Diagnostic and Prognostic Significance of Serum and Tissue Galectin 3 Expression in Patients with Carcinoma of the Bladder

Hoda El Gendy^a Bothina Madkour^b Sara Abdelaty^b Fayza Essawy^b
Dina Khattab^a Olfat Hammam^c Hani H. Nour^d

^aDepartment of Clinical and Chemical Pathology, Ain Shams University, Cairo; Department of ^bClinical and Chemical Pathology, ^cPathology and ^dUrology, Theodor Bilharz Research Institute, Guiza, Egypt

Key Words

Galectin 3 • Bladder cancer • Transitional cell carcinoma • Squamous cell carcinoma • Schistosomiasis

Abstract

Background: Galectins are group of proteins found in the cytoplasm, nucleus, cell surface and extracellular matrix. Galectin 3 (Gal-3) displays pathological expression in a variety of processes such as tumorigenesis. **Patients and Method:** 70 patients classified into the control group, cystitis group, transitional cell carcinoma group, and squamous cell carcinoma group were enrolled in this study which aimed to detect the serum level and the intensity of tissue expression of Gal-3. **Results:** Both serum level and tissue expression of Gal-3 were statistically higher in bladder cancer patients compared to the other groups. Gal-3 level expression increased from low to high grade urothelial tumors, with a statistically significant increase of its level and expression between muscle invasive and non-muscle invasive Ta urothelial tumors. **Conclusion:** The serum Gal-3 level is sensitive and specific for the diagnosis of bladder cancer. The prognostic significance of tissue expression is to be confirmed.

Introduction

Carcinoma of the bladder represents a worldwide health problem ranked ninth in cancer incidence [1]. Several urine and serum tumor markers have been studied for its diagnosis, yet histopathological examination of a transurethral resected bladder tumor is the corner stone in the diagnosis of bladder cancer allowing both histological diagnosis and tumor staging [2].

Radical cystectomy with urinary diversion is the treatment of choice for muscle invasive (MI) transitional cell carcinoma (TCC) of the bladder, together with other histological types of bladder cancer including squamous cell carcinoma (SCC) which account for about 40% of bladder cancer in Egypt [3]. It is also the treatment of choice for non-muscle invasive (NMI) TCC refractory to intravesical treatments. The overall tumor free 5-year survival following radical cystectomy (all stages combined) is around 60% with most of relapses occurring in the first 24 months [4].

Patients' prognosis depends independently on histological type, final pathological stage, and tumor grade. Several tissue markers have been studied to enhance the prognostic accuracy, but the results are conflicting [5].

Galectins are group of proteins identified in the cytoplasm, nucleus, cell surface, and extracellular matrix, which suggest that they have several functions. Studied

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showed that galectin-3 (Gal-3) plays a role in the regulation of various biological cycles including inflammation, cell growth, cell differentiation, cell adhesion, and apoptosis [6]. Gal-3 displays pathological expression in a variety of process including tumorigenesis e.g. colon and bladder cancer and inflammatory conditions e.g. rheumatoid arthritis, heart disease and stroke [7, 8]. The prognostic significance of Gal-3 expression in several human cancer tissues has been studied with intensity of expression correlating with tumor progression, invasiveness, and metastatic potential [9].

High circulating levels of Gal-3 were also found in the serum of patients with bladder cancer, and these levels correlate with the antiapoptotic activity of Gal-3 as a possible cause of carcinogenesis [10].

The aim of the work was to measure the serum Gal-3 level as well as its expression in bladder tissues of bladder cancer patients, patients with cystitis, and normal individuals. The results will test the potential role of serum Gal-3 in the diagnosis of bladder cancer. In addition, the prognostic significance of Gal-3 expression in different stages and grades of tumor will be evaluated as well as the effect of schistosomal infestation on its expression.

