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ORIGINAL ARTICLE

R. Venkatraman · S. Singh · R.W. Minz

Study of the autoantibody profile after the acute phase of Kawasaki disease in a cohort of children from North India

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Abstract A study of organ-specific and organ-nonspecific autoantibodies was carried out in 21 children being followed up after a diagnosis of Kawasaki disease at a tertiary care center of North India. Anti-nuclear antibodies were detected in 9.5% patients while anti-thyroid microsomal antibodies were detected in 23.9% patients. Other autoantibodies (e.g. anti-parietal cell antibody, anti-liver kidney microsomal antibody anti-neutrophil cytoplasmic antibody, anti-mitochondrial antibody and anti-smooth muscle antibody) were not detected in any of our patients. Children with Kawasaki disease need to be monitored for the development of autoantibodies during follow-up.

Keywords Kawasaki disease · India · Autoantibodies

Introduction

Kawasaki disease (KD) is a vasculitic syndrome of unknown etiology and variable clinical presentation, which predominantly affects the medium-sized vessels, most notably the coronary arteries [1]. The diagnosis of KD is difficult because it is based entirely on the recognition of a typical temporal sequence of a constellation of clinical features, with none of the features taken individually being of any diagnostic significance. Although the etiology of KD is not known, various investigators have demonstrated organ-specific and nonspecific autoantibodies in KD [2–11]. The rationale for doing this study was that there are conflicting data on autoantibody levels in KD reported by various authors. Further, there are no studies on this aspect of KD from India or any other developing country. Moreover, there is paucity of information regarding the levels of anti-mitochondrial (AMA), anti-liver kidney microsomal (LKM), anti-parietal cell (PCA) and antithyroid microsomal (TMA) antibodies in patients with KD.

R. Venkatraman · S. Singh (✉) · R.W. Minz
Department of Pediatrics and Immunopathology, Advanced Pediatric Center, Postgraduate Institute of Medical Education and Research (PGIMER), 160012 Chandigarh, India
E-mail: surjitsinghpgi@rediffmail.com
Tel.: +91-172-2747585
Fax: +91-172-2744401

Patients and methods

Twenty-one children diagnosed with Kawasaki disease and having been followed up for a minimum period of 3 months in the Pediatric Rheumatology and Immunology Clinic of Advanced Pediatric Center, Post Graduate Institute of Medical Education and Research, Chandigarh, comprised the study sample. KD was diagnosed in our patients on the basis of the standard criteria outlined in Table 1 [12]. Nineteen of the 21 study patients were treated with IVIG according to the standard protocols [12–14]. There was rapid defervesence of fever within 48 h of therapy. Two patients who were presented 1 month after the onset of the illness were not given IVIG because such a therapy is believed to be useful only in the acute phase of the disease.

The organ-specific autoantibodies, which were studied, included Anti-thyroid microsomal antibody (TMA), Anti-parietal cell antibody (PCA) and Anti-liver kidney microsomal antibody (anti-LKM). The organ-nonspecific autoantibodies that were studied included anti-nuclear antibody (ANA), anti-neutrophil cytoplasmic antibody (ANCA), anti-mitochondrial antibody (AMA) and anti-smooth muscle antibody (SMA). We had used the technique of indirect immunofluoresence (IIF) to assess ANA, AMA, SMA, anti-LKM and PCA. Herein, we used cryostat sections of a composite of rat stomach, liver and kidney tissues. The secondary antibody (i.e. fluorescein isothiocyanate labeled polyspecific antihuman immunoglobulin—IgG, IgA and IgM) was purchased from Sevapharma, Czech Republic. It was used in a dilution of 1:16. The sera dilution used in this assay was 1:20. After washing the excess secondary antibody, the slides were mounted in buffered glycerine and viewed under a Nikon fluorescence microscope. A semiquanti-

Table 1 Kawasaki disease: frequency and characteristics of diagnostic criteria

Criterion	Frequency in western population (%) [1]	Frequency in our study population (%)	Characteristics
Fever	100	100	Duration 5 days or more; 39–40°C; unresponsive to antibiotics
Conjunctivitis	85	57	Bilateral, bulbar, nonsuppurative
Lymphadenopathy	70	90	Cervical, tender, often unilateral, acute nonpurulent, >1.5 cm in diameter
Rash	80	95	Polymorphous, no vesicles or crusts
Changes in lips or oral mucosa	90	100	Dry, swollen, red, vertically cracked lips; "strawberry" tongue Diffusely erythematous oropharynx
Changes in extremities	70	95	*Acute*: erythema of palms or soles; indurative edema of hands or feet *Convalescent:* membranous desquamation from fingertips

tative grading of the positivity of immunofluorescence was used from 1+ to 4+.

