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Antiheart antibody in idiopathic hypertrophic subaortic stenosis[1]

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The prevalence of antiheart antibody using an indirect immunofluorescent technique with infant heart muscle as substrate was determined in 11 patients with idiopathic hypertrophic subaortic stenosis. For comparison, sera from patients with congestive cardiomyopathy, coronary artery disease, congenital heart disease, chronic rheumatic heart disease, and normal subjects were studied. There was a significant increase in the prevalence of antiheart antibody in patients with idiopathic hypertrophic subaortic stenosis (82%) compared to the other groups. The degree of intensity of fluorescence of the patients' sera correlated with periods of increased symptomatology. Conversely, with improvement and a stable clinical course there was a qualitative reduction in reactivity of sera with heart tissue. The mechanisms for the development of antiheart antibody are unknown. It may result from one or more insults or injuries to heart tissue. Upper respiratory infections may be associated with onset and subsequent aggravation of cardiac symptoms in some patients with idiopathic subaortic stenosis.

Idiopathic hypertrophic subaortic stenosis is a form of cardiomyopathy which has been recognized in both an acquired sporadic form and a familial form (Frank and Braunwald, 1968). Aetiological events associated with it have not been elucidated, though pathophysiological mechanisms and natural history are becoming increasingly clear (Burchell, 1971). Factors which account for the aggravation of symptoms in a given patient with an otherwise stable course are likewise unknown. Whether an underlying immunological disturbance plays a role in initiation of disease is unclear. Our purpose in this study was to determine the prevalence of antiheart antibody in idiopathic subaortic stenosis and to examine its relation to the clinical state of the patient. Furthermore, we would like to illustrate that upper respiratory infections may have been responsible for initiation of cardiac symptoms in one of our patients and for aggravating symptomatology in another.

Subjects and methods

Eleven consecutive patients with idiopathic subaortic stenosis seen at the University of Michigan Medical Center during the 2-year period 1968 to 1969 were studied. There were 4 women, 6 men and a boy, whose ages ranged from 8 to 58 years (Table 1). Diagnosis was confirmed in each at cardiac catheterization and in all but one with cineangiocardiography. Typical responses of the subaortic gradient to physiological and pharmacological manoeuvres were observed in each patient. Seven of these patients were symptomatic at the time of their initial visit. Based on the New York Heart Association Classification, 1 was in Class IV, 4 in Class III, 2 in Class II, and 4 in Class I. In one patient an upper respiratory tract infection initiated cardiac symptoms, and in another respiratory infections aggravated preexisting dyspnoea on exertion, palpitations, and angina pectoris. Both of these patients improved on subsequent evaluations. Five other symptomatic patients were seen during exacerbations without evident precipitating causes. The remaining 4 patients have maintained a stable course. For comparison, 35 patients with idiopathic congestive cardiomyopathy, 17 with coronary artery disease, 11 with congenital heart disease, 24 with chronic rheumatic heart disease, and 50 normal subjects were studied. Sera from all of the above patients were assayed for antiheart antibody. In addition, during the 2-year period, sera from 7 patients were reassayed on one or more occasions.

An indirect immunofluorescent technique was employed to detect antiheart antibody. Heart tissue was obtained within 6 hours of death from infants who died of non-cardiac causes. The tissue was frozen immediately in a mixture of dry ice and acetone and stored at

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TABLE 1 *Clinical, haemodynamic, and serological data in 11 patients with idiopathic hypertrophic subaortic stenosis*

Case No.	*Age (yr)*	*Sex*	*Functional class (NYHA)*	*Electrocardiogram*	*LV end-diastolic pressure (mmHg)*	*LV outflow gradient at rest (mmHg)*	*Mitral insufficiency (cineangiography)*	*Antiheart antibody*
1	44	M	IV	LV hypertrophy	27	103	Moderate	4+
2	58	F	III	,, ,,	14	130	Slight	3+
3	33	F	III	,, ,,	12	30	None	2+
4	37	F	III	,, ,,	10	20	Minimal	3+
5	58	F	III	,, ,,	18	80	Minimal	3+
6	49	M	II	,, ,,	15	35	Minimal	Negative
7	38	M	II	,, ,,	16	75	Minimal	1+
8	44	M	I	,, ,,	38	40	Minimal	1+
9	8	M	I	,, ,,	10	46	Unknown	Negative
10	52	M	I	,, ,,	5	75	Minimal	1+
11	22	M	I	,, ,,	17	55	Minimal	1+

−70°C. Four micra sections were cut in a cryostat, placed on glass slides, air dried, and fixed in acetone for 15 minutes at 4°C. The sections were washed 3 times for 10 minutes each in phosphate buffered saline at *p*H 7·4. Serum was diluted 1:5 before testing and was incubated on a tissue section in moist air for 45 minutes at room temperature. The sections were then washed as before and incubated with fluorescein labelled rabbit antihuman IgG, IgA, or IgM for 30 minutes. The slides were washed again, counterstained quickly with eriochrome black, air dried, and mounted in Elvanol. Normal serum and an antiheart antibody positive serum before and after adsorption with human heart muscle powder and unlabelled rabbit anithuman serum were employed as controls in each experiment. The slides were coded by number and read with a Zeiss ultraviolet microscope with a 200-watt mercury light source and were graded 0 to 4+ on the basis of strength of fluorescence.

