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Anticardiolipin and Anti-β_2-Glycoprotein I Antibodies in Behcet's Disease

To investigate prevalence of anticardiolipin antibodies (aCL) in patients with Behcet's disease (BD) and to determine whether they are related to anti-β_2-glycoprotein I antibodies (aGPI), we measured aCL and aGPI in 47 patients of BD and 14 patients of systemic lupus erythematosus (SLE). The levels of aCL and aGPI were determined by conventional enzyme immonoassay for both IgG and IgM classes. Twelve (25.5%) patients with BD were positive for IgG or IgM aCL and no patient was positive for aGPI. Eleven (78.6%) patients with SLE were also positive for aCL and among them, 8 (72.7%) patients were positive for aGPI. Positive IgG aCL patients with BD showed lower level of IgG aCL than those with SLE (15.7±7.3 vs 34.1±16.0 GPL, $p<0.05$). There was no relation between the presence of aCL in BD and either clinical activity or clinical features. In the patients with BD, aCL are found but it would not be associated with aGPI as they are in patients with SLE. In patients with BD, aCL seem to be authentic aCL unlike those in patients with SLE and may not be related with vascular complications in BD.

Key Words : *Behcet's syndrome; Antibodies, anticardiolipin*

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INTRODUCTION

Anticardiolipin antibodies (aCL) are autoantibodies detected in sera from patients with systemic lupus erythematosus (SLE), infectious diseases, malignancies, and primary antiphospholipid antibody syndromes. The appearance of the autoantibodies is known to be associated with thromboembolic manifestations such as cerebral or myocardial infarctions, intrauterine fetal death due to placental infarction, and neurological defects.

Recent studies have shown that serum or plasma cofactor was required for aCL induced in SLE to bind to cardiolipin. This cofactor was identified as β_2-glycoprotein I (β_2-GPI). Various physiological functions of β_2-GPI have been reported: inhibition of the intrinsic coagulation pathway, the adenosine diphosphate mediated platelet aggregation, and prothrombinase activity of activated platelets.

It has also been reported that the behavior of aCL in relation to β_2-GPI is heterogeneous. Two different types of aCL are known to exist; infection associated aCL which bind directly to anionic phospholipids (1, 2) and autoimmune type aCL which do not recognize anionic phospholipids alone, but a complex of anionic phospholipids and β_2-GPI (3). It has been reported that antibodies binding directly to β_2-GPI (aGPI) in enzyme linked solid phase immunoassay (ELISA) were associated with thrombosis in patients with SLE and primary antiphospholipid syndrome (4, 5).

The reported prevalence of aCL in Behcet's disease (BD) has varied widely and the association of aCL with clinical and serological features of BD has not yet been determined. Furthermore, relation of aCL in BD with aGPI has not been clarified. In this study, we investigated prevalence of aCL in patients with BD and determined whether they are related with aGPI which are suggested as a new marker of thromboembolic complications.

MATERIALS AND METHODS

Serum samples were obtained from 47 patients with BD, 14 patients with SLE and 20 healthy control subjects. The patients with BD (21 males, 26 females; aged 21-61, median 40.5 years) fulfilled the criteria of the International Study Group for Behcet's Disease (6) and

did not have concurrent infection or other medical problems. The age of onset ranged from 18 to 48 years (median 32), with duration of 1-20 years (median 9). Clinical and laboratory data were reviewed at the time when blood samples were collected. The clinical features included oral ulcer, genital ulcer, skin manifestations, eye involvement, arthritis, thrombophlebitis and neurologic manifestations. One point was assigned for each clinical feature, and the sum was defined as the score of clinical activity (7). To determine vascular involvement, we performed color Doppler sonography or skin biopsy in the patients who complained pain with physical findings such as swelling, redness and tenderness. The color Doppler sonography was performed in two patients and skin biopsy in one patient. Two of 21 patients were positive for Pathergy test. The collected sera were also tested for VDRL and fluorescent antinuclear antibodies (ANA). The latter were tested at 1:40 dilution.

The levels of aCL and aGPI of both IgG and IgM classes were measured using commercially available enzyme immonoassay kits (Quanta Lite™, INNOVA Diagnostics Inc., USA). For aGPI test, γ irradiated polystyrene 96-microwell plate coated with purified β_2-GPI, and for aCL test, polystyrene 96-microwell plate coated with purified cardiolipin were used. The results of aCL were expressed as standard units of either IgG (GPL) and IgM (MPL) (8) and considered negative (<10 GPL or MPL), low positive (10 to 40 GPL or MPL), or highly positive (>40 GPL or MPL) (9). We considered positive more than 20 units for IgG aGPI (10) and 20 Standard IgM β_2 GPI Units (SMU) for IgM aGPI.

