PREVALENCE OF COXSACKIEVIRUS B ANTIBODY IN PATIENTS WITH SUSPECTED RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE

L Tay
M Yin-Murphy
P H Chua
L H Koh

SYNOPSIS

A preliminary study was carried out to determine the prevalence and to evaluate the significance of coxsackievirus B antibody in sera from 332 patients suspected of having rheumatic fever. Results showed that 71.3% of 160 patients with carditis and 61.5% of 169 patients without carditis were found to have antibody titres of ≥ 16 coxsackievirus B. 46.9% of carditis patients had coxsackievirus B neutralising antibody titres of 64 to ≥ 256 compared to 16.0% of patients without carditis. It was also noted that 18.8% of patients with carditis had both antistreptolysin 0 titres of ≥ 200 Todd units and coxsackievirus B antibody titres of ≥ 64 compared to 3.0% of patients without carditis. The marked increase in the number of patients with carditis manifesting high coxsackievirus antibody titres of ≥ 64 suggestive of recent or current infection with this agent, supports the contention that a viral etiology should also be considered in patients with symptoms and signs of "Rheumatic Heart Disease".

INTRODUCTION

Acquired valvular heart disease is widely thought to be associated with rheumatic fever (RF), a sequel to group A streptococcal infection. Epidemiological studies on the relationships of streptococcal infection to RF showed that approximately half of the patients examined have no history of an acute streptococcal infection or RF preceeding carditis. The fact that viruses can cause illness with symptoms and signs similar to those described for streptococcal RF and RHD is well known. The majority of pharyngitis is caused by viruses some of which particularly the coxsackievirus B are frequently associated with human carditis. Apart from pericarditis and myocarditis, acute valvulitis and mural endocarditis often accompanied coxsackievirus B infections (1 — 10).

Coxsackievirus B antigens were demonstrated in cardiac myocytes and fibrocytes of the valves, pericardium, myocardium and endocardium in autopsy specimens (11). Furthermore, coxsackie B viruses readily produce cardiac lesions in monkeys and mice resembling those seen in man (12 — 17). Data supporting a virus and streptococcal relationship in carditis where the streptococcus acts as a conditioning factor exerting an immune suppressed effect which enables the release and reactivation of latent viruses in valvular and myocardial lesions have also been recorded (18, 19).

A study was performed to determine the prevalence of coxsackievirus B in parallel with antistreptolysin 0 (ASO) antibody in patients with suspected streptococcal RF with and without carditis, whose sera were sent for laboratory confirmation of preceeding streptococcal group A infection, and to evalute the significance of coxsackievirus B in association with these illnesses.

Bacteriology Section
Department of Pathology
Singapore General Hospital
Outram Road
Singapore 0316

L Tay, MBBS, Dip Bact
Bacteriologist

L H Koh
Laboratory Technician

Department of Microbiology
Faculty of Medicine
National University of Singapore
Kent Ridge
Singapore 0511

M Yin-Murphy, MA, PhD, Dip Bact
Associate Professor

P H Chua
Laboratory Technician

Table 1a. Prevalence of Coxsackievirus B Antibody in Patients with Carditis

			Male		Female		Total	
			No.	%	No.	%	No.	%
<16* CB1 to CB6			25	15.6	21	13.1	46	28.8
Antibody Titre	16 — 32	CB1	5	3.1	6	3.8	11	6.9
		CB2	15	9.4	11	6.9	26	16.3
		CB3	13	8.1	9	5.6	22	13.7
		CB4	18	11.3	15	9.4	33	20.6
		CB5	6	3.8	6	3.8	12	7.6
		CB6	2	1.3	5	3.1	7	4.4
	64 — ≥ 256	CB1	3	1.9	4	2.5	7	4.4
		CB2	17	10.6	29	18.1	46	28.8
		CB3	5	3.1	4	2.5	9	5.6
		CB4	24	15.0	23	14.4	47	29.4
		CB5	1	0.6	0	0	1	0.6
		CB6	0	0	0	0	0	0
(a)	16 — ≥ 256 >1CB**		28	17.5	36	22.5	64	40.0
(b)	16 — 32 1CB		18	11.3	10	6.3	28	17.5
(c)	64 — ≥ 256 1CB		11	6.9	11	6.9	22	13.8
Total of (a) + (b) + (c)			57	35.6	57	35.6	114	71.3

