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Urological Research

Beta-D-glucan concentrations detected by Toxicolor and Endospecy tests in the urine of patients with urinary fungal infections

Tetsuro Matsumoto, Masashi Haraoka, Shuta Kubo, Koichi Takahashi, Masatoshi Tanaka, Nobuo Ogata, Joichi Kumazawa

Department of Urology, Faculty of Medicine, Kyushu University, Fukuoka, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812, Japan

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Summary. Beta-D-glucan is an essential component of the cell wall of fungi. We measured its concentration in the urine of patients with funguria using the chromogenic endotoxin assay kits, Toxicolor and Endospecy. These assay systems use the same *Limulus* coagulation enzymes. Since the Endospecy test detects endotoxin but not factor G, which is activated by beta-D-glucan, the beta-D-glucan concentration can be calculated by subtracting the Endospecy value from the Toxicolor value. Concentrations of beta-D-glucan were found to be significantly higher in urine samples from patients with funguria ($\geq 10^3$ colony-forming units/ml) than in non-infected samples.

Key words: Beta-D-glucan, Endospecy – Endotoxin G – Funguria – Toxicolor

Fungal infections of the genitourinary tract have shown a recent increase in incidence. The increase in both systemic and genitourinary fungal infections is attributable in part to the widespread use of antibiotics, corticosteroids, immunosuppressive drugs and antineoplastic agents, as well as to the ability to prolong the lives of patients with serious underlying diseases whose immunity is compromised [4, 7].

In addition to the conventional culture method, some diagnostic methods are now available for detecting fungal infections, such as the Labofit test for D-arabinitol and the CAND-TEC test for *Candida* antigen. Beta-D-glucan, an essential component of the cell wall of fungi, is an important finding for the diagnosis of fungal infections.

Chromogenic endotoxin assay kits have been developed and are now commercially available. The Toxicolor test uses recombined *limulus* coagulation enzymes that include factor G, which is activated by 1,3-beta-D-glucan. The Endospecy test, which uses the same recombined *Limulus* coagulation enzymes without factor G, detects endotoxin only (Fig. 1) [5]. Therefore, beta-D-glucan concentration can be calculated by subtracting the Endospecy value from the Toxicolor value. This value, known as the Fungal Index, is one of the diagnostic indicators of a deep-seated fungal infection. A significant value of this index is reported to be more than 20 or 60 [1].

We measured the urinary concentrations of beta-D-glucan using this assay system, and discuss here the significance of such measurements in cases of fungal urinary tract infections.

Materials and methods

Patients and urine samples

We evaluated 62 urine samples obtained from 38 patients who were attending an outpatient clinic for the diagnosis and treatment of a urinary tract infection caused by fungi. A clean-voided midstream urine specimen (10–50 ml) was collected from each patient and divided into three glass tubes previously heated to 250°C to avoid contamination with endotoxin. One sample per patient was evaluated by the Toxicolor test, one by the Endospecy test, and one by a conventional culture method using the dip-slide technique (BCB slide; Roche, Basel, Switzerland). The fungal count was considered to be significant if it exceeded 10^3 colony-forming units (cfu)/ml.

Toxicolor and Endospecy tests

The endotoxin-specific chromogenic assay was performed as described below [5]. A factor-G-containing kit, the Toxicolor (TX) test (Seikagaku Kogyo, Tokyo, Japan), and a factor-G-free endotoxin assay kit, the Endospecy (ES) test (Seikagaku Kogyo, Tokyo, Japan), both of which contain the chromogenic substrate Boc-Leu-Gly-Arg-pNA, were used (Fig. 1). A volume of 0.1 ml urine was added to 0.1 ml of the test kit and dissolved in TRIS-HCl buffer, 0.2 mmol·l^{-1}, pH 8.0. This mixture was incubated at 37°C for 30 min. Absorbance was measured at 545 nm using a double-beam spectrophotometer following diazotrization to avoid interference by yellow pigment. This method takes only 40–50 min. Since the