Patients and Method

This prospective non-randomized study followed the tenets of the Declaration of Helsinki (1975) and had the approval of the ethical committee of Theodor Bilharz Research Institute (TBRI). It included patients with radiologically diagnosed bladder growth enlisted for transurethral resection of bladder tumor (TURB) in the Urology Department of TBRI between November 2012 and January 2013, together with patients enlisted for cystoscopy for persistent lower urinary tract symptoms (LUTs). Ten patients served as a control. They had to undergo cystoscopy as a part of their urological workup and/or treatment. The final study included 70 patients, 58 males and 12 females with a median age of 58 years (range 30–84 years). Patients were divided into the control group (n = 10), cystitis group (n = 15), SCC group (n = 10), and TCC group (muscle invasive and non-invasive) (n = 35). After informed consent, a blood sample was taken from all patients the night before cystoscopy. Transurethral resection of bladder tumors was done to all patients with an apparent tumor, while cold cup biopsies were taken from bladder mucosa of patients with LUTs and the control group.

Histopathological evaluation was made by an uropathologist in the Pathology Department of TBRI according to the international histological classification of urinary bladder tumors proposed by the WHO in 2004. Immunohistochemical staining, scoring, and interpretation of Gal-3 was done by the same uropathologist. Expression of Gal-3 was evaluated using light microscopy at 400 x magnification and the percentage of the positively stained cells was calculated and scored according to Sakaki et al. [11] as:

- Weak expression (0–20% stained cells).

- Moderate expression (21–50% stained cells).
- Strong expression (51–100% stained cells).

About 2 ml of blood samples were collected under aseptic conditions the night before surgery and were delivered to a plain tube and allowed to clot. Then serum was aliquoted in clean vials and stored frozen at -20°C. Enzyme immunoassay technique was used for measurement of serum Gal-3 concentration – ELISA method (eBioscience, Vienna, Austria) [12].

Results were expressed as the median value. Comparison between different parameters in the two groups was done using the Mann-Whitney U test while comparison between the three groups was performed using the Kruskal-Wallis test followed by the Mann-Whitney U test. The Receiver Operating Characteristic (ROC) curve was used to determine the sensitivity and specificity of serum Gal-3. A P value less than 0.05 was considered significant and 0.01 was considered highly significant. Statistical analysis was performed with the aid of the SPSS computer program (version 16 windows).

Results

The clinicopathological distribution of study groups is showed in table 1. For an accurate evaluation of the Gal-3 serum level and tissue expression, patients with non-muscle invasive (NMI) TCC were classified according to the depth of invasion into patients with Ta tumors (n = 6) and patients with T1 tumors (n = 11). Associated schistosomal infestation based on the detection of schistosomal eggs in the tissues was present in 40% of the cases (28/70), 6 in the cystitis group, 7 in SCC group, and 15 in the TCC group.

The median serum Gal-3 level was 100 pg/ml and 380 pg/ml in the control and cystitis groups respectively, with a statistically significant increase in Gal-3 expression in the cystitis group. There were weak tissue expressions of Gal-3 in the control and cystitis groups. Bilharzial infestation did not affect the level or expression of Gal-3 in either the serum or tissue in the cystitis group (table2).

The serum Gal-3 concentration showed a highly significant increase in the TCC and SCC groups when compared to the control and cystitis groups ($p < 0.01$) with no statistical difference when the TCC group was compared to the SCC group ($p > 0.05$). There was moderate to strong tissue expression of Gal-3 in all bladder cancer patients either TCC or SCC in relation to the control and cystitis patients, with a significant increase of G3 expression in patients with SCC (fig. 1) in relation to patients with TCC ($p < 0.05$). Schistosomal infestation did not affect the results of serum Gal-3 level in patient with either TCC or SCC, but patients with bilharzial associated SCC showed a highly significant Gal-3 tissue expression in relation to TCC patients.

Table 1. Clinicopathological distribution

	Number	Sex	Age, median (range)	Bilharzial infestation, %
Control	10	M = 9, F = 1	56.5 (30–70)	0 (0%)
Cystitis	15	M = 12, F = 3	50 (10–80)	6 (40%)
SCC	10	M = 8, F = 2	50 (45–65)	7 (70%)
TCC	35	M = 29, F = 6	60 (45–84)	15 (42.8%)
NMI	17	M = 14, F = 3		
MI	18	M = 15, F = 3		
TCC	35	M = 29, F = 6	60 (45–84)	
Low grade	17	M = 13, F = 4		
High Grade	18	M = 16, F = 2		
Total	70	M = 58, F = 12	58 (30–84)	28 (40%)

M = Male; F = female; SCC = squamous cell carcinoma; TCC = transitional cell carcinoma; NMI = non-muscle invasive; MI = muscle invasive.