Results

Two types of fluorescence were encountered in these sera: sarcolemmal and subsarcolemmal. Sarcolemmal staining occurred along the margins and periphery of the cardiac fibres. Subsarcolemmal immunofluorescence occurred in deposits immediately within the fibre (Fig.). Both of these patterns were usually observed in the patients with idiopathic subaortic stenosis. Sarcolemmal staining, when it occurred alone, was generally 1 to 2+ in intensity with a titre of 1:10 or less. Subsarcolemmal staining was seen with more intense immunofluorescense graded as 3 to 4+. Sera with subsarcolemmal staining were positive to a titre of 1:25 or less. IgG was the most frequent immunoglobulin associated with antiheart antibody positivity and occurred in all 9 patients with idiopathic hypertrophic subaortic stenosis, 5 of 6 with idiopathic congestive cardiomyopathy, and 3 of 4 with rheumatic heart disease.

Table 2 shows the results of these tests observed in the patient groups. Nine of 11 patients with idiopathic hypertrophic subaortic stenosis, 6 of 35 with cardiomyopathy, 4 of 24 with rheumatic heart disease, none of 17 with coronary artery disease, none of 11 with congenital heart disease, and 1 of 50 normal subjects were positive for antiheart antibody. Sarcolemmal and subsarcolemmal staining were observed in 6 of 7 symptomatic patients; the other mildly symptomatic patient (Class II) showed a negative response. In contrast, sera from 3 of 4 non-symptomatic patients with idiopathic hypertrophic subaortic stenosis were only faintly positive (1+) with predominantly sarcolemmal staining; the serum of the fourth patient was negative. Sarcolemmal staining was observed in 4 of 6 patients with cardiomyopathy, and in the other 2 only slightly increased intensity of staining (2+) of both sarcolemmal and subsarcolemmal types was observed. The normal subject with positive antiheart antibody showed only sarcolemmal staining.

It was possible in a follow-up study to examine sera from 7 patients with idiopathic hypertrophic subaortic stenosis, all initially positive for antiheart antibody. In 3 of the 7, the tests were negative, and in 2 the degree of fluorescence was conspicuously reduced compared to earlier results. A negative test or a decrease in degree of fluorescence in the follow-up study was associated clinically with symptomatic improvement in all but 1 patient (Table 2). In 1 man (Case 1) an upper respiratory tract infection triggered the initial cardiac symptoms and he remained severely disabled for several weeks. His serum was intensely positive for antiheart antibody at that time. With clinical improvement there was

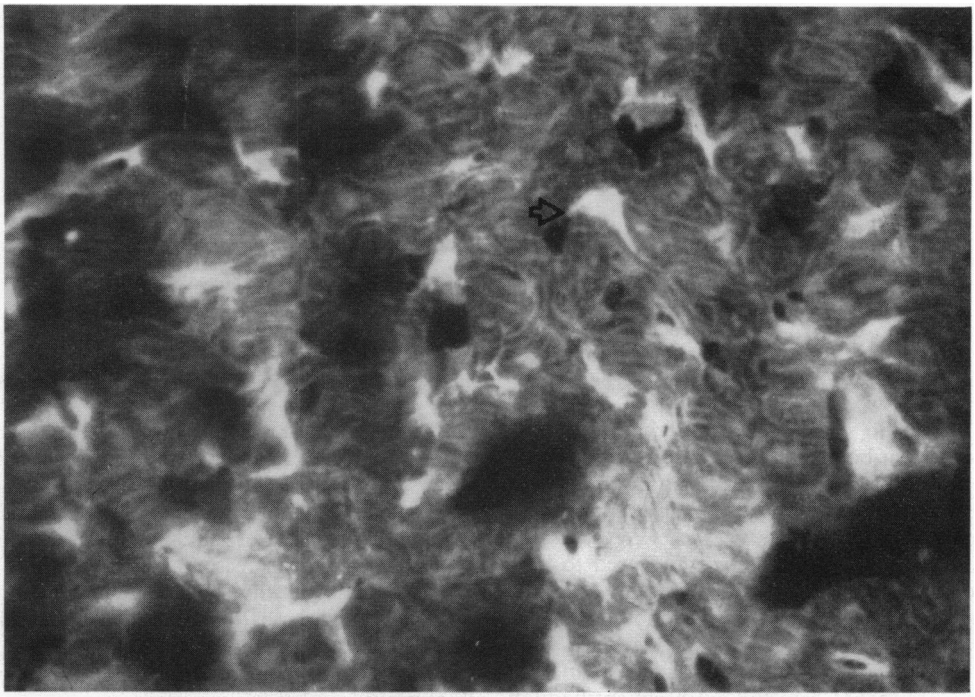

FIG. *Example of a positive antiheart antibody test with serum from Case 1 with idiopathic hypertrophic subaortic stenosis showing predominantly subsarcolemmal staining* (arrow). (× *312.*)

reduction in the degree of fluorescence for antiheart antibody. Clinical improvement was maintained and 2 years later he showed no evidence of circulating antiheart antibody. The other patient's course (Case 4) was aggravated by frequent chest infections. A programme of regular prophylactic antibiotic therapy was instituted, and for the ensuing 3 years she was free of infections and has maintained a clinically stable cardiac course. Serological studies periodically during this time showed no recurrence of antiheart antibody.

