

ORIGINAL ARTICLE

## Serum Anti-*Yersinia* Antibody in Chinese Patients with Kawasaki Disease

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**Background.** Many infectious agents have been implicated as an etiology to develop Kawasaki disease (KD). In Taiwan, studies on the relationship between *Yersinia* and KD have not been reported.

**Methods.** We measured sera for anti-*Yersinia* antibodies by using enzyme immunoassay (EIA) in 31 patients with KD and 60 healthy children (HC). *Yersinia* strains included *Y. pseudotuberculosis* I, II, III, IV, V, VI and *Y. enterocolitica* O3, O8 and O9.

**Results.** Data of 31 patients with KD showed that for the IgG antibody, serum anti-*Y. pseudotuberculosis* II, III, Y. O8 and O9 antibody were significantly higher when compared to the HC. Except for *Y. pseudotuberculosis* IV, all other *Yersinia* strains of either IgA or IgM antibodies increased significantly in patients with KD vs. the HC. If we compared the number of patients who had significant elevation of OD and those of HC, we found IgA anti-*Yersinia* antibodies (PST I, PST II, O3, O8, O9), IgM (PST VI, O8) and IgG (PST II, O8, O9) were significantly elevated in KD patients than in HC. A significant relationship was present between KD with myocarditis and increased anti-*Yersinia* antibody titer.

**Conclusions.** The findings in this study suggest that preceding *Yersinia* infection may play a role in the pathogenesis of KD. Further study of the relationship between KD with myocarditis and increased anti-*Yersinia* antibody is needed. © 2005 IMSS. Published by Elsevier Inc.

**Key Words:** *Yersinia* infection, anti-*Yersinia* antibody, Kawasaki disease, Enzyme immunoassay.

### Introduction

Kawasaki disease (KD) was originally named mucocutaneous lymph node syndrome by Kawasaki (1–4). Until 1991, more cases were found in Japan than any other country (1). The disease mainly affects young children who present fever, lymph node enlargement, conjunctivitis, red lips and red palms and soles, and a strawberry tongue. Associated features of KD include a variety of disorders, for instance,

arthritis or arthralgia, coronary artery aneurysm or thrombosis, increased serum transaminase, aseptic meningitis, diarrhea and pneumonitis, etc. (1–3). The annual incidence of this disease among children <5 years of age has increased from 2.1 cases/100,000 in 1979 to 10.3 cases/100,000 children in 1983. Data from a nationwide incidence survey of KD in Japan from 1985 to 1986 showed 7611 cases in 1985 and 12847 in 1986 (4). Salo surveyed KD in Finland from 1982 to 1992 and found the annual attack rate varied from 3.1 to 7.2 per 100,000 children <5 years of age (5). In Taiwan, KD cases were collected from 18 hospitals and showed the annual incidence after 1986 to be estimated at 20 per 100,000 (6).

The etiology of KD is still unknown, but preceding infections have been suggested to contribute to the disease (3). Many infectious agents including superantigen-producing

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*Staphylococcus aureus* or Group A  $\beta$ -hemolytic *Streptococcus* (7,8), parvovirus B19 (9,10) and retrovirus (11) etc. were reported to be associated with KD.

Gastrointestinal infection in children due to *Yersinia enterocolitica* or *Y. pseudotuberculosis* is common in Scandinavia and European countries (12,13). Like enteritis, yersinosis may cause fever, arthritis, scarletiform skin rash, conjunctivitis, erythema nodosum and even complications including uveitis and aortic aneurysm (13–18). Those clinical features showed very similar findings as KD.

In Taiwan, *Yersinia* has been isolated from stools of a few patients with enteritis (19). Furthermore, Ding et al. found that of 187 food samples analyzed, 3 (1.6%) displayed the presence of *Yersinia enterocolitica* (20). Konishi et al. in their previous studies demonstrated that 55/208 KD patients had either a positive *Y. pseudotuberculosis* antibody titer elevation and/or a positive stool culture (21,22). In Taiwan, studies on the relationship between *Yersinia* infection and KD have not been reported. In this study, we used enzyme-linked immunoassay (EIA) to measure serum anti-*Yersinia* antibodies in Chinese patients with KD in order to understand the *Yersinia* infection may play a role in patients with KD.

## Patients and Methods

### Patients

According to the proposal suggested by the Research Committee on Kawasaki disease (22), at least five of the six principal symptoms (fever, palm erythema and edema, polymorphous exanthema, conjunctivitis, lip erythema or strawberry tongue, cervical lymphadenopathy) should be present for diagnosis of KD. Venous blood samples were taken from 31 cases with definite KD (22) from either the outpatient clinic or the ward. Two-dimensional echocardiography was performed for any case suspected of having a coronary artery aneurysm.

