzial and non-bilharzial, and group C into those with TCC or squamous cell carcinoma (SCC).

All patients had a detailed history taken, a full clinical examination, routine laboratory investigations, urine cytology, and imaging in the form of abdominal and pelvic ultrasonography, intra-venous urography (IVU) and computed tomography scan (CT) of the abdomen and pelvis in selected cases. There were no patients with associated upper tract TCC in the study.

After signing an informed consent, all patients underwent cysto-urethroscopy and biopsy. For groups A and B the biopsies were taken from the urothelium using a cold-cup biopsy forceps, while for group C we used a resectoscope to take biopsies from the tumour and from the surrounding apparently normal urothelium. In patients with invasive bladder cancer who had a radical cystectomy, the biopsy was taken from the cystectomy specimen after surgery.

The biopsy specimens were immediately fixed with formalin 10%, and assessed histopathologically after staining with haematoxylin and eosin, and for the immunohistochemical study of p63 expression. Schisto-

somal infestation was diagnosed by detecting *Schistosoma* eggs in the tissues.

Although there was a discrepancy in the number of patients in the three groups we assessed the results using statistical analysis, computing the mean (SD) of the variables and using Student's *t*-test to compare two means, and for more than two means we used a one-way anova with the least-significant difference test. The correlation between the p63 tissue expression and histopathological stage, grade and clinical data was assessed by Spearman's correlation coefficient.

## Results

The study included 60 patients with different bladder lesions (neoplastic and non-neoplastic) and 10 with a normal urothelium, classified into three groups. The mean (SD) ages of groups A, B and C were 45.2 (9.5), 50.5 (11.7) and 60.5 (9.9) years, respectively. Five of the 10 patients in group A, 12 of 20 (60%) in group B and 37/40 (93%) in group C were men.

For group A, the original indication for cystoscopy was ureteroscopy for a stone in the lower ureter (six), retrograde ureteropyelography (three) and transurethral resection of the prostate (TURP) (one).

In group C (40 patients), 25 (63%) and 15 (38%) had TCC and SCC, respectively. Using the classification of urothelial tumours outlined by Eble et al. [12], five (13%), seven (18%), 17 (43%) and 11 (28%) were staged Ta, T1, T2 and T3, respectively. Eight (20%), 23 (58%) and nine (23%) had Grades I, II and III, respectively (Table 1). There were no cases of carcinoma *in situ* (CIS) in this group.

Among all groups (70 patients), 40 (57.1%) were associated with bilharziasis, i.e. 10 of 20 (50%) in group B and 30 of 40 (75%) in group C, of which, 20 of 30 (67%) were invasive tumours and 23 (77) were both Grade II and Grade III tumours (Table 2).

### Immunoeexpression of p63

The non-neoplastic urothelium showed nuclear immunoreactivity with a slightly decreasing gradient from the basal to the luminal cells (with the superficial umbrella cells remaining unstained) and with no staining of the stromal cells. In malignant lesions all layers showed the expression of the p63.

The expression was positive in all 10, all 20 and in 33 of the 40 patients in groups A, B and C, respectively. Strong immunoreactivity decreased significantly from all 10 in the group A, to 17/20 (85%) in group B ( $P = 0.04$ ), to 16/40 (40%) in group C, with a statistically significant difference ( $P < 0.01$ ; Table 3). Also, negative expression increased significantly from none in both groups A and B to 18% of group C ( $P < 0.01$ ; Table 3).

**Table 1** The stages and grades of different malignant lesions in group C.

Stage or grade	n or n (%)		
	TCC	SCC	Total
Total	25	15	40
<b>Stage</b>			
Ta	5 (20)	0	5 (13)
T1	7 (28)	0	7 (18)
T2	10 (40)	7	17 (43)
T3	3 (12)	8	11 (28)
<b>Grade</b>			
I	8 (32)	0	8 (20)
II	14 (56)	9	23 (58)
III	3 (12)	6	9 (23)

