COXSACKIE VIRUS AND UROGENITAL PATHOLOGY

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Summary: In 43 women (average age 49.6 years), and 70 men (average age 55.2 years) with pathology of the genito-urinary apparatus, seroantibodies to Coxsachie virus B were measured using the passive hemoagglutination method and the virus was isolated in the uterine. Viral isolation test was negative in all urine samples tested. Seropositivity for Coxsackie virus was reported in 26 women (60.46%) and in 51 men (72.85%). Positivity to B1 was 37.16% (42 cases), B2 in 38.05% (43 cases), and to B4 in 35.39% (40 cases). 17.69% (20 cases) of patients were seropositive to only one serotype, 17.69% (20 cases) to 5 serotypes, 14.15% (16 cases) to 3 serotypes, 9.73% (11 cases) to 2 serotypes, and 8.84% (10 cases) to 4 serotypes. B1, 2, 3, 4, 5 (25.97%; 20 cases) and B2, 3, 4, 5 (7.79%; 6 cases) were the most frequent associations. Seropositivity to Coxsackie virus was reported in 100% of patients (4 cases) with urethral caruncola, in 84.21% (16 cases out of 19) with cancer of the bladder, in 81.81% with cystitis (9 cases out of 11) and in 80% with prostatitis (8 out of 10 cases). In relation to sex, seropositivity was higher in males in cases of calculosis (75%; 9 cases out of 12 against 28.57%; 2 cases out of 7) and in cystitis (100%; 6 cases against 60%; 3 cases out of 5). Further studies are necessary to determine the clinical significance of serum Coxsackie virus antibodies in patients with urological pathology in the absence of urinary elimination of Coxsackie virus.

Key words: coxsackie; virus; cystitis; bladder calculosis; cancer.

INTRODUCTION

Correlation between the Coxsackie virus and pathology of the urogenital tract has been hypothesized from the observation that some Coxsackie type B viruses multiply in the renal tissue of newborn mice, dogs and monkeys ([1, 2, 3, 4, 5]).

In 1960 Papperheimer *et al.*, demonstrated that the Coxsackie virus causes renal lesions in man, and subsequently Benyesh-Melnick *et al.* ([6]), demonstrated the presence of Coxsackie B1 antigen in urinary tract epithelium present in the urinary sediment of patients who died of aseptic meningitis caused by type B1 Coxsackie. Date *et al.*, ([7]) demonstrated that 7 out of 20 sera of patients with non systemic glomerular disease had a high Coxsackie B2 or B4 antibody titres, and Dunnet *et al.*, ([8]) reported 3 patients with acute renal failure after rhabdomyolysis due to Coxsackie virus infection; similar observations were made by Aronson and Phillips ([9]), and Austin and Ray ([10]), Bayatpour *et al.*, ([11]), and Birch ([12]) reported an immune-complex mediated acute glomerulonephritis in Coxsackie B4 infections. Coxsackie virus infections has also been described in patients with renal transplantation ([13]).

Brown and Karunas ([14]) have demonstrated that Coxsackie B2 and B4 were associated with fetal urogenital anomalies. Other studies have shown a possible correlation between orchitis or orchiepididymitis and infection from types B2 and B3 coxsackie virus which were in large part reported as complications of Bornholm's disease ([4, 15, 16, 17, 18, 19, 20, 21, 22]).

The aim of this study was to evaluate positivity of type B Coxsackie virus in pa-

tients admitted to the Urology Institute of Catania University Medical School, Catania, Italy who were affected by pathology of the urogenital apparatus.

MATERIALS AND METHODS

113 patients (43 women and 70 men) affected by pathologies of various etioilogies of the urogenital apparatus were involved in this study. These patients were admitted to the Urology Institute of Catania University Medical School, Catania, Italy, during 1987.

Serum and urine samples were obtained from all patients. The urine was immediately buffered to pH 7.2±0.2 and inoculated with LLCMK2 monkey renal cells for viral isolation. In cases where contamination was detected immediately the urine was conserved at −20° C. Qualitative analysis was performed on the serum samples to detect the presence of coxsackie B1, B2, B3, B4, and B5 antibodies utilizing passive or indirect hemoagglutinization.

This technique is based on the surface sensitization of erythrocytes with viral antigens. When sensitized red cells come into contact with specific antibodies for the sensitized antigen the red cells passively agglutinate. In some agglutination may be caused by antigens common to more than one type of Coxsackie virus, a characteristic common in the enterovirus.

Human type O red cells were sensitized by suspending an equal volume of erythrocytes, viral suspension of each type of Coxsackie, and a solution of 1% $CrCl_3$ diluted at 1:20 in physiologic solution. This mixture was left to react for 5 minutes and then repeatedly washed in saline phosphate buffer (PBC) and pH 7.2±0.2, being centrifuged in between washes.

Red cells in the sediment were removed and resuspended in 1% PBS containing 0.5% bovine albumin and 0.1% gelatin (PBS-A-G). The sera were diluted 1:10 in PBS-A-G and 25 ml of the solution was placed in the culture plate wells, the bottom of which were shaped in the form of a V.

Twentyfive ml. of sensitized erythrocytes was added to each well and this culture plate was incubated for one night at room temperature. The following day agglutination was evaluated using a system based on arbitrary evaluation: − (negative), ± (borderline), + (low positive), ++ (medium positive), and +++ (high positive). The patients were divided into seronegative (−) and seropositive (±, +, ++, and +++).

Statistical analysis of the results was performed utilizing the chi square test and p values ≤ 0.05 were considered significant.