< 16* = Reciprocal of serum dilution
>1CB** = More than one coxsackievirus B type

Table 1b. Prevalence of Coxsackievirus B Antibody in Patients without Carditis

			Male		Female		Total	
			No.	%	No.	%	No.	%
<16* CB1 to CB6			32	18.9	33	19.5	65	38.5
Antibody Titre	16 — 32	CB1	3	1.8	4	2.4	7	4.2
		CB2	32	18.9	21	12.4	53	31.4
		CB3	15	8.9	5	3.0	20	11.8
		CB4	31	18.3	24	14.2	55	32.5
		CB5	5	3.0	2	1.2	7	4.1
		CB6	2	1.2	1	0.6	3	1.8
	64 — ≥ 256	CB1	0	0	0	0	0	0
		CB2	4	2.4	3	1.8	7	4.2
		CB3	2	1.2	1	0.6	3	1.8
		CB4	13	7.7	6	3.6	19	11.2
		CB5	0	0	1	0.6	1	0.6
		CB6	0	0	0	0	0	0
(a)	16 — ≥ 256 >1CB**		33	19.5	20	11.8	53	31.4
(b)	16 — 32 1CB		25	14.8	14	8.3	39	23.1
(c)	64 — ≥ 256 1CB		7	4.1	5	3.0	12	7.1
Total of (a) + (b) + (c)			65	38.5	39	23.1	104	61.5

<16* = Reciprocal of serum dilution
>1CB** = More than one Coxsackievirus B type

MATERIALS AND METHODS

Serology

332 sera submitted to the Bacteriology Section, Government Department of Pathology, were separated into two groups. One group comprised of sera from patients with suspectd rheumatic carditis such as pericarditis, myocarditis, valvulitis and endocarditis. The second group included patients with suspected rheumatic fever but without signs of carditis.

Each serum was divided into two aliquots, one for ASO determination by the spectrophotometric method based on modification of Liao's method (20), and the other for estimation of neutralising antibody to coxsackievirus B1 to B6 by micrometabolic-inhibition test (21). Sera for ASO were tested from 1/100 to 1/1600 serum dilutions. Sera for coxsackievirus B antibody were titrated from 1/16 to 1/256 serum dilutions.

RESULTS

The prevalence of type specific neutralising antibody to coxsackievirus B1 to B6 in patients with carditis and patients without carditis is shown in Tables, 1a, 1b and 1c. A 71.3% of 160 carditis patients and 61.5% of 169 patients without carditis had antibody of $\geq$ 16 to coxsackievirus B. Forty percent of carditis patients and 31.4% without carditis had neutralising antibody to more than one coxsackievirus B type. In both these clinical groups, coxsackievirus B4 antibody was the most commonly encountered of the six coxsackievirus B types followed by B2 and B3. Whereas coxsackievirus B1 antibody was fourth in prevalence followed by B5 and B6 in the carditis group, B5 was fourth followed by B1 and B6 in the non-carditis group. The prevalence of coxsackievirus B antibody were 35.6% and 35.6% in male and female patients with carditis and 38.5% and 23.1% in patients without carditis respectively. In both these clinical groups, prevalence of ASO and coxsackievirus B antibody were seen in the 15-24 years age group. Only 28.8% of these patients with carditis and 38.5% without carditis had no ($<$ 16) neutralising antibody to all six coxsackievirus B types.

46.9% of patients with carditis had coxsackievirus B neutralising antibody titres of 64 to $\geq$ 256 compared to 16% of patients without carditis. A 18.8% of carditis group compared to 3.0% without carditis had both coxsackievirus B antibody titres of 64 and $\geq$ 256 together with ASOT of $\geq$ 200 units. Tables 2a and 2b show that only 35.6% of 160 patients with carditis and 32.5% of 169 patients without carditis had ASOT of $\geq$ 200 Todd units.