**Table 2** The effect of a bilharzial association on the tumour stage and grade in group C.

Category	Non-bilharzial	Bilharzial	Total
No. of patients	10	30	40
<b>Stage</b>			
Superficial	2	10 (33)	12 (30)
Invasive	8	20 (67)	28 (70)
Total	10 (25)	30 (75)	40 (100)
<b>Grade</b>			
I	1	7 (23)	8 (20)
I and II	9	23 (77)	32 (80)
Total	10 (25)	30 (75)	40 (100)

Although the positive expression was similar in both TCC and SCC tumours (84% vs. 80%) the immunoreactivity showed a statistically significant decrease, from strong and moderate in 16/25 (64%) of patients with TCC, to five of 15 (33%) of those with SCC, respectively ( $P = 0.03$ ). Also, there was a statistically significant increase in immunoreactivity, from negative and moderate in nine of 25 patients with TCC, to 10/15 with SCC, respectively ( $P = 0.03$ ; Table 3).

With increasing tumour stage there was a significant decrease in both the expression and immunoreactivity. The positive expression decreased from all five to 22/28 (79%) of Ta and T2 + T3 tumours, respectively ( $P = 0.01$ ). Strong immunoreactivity decreased from four of five to 9/32 (32%) of Ta and T2 + T3 tumours, respectively ( $P = 0.04$ ), and negative immunoreactivity increased from none of five to 6/28 (21%) of Ta and T2 + T3 tumours, respectively ( $P = 0.01$ ; Table 3).

In patients with TCC there was a significant decrease in both expression and immunoreactivity from non-muscle-invasive to muscle-invasive tumours. The positive expression decreased from 11/12 to 10/13, with decreasing strong and moderate immunoreactivity from 10/12 to six of 13, and increasing negative and mild immunoreactivity from two of 12 to seven of 13 in

**Table 3** The expression of p63 in the three groups.

Category (n)	Nuclear p63 immunoreactivity, n (%)						Total +ve
	Negative (0–10%)	Mild (11–30%)	Negative + Mild	Moderate (31–60%)	Strong (61–100%)	Moderate + Strong	
Group A (10)	0	0	0	0	10	10	10
Group B (20)							
Non-bilharzial (10)	0	0	0	0	10	10	10
Bilharzial (10)	0	0	0	3 <sup>a</sup>	7 <sup>a</sup>	10	10
Total (20)	0	0	0	3 (15) <sup>b</sup>	17 (85) <sup>b</sup>	20 (100)	20 (100)
Group C (40)							
Non-bilharzial (10)	3 <sup>a</sup>	3 <sup>a</sup>	6 <sup>l</sup>	2	2 <sup>j</sup>	4 <sup>l</sup>	7 <sup>a</sup>
Bilharzial (30)	4 <sup>l</sup>	9 <sup>k</sup>	13 <sup>m</sup>	3	14	17 (57) <sup>m</sup>	26 (87) <sup>l</sup>
TCC bilharzial (15)	1	2 <sup>c</sup>	3 <sup>cde</sup>	2	10 <sup>cdi</sup>	12 <sup>cde</sup>	14
SCC (15)	3	7	10 <sup>f</sup>	1	4	5 <sup>f</sup>	12
Total	7 <sup>h</sup>	12 <sup>h</sup>	19 (48) <sup>ab</sup>	5 <sup>g</sup>	16 (40) <sup>fn</sup>	21 (53) <sup>ab</sup>	33 (83) <sup>fn</sup>
Group C (40)							
TCC (25)	4	5	9	4	12	16 (64)	21 (84)
Superficial (12)	1	1	2	3	7	10	11
Invasive (13)	3	4	7 <sup>g</sup>	1	5	6 <sup>g</sup>	10
SCC (15)	3	7	10	1	4	5	12
Total (40)	7	12	19 (48)	5	16 (40)	21 (53)	33 (83)
Stage (40)							
Ta (5)	0	0	0	1	4	5	5
T1 (7)	1	1	2	2	3	5	6
T2 – T3 (28)	6 <sup>a</sup>	11 <sup>c</sup>	17 (61) <sup>h</sup>	2	9 <sup>b</sup>	11 <sup>h</sup>	22 (79) <sup>a</sup>
Grade (40)							
I (8)	0	1	1	1	6	7	8
II (23)	3 <sup>d</sup>	9	12 <sup>i</sup>	3	8 <sup>e</sup>	11 <sup>i</sup>	20 (87) <sup>d</sup>
III (9)	4 <sup>f</sup>	2	6 <sup>i</sup>	1	2 <sup>f</sup>	3 <sup>i</sup>	5 <sup>f</sup>
Normal adjacent mucosa							
TCC (25)			1 <sup>j</sup>			24 (96) <sup>j</sup>	
Urothelial hyperplasia (7/25)	0	0		1	6		
Low-grade dysplasia (10/25)	0	0		3	7		
High-grade dysplasia (8/25)	0	1		3	4		
Total	0 <sup>b</sup>	1 <sup>c</sup>		7 <sup>a</sup>	17 (68) <sup>a</sup>		25 (100) <sup>b</sup>
SCC			0 <sup>k</sup>			15 (100) <sup>k</sup>	
Squamous metaplasia (10/15)	0	0		2	8		
Low-grade dysplasia (2/15)	0	0		1	1		
High-grade dysplasia (3/15)	0	0		1	2		
Total	0 <sup>e</sup>	0 <sup>f</sup>		4 <sup>d</sup>	11 <sup>df</sup>		15 (100) <sup>e</sup>