In this study, 60 age- and sex-matched normal healthy children (HC) were used as controls. All serum samples were stored at  $-70^{\circ}\text{C}$  until use.

### Determination of Serum Yersinia Antibody with EIA Method (23,24)

Different *Yersinia* strains [*Yersinia pseudotuberculosis* (PST) I, II, III, IV, V, VI; *Yersinia enterocolitica* O3, O8 and O9] were treated with sodium dodecyl sulfate (SDS) and the extracted antigens were coated on 96 microtiter plates by overnight incubation at  $37^{\circ}\text{C}$ . After saturation with 3% bovine serum albumin, plates were incubated with diluted serum samples (1:250) for 2 h at  $37^{\circ}\text{C}$ . The plates were washed three times with phosphate buffered saline (PBS) containing 0.05% Tween 20. Then, alkaline phosphatase-conjugated swine anti-human IgG, IgM or IgA (Orion Diagnostic, Helsinki, Finland) with dilutions of 1:300 1:250, and

1:250 were added (75  $\mu\text{L}$ /well) and incubated on the plates overnight at room temperature. After washing, a substrate of p-nitrophenyl phosphate in diethanolamine-MgCl<sub>2</sub>-buffer solution (1 mg/mL, Orion Diagnostic) was added and incubated for 30 min at  $37^{\circ}\text{C}$ . The final reaction was stopped with 1 M NaOH. Optical density (OD) was measured with a Titertek Multiscan Photometer (Labsystems, Helsinki, Finland) at a wavelength of 405 nm.

### Statistics

Unpaired Student t test and chi-square test were used for the statistical analysis between the OD value in patients with KD and healthy subjects. Significance was considered when  $p < 0.05$ .

## Results

From January 1994 to December 1995, we had the opportunity to study 31 patients with KD at China Medical College Hospital. Demographic data of these patients are shown in Table 1. Diagnosis and long-term follow-up was completed by only one pediatrician. Among these 31 patients, 16 were male and 15 were female. Ages ranged from 3 months to 5 years with a mean of 18.4 months. All patients had fever whose duration ranged from 3 to 10 days. Except for two patients, all had conjunctivitis. Either lip erythema or strawberry tongue was present in all patients. Palm erythema or edema occurred in 27 of 31 patients. All patients had polymorphous exanthema. Six patients had no apparent cervical lymph node enlargement during the physical examination. Six of the 27 patients who received echocardiogram had either right or left coronary aneurysm. Myocarditis was found in six patients. All patients had elevated erythrocyte sedimentation rate (ESR). Serum anti-streptolysin O titer (ASOT) was measured in 31 patients but all data were within

**Table 1.** Clinical features and laboratory findings of the patients with Kawasaki disease

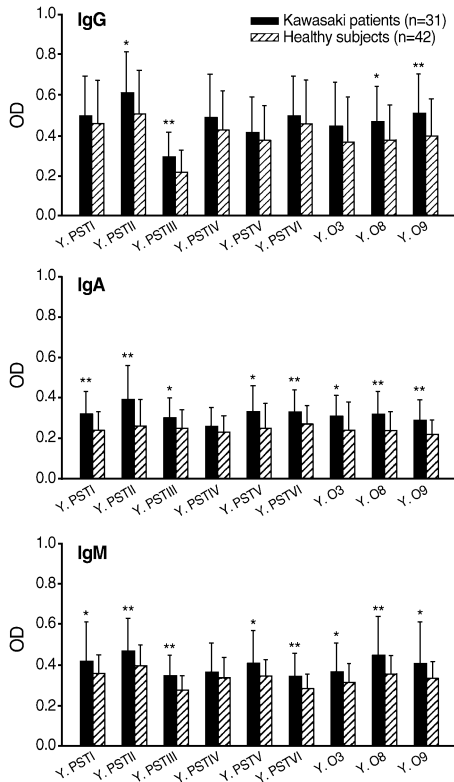
Clinical findings	Frequency (%)
Fever	100
Conjunctiva injection	96.8
Lip erythema or strawberry tongue	100
Polymorphous exanthema	93.8
Cervical lymphadenopathy	78.1
Coronary aneurysm	22.2
Myocarditis	18.5
ESR elevation ( $>25$ mm/h)	100
CRP elevation ( $>0.8$ g/dL)	98
Leukocytosis ( $>10000/\text{mm}^3$ )	95.5
ASOT elevation ( $>200$ )	0.0

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; ASOT: anti-streptolysin O.

normal range. Peripheral blood leukocyte counts increased in 29 patients at the early time.