Discussion

Antiheart antibody has been observed in a number of cardiac and noncardiac conditions (Kaplan and Frengley, 1969). These authors have emphasized the nonspecificity of antiheart antibody and have suggested that the variable frequency of occurrence of antiheart antibody in published reports was related partly to the nonuniformity of antigenic composition of the substrates used and the different methods employed.

The mechanism for its production in idiopathic hypertrophic subaortic stenosis is not understood. It may be a consequence of injury to heart tissue as in its occurrence after severe ischaemia or infarction (Heine *et al.*, 1966). Since antiheart antibody has not been found in the sera of all of our patients, it lacks specificity from a pathogenic or diagnostic standpoint. Unique among our patients with hyper-

TABLE 2 *Study groups and results of immunofluorescent test for antiheart antibody*

Diagnosis	*No.*	*Women*	*Men*	*Average age (yr)*	*No. with positive test*	*Per cent positive*
Idiopathic hypertrophic subaortic stenosis	11	4	7	36	9	82
Cardiomyopathy	35	7	28	43	6	17
Coronary artery disease	17	4	13	55	0	0
Congenital heart disease	11	7	4	38	0	0
Rheumatic heart disease	24	18	6	50	4	17
Control subjects	50	23	27	24	1	2

TABLE 3 *Change in reactivity to antiheart antibody and in functional class (New York Heart Association, I–IV) in clinical course of 7 patients with idiopathic hypertrophic subaortic stenosis*

Case No.	*Initial evaluation*		*Follow-up evaluation*	
	Antiheart antibody	*Functional class*	*Antiheart antibody*	*Functional class*
1	++++	IV	−	II (improved)
2	+++	III	+	III (unchanged)
4	+++	III	−	II (improved)
5	+++	III	+	II (improved)
3	++	III	−	II (improved)
7	+	II	+	II (unchanged)
8	+	I	+	I (unchanged)

trophic subaortic stenosis is the higher frequency of antiheart antibody and the enhanced antiheart muscle fluorescence observed during periods of increased symptomatology, unlike that in cardiomyopathy patients (Das, Cassidy, and Petty, 1972). With clinical improvement there appeared to be a corresponding decrease in detectability of antiheart antibody and in intensity of immunofluorescence (Table 3).

The reasons for these phenomena are unclear. Van der Geld (1964) detected antiheart antibody in patients with postcardiotomy and postmyocardial infarction syndromes particularly when they were symptomatic, but not during symptom-free periods. Similarly, we have recently observed a higher prevalence of antiheart antibody in patients with infective endocarditis complicated by heart failure (Das, Cassidy, and Willis, 1971). Persistence of antibody during treatment was associated with an ominous outcome. On the contrary, Bauer, Waters, and Talano (1972) did not encounter any difference in prevalence of antiheart antibody in complicated versus uncomplicated patients with coronary artery disease. Thus, a clear relation between presence of antiheart antibody and symptoms, on the one hand, and the severity of symptoms and titre of antiheart antibody response on the other has not been established. Kaplan, Meyeserian, and Kushner (1961) and Hess *et al.* (1964) found a high prevalence of circulating antiheart antibody in acute rheumatic fever and particularly in patients with active carditis. The high frequency of antiheart antibody in rheumatic fever might be related in susceptible subjects to streptococcal antigens capable of cross-reacting with heart muscle (Kaplan, 1963). Recently Das *et al.* (1972) found a high frequency of antinuclear antibody in patients with cardiomyopathy and idiopathic hypertrophic subaortic stenosis. Age did not materially influence the prevalence of antinuclear antibody. The latter did not persist as a constant feature during the follow-up study. In addition there was a significant rise in the levels of IgM among women with hypertrophic subaortic stenosis. Observations by Olson (1971) which described bizarre shaped nuclei with perinuclear haloes along with whorl formation of muscle fibres as typical histological features of idiopathic hypertrophic subaortic stenosis may be relevant. It is possible that these pathological alterations might be associated with the high frequency of serum factors (antiheart and antinuclear antibody) in idiopathic hypertrophic subaortic stenosis.

Further studies would be necessary to determine if autoimmune mechanisms were indeed involved in hypertrophic subaortic stenosis. Virus infection might also be related to the development of autoimmunity (Lachmann, 1968). Upper respiratory infection, though aetiologically unclarified, appeared to play an important role in the clinical course of the disease in 2 of our patients.

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