Statistical analysis was carried out using Fisher's exact probability test for analysis of frequencies and Wilcoxon rank sums test for comparison of means. Two tailed p values <0.05 were considered significant.

RESULTS

As shown in Table 1, aCL were detected in 12 of 47 patients with BD (25.5%), 11 of 14 patients with SLE (78.6%) and 3 of 20 healthy control subjects (15%). The frequency of aCL in patients with BD was significantly lower than in those with SLE (p=0.001). Positive aCL patients with BD showed lower level of serum aCL than those with SLE (IgG 15.7±7.3 vs 34.1±16.0 GPL, p=0.004; IgM 11.5±1.8 vs 17.5±4.5 MPL, p=0.03). The levels of aCL IgG and IgM in the serum samples are shown in Fig. 1.

In 9 patients with SLE (64.3%), aGPI were detected, but neither IgM nor IgG aGPI was detected in patients with BD and healthy control subjects. Among aCL positive patients, aGPI were more frequently detected in patients with SLE than those with BD (8/11 vs 0/12, p=0.0003).

Table 1. Frequencies of anticardiolipin antibodies (aCL) and anti-β_2-glycoprotein I antibodies (aGPI)

	Behcet's disease (N=47)	SLE (N=14)	Control (N=20)
Positive aCL	12 (25.5%)*	11 (78.6%)	3 (15.0%)
IgG	11 (23.4%)	7 (50.0%)	1 (5.0%)
IgM	2 (4.3%)	10 (71.4%)	2 (10.0%)
IgG & IgM	1 (2.1%)	6 (42.9%)	0 (0.0%)
Positive aGPI	0 (0.0%)†	9 (64.3%)	0 (0.0%)
IgG	0 (0.0%)	9 (64.3%)	0 (0.0%)
IgM	0 (0.0%)	8 (57.1%)	0 (0.0%)
IgG & IgM	0 (0.0%)	8 (57.1%)	0 (0.0%)

*p=0.001 compared to SLE; †p<0.0001 compared to SLE.

There was no significant association of aCL with any clinical feature with BD (Table 2). Two patients had thrombophlebitis; one was confirmed by skin biopsy and the other showed venous thrombosis on color Doppler sonography with typical symptoms and findings. The VDRL and ANA tests were negative in all patients.

Table 3 shows the frequencies of aCL in patients with BD according to clinical activities. The mean score of clinical activity was 2.0±1.12 in positive aCL and 2.2±0.88 in negative aCL patients with BD, which did not show any statistical difference.

Table 2. Correlation of anticardiolipin antibodies with the clinical features of patients with Behcet's disease

Clinical feature	ACL negative (n=35)	aCL positive (n=12)	p*
Oral ulcer	35 (100.0%)	12 (100.0%)	
Genital ulcer	34 (97.1%)	11 (91.7%)	0.45
Skin involvement			
Erythema nodosum	14 (40.0%)	6 (50.0%)	0.73
Papular pustular lesion	7 (20.0%)	0 (0.0%)	0.16
Pseudofolliculitis	2 (5.7%)	0 (0.0%)	1.00
Acneiform eruption	4 (11.4%)	1 (8.3%)	1.00
Eye lesion			
Uveitis	11 (31.4%)	5 (41.7%)	0.73
Cells in vitreous	4 (11.4%)	3 (25.0%)	0.35
Retinal vasculitis	2 (5.7%)	1 (8.3%)	1.00
Joint			
Arthirits	15 (42.9%)	4 (33.3%)	0.74
Enthesitis	2 (5.7%)	1 (8.3%)	1.00
Thorombophlebitis	1 (2.9%)	1 (8.3%)	0.45
CNS involvement	2 (5.7%)	0 (0.0%)	1.00
Gastrointestinal features	6 (17.1%)	2 (16.7%)	1.00

*Fisher's exact probability test.