Correspondence to: Tetsuro Matsumoto, MD, PhD; FAX: 81/92 651-0165

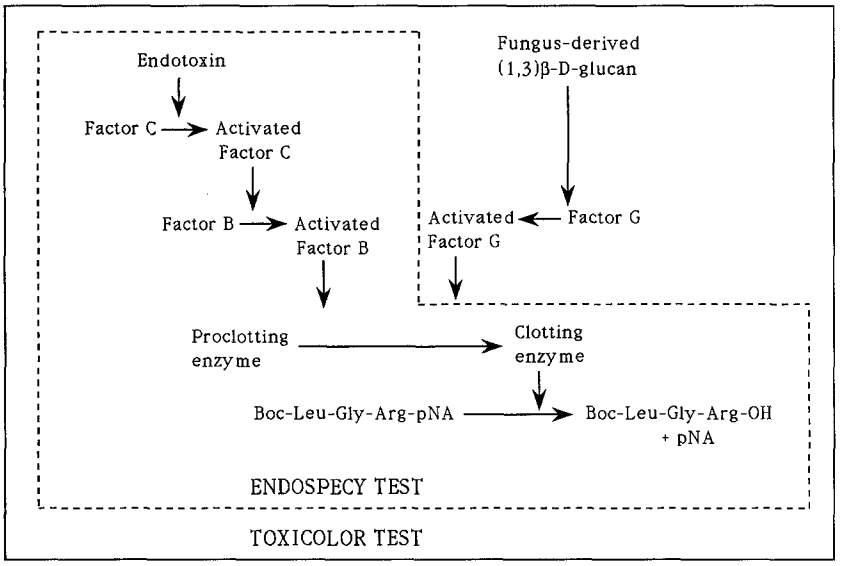

Fig. 1. Schema of detection of fungus-derived beta-D-glucan by the Endospecy and Toxicolor tests

Table 1. Strains of fungi isolated from urine

Species	Number of isolates		Total
	Pure infection	Mixed bacterial + fungal	
Candida albicans	6	4	10
Candida glabrata	5	8	13
Candida lusitaniae	1	0	1
Candida parapsilosis	1	2	3
Candida tropicalis	2	2	4
Candida spp.	2	2	4
Trichosporon spp.	2	2	4
Total	19	20	39

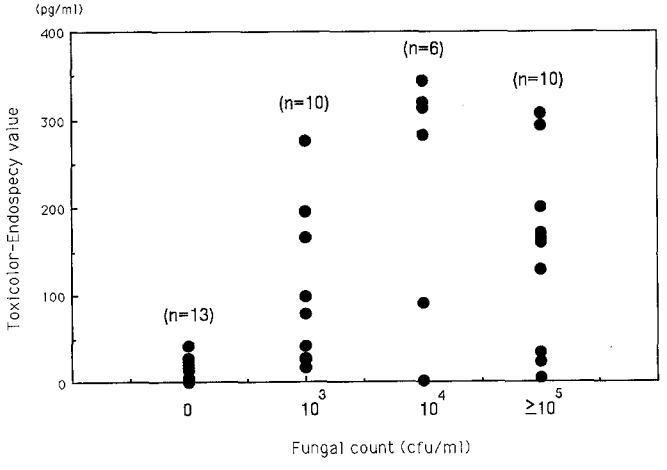

Fig. 2. Relationship between the urinary beta-D-glucan concentrations (calculated by subtracting the Endospecy value from the Toxicolor value) and fungal count in urine samples with pure funguria

maximum value of both the TX and ES tests was 350 pg/ml, any amount in excess of this could not be quantified. Both tests exceeded 350 pg/ml in samples with significant Gram-negative bacteriuria ($\geq 10^4$ cfu/ml) or contamination with endotoxin. In this study, samples with a value of more than 350 pg/ml were not included.

Statistical method

Statistical analysis was performed using Student's *t*-test; differences were considered to be significant at a probability level of < 0.05.

Results

Strains of fungi isolated from urine

Thirty-nine strains of fungi were isolated from 62 urine samples from 38 patients. *Candida* species ($n = 35$) was the most common; four strains of *Trichosporon* species were also isolated (Table 1). Thirteen urine samples in which neither bacteria nor fungi were found as controls. The culture method detected fungi only in 26 samples, and a mixed bacterial and fungal infection in 23.

Relationship between beta-D-glucan concentration and fungal count in pure fungal infections

Figure 2 shows the beta-D-glucan concentrations in 26 urine samples with pure fungal infections. Urine samples with funguria greater than 10^3 cfu/ml showed higher values than the non-infected controls. Table 2 gives the mean values of the TX test, ES test and Fungal Index in control samples and samples with fungal counts of 10^3, 10^4 and $\geq 10^5$ cfu/ml. TX values were significantly higher in samples with fungal counts of 10^3, 10^4 and $\geq 10^5$ cfu/ml compared with the controls. Mean concentration of beta-D-glucan measured by the ES test did not differ significantly among samples with differing fungal counts. The

Table 2. Concentration of beta-D-glucan in the urine samples from patients with pure funguria

Fungal count	Bacterial count	Sample No. (*n*)	Concentration (pg/ml)		
			Toxicolor	Endospecy	Fungal Index
–	$\leq 10^3$	13	19.8± 11.7	8.3± 5.5	15.9± 12.3
10^3	$\leq 10^3$	10	106.0± 89.9[a]	3.8± 3.4	111.7± 89.7[a]
10^4	$\leq 10^3$	6	240.0±145.6[a]	17.6±19.5	225.8±143.1[a]
$\geq 10^5$	$\leq 10^3$	10	155.1±108.8[a]	5.0± 6.3	149.1±105.5[a]