For initial group A–C comparisons: <sup>a</sup> $P = 0.03$  vs. chronic non-bilharzial cystitis; <sup>b</sup> $P = 0.04$  vs. control; <sup>c</sup> $P = 0.01$  vs. non-bilharzial malignant lesion; <sup>d</sup> $P = 0.01$  vs. SCC; <sup>e</sup> $P = 0.02$  vs. SCC; <sup>f</sup> $P < 0.01$  vs. control; <sup>g</sup> $P = 0.01$  vs. control; <sup>h</sup> $P < 0.01$  vs. cystitis; <sup>i</sup> $P = 0.04$  vs. total tumour; <sup>j</sup> $P < 0.01$  vs. chronic non-bilharzial cystitis; <sup>k</sup> $P < 0.01$  vs. chronic bilharzial cystitis; <sup>l</sup> $P = 0.02$  vs. chronic bilharzial cystitis.

For group C section: <sup>a</sup> $P = 0.01$  vs. Ta; <sup>b</sup> $P = 0.04$  vs. Ta; <sup>c</sup> $P < 0.01$  vs. Ta; <sup>d</sup> $P = 0.04$  vs. Grade I; <sup>e</sup> $P = 0.03$  vs. Grade I; <sup>f</sup> $P = 0.02$  vs. Grade I. For normal adjacent mucosa: <sup>a</sup> $P < 0.01$  vs. control; <sup>b</sup> $P = 0.02$  vs. total TCC; <sup>c</sup> $P = 0.04$  vs. total TCC; <sup>d</sup> $P = 0.02$  vs. control; <sup>e</sup> $P = 0.04$  vs. SCC; <sup>f</sup> $P < 0.01$  vs. SCC.

For combined groups Negative + Mild and Moderate + Strong: <sup>a</sup> $P < 0.01$  vs. control; <sup>b</sup> $P < 0.01$  vs. chronic cystitis; <sup>c</sup> $P = 0.03$  vs. non-bilharzial tumour; <sup>d</sup> $P = 0.02$  vs. total tumour; <sup>e</sup> $P < 0.01$  vs. SCC; <sup>f</sup> $P = 0.03$  vs. TCC; <sup>g</sup> $P = 0.03$  vs. superficial TCC; <sup>h</sup> $P < 0.01$  vs. Ta; <sup>i</sup> $P = 0.01$  vs. Grade I; <sup>j</sup> $P < 0.01$  vs. TCC; <sup>k</sup> $P < 0.01$  vs. SCC; <sup>l</sup> $P < 0.01$  vs. chronic non-bilharzial cystitis; <sup>m</sup> $P < 0.01$  vs. chronic bilharzial cystitis.

non-muscle-invasive and muscle-invasive tumours, respectively ( $P = 0.03$ ; Table 3).