ANCA was also detected by the standard IIF assay. Neutrophils were isolated from O group whole blood, washed twice in PBS buffer saline (pH 7.4) and then spun down on glass slides using a Shandon cytocentrifuge. The slides were dried and fixed in absolute ethanol for 10 min. Serum was diluted 1:20 and layered over the neutrophils. In the last step, the secondary antibody (same as the one used in IIF screening for organ-non-specific autoantibodies) was layered over the neutrophils. Interpretations were made as per the standard international guidelines.

TMA was tested by a commercial kit (Serodia-AMC, Japan) based on the principle of particle agglutination. The test was performed by the method of doubling dilutions, using 1/20 and 1/40 serum dilutions [11]. Positive and negative controls were run along with the test sera. Sera that tested positive in the 1:20 dilution were quantitated as 1+, while those that tested positive in 1:40 dilutions were quantitated as 2+.

All the tests were carried out under the direct supervision of an experienced immunopathologist (RWM). These techniques have been in routine use in our institute for the last 25 years.

Results

The basic clinical and laboratory data are given in Table 2 and the autoantibody profile is given in Table 3. The patients were sampled in the convalescent phase of the disease, and at least 3 months after having received intravenous immunoglobulin (IVIG). This was done in order to avoid interference in the study by antibodies passively acquired through the IVIG. IVIG has a half-life ($t½$) of 20–22 days and passively acquired antibodies are likely to disappear after four half-lives (i.e. approximately 3 months).

Table 2 Clinical and laboratory data

Age (years)	6.8 ± 3.2 (1–16 Years)
Male/Female	17:4 (81:19%)
Treatment given	
Aspirin + IVIG Aspirin alone	19/21 (90.5) 2/21 (9.5)
Interval between presentation and sampling	
<6 months	7/21 (33.3)
6 months to 1 year	2/21 (9.5)
1–2 years	4/21 (19.0)
2–3 years	3/21 (14.2)
3–4 years	2/21 (9.5)
>4 years	1/21 (4.8)

ANA was detected in two patients. While one had a faint speckled pattern, the other had a 3+ nucleolar pattern (both in 1:40 dilution). Also, ANA was positive on repeat testing in both these patients. Five out of the 21 patients (23.9%) had a positive TMA. While three of these (14.3%) had a positive TMA in 1:20 dilution, the remaining two patients (9.5%) had positive TMA in 1:40 dilution. One of the patients with a positive ANA also had a positive TMA. None of the other autoantibodies were detectable in any of our patients (Table 3).

All the patients included in our study had a normal echocardiography examination on follow-up. As part of patient care, Thallium dobutamine stress scintigraphy was carried out on ten of the 21 study patients. Abnormal myocardial perfusion was detected in four of the ten patients who had undergone these scans, indicative of underlying myocardial pathology secondary to KD. Both the patients with positive ANA also had an abnormal thallium scan.

Discussion

The etiology of KD remains unknown [14, 15]. Although clinical and epidemiologic features of the illness do suggest an infectious etiology, exhaustive microbiologic and serologic investigations have failed to yield an infectious cause [1]. There is, however, reason to believe that streptococcal and/or staphylococcal superantigens may act as a trigger in at least some children with KD [1]. Perturbations of the immune system may also have a role to play in the pathogenesis of the disease [16–25].

The role of antibodies against self-antigens (i.e. autoantibodies) in KD has been investigated by various researchers previously [2–10, 26–30], but the results have been conflicting. Our study was conducted to look at the profile of some of the common organ specific and organ-

Table 3 Autoantibody profile

Autoantibody	Percentage positive	Historical controls (36, 38–41)
ANA	2/21 (9.5)	1–2%
TMA	5/21 (23.9)	2.17%
SMA	0/21 (0)	< 2%
AMA	0/21 (0)	< 1%
PCA	0/21 (0)	2–16%
ANCA	0/21 (0)	< 1%
Anti LKM	0/21 (0)	< 1%

