Serum levels of anti-β_2-glycoprotein-I antibodies and anti-cardiolipin antibodies in children with systemic lupus erythematosus

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Original article

***Background*: There are few reports on the relationship of anti-β_2-glycoprotein-I antibodies and anti-cardiolipin antibodies in children with systemic lupus erythematosus (SLE). This study was undertaken to compare the serum levels of anti-β_2-glycoprotein I (β_2-GPI) antibodies with those of anticardiolipin (aCL) antibodies in SLE patients with secondary antiphospholipid syndrome (SAPS) and without SAPS (WSAPS).**

***Methods*: Forty-two SLE patients with SAPS and 68 without SAPS were studied. Serum aCL antibodies and anti-β_2-glycoprotein I antibodies were measured by ELISA.**

***Results*: The serum level of anti-β_2-GPI antibodies in 57.1% (24/42) of the patients in the SAPS-SLE group was higher than that in the control group, whereas it was only 1.5% (1/68) in the WSAPS-SLE group ($P<0.01$). The serum level of aCL antibodies was higher in 6.68% (28/42) of the patients in the SAPS-SLE group and in 42% (29/68) in the WSAPS-SLE group ($P<0.01$).**

***Conclusions*: Anti-β_2-glycoprotein I (β_2-GPI) antibodies are not only strongly associated with SAPS in children with SLE but also highly specific in predicting SAPS-SLE in comparison with aCL antibodies.**

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***Key words*: anti-β_2-glycoprotein I; platelet; anticardiolipin antibodies; thrombopeny; hemolysis; children; SLE**

Introduction

Antiphospholipid syndrome (APS) is often found in patients with thrombus, platelet counts dropping, hemolysis, spontaneous abortion during metaphase, late pregnancy or increased level of antiphospholipin antibodies (aCL).[1] The syndrome is divided into primary and secondary ones. The patients with systemic lupus erythematosus (SLE) often present symptoms with secondary APS. Further research into SLE has indicated that a kind of negatively charged anticardiolipin antibodies and lupus anticoagulants in the sera of patients with secondary antiphospholipid syndrome (SAPS)-SLE (LAC)[2] are associated with lipid proteins. Additionally, another kind of blood plasma lipid protein, namely β_2-glycoprotein I (β_2-GPI), can also react with blood plasma lipid protein. It is indicated that anti-β_2-GPI antibodies are closely related to a variety of clinical symptoms and progression of the disease.[3,4] In the present study, we investigated the isotypes of anti-β_2-GPI antibodies in 110 children with SLE by the method of ELISA, and then analyzed a possible role of the isotypes of anti-β_2-GPI antibodies in children with SAPS-SLE.

Methods

Subjects

A total of 110 children with SLE were recruited at our hospital from January 2000 to September 2003, including 14 males and 96 females, aged from 5 to 17 years. They were all diagnosed according to the diagnostic criteria for SLE and APS established by the American Rheumatism Academy (ARA) in 1998. Based on their clinical symptoms and laboratory findings, the children were grouped into two groups. One group consisted of 42 children with platelet count dropping, thrombosis, hemolysis, anti-aCL antibodies and anti-LAC antibodies positive or negative, and another group comprised 68 children with SLE but SAPS-SLE

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and the above manifestations.[1]

Control group

Normal control group was composed of 37 children aged from 3 to 15 years who were scheduled to perform operation. They had normal liver function and normal blood coagulation. Rheumatoid arthritis control group consisted of 28 children aged from 4 to 12 years with all kinds of rheumatoid arthritis enrolled from inpatient and outpatient departments and follow-up patients.

Methods

Detection of anti-β_2-GPI antibodies

Purified β_2-GPI was diluted into 10 mg/L by PBS buffer. Seventy-five μl of the solution was placed into every well in a 96-well polythylene plate, which were incubated subsequently with 2% BSA. Blood serum at 1:100 dilution, standard substances 2, 20, 200 RU/ml (RU/ml indicates relative unit per ml), and positive and negative controls were all added into the reaction plate. After incubation for an hour at room temperature, reaction liquid was covered by anti-human IgG, IgM, IgA signed by enzyme, substrate and terminating liquid. Optical densitometer was read at the wavelength of 450 nm. The concentration of standard substance was made for x-axis and absorbency for y-axis. Cut-off values of anti-β_2-GPI IgG, IgM, IgA were all 20 RU/ml.

Test of aCL antibodies

All samples were determined for aCL antibodies IgG, IgM, IgA by ELISA. Anti-aCL antibodies IgG, IgM, IgA were typed in all the subjects. Standard curves were made respectively according to the concentration of standard substances 2, 12, 120 U/ml for x-axis and absorbency for y-axis. The absorbency of detected sample was read from the standard curve. aCL antibodies IgG, IgM, IgA were grouped into 4 groups according to their values: negative for value less than 12, low positive for 12-19, moderate positive for 20-79, and strongly positive for higher than or equal to 80.