Similarly, there was a statistically significant decrease in the expression and immunoreactivity with increasing tumour grade. The positive expression decreased from all eight to five of nine ( $P = 0.04$ ), and strong immunoreactivity decreased from six of eight to two of nine in Grade I and III tumours, respectively ( $P = 0.03$ ). Also, negative immunoreactivity increased from none of eight to four of nine for Grade I and III tumours, respectively ( $P = 0.04$ ; Table 3).

#### Effect of bilharziasis on p63 immunoreactivity

Although all 20 patients of group B (bilharzial and non-bilharzial) showed positive expression, there was a difference in the immunoreactivity. All 10 non-bilharzial patients showed strong expression, which decreased to seven of 10 and three of 10 for strong and mild expression in the bilharzial patients, respectively ( $P = 0.04$ ; Table 3).

In group C, there was an increase in the expression with bilharzial association, but with varied immunoreac-

tivity. The positive expression increased significantly from seven of 10 to 87% of non-bilharzial and bilharzial malignant tumours, respectively. Although strong immunoreactivity increased significantly from two of 10 to 14/30 (47%) of non-bilharzial and bilharzial malignant tumours, respectively ( $P = 0.01$ ), there was a significant decrease in the negative expression from three of 10 to 4/30 (13%) in non-bilharzial and bilharzial malignant tumours, respectively ( $P = 0.03$ ; Table 3).

As all of the non-bilharzial malignant tumours were TCC, on comparing the non-bilharzial- and the bilharzial-associated TCC there was a significant increase in strong expression, from two of 10 to 10/15, respectively ( $P = 0.01$ ; Table 3). There was a significant increase in strong and moderate expression, from four of 10 to 12/15, in the non-bilharzial and the bilharzial-associated TCC, respectively ( $P = 0.03$ ), and a significant decrease in negative and mild expression, from six of 10 to three of 15 in the non-bilharzial and the bilharzial-associated TCC, respectively ( $P = 0.03$ ; Table 3).

All of the SCC tumours were associated with bilharziasis, so we compared the bilharzial-associated TCC to SCC, finding a significant decrease in the strong immunoreactivity in the SCC tumours, from 10/15 to four of 15, respectively ( $P = 0.01$ ; Table 3). There was a significant decrease in strong and moderate expression, from 12/15 to five of 15, in the bilharzial-associated TCC and SCC, respectively ( $P < 0.01$ ), and a significant increase in negative and mild expression, from three of 15 to 10/15, in the bilharzial-associated TCC and SCC, respectively ( $P < 0.01$ ; Table 3).

#### *Expression of p63 in the apparently healthy mucosa adjacent to the malignant tumours*

There was a change in the expression of p63 in the adjacent mucosa. In TCC tumours there was urothelial hyperplasia, low-grade and high-grade dysplasia in 7/25, 10/25 and 8/25 patients, respectively. There was a significant decrease in the strong and moderate expression, from 10/10 and none of 10 in the control cases, to 17/25 (68%) and 7/25 (28%) in the adjacent mucosa of TCC cases, respectively ( $P < 0.01$ ; Table 3). In SCC tumours there was squamous metaplasia, low-grade and high-grade dysplasia in 10/15, 2/15 and 3/15 patients, respectively. There was a significant decrease in the strong and moderate expression, from 10/10 and none of 10 in the control cases, to 11/15 and four of 15 in the adjacent mucosa of SCC cases, respectively ( $P = 0.02$ ; Table 3).

#### **Discussion**

The immunohistochemical expression of p63 was assessed in the urothelium of 60 patients with different bladder lesions, and in 10 with an apparently normal

healthy bladder, to evaluate its diagnostic value in bladder cancer and whether bilharziasis (which is endemic in Egypt) has an effect on its expression.

All of the non-neoplastic urothelium showed strong nuclear immunoreactivity with a decreasing gradient from the basal to the luminal cells, with no staining of the stromal cells. Comp erat et al. [13] stated that staining was always nuclear and there was no staining in the smooth muscle cells, adipocytes or the neural cells.