AMA: anti-mitochondrial
LKM: anti-liver kidney microsomal
PCA:antiparietal cell
TMA: antithyroid microsomal

North India. Previous reports on the subject have all emanated from the developed world. Many pediatric rheumatological disorders like systemic lupus erythematosus, Henoch-schonlein purpura, juvenile dermatomyositis and juvenile rheumatoid arthritis have a different presentation in our population when compared to the western world [31–34]. We, therefore, thought that it would be important to find out if there were some differences as regards KD as well. Previous studies on the subject have also not commented on the presence of anti-mitochondrial antibody (AMA), anti-liver kidney microsomal (LKM) antibody and anti-parietal cell antibody (PCA) in patients with KD. The present study was planned as a more comprehensive investigation and included the aforementioned antibodies in addition to the anti-nuclear antibody (ANA), anti-neutrophil cytoplasmic antibody (ANCA) and anti-smooth muscle antibody (SMA). We have, therefore, attempted to fill up this lacuna in the literature. Our study also intended to find out whether patients with KD are more prone to develop other autoimmune diseases and if these could be picked up early.

IIF is the most common test for the detection of autoantibodies because of its wide range of applications. It can detect multiple antibodies on a single tissue block and thus, has considerable value as a screening test. It is especially appropriate for testing autoantibodies against antigens, which have not yet been characterized [35–41]. In our hands IIF is also a very inexpensive test as compared to tests based on commercial ELISA kits. However, ELISA and radioimmunoassay (RIA) are believed to be more sensitive than IIF when the antigen in question has been definitely identified [35, 41].

Under ideal circumstances, children of the same age distribution as our study subjects should have been taken as controls so as to compare the prevalence of autoantibodies among them. Because of ethical considerations being involved in sampling normal children, we were unable to take controls. Also, data on the presence of autoantibodies in the normal population already exists both in the western literature and in our own population [35, 36, 38–41].

Our study has shown that the prevalence of ANA in children with KD is 9.5%, which is high when compared to the prevalence of 1–2% in the normal adult population [36]. The prevalence of ANA in KD has varied from 0% [9] to 86% [26] in previous studies. A higher prevalence of ANA has been demonstrated in the acute phase of KD when compared to the convalescent phase [26].

Twenty-fourpercent of our patients had a positive TMA, which is considerably high compared to the reported prevalence of 2.17% in the normal adult population [38, 41]. To the best of our knowledge, there are currently no data available on the presence of TMA in KD. We were unable to demonstrate the presence of AMA, SMA, LKM, PCA and ANCA in our study patients. Previous studies on the subject have not commented on the occurence of AMA, LKM and PCA in children with KD. These autoantibodies are seen in less than 1–2% of the normal population [38, 40–41]. Prevalence of ANCA has varied from 28–73% [2, 28] during the acute phase of KD to 0–89% [3, 28] in the convalescent phase. The wide variation in ANCA positivity may be related to the technique used, the phase of the illness when the patients were sampled, therapy with IVIG and genetic variations. Our study shows a significant ANA positivity in KD patients in the convalescent phase when compared to the normal population.

Both the patients with ANA positivity had an abnormal thallium scan, but a normal echocardiography examination. This observation is important as it suggests that ANA may be predictive of subtle myocardial dysfunction in patients with KD, which may not be picked up by echocardiography alone. However, as only ten out of the 21 patients in our study had undergone a thallium scan, it would be presumptuous on our part to suggest a possible incriminating role of ANA in the evolution of myocardial dysfunction in children with KD. Undoubtedly, further studies are needed to clarify this aspect. None of our patients had a demonstrable coronary artery abnormality (CAA) by echocardiography. Western literature reports the incidence of CAA to be less than 1% in patients who have been treated with intravenous immunoglobulin [42]. The majority (19/21) of the patients in our study had received treatment with intravenous immunoglobulin within 10 days of fever.

Our finding of positive TMA in approximately 24% of our patients is also significant. Autoimmune thyroid dysfunction has not been previously described with KD. Further studies to assess the thyroid function need to be done on such patients both in the acute and convalescent phases of the disease.

Conclusions

We were able to demonstrate organ-specific (TMA) as well as organ-nonspecific (ANA) autoantibodies in a significant number of children with KD. While the prevalence of ANA was similar to previously published studies on the subject, there are no comparable data available on the prevalence of TMA in KD. We were unable to detect the presence of SMA, LKM, AMA, and ANCA in any of our study patients. Children with

Kawasaki disease need to be monitored for the development of autoantibodies during follow-up.