Table 2. Clinicopathological distribution

	Number	Gal-3 expression (%), median (range)	Gal-3 (pg/ml), median (range)
Control	10	0 (0–10)	100 (50–220)
Cystitis	15	10 (0–20)	380 (142–1,200)
SCC	10	80 (70–90 ^a)	925 (520–3,200 ^{ac})
TCC	35	55 (21–95 ^a)	720 (500–4,000 ^a)
Ta	6	32.5 (21–40)	515 (515–3,120 ^a)
T1	11	50 (40–90 ^a)	1250 (500–2,080 ^a)
T2–3	18	70 (45–95 ^{ab})	840 (530–4,000 ^{ab})
TCC	35	55 (21–95)	720 (500–4,000)
Low grade	18	50 (21–90)	590 (500–3,160)
High Grade	17	70 (45–95 ^c)	840 (530–4,000 ^c)

^ap < 0.05 relative to normal urothelium group; ^bp < 0.05 relative to Ta group; ^cp < 0.05 relative to TCC; *p < 0.05 = significant.

Both serum Gal-3 level and tissue Gal-3 expression were statistically higher in muscle invasive tumors compared to papillary non-invasive tumors (Ta) ($p < 0.05$), while there was no statistical significance observed between muscle invasive and tumors that invaded sub-epithelial connective tissue (T1) nor between papillary non-invasive tumors (Ta) and (T1) ($p > 0.05$). From low to high grade TCC, there was a statistically significant increase in the serum and tissue level of Gal-3 expression ($p < 0.05$).

ROC curve analysis in order to detect a cutoff serum Gal-3 level in the diagnosis of bladder cancer in relation to normal controls and cystitis patients was applied. The level of serum Gal-3 at cutoff point ≥ 220 pg/ml provided

the best specificity (100%) for bladder cancer diagnosis in relation to normal urothelium (either TCC or SCC) with (100%) sensitivity and (1.00%) diagnostic accuracy (fig. 2). A cutoff level of > 420 pg/ml provided a 73% specificity and 100% sensitivity (0.957 diagnostic accuracy) for discrimination of bladder cancer patients from patients with cystitis (fig. 3).

Discussion

In 2010, bladder cancer was responsible for about 170,000 deaths, with more than 55% of newly diagnosed bladder cancer patients occurring in developed countries

Fig. 1. Poorly differentiated squamous cell carcinoma, GIII, with strong expression of Gal-3 monoclonal antibody (IHC, DAB x200).

Fig. 2. ROC curve between control and bladder cancer.

[13]. This rate is rapidly changing as bladder cancer is rapidly rising in underdeveloped countries where industrialization has led to carcinogenic exposure [14]. Among the Egyptian population, bladder cancer accounts for 29.8% of all malignant diseases and it is the most common cancer among males [14].

Fig. 3. ROC curve between cystitis and bladder cancer.

In a trial to improve the detection rate of bladder cancer using non-invasive techniques, several tumor markers were studied including detection of urinary bladder tumor antigen (BTA), nuclear matrix protein 22 (NMP-22), fluorescence in situ hybridization DNA probe technology, serum carcinoembryonic antigen (CEA), and carbohydrate-antigen 19.9 (CA19.9) [15, 16]. But none of these have taken the place of urine cytology which showed up to 85% sensitivity and 87% specificity in high grade tumors [17].

Several tissues markers were tested for their prognostic significance, yet tumor stage and grade together with histological type are still the most reliable prognostic factors upon which patient's prognosis and the need for adjuvant treatment post radical cystectomy depends [5].