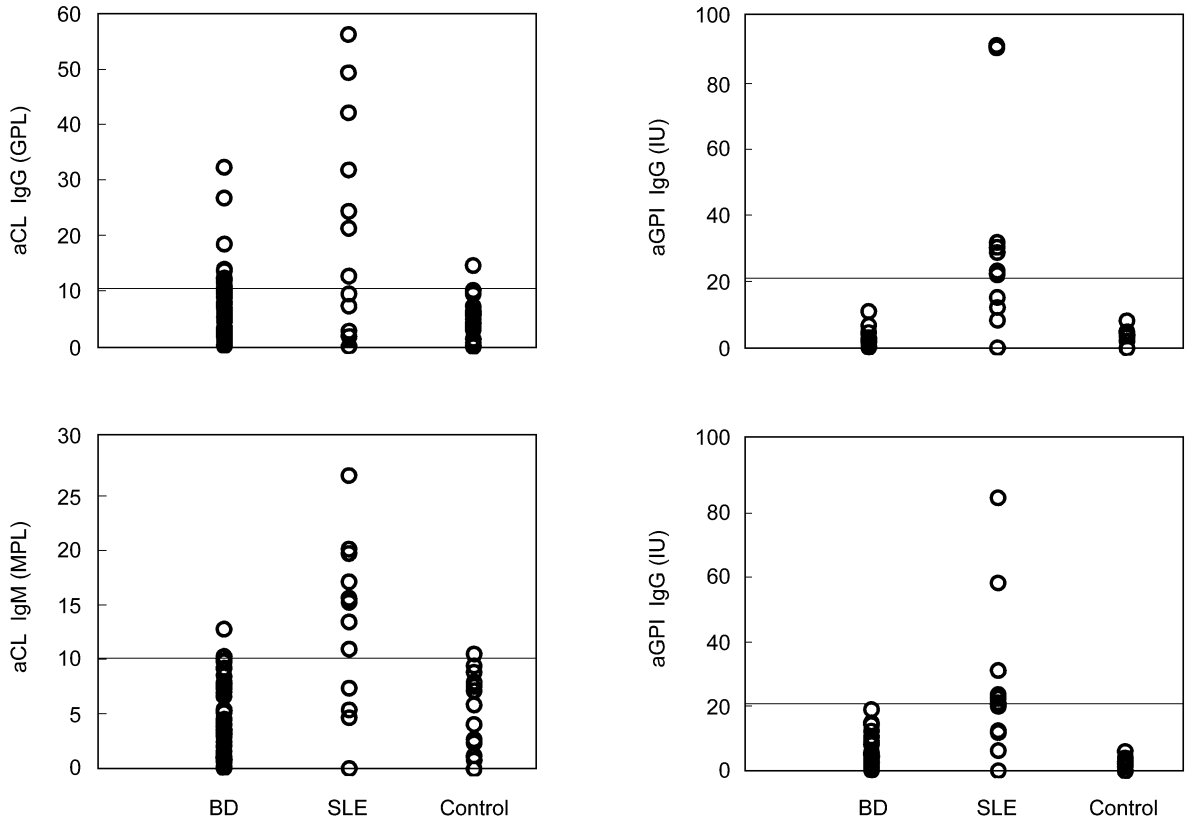

Fig. 1. Levels of IgG and IgM anticardiolipin antibodies (aCL) and anti-β_2-glycoprotein I antibodies (aGPI) in sera from patients with Behcet's disease (BD), systemic lupus erythematosus (SLE), and healthy control subjects. The horizontal lines indicate the cut-off level.

Table 3. Frequencies of anticardiolipin antibodies (aCL) in Behcet's disease according to clinical activities

Score of clinical activities	Positive aCL (%)		
	IgG	IgM	IgG &/or IgM
1 (n=13)	6 (46)	0 (0)	6 (46)
2 (n=17)	1 (6)	0 (0)	1 (6)
3 (n=13)	4 (31)	1 (8)	4 (31)
4 (n=4)	0 (0)	1 (25)	1 (25)

DISCUSSION

In patients with infections, aCL have been detected, as a drug- induced condition, and in up to 10% of population having no predisposing factors, as well as patients with autoimmune conditions (11). The presence of aCL is associated with multiple arterial or venous thrombosis, recurrent fetal loss, and thrombocytopenia in some patients. However, these clinical features are not associated with aCL occurring in syphilis or other infectious diseases. Assessment of the clinical importance of these autoantibodies have been hampered by the extreme heterogeneity. It has been demonstrated that β_2 GPI is required for binding of aCL to cardiolipin (12, 13). Additional interesting findings by other authors (13, 14, 15) suggest that β_2 GPI may be the required cofactor of some lupus anticoagulant and aCL for the expression of their anticoagulant activity. This protein has been considered as the true antigen for aCL (3, 16) or a cofactor that enhances the binding to cardiolipin (17). Wagenknecht and McIntyre (18) postulated that β_2 GPI undergoes conformational changes when it binds to anionic phospholipids. It was also postulated that a neoepitope can be exposed when β_2 GPI is bound to phospholipids (12, 19, 20) or to a negatively charged surface, irradiated polystyrene (21). It has been demonstrated recently (22) that aCL detected in patients with infection bind directly to phospholipids without requirement of β_2 GPI, but aCL present in autoimmune diseases do not bind phospholipids alone — they require β_2 GPI for binding. Recent findings have shown that aGPI differentiate aCL associated with infectious disease from autoimmune aCL (1, 2).

The associations of positive aCL with BD have been investigated with conflicting results. In 1984, Hull et al.

detected 13 patients positive for aCL out of 70 (19%) with BD (seven IgG, three IgM