References

1. Cassidy JT, Petty RE (2001) Vasculitis. Kawasaki disease. In: Cassidy JT, Petty RE (eds) Textbook of pediatric rheumatology, 4th edn. W.B. Saunders Company, Philadelphia
2. Soppi E, Salo E, Pelkonen P (1992) Antibodies against neutrophil cytoplasmic components in Kawasaki disease. Acta Pathol Microbiol Immunol Scand 100(3):369–372
3. Savage CO, Tizard J, Jayne D, Lockwood CM, Dillon MJ (1989) Antineutrophilic cytoplasmic antibodies in Kawasaki disease. Arch Dis Child 64:360–363
4. Tizard EJ, Baguley E, Hughes GR, Dillon M (1991) Antiendothelial cell antibodies detected by a cellular based ELISA in Kawasaki disease. Arch Dis Child 66:189–192
5. Salo E, Kekomaki R, Pelkonen P, Ruuskanen O, Viander M, Wagner O (1988) Kawasaki disease: monitoring of circulating immune complexes. Eur J Pediatr 147:377–380
6. Cunningham MW, Meissner CH, Heuser JS, Pietra BA, Kurahara DK, Leung DYM (1999) Anti-human cardiac myosin autoantibodies in Kawasaki syndrome. J Immunol 163:1060–1065
7. Guzman J, Fung M, Petty RE (1994) Diagnostic value of antineutrophil cytoplasmic and anti-endothelial cell antibodies in early Kawasaki disease. J Pediatr 124:917–920
8. Gupta M, Johann-Liang R, Bussel JB, Gersony WM, Lehman TJ (2002) Elevated IgA and IgM anticardiolipin antibodies in acute Kawasaki disease. Cardiology 97:180–182
9. Lee LA, Burns J, Glode M, Harmon C, Weston WL (1983) No autoantibodies to nuclear antigens in the Kawasaki disease. N Engl J Med 308:1034
10. Birdi N, Laxer RM, Silverman ED (1991) Antineutrophil cytoplasmic antibodies in Kawasaki disease. Arthritis Rheum 34(Suppl):70
11. Serodia-AMC. Semi-quantitative microtiter particle agglutination test for in vitro diagnostic detection and titration of microsomal antibodies in human serum. Fujirebio Inc., Tokyo, Japan
12. Shulman ST, Bass JL, Bierman F, Burns JC, Chung K, Dillon MJ, et al (1989) Management of Kawasaki syndrome: a consensus statement prepared by North American participants of the third international Kawasaki disease symposium, Tokyo, Japan, December 1988. Pediatr Infect Dis J 8(Suppl):663–667
13. Kato H, Inoue O, Akagi T (1988) Kawasaki disease: cardiac problems and management. Pediatr Rev 9:209–217
14. Leung DY (1989) The immunologic effects of IVIG in Kawasaki disease. Int Rev Immunol 5:197–202
15. Rowley AH, Shulman ST (1987) The search for the etiology of Kawasaki disease. Pediatr Infect Dis J 6:506–508
16. Leung DY, Siege RL, Grady S, Krensky A, Meade R, Reinherz EL, Geha RS (1982) Immunoregulatory abnormalities in mucocutaneous lymph node syndrome. Clin Immunol Immunopathol 23:100–112
17. Leung DY, Collins T, Lapierre LF, et al (1986) IgM antibodies in the acute phase of KD lysed cultured vascular endothelial cells stimulated by gamma interferon. J Clin Invest 77:1428–1435
18. Suzuki H, Noda E, Miyawaki M, Takeuchi T, Uemura S, Koike M (2001) Serum levels of neutrophil activation cytokines in Kawasaki disease. Pediatr Int 43(2):115–119
19. Falcini TF, Trapani S, Turchini S, et al (1997) Immunological findings in Kawasaki disease: an evaluation in a cohort of Italian children. Clin Exp Rheumatol 15(6):685–689
20. Nonoyama S (1991) Immunological abnormalities and endothelial cell injury in Kawasaki disease. Acta Pediatr Jpn 33:752
21. Lin CY, Hwang B (1987) Serial immunologic studies in patients with mucocutaneous lymph node syndrome (Kawasaki disease). Ann Allergy 59:291–297