RESULTS

Of the 113 subjects examined, 77 (68.14%) were seropositive for type B Coxsackie antibodies Of these 26 (60.46%) were from the group of 43 women, and 51 (72.85%) were from the group of 70 men (Table 1).

Table 1. – *Seropositivity for Coxsackie B. virus in sera and urine of 113 patients admitted to the Urology Institute of Catania. University Medical School, between January and March 1987.*

	female	male	total
Cases	43	70	113
Age	49.6 (range 16 to 81)	55.2 (range 9 to 88)	53.3 (range 9 to 88)
Coxsackie-positivity	26 (60.46%) *	51 (72.85%) *	77 (68.14%)

* $p > 0.05$

The average age of the women was 49.6 years (range 16 to 81), while the average age of the men was 55.2 (range 9 to 88 years).

The viral isolation test was negative in all urine samples tested.

Table 2 shows that males are more frequently positive to B1, B2, and B5 virus; in all patients positivity to the B5 virus was 47.78% (54 cases) and to B3 43.13% (51 cases); positivity to B1 was 37.16% (42 cases), to B2 38.05% (43 cases), and to B4 35.39% (40 cases).

Table 2. – *Frequency of seropositivity to Coxsackie B1, B2, B3, B4, B5 virus.*

Serotype	female		male		total	
	43 cases		70 cases		113 cases	
	cases	%	cases	%	cases	%
B1	12	27.90	30	42.85	42	37.16
B2	15	34.88	28	40.00	43	38.05
B3	17	39.53	34	48.57	51	45.13
B4	15	34.88	25	35.71	40	35.39
B5	18	41.86	36	51.42	54	47.78

17.69% (20 cases) of all patients were seropositive to only one serotype, 17.69% (20 cases) to 5 serotypes, 14.15% (16 cases) to 3 serotypes, 9.73% (11 cases) to 2 serotypes, and 8.84% (10 cases) to 4 serotypes (Table 3).

Table 3. – *Seropositivity combination for 1 or more serotypes of Coxsackie B virus in relation to sex.*

Positivity to	female		male		total	
	43 cases		70 cases		113 cases	
	cases	%	cases	%	cases	%
1 serotype	7	16.27	13	18.57	20	17.69
2 serotype	4	9.30	7	10.00	11	9.73
3 serotype	5	11.62	11	15.71	16	14.15
4 serotype	3	6.97	7	10.00	10	8.84
5 serotype	7	16.27	13	18.57	20	17.69

Table 4a and 4b compare positivity to one or more types of Coxsackie virus in relation to pathology diagnosed in the two sexes. Seropositivity to Coxsackie virus was reported in 100% of patients (4 cases) with urethral caruncola, in 84.21% (16 cases out of 19) with bladder cancer, in 81.81% with cystitis (9 cases out of 11) and in 80% with prostatitis (8 out of 10 cases). There is an evident association between cancer of the bladder and B4, and B5 serocoxsackie virus positivity.

Table 4 a. – *Pathology of the upper and lower urinary tract in relation to seropositivity of female subjects.*

Pathology of lower Urinary tract	Total cases	Sero-positive cases	Positivity to				
			B1	B2	B3	B4	B5
Pyelonephritis	6	2	1	–	1	–	2
Renal or ureteral calculosis	14	7	4	3	4	4	4
Other	12	9	4	4	5	4	4
Cystitis	11	9	7	5	8	7	7
Cancer of the bladder	19	16	9	10	13	12*	15**
Bladder calculosis	5	4	4	2	3	2	3
Prostatitis	10	8	3	2	5	–	6
Other	28	18	9	12	14	14	13

* $p = 0.001$
** $p < 0.001$

Table 4. b. – *Pathology of the upper and lower urinary tract in relation to seropositivity of male subjects.*

Pathology of lower Urinary tract	Total cases	Sero-positive cases	Positivity to				
			B1	B2	B3	B4	B5
Pyelonephritis	1	1	1	–	1	–	1
Renal or ureteral calculosis	7	5	2	1	2	2	2
Other	2	1	1	1	1	–	–
Cystitis	6	6	4	3	6	5	5
Bladder calculosis	5	4	4	2	3	2	3
Cancer of the bladder	16	13	7	8	11	10*	12**
Prostatitis	10	8	3	2	5	–	6
Prostatic adenoma	27	22	14	13	14	7	16
Other	14	8	6	5	6	6	6

* $p = 0.001$
** $p < 0.001$

The category "other" comprises various pathologies which occured in numbers to low to categorize them separately (varicocoele, cystocoele, stress incontinence, urethral caruncola, and urinary tuberculosis).

DISCUSSION

68.14% of the subjects in our study were seropositive for type B Coxsackie virus with a higher frequency in males (72.85%) compared to females (60.46%) ($p > 0.005$). Failure to isolate the virus in urine samples indicated the absence of active elimination of the virus in uterine.

The high seropositivity rate was consistent with data obtained in a similar study of 400 pregnant women admitted to the

First Clinic of Obstetrics and Gynecology of Catania University Medical School, Catania, Italy (56.75%; 227 cases) ([23]).

Seropositivity was present in all age groups with a higher frequency in the 51 to 80 year old group and the seropositivity was usually for more than one type of Coxsackie virus, probably due to the antigen that the various Coxsackie virus types have in common.

We have reported a significant association between some pathologies of the urinary tract (calculosis and cancer of the bladder) and seropositivity to the Coxsackie virus, but failure to isolate the virus in the urine samples indicates the necessity for further studies. Patients with neoplasia, i.e, are immunodepressed and thus more frequently subject to infections. For a correct evaluation of the clinical significance of our data the modification of serum antibody concentrations must be considered in follow-up research projects, while the isolation of the virus from other body fluids, as well as feces, is necessary.

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