[a] $P<0.01$

Table 3. Concentrations of beta-D-glucan in urine samples from patients with mixed fungal and bacterial infection

Fungal count	Bacterial count	Sample no. (*n*)	Concentration (pg/ml)		
			Toxicolor	Endospecy	Fungal Index
–	$\geq 10^4$	13	19.8± 11.7	8.3± 5.5	15.9± 12.3
10^3	$\geq 10^4$	10	99.1± 79.9[a]	4.1± 4.1	76.0± 76.4[a]
10^4	$\geq 10^4$	5	168.1± 50.3[b]	6.8± 3.3	133.5±111.4[b]
$\geq 10^5$	$\geq 10^4$	8	155.4±101.1[b]	12.8±11.7	142.6±101.2[b]

[a] $P<0.05$; [b] $P<0.01$

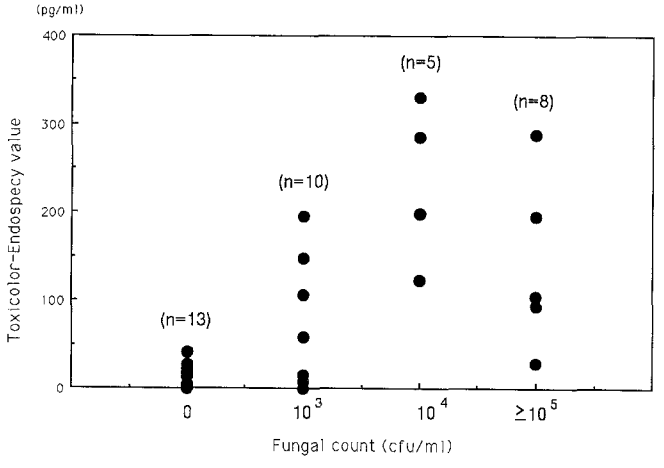

Fig. 3. Relationship between the urinary beta-D-glucan concentrations (calculated by subtracting the Endospecy value from the Toxicolor value) and fungal count in urine samples with a mixed infection of fungi and bacteria

beta-D-glucan concentration was significantly higher in urine samples with counts exceeding 10^3 cfu/ml than in non-infected controls.

Relationship between beta-D-glucan concentration and fungal count in mixed fungal and bacterial infections

Figure 3 shows the beta-D-glucan concentrations in 23 urine samples with mixed bacterial and fungal infections. Samples with mixed infections had higher beta-D-glucan concentrations than non-infected controls. Table 3 gives the mean values of the TX test, ES test and Fungal Index of samples with mixed infections. TX values were significantly higher in samples with counts of 10^3 cfu/ml or more. There were no significant differences in the ES test values among the different groups. Beta-D-glucan concentrations, calculated by subtracting the ES value from the TX value, were significantly higher in samples with counts exceeding 10^3 cfu/ml.

Discussion

Fungal infection of the urinary tract is poorly understood. The reported incidence of funguria in routinely collected clean urine specimens has ranged from 0 to 4% [4]. It has been suggested that a count of greater than 10^3 or 10^4 cfu/ml indicates a significant infection [2, 6]. Fungal counts are routinely detected by the conventional culture method, which requires more than 24 h before results are known. The TX and ES tests, which detect endotoxin, are now commercially available. The TX test uses recombined *Limulus* coagulation enzymes including factor G, whereas the ES test does not include factor G. Therefore, subtracting the ES value from the TX test value provides a value for the concentration of beta-D-glucan. This method requires only 40–50 min for the detection of funguria.

We measured the beta-D-glucan concentration in urine samples with fungal infections. Concentrations were significantly higher in urine samples from patients with more than 10^3 cfu/ml of fungi than in samples from non-

infected controls. These findings indicate that the detection of beta-D-glucan provides a means for the rapid diagnosis of any fungal infection of the urinary tract.

However, TX and ES tests do not indicate the true extent of funguria when the urine or test tubes are heavily contaminated with endotoxin. Sampling of urine and decontamination of test tubes are therefore both important when detecting funguria by this method. We previously reported that the endotoxin concentration detected by the ES test was not elevated in urine samples with 0–10^3 cfu/ml of Gram-negative bacteriuria [3]. Here we considered the level of bacteriuria to be significant when more than 10^4 cfu/ml of bacteria were detected.

Beta-D-glucan concentrations were significantly higher in the urine samples from patients with funguria than in the non-infected controls. The Toxicolor and Endospecy tests are both rapid, simple methods for detecting fungal and bacterial urinary tract infections.

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