22. Ohshio G, Furukawa F, Khine M, Yoshioka H, Kudo H, Hamashima Y (1987) High levels of IgA-containing circulating immune complexes and secretory IgA in Kawasaki disease. Microbiol Immunol 31:891–898
23. Li C, Yang X, Shen J, Li Y, Jiang L (1990) Immunoglobulin G subclasses in serum and circulating immune complexes in patients with Kawasaki syndrome. Pediatr Infect Dis J 9:544–547
24. Takeshita S, Kawase H, Shimizu T, Yoshida M, Sekine I (2002) Increased production of serum IgA—class antibody to lipid A in Kawasaki disease. Pediatr Int 44:5–11
25. Rowley AH, Shulman ST, Spike BT, Mask CA, Baker SC (2001) Oligoclonal IgA response in the vascular wall in acute Kawasaki disease. J Immunol 166(2):1334–1343
26. Krasovec S, Bezrodnik L, Gaillard MI, et al (2001) Kawasaki disease. Immunological evaluation of 26 cases. Medicina 61(1):8
27. Nash MC, Shah V, Reader JA (1995) Anti-neutrophil cytoplasmic antibodies and anti-endothelial cell antibodies are not increased in Kawasaki disease. Br J Rheumatol 34(9):882–887
28. Rider LG, Wener MH, French J, et al (1993) Autoantibody production in Kawasaki syndrome. Clin Exp Rheumatol 11(4):445–449
29. Vaarala O, Salo E, Pelkonen P, Palosuo T, Aho K (1990) Anticardiolipin response in Kawasaki disease. Acta Pediatr Scand 79:804–809
30. Nomura Y, Yoshinaga M, Oku S, Kono Y, Yuasa Y, Noda T (1993) Estimation of myocardial damage in Kawasaki disease using antimyosin antibody. Acta Paediatr Jpn 35(5):412–417
31. Singh S, Kumar L, Khetarpal R, Aggarwal P, Marwaha RK, Minz RW, Sehgal S (1997) Clinical and immunological profile of SLE: some unusual features. Indian Pediatr 34:979–986
32. Kumar L, Singh S, Goraya JS, Uppal B, Kakkar S, Walker R, Sehgal S (1998) Henoch-Schonlein purpura: the Chandigarh experience. Indian Pediatr 35(1):19–25
33. Singh S, Salaria M, Kumar L, Minz R, Datta U, Sehgal S (1999) Clinico-immunological profile of juvenile rheumatoid arthritis at Chandigarh. Indian Pediatr 36(5):449–454
34. Singh S, Kumar L, Shankar KR (1997) Juvenile dermatomyositis in North India. Indian Pediatr 34(3):193–198
35. Rose NR, Macario EC, Fahey JL, Fereidman H (1992) Immunofluroscent antinuclear antibody test. In: Manual of clinical laboratory immunology, 4th edn. American Society of Microbiology, WA, pp724–729
36. Sehgal S, Aikat BK, Pasricha N (1978) Diagnostic significance of antinuclear antibodies. Indian J Med Res 67:116–128
37. Rose NR, Macario EC, Fahey JL, Fereidman H (1992) Antibodies to tissue specific endocrine, gastrointestinal and surface receptor antigen. In: Manual of clinical laboratory immunology, 4th edn. American Society of Microbiology, WA, pp765–774
38. Rose NR, Macario EC, Fahey JL, Fereidman H (1992) Antineutrophil cytoplasmic autoantibodies. In: Manual of clinical laboratory immunology, 4th edn. American Society of Microbiology, WA, pp781–785
39. Burek CL (1992) Autoantibodies, test for. In: Roitt IM, Delves PJ (eds) Encyclopedia of immunology, Academic Press, London, pp176–180
40. Lahita RG, Chiorazzi N, Reeves WH (2000) How to interpret autoimmune tests. In: textbook of autoimmune disease. Lippincot Williams and Wilkins, Philadelphia, pp727–752
41. Savige J, Gillis D, Benson E, Davies D, Esnault V, Falk RJ, et al (1999) International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). Am J Clin Pathol 111(4):507–513
42. Newburger JW, Sanders SP, Burns JC, Parmess IA, Beiser AS Colan SD (1989) Left ventricular contractility and function in Kawasaki syndrome. Effect of intravenous γ-globulin. Circulation 79:1237–1246