DISCUSSION

Of 332 patients with symptoms and signs suggestive of rheumatic fever with and without carditis, approximately a third had antistreptolysin 0 titre of $\geq$ 200 units and about two thirds had coxsackievirus B neutralising antibody titires of $\geq$ 16. The prevalence of antistreptolysin 0 was not apreciably different in patients with carditis and those without carditis. A 35.6% of 160 patients with carditis and 32.5% of 169 patients without carditis had ASOT of $\geq$ 200 units. Similarly, the prevalence of coxsackievirus B antibody of $\geq$ 16 did not differ significantly in these two clinical groups, being 71.3% in the carditis group and 61.5% in the non-carditis group (Table 1a and 1b). However, there was a marked increase in the number of patients with carditis with high coxsackievirus B antibody titres of $\geq$ 64 suggestive of recent or current infection with coxsackievirus B. A 46.9% of patients with carditis as compared with 16.0% of patients without carditis had coxsackievirus B antibody titres of 64 to $\geq$ 256. This difference is statistically significant. (Tables 2a and 2b). Generally speaking, the higher the antibody titre, the higher the probability of recent infection and although titres of $\geq$ 64 is considered significant, varied immune response in different individuals makes the decision on a "diagnostic" titre difficult. In young children and patients where infection with coxsackievirus B occurred for the first time, a titre as low as 16 could be highly significant. Both the antistreptolysin 0 and coxsackievirus B antibody were most commonly observed in the 15 to 24 years old age group.

Of the six coxsackievirus B serotypes, neutralising antibody to coxsackievirus B4 was most commonly encountered followed by B2 and B3. This is similar to our experience of viral pericarditis and myocarditis in children where high or significant rising antibody titres to coxsackievirus B4, B2 and B3 in order of prevalence were observed (22).

It was also noted that 18.8% of 160 patients with carditis compared to 3.0% of 169 patients without carditis had both antistreptolysin 0 titres of $\geq$ 200 units and coxsackievirus B antibody titres of $\geq$ 64. This difference in antistreptolysin 0 titres and coxsackievirus B antibody titres between patients with and without carditis is statistically highly signficiant ($P = < 0.001$). The immune response to both these agents could be supportive of a possible streptococcal and coxsackievirus B relationship in the pathogenesis of carditis in these patients. Burch et al (8, 10) contended that viruses carried to heart tissue cells following systemic infection can remain dormant for long period and become reactivated when resistance of host is reduced by any factors including bacterial infection. These latent viruses could under certain circumstances develop into an acute fulminating infection with rapidly progressive myocardial disease and death. Group A Streptococci were shown to have strong immune suppresent effect which could release dormant viruses in experimental animals (19).

Whereas viral myocarditis and viral pericarditis have received recognition among clinicians, the concept of viral valvulitis and viral edocarditis have not been as widely acknowledged. Experiments in laboratory animals proved that viruses can damage the valves and mural endocardium (14 — 17). Furthermore, virus antigens especially the coxsackievirus B antigens have been demonstrated in tissue cells of pericardium, myocardium, valves and endocardium in autopsy specimens (11).

Results of our study support the contention that although carditis such as acquired valvular heart disease has traditionally been thought to be sequel to group A streptococcal infection, it could also be caused by cardiotropic viruses which in some instances might have been activated by streptococci.