Test of LAC

This test was performed according to the reported method.[2]

Dynamic observation

Six hospitalized SAPS-SLE children were randomly chosen for observation of dynamic changes of aCL antibodies and anti-β_2-GPI antibodies from 0 to 10 days.

Statistical analysis

Data in the present study were analyzed by student's *t* test and SPSS 10.0 software. A *P* value of less than 0.05 was considered statistically significant.

Results

Comparison of antibodies of aCL and anti-β_2-GPI among the groups

Twenty-five (22.7%) of the 110 children with SLE and 24 (57.1%) of the 42 children with SAPS-SLE showed an increase of anti-β_2-GPI antibodies. Fifty-seven (51.8%) of the 110 children and 28 (66.7%) of the 42 children with SAPS-SLE showed an increase of aCL antibodies.

Twenty-four (57.1%) of the 42 patients with anti-β_2-GPI antibodies positive showed SAPS-SLE with aCL antibodies positive, while none of the patients with aCL antibodies negative SAPS-SLE and WSAPS-SLE showed anti-β_2-GPI antibodies positive; but β_2-GPI antibodies were significantly related to aCL antibodies ($P<0.01$).

In the WSAPE-SLE group, one (1.5%) of the 68 patients showed an increased level of antibodies of β_2-GPI, and 29 (42.7%) an increased level of aCL antibodies. The specificity of anti-β_2-GPI antibodies increased to 98.5%, whereas the specificity of aCL antibodies was only 57.3% ($P<0.01$).

Table 1. Comparison of SAPS, WSAPS and control groups

Group	*n*	Positive (*n*)	Rate of positivity (%)
SLE			
SAPS	42	28	66.7 *△
WSAPS	68	29	42.7 *△
Normal control	37	0	0
Rheumatoid arthritis control	28	9	32.1 *

Comparison of aCL antibodies; *: compared with normal control, $P<0.01$; △: compared with rheumatoid arthritis control group, $P<0.01$.

Table 2. Dynamic comparison of isotypes of anti-aCL and anti-β_2-GPI antibodies in 6 SAPS-SLE patients

Patients	aCL						Anti-β_2-GPI					
	Day 0			Day 10			Day 0			Day 10		
	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA
1	−	++	−	+	+	−	−	++	−	+	++	−
2	−	+++	−	+	+	−	+	++	−	++	+	−
3	+	++	−	++	+	−	+	+++	+	++	+	−
4	−	+	−	+	−	−	−	++	+	+	++	+
5	−	+	+	−	++	+	+	++	+	++	−	−
6	−	+	−	+	−	−	−	+	−	++	−	−

−: negative; +: low positive; ++: moderate positive; +++: strongly positive.

Isotypes of anti-aCL and anti-β_2-GPI antibodies

Isotypes of anti-aCL and anti-β_2-GPI antibodies were determined in 6 children on day 0 and 10 (Table 2). All the 6 patients showed an increased level of IgM of anti-aCL and anti-β_2-GPI antibodies on day 0, whereas

the level of IgG was negative or low positive. On day 10, the level of IgG increased while the level of IgM decreased. The level of IgA was not changed between the 10 days.

Discussion

We determined the isotypes of anti-β_2 GPI antibodies of SLE patients and SLE patients with SAPS. The level of anti-β_2 GPI antibodies increased in the SAPS-SLE patients with aCL antibodies positive, and the level of β_2 GPI antibodies was related to aCL antibodies.[5] As a seromarker, however, anti-β_2 GPI antibodies were more specific than aCL antibodies to judge whether the patients have APS.[6]

Recent studies[7-10] have shown that β_2-GPI is essential to APS. A special bound site of phospholipid exists in the position cystines 281-288 of β_2-GPI peptide chain, which can cognate with aCL antibodies. Cardiolipin monoclonal antibodies purified from a SLE mice model and serum of patients who are aCL antibodies positive can be bound with β_2-GPI in the absence of cardiolipin, confirming that β_2-GPI contributes to the development of APS.[11-15]