For the EIA test, different *Yersinia* strains including PST I, II, III, IV, V, VI and *Y. enterocolitica* O3, O8 and O9 were used as antigens to measure serum antibodies in patients and controls. The results are shown in Figure 1. For the IgG anti-*Yersinia* antibody, serum anti-*Y. pseudotuberculosis* II, III, and *Y. enterocolitica* O8 and O9 antibody titer were significantly higher when compared to HC ( $p < 0.05$  or  $p < 0.01$ ). In contrast, except for *Y. pseudotuberculosis* IV, all the other *Yersinia* strains of IgM anti-*Yersinia* antibody titers increased significantly in patients with KD vs. the HC ( $p < 0.05$  or  $p < 0.01$ ). Like IgM antibody, the IgA anti-*Yersinia* antibody level was significantly elevated in all *Yersinia* strains except *Y. pseudotuberculosis* IV, in patients with KD when compared to the HC. Overall, only 4 (*Y.* PST II, III, *Y.* O:8 and O:9) of a total nine *Yersinia* strains showed a statistical difference of IgG anti-*Yersinia* antibody between patients with KD and the HC. IgM anti-*Yersinia* antibody levels against the nine different *Yersinia* strains had similar results to the IgA anti-*Yersinia* antibody.

Apart from the comparison of OD between KD and HC, we also compared the number of patients who had significant elevation of OD and those of HC. In this study, for KD and HC, the OD value exceeded mean + 1 SD of 60 HC



**Figure 1.** The IgG, IgA and IgM anti-*Yersinia* antibody in Chinese patients with Kawasaki disease and healthy subjects. \* $p < 0.05$ , \*\* $p < 0.01$ .

was counted as a significant elevation. Table 2 shows the numbers and percentage of patients who had significant elevation of OD. Obviously, 5 IgA anti-*Yersinia* antibodies (PST I, II, O3, O8, O9) in KD were significantly elevated when compared to HC. Other antibodies, including IgG PST II, O8, O9 and IgM PST VI, O8, also showed a significant difference between KD and HC.

Finally, five clinical parameters including gender (male vs. female), aneurysm (presence vs. absence), myocarditis (presence vs. absence), ESR ( $\geq 100$  vs.  $< 100$ ) and CRP ( $\geq 100$  vs.  $< 100$ ) were used to look for the difference of anti-*Yersinia* antibody titer (OD) between patients with KD and HC. Only IgG class in KD with myocarditis had a significant elevation of antibody titer (IgG anti-PST I, IgG anti-PST II, IgG anti-PST III, IgG anti-PST IV, IgG anti-PST VI, IgG anti-O3, IgG anti-O8,  $p < 0.05$ ) when

**Table 2.** Comparison of the number of patients who had significant elevation of OD vs. those of healthy control (all data are  $p$  value)

<i>Yersinia</i> strain	Patient (31)	Control (60)	$p$ value
(1) PST I			
IgG >0.67	6 (19.4%)	14 (23.3%)	0.664
IgA >0.33	11 (35.5%)	7 (11.7%)	0.007 <sup>Δ</sup>
IgM >0.44	11 (35.5%)	12 (20.0%)	0.107
(2) PST II			
IgG >0.72	9 (29.0%)	9 (15.0%)	0.111
IgA >0.39	13 (41.9%)	6 (10.0%)	<0.001 <sup>Δ</sup>
IgM >0.49	11 (35.5%)	13 (21.0%)	0.156
(3) PST III			
IgG >0.33	13 (41.9%)	8 (13.5%)	0.002 <sup>Δ</sup>
IgA >0.34	9 (29.0%)	8 (13.3%)	0.069
IgM >0.35	11 (35.5%)	12 (20.0%)	0.107
(4) PST IV			
IgG >0.62	9 (29.0%)	9 (15.0%)	0.111
IgA >0.31	7 (22.6%)	10 (16.7%)	0.493
IgM >0.43	8 (25.8%)	8 (13.3%)	0.139
(5) PST V			
IgG >0.55	5 (16.1%)	10 (16.7%)	0.948
IgA >0.37	8 (25.8%)	8 (13.3%)	0.139
IgM >0.43	12 (38.7%)	13 (21.7%)	0.084
(6) PST VI			
IgG >0.67	5 (16.1%)	10 (16.7%)	0.948
IgA >0.36	8 (25.8%)	7 (11.7%)	0.085
IgM >0.36	11 (35.4%)	9 (15.0%)	0.025*
(7) O3			
IgG >0.59	9 (29.0%)	13 (21.7%)	0.111
IgA >0.38	13 (41.9%)	7 (11.7%)	<0.001 <sup>Δ</sup>
IgM >0.41	9 (29.0%)	10 (16.7%)	0.156
(8) O8			
IgG >0.55	12 (38.7%)	11 (18.3%)	0.034*
IgA >0.34	13 (41.9%)	7 (11.7%)	<0.001 <sup>Δ</sup>
IgM >0.44	11 (35.4%)	8 (13.3%)	0.014*
(9) O9			
IgG >0.59	11 (35.4%)	10 (16.7%)	0.043*
IgA >0.29	12 (38.7%)	7 (11.7%)	0.003 <sup>Δ</sup>
IgM >0.42	9 (29.0%)	13 (21.7%)	0.437