All 10 patients in the control group had a strong positive expression of p63. Similar results were reported by others who studied the expression in the non-neoplastic upper urinary tract [10,14–16]. Also, the expression was positive in all 20 patients in group B (cystitis) but the immunoreactivity decreased with bilharzial association, from strong in all non-bilharzial patients, to strong and moderate in seven of 10 and three of 10 of the bilharzial patients, respectively. We are not sure whether this decrease in the immunoreactivity from the normal pattern is attributed to the local inflammatory effect of bilharziasis, but we recommend more studies to address the effect of bilharziasis, as the published studies are deficient in this point.

In group C (malignant) there was 83% positive expression (33/40) with varied immunoreactivity, being strong and negative in 40% (16/40) and 18% (7/40), respectively. Ud Din et al. [17] reported an 88% positive expression (44/50), and Comp erat et al. [14] reported 81.2% positive expression (39/48), with 41.6% (20/48) having strong immunoreactivity. Although we had no patients with an associated upper tract TCC, reviewing the results of Langner et al. [15], who assessed the upper urinary tract TCC, they had 96.2% (51/53) positive expression, with strong and negative immunoreactivity in 45.3% (24/53) and 3.8% (2/53), respectively. This shows that the whole urothelium behaves in the same way concerning the expression of p63.

In group C there was a statistically significant decrease in immunoreactivity with increasing clinical stage of the TCC tumours. The immunoreactivity decreased from being strong and moderate in all patients with Ta disease to 11/28 of those with T2 – T3 ( $P < 0.01$ ). Also, the immunoreactivity increased from being negative and moderate in none of the patients with Ta tumours, to 17/28 (61%) of those with T2 – T3 disease ( $P < 0.01$ ). Studying 160 patients with TCC, Urist et al. [11] showed that invasive tumours expressed low levels of p63, with only an average of 16% of cells positive (mild immunoreactivity), and the difference between non-muscle-invasive and invasive TCC was significant.

There was a decrease in p63 expression with increasing tumour stage, from Ta to T1 and from T1 to T2 + T3, and a statistically significant decrease from Ta to T2 + T3 ( $P = 0.01$ ). The expression decreased from all five, to six of seven, and to 22/28 (79%) in

Ta, T1, and T2 + T3, respectively. In Ta tumours the immunoreactivity was strong and moderate in four of five and one of five, respectively. In the T1 group of seven cases, the immunoreactivity decreased to be strong, moderate, mild, and negative in three, two, one and one, respectively, with a greater decrease in 28 invasive tumours (T2 + T3) of nine (32%), two (7%), 11 (39%) and six (21%), respectively.

Comp erat et al. [14] reported a similar decrease in the expression from all 12 tumours, four of five, to 22/31 (70%) of Ta, T1, and T2 – T3 cases, respectively. In the present Ta group, the immunoreactivity was strong and mild in 10 of 12 and two of 12, respectively. The immunoreactivity decreased in the T1 group of five to be strong, mild, and negative in three, one and one, respectively, with a greater decrease in the immunoreactivity in the invasive group of 31 (T2 + T3) tumours of seven (22%), 15 (48%) and nine (29%), respectively.

In 2006, Comp erat et al. [13] studied retrospectively 158 patients who had either endoscopic resection of non-invasive tumours or cystectomy for invasive tumours. They found a statistically significant difference between the stages of pTa, pT1, and  $\geq$ pT2 cases. In 93% (52/56) of pTa tumours the expression was homogenous and strong. The expression was more heterogeneous, with negative, mild and strong staining in 6/45 (13%), 15/45 (34%) and 24/45 (53%), and in 13/57 (23%), 26/57 (46%) and 18/57 (31%) of pT1 and  $\geq$ pT2 cases, respectively.

For tumour grade there was a statistically significant decrease in both the expression and immunoreactivity with increasing grade. All eight Grade I tumours showed a positive expression, which decreased to 20/23 (87%) ( $P = 0.04$ ) and five of nine ( $P = 0.02$ ) of Grade II and III tumours, respectively. Also, the immunoreactivity decreased from strong and negative in six and none of the eight Grade I tumours, respectively, to mild and negative in two and four of the nine Grade III tumours, respectively ( $P = 0.02$ ). Urist et al. [11] reported a statistically significant inverse association between the immunoreactivity and the increase in the grade within non-muscle-invasive tumours. Low-grade papillary tumours expressed p63 strongly in 93% of the tumour cells, with a significant reduction in the positivity to 68% (moderate immunoreactivity) in intermediate- to high-grade non-muscle-invasive tumours.