aCL antibodies positive are markers of SLE diagnosis.[16-18] In the present study, 25 patients also showed elevated levels of β_2-GPI antibodies and aCL antibodies. About 96% of the patients had SAPS-SLE. In the group of aCL antibodies negative (LAC positive and have or not SAPS), β_2-GPI antibodies were not detected. In the groups of SLE and WSAPS (with or without antibodies), Only 1 patient had an increased level of β_2-GPI antibodies. The results showed that β_2-GPI antibodies are not only closely related to aCL antibodies but also to the development of APS and other clinical symptoms such as thrombus, thrombocytopenia and hemolysis. In the group of WSAPS-SLE, 1.5% of them were β_2-GPI antibodies positive, but 42.8% were aCL antibodies positive. Thus, β_2-GPI antibodies are better than aCL antibodies in judging whether or not SLE patients have SAPS.[19-21] The finding may be explained that polyclonal B cell is activated to produce some "non-specific anti-phospholipid antibodies", which are not related to SLE pathogenesis. In the control group of this study, 32.1% showed a slightly increased level of aCL antibodies (12-19 U/L). We speculate that the increased level of aCL antibodies in the group of WSAPS-SLE may be related to the damage of cell membrane.[22,23]

We found that β_2-GPI could interact negatively with charged proteins and play an important role in APS with thrombosis, thrombocytopenia, hemolysis and spontaneous abortion during metaphase or later.[24] Under normal conditions, β_2-GPI can suppress ADP-dependent platelet aggregation, triggering of blood coagulation factor XII, and blood coagulation.[25] The production of anti-β_2-GPI antibodies either for extrinsic or intrinsic factors may change the structure of β_2-GPI or expose the bounding site.[26]

In the present study, we focused on the relationship between β_2-GPI antibodies and the symptoms of thrombosis, thrombocytopenia, and hemolysis. Because the patients were all under 18 years old, we could not find the correlation of β_2-GPI antibodies and abortion.[27]

Moreover, 14.3% of the SAPS-SLE patients with aCL antibodies positive were not detected with an increased level of anti-β_2 GPI antibodies. We suggested that aCL antibodies tested by ELISA are useful to detect APS.[28,29] Despite non-specific deficiency, the method is very sensitive. aCL antibodies test should be taken as a routine method for the diagnosis of APS, and β_2-GPI antibodies for determination of risk rate of patients with APS.[30]

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References

1 He XH. The antiphospholipid syndrome. In: Hu YM, Jiang ZF, eds. Fu-Tang Zhu Practical Pediatrics. Beijing: People's Medical Publishing House, 2002:711-713.
2 Teng Q, Hou LY, He XH. The determination of Lupus anticoagulant and anticardiolipin antibodies and its clinical application. Chin J Lab Med 1996;19:157-159.
3 Mastuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. Lancet 1990;336:177-178.
4 Arvieux J, Roussel B, Jacob MC, Colomb MG. Measurement of antiphospholipid antibodies by ELISA using β_2-glycoprotein I as an antigen. J Immunol Methods 1991;143:223-230.
5 Bhattacharyya M, Kannan M, Biswas A, Kumar A, Saxena R. Beta2 glycoprotein 1 in Indian patients with SLE. Clin Appl Thromb Hemost 2005;11:223-226.
6 Yang CD, Chen SL, Shen N, Gu YY, Bao CD. Cloning and expression of β_2-GPI recognized by antiphospholipid antibodies and its clinical investigation. Chin J Rheumatol 1998;2:10-13.
7 McLaurin EY, Holliday SL, Williams P, Brey RL. Predictors of cognitive dysfunction in patients with systemic lupus erythematosus. Neurology 2005;64:297-303.
8 Bizzaro N, Tonutti E, Villalta D, Tampoia M, Tozzoli R. Prevalence and clinical correlation of anti-phospholipid-binding protein antibodies in anticardiolipin-negative patients with sys-