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Table 1c. Prevalence of Coxsackievirus B Antibody by Age

Age Group Years	With Carditis: Number with Antibody Titre					Without Carditis: Number with Antibody Titre				
	<16* CB1-CB6	(a) 16 — ≥ 256 ICB**	(b) 16 — 32 ICB	(c) 64 — ≥ 256 ICB	Total of (a) + (b) + (c)	16* CB1-CB6	(a) 16 — ≥ 256 > 1Cb**	(b) 16 — 32 1CB	(c) 64 — ≥ 256 1CB	Total of (a) + (b) + (c)
0 — 4	1	0	0	1	1	1	1	0	0	1
5 — 14	12	13	5	7	25	14	14	11	3	18
15 — 24	15	23	10	9	42	29	17	14	7	38
25 — 34	11	12	6	1	19	12	14	9	1	24
35 — 44	0	3	0	0	3	0	1	1	0	2
45 — 54	1	1	1	2	4	1	1	1	1	3
≥ 55	2	3	2	1	6	2	2	0	0	2
Unknown	4	9	4	1	14	6	3	3	0	6
Total	46	64	28	22	114	65	53	39	12	104

< 16* = Reciprocal of serum dilution
> ICB** = More than one Coxsackievirus B

Table 2a Prevalence of Coxsackievirus B Antibody and Antistreptolysin 0 in Patients with Carditis

Clinical Groups Age Group Years	No. Tested No.	%	Coxsackievirus B Antibody Titre + <16* No.	%	16 — 32 No.	%	64 — ≥ 256 No.	%	Antistreptolysin < 200 No.	%	0 Titre ≥ 200 No.	%	Cox B AB ≥ 64 and ASOT ≥ 200 units No.	%
0 — 4	2	1.3	1	0.6	0	0	1	0.6	1	0.6	1	0.6	1	0.6
5 — 14	37	23.1	12	7.5	8	5.0	17	10.6	18	11.3	19	11.9	10	6.3
15 — 24	57	35.6	15	9.4	16	10.0	26	16.3	38	23.8	19	11.9	11	6.9
25 — 34	30	18.8	11	6.9	7	4.4	12	7.5	20	12.5	10	6.3	4	2.5
35 — 44	3	1.9	0	0	1	0.6	2	1.3	3	1.9	0	0	0	0
45 — 54	5	3.1	1	0.6	1	0.6	3	1.9	5	3.1	0	0	0	0
≥ 55	8	5.0	2	1.3	2	1.3	4	2.5	8	5.0	0	0	0	0
Unknown	18	11.3	4	2.5	4	2.5	10	6.3	10	6.3	8	5.0	4	2.5
Total	160	100	46	28.8	39	24.8	75	46.9	103	64.4	57	35.6	30	18.8

<16* = Reciprocal of serum dilution.
Coxsackievirus B Antibody Titre+ = Number of patients with CB antibody titres indicated irrespective of CB Type.

Table 2b Prevalence of Coxsackievirus B Antibody and Antistreptolysin 0 in Patients without Carditis

Clinical Groups Age Group Years	No. Tested No.	%	Coxsackievirus B Antibody Titre + <16* No.	%	16 — 32 No.	%	64 — ≥ 256 No.	%	Antistreptolysin < 200 No.	%	0 Titre ≥ 200 No.	%	Cox B Ab ≥ 64 and ASOT ≥ 200 units No.	%
0 — 4	2	1.2	1	0.6	0	0	1	0.6	2	1.2	0	0	0	0
5 — 14	42	24.9	14	8.3	18	10.7	10	5.9	24	14.2	18	10.7	3	1.8
15 — 24	67	39.6	29	17.2	26	15.4	12	7.1	41	24.3	26	15.4	2	1.2
25 — 34	36	21.3	12	7.1	22	13.0	2	1.2	31	18.3	5	3.0	0	0
35 — 44	2	1.2	0	0	1	0.6	1	0.6	2	1.2	0	0	0	0
45 — 54	4	2.4	1	0.6	2	1.2	1	0.6	4	2.4	0	0	0	0
≥ 55	4	2.4	2	1.2	2	1.2	0	0	4	2.4	0	0	0	0
Unknown	12	7.1	6	3.6	6	3.6	0	0	6	3.6	6	3.6	0	0
Total	169	100	65	38.5	77	45.6	27	16.0	114	67.5	55	32.5	5	3.0

<16* = Reciprocal of serum dilution.
Coxsackievirus B Antibody Titre+= Number of patients with CB antibody titres indicated irrespective of CB Type.

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