Gal-3 is a member of the galactin gene family that is expressed at elevated levels in a variety of neoplastic cell types and has been associated with cell growth, cellular adhesion process, cell proliferation, transformation, metastasis, and apoptosis [18]. It can be expressed in the nucleus, cytoplasm and on the cell surface, and furthermore it can be secreted into the stroma [8]. Gal-3 is involved in the regulation of inflammatory processes through its role in macrophage activation and migration [19].

In this study the serum Gal-3 in bladder cancer patients either TCC or SCC (all stage combined) was statistically higher than in the control group ($p < 0.05$) and cystitis patients ($p < 0.05$), where the median value of

Gal-3 in serum was 840 pg/ml, 100 pg/ml, and 380 pg/ml in bladder cancer, the control group, and cystitis patients respectively. Schistosomal infestation did not show any statistically significant value. These results confirm the diagnostic utility of serum Gal-3 in patients with bladder cancer as demonstrated by Waalkes et al. [6], who found that patients with a bladder tumor showed an elevated serum level of Gal-3. No statistical difference was found in Gal-3 serum level between TCC and SCC.

Immunohistochemical staining of Gal-3 showed a statistically significant increase of Gal-3 expression in bladder cancer patients (TCC and SCC) in relation to control and cystitis patients ($p < 0.05$), which confirmed the work of Sakaki et al. [11], but in his study the effect of urothelial inflammation was not taken into consideration.

Patients with SCC showed a statistically significant higher Gal-3 expression in relation to patients with TCC. This may be attributed to schistosomal infestation which induced an immune response present in approximately 70% of SCC patients in comparison to only 43% in TCC patients [20]. The role of Gal-3 in inducing an immune reaction was reported by Sano et al. in 2010 [12].

In 2008, Sakaki et al. [11] reported no significant statistical difference in serum Gal-3 levels in patients with NMI and MI TCC of the bladder. In our study, the serum Gal-3 level and tissue Gal-3 expression were statistically higher in patient with MI TCC in comparison to Ta tumors. However, this significance was lost when comparing MI tumors to tumors invading the lamina propria (T1), and also when comparing T1 to Ta. This may be because that in our study we separately investigated Gal-3 levels and expression in Ta, and T1 patients, but Kramer et al. [21] were not able to demonstrate a difference in Gal-3 expression between Ta and T1 tumors. We propose 2 hypotheses for this: either these tumors were muscle invasive and we had understaged them, or they represent an aggressive form of T1. The prognostic significance of Gal-3 expression in T1 tumors must be evaluated as it may help identifying aggressive T1 tumors which may need early radical treatment.

There was a statistically significant increase of serum Gal-3 level and Gal-3 tissue expression from low to high grade TCC of the bladder confirming the results of Canesin et al. [22]. However, Sakaki et al. [11] failed to demonstrate this relation. The present results need to be validated by large scale studies taking into consideration the limitations of the present study to confirm this prognostic significance.

In an attempt to evaluate the diagnostic performance of serum Gal-3 as a diagnostic marker in bladder cancer

patients, we found that the level of Gal-3 at a cutoff point of 420 pg/ml provided the best sensitivity (100%) and specificity (73.3%) for discriminating bladder cancer patients from patients with cystitis.

This study had several limitations. Although it was prospective, it was a non-randomized study, as it included patient with first time diagnosed bladder cancer we did not study the pattern of Gal-3 level in patients who received intravesical treatment for NMI TCC.

Conclusion

In our study we evaluated the level of Gal-3 in serum of patients with different types of bladder cancer and compared them to normal individuals. The serum Gal-3 level proved to be as highly sensitive as specific for the diagnosis of both TCC and SCC of the bladder.

Tissue expression of Gal-3 was higher in patients with bladder cancer in relation to normal urothelium. Bilharzial infestation seen more in SCC, may be responsible for the statistically higher expression of Gal-3 in patients with SCC in relation to TCC. As high grade TCC exhibits more Gal-3 tissue expression, its prognostic value needs to be confirmed.

A large scale randomized study needs to be done and it should include a separate group for patients previously treated for NMI bladder cancer. A close follow-up should be given to patients with T1 TCC and high levels of serum Gal-3 as they may be either under-staged or represent an aggressive tumor with high probability of progression.

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