<sup>Δ</sup> =  $p < 0.01$ ; \* =  $p < 0.05$ .

compared to KD without myocarditis. There was no significant difference for other four items.

## Discussion

In this study, 7 of 31 (22.6%) KD patients were complicated with coronary artery aneurysm, these results are close to the incidence of 20 to 40% reported by Rowley et al. (25) and Holm et al. (10). Echocardiographic examinations in those cases within the first week of illness presented either left ventricular dilatation or pericardial effusion. The clinical feature of myocarditis with congestive heart failure was seen occasionally. No fatal cases in our 31 patients demonstrated during the one year follow-up period, which is also similar to the report by Rowley et al. (25). Early diagnosis of coronary artery aneurysm in patients with KD and early application of IVIG may largely reduce the incidence of cardiovascular complication to approximately 10% (26). The complication ratio in this study was equal to that in decade.

The etiology of KD is unknown. Many infectious agents, particularly *Staphylococcus* and *Streptococcus* (7,8), may be related to KD. Previous studies demonstrated *Streptococcus pyogenes* and *Staphylococcus aureus* possessing superantigenic activity that might play a pathogenic role in KD (7,8). Curtis et al. (8) found V $\beta$ 2 expressing T-cell significantly increased in patients with KD when compared to the HC. The potent T cells activation in patients with KD probably resulted from superantigen-mediated T-cell stimulation. However, Morita et al. (27) and Terai et al. (28) failed to prove the involvement of Streptococcal and Staphylococcal superantigen in the pathogenesis of KD. To further identify the association of intestinal bacteria infection and KD, we measured serum anti-*Yersinia* antibodies. In this study, surprisingly, all the IgM and IgA anti-*Yersinia* antibodies including *Y. pseudotuberculosis* I, II, III, V, VI and *Y. enterocolitica* O3, O8 and O9 strains significantly increased when compared to the HC. However, only four strains of the *Yersinia* antibody in IgG type were elevated. The discrepancy between the immunoglobulin isotypes in patients with KD is unknown.

On the other hand, if we compared the number of patients who had significant elevation of OD and those of HC, only three strains of the *Yersinia* antibody in IgG class, two in IgM class and five in IgA class were significantly elevated. Among them, all O8 strains (IgG, IgM, IgA class) and two O9 strains (IgG, IgA class) showed a statistical difference. Taken together, elevation of anti-*Yersinia* O8 and O9 may have a role in KD. More IgA class may indicate a chronic infection in KD. Since *Yersinia* enteric infection is not a popular disease in Taiwan, the increased anti-*Yersinia* antibody in Chinese patients with KD may either be due to subclinical enteric infection or the unavailability of stool cultures from patients with unknown diarrhea. In fact, it cannot be ruled out that the increased antibacteria antibody

in KD patients may be an epiphenomenon or the cross-reactive antigen between *Yersinia* and other bacteria (e.g., *Salmonella*, *E. coli*).

In this study, the finding of increased anti-*Yersinia* antibody in KD with myocarditis has not been reported by other investigators. The pathogenesis is unknown. Further study may be needed to explore the relationship between myocarditis and anti-*Yersinia* antibody.

The similarity of clinical manifestations between KD and *Yersinia* enteric infection and the increased anti-*Yersinia* antibodies in our patients with KD suggest that preceding *Yersinia* infection may play a role in the pathogenesis of KD. Long-term follow-up of children who have proven *Yersinia* infection would give information about the level, time course and specificity of antibody production and the prevalence of KD.

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