Zigeuner et al. [10] investigated 53 upper urinary tract TCC specimens and noted a normal strong pattern of expression in one of 22 (4.5%) pT3 cases, compared to 13/31 (42%) of pT1–T2 cases. There was a normal pattern of expression also in 10/28 (36%) and 4/25 (16%) Grade II and III tumours, respectively, with a trend to decreased immunoreactivity in the poorly differentiated tumours. Their results are similar to ours, indicating a similarity in the behaviour of the whole urothelium in this respect.

As to the histopathological type of malignancy (TCC vs. SCC), the expression was similar (84% vs. 80%) but the difference was in the immunoreactivity, which decreased from being strong and moderate in 16/25 (64%) patients with TCC, to five of 15 (33%) with SCC ( $P = 0.03$ ). There was also a statistically significant increase in immunoreactivity, from being negative and moderate in nine of 25 (36%) with TCC, to 10/15 with SCC ( $P = 0.03$ ). To our knowledge, there are no reports on the expression of p63 in either SCC or bilharzial-associated bladder cancer.

Comparing the expression in the bilharzial- and non-bilharzial-associated TCC, there was a statistically significant decrease in strong and moderate immunoreactivity, and a statistically increase in negative and mild immunoreactivity, respectively ( $P = 0.03$ ).

All of the SCC cases were associated with bilharziasis; thus, when comparing the expression in the bilharzial-associated TCC to SCC, there was a statistically significant decrease in the immunoreactivity from strong and moderate in 12/15 of the TCC cases, to mild and negative in 10/15 of the SCC cases, respectively ( $P < 0.01$ ).

That the pattern of expression was higher in the bilharzial-associated TCC than in the non-bilharzial-associated TCC contradicts the results in group B, where there was a decrease in the immunoreactivity in the bilharzial cases, which might be attributed to the grade and stage of the tumour rather than bilharzial association. Also, the pattern of decreased immunoreactivity in the SCC (all associated with bilharziasis) in relation to the bilharzial-associated TCC is similar to that of the histopathological type, i.e. TCC vs. SCC and hence, it might be attributed to the histopathological type of the tumour rather than the bilharzial association. Hence, according to the present results, but because of the discrepancy in the number of patients in the different groups, we recommend more studies to address the effect of bilharziasis on the expression of p63 in the healthy and diseased bladders.

As to the expression in the apparently normal mucosa adjacent to the TCC tumours, the immunoreactivity was strong and moderate in six of seven and one of seven, and three of 10, four of eight and three of eight for urothelial hyperplasia, low-grade and high-grade dysplasia specimens, respectively. In the SCC patients, the immunoreactivity was strong and moderate in eight and two of 10, one and one of two, and two and one of three with squamous metaplasia, low-grade and high-grade dysplasia, respectively (Table 3).

Comparing the adjacent mucosa in the patients with TCC or SCC to that in the control patients, there was a significant decrease in both strong and moderate expression in the adjacent mucosa of patients with TCC ( $P < 0.01$ ), and in those with SCC, respectively ( $P = 0.02$ ; Table 3).

To our knowledge, this is the first study to investigate the pattern of p63 expression in the apparently normal mucosa adjacent to the malignant tumour, and these changes in the expression might imply a field change that can be used in the follow-up of resected non-muscle-invasive tumours.

In conclusion, although the histopathology of the tumour cells remains the standard method in the diagnosis of bladder cancer, the expression of p63 might have a role in assessing the pathogenesis and progression of bladder cancer. We recommend further research on p63 to confirm its usefulness as a predictor and as a prognostic marker. The diagnostic value of using p63 as part of the routine follow-up of patients after complete resection of high-risk non-muscle-invasive TCC needs to be accurately addressed. Also, additional studies are needed on the possibility of malignant transformation in patients with a bilharzial bladder.

### Conflict of interest

None.

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