temic lupus erythematosus and women with unexplained recurrent miscarriages. Arch Pathol Lab Med 2005;129:61-68.
9 Cucnik S, Kveder T, Krizaj I, Rozman B, Bozic B. High avidity anti-beta2-glycoprotein I antibodies in patients with antiphospholipid syndrome. Ann Rheum Dis 2004; 63: 1478-1482.
10 Sanmarco M, Roll P, Gayet S, Oksman F, Johanet C, Escande A, et al. Combined search for anti-beta2-glycoprotein I and anticardiolipin antibodies in antiphospholipid syndrome: contribution to diagnosis. J Lab Clin Med 2004;144:141-147.
11 Levine JE, Branch DW, Rauch J. The antiphospholipid syndrome. N Engl J Med 2002;346:752-757.
12 Ishikura K, Wada H, Kamikura Y, Hattori K, Fukuzawa T, Yamada N, et al. High prevalence of anti-prothrombin antibody in patients with deep vein thrombosis. Am J Hematol 2004;76:338-342.
13 Lopez LR, Dier KJ, Lopez D, Merrill JT, Fink CA. Anti-beta2-glycoprotein I and antiphosphatidylserine antibodies are predictors of arterial thrombosis in patients with antiphospholipid syndrome. Am J Clin Pathol 2004;121:142-149.
14 Loizou S, Singh S, Wypkema E, Asherson RA. Anticardiolipin, anti-beta2-glycoprotein I and antiprothrombin antibodies in black South African patients with infectious disease. Ann Rheum Dis 2003;62:1106-1111.
15 Doria A, Shoenfeld Y, Wu R, Gambari PF, Puato M, Ghirardello A, et al. Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. Ann Rheum Dis 2003;62:1071-1077.
16 Nojima J. Association between anti-phospholipid antibodies and thrombotic complications in systemic lupus erythematosus. Rinsho Byori 2003;51:239-247.
17 Musial J, Swadzba J, Motyl A, Iwaniec T. Clinical significance of antiphospholipid protein antibodies. Receiver operating characteristics plot analysis. J Rheumatol 2003; 30: 723-730.
18 Meroni PL, Tincani A, Sepp N, Raschi E, Testoni C, Corsini E, et al. Endothelium and the brain in CNS lupus. Lupus 2003;12:919-928.
19 Koskenmies S, Vaarala O, Widen E, Kere J, Palosuo T, Julkunen H. The association of antibodies to cardiolipin, beta2-glycoprotein I, prothrombin, and oxidized low-density lipoprotein with thrombosis in 292 patients with familial and sporadic systemic lupus erythematosus. Scand J Rheumatol 2004; 33:246-252.
20 Delgado Alves J, Ames PR, Donohue S, Stanyer L, Nourooz-Zadeh J, Ravirajan C, et al. Antibodies to high-density lipoprotein and beta2-glycoprotein I are inversely correlated with paraoxonase activity in systemic lupus erythematosus and primary antiphospholipid syndrome. Arthritis Rheum 2002; 46: 2686-2694. Erratum in: Arthritis Rheum 2003;48:284.
21 Antolin J, Almeida D, Amerigo MJ, Cantabrana A, Roces A, Hayeck M. Anti-beta2-glycoprotein I antibodies and systemic lupus erythematosus. Med Clin (Barc) 2002;119:331-332.
22 Ong SG, Cheng HM, Soon SC, Goh E, Chow SK, Yeap SS. IgG anti-beta2 glycoprotein I antibodies in Malaysian patients with antiphospholipid syndrome and systemic lupus erythematosus: prevalence and clinical correlations. Clin Rheumatol 2002;21:382-385.
23 von Scheven E, Glidden DV, Elder ME. Anti-beta2-glycoprotein I antibodies in pediatric systemic lupus erythematosus and antiphospholipid syndrome. Arthritis Rheum 2002; 47: 414-420.
24 Nojima J, Kuratsune H, Suehisa E, Futsukaichi Y, Yamanishi H, Machii T, et al. Association between the prevalence of antibodies to beta2-glycoprotein I, prothrombin, protein C, protein S, and annexin V in patients with systemic lupus erythematosus and thrombotic and thrombocytopenic complications. Clin Chem 2001;47:1008-1015.
25 Loizou S, Samarkos M, Norsworthy PJ, Cazabon JK, Walport MJ, Davies KA. Significance of anticardiolipin and anti-beta2-glycoprotein I antibodies in lupus nephritis. Rheumatology (Oxford) 2000;39:962-968.
26 Davies ML, Young SP, Welsh K, Bunce M, Wordsworth BP, Davies KA, et al. Immune responses to native beta2-glycoprotein I in patients with systemic lupus erythematosus and the antiphospholipid syndrome. Rheumatology (Oxford) 2002;41:395-400.
27 Carmo-Pereira S, Bertolaccini ML, Escudero-Contreras A, Khamashta MA, Hughes GR. Value of IgA anticardiolipin and anti-beta2-glycoprotein I antibody testing in patients with pregnancy morbidity. Ann Rheum Dis 2003;62:540-543.
28 Caronti B, Pittoni V, Palladini G, Valesini G. Anti-beta2-glycoprotein I antibodies bind to central nervous system. J Neurol Sci 1998;156:211-219.
29 Reshetniak TM, Derksen RV, Alekberova ZS, Horbach D, De Groot F, Nasonov EL, et al. Antibodies to beta2-glycoprotein I in systemic lupus erythematosus: new laboratory marker of antiphospholipid syndrome. Klin Med (Mosk) 1998;76:36-40.
30 Guerin J, Casey E, Feighery C, Jackson J. Anti-Beta2-glycoprotein I antibody isotype and IgG subclass in antiphospholipid syndrome patients. Autoimmunity 1999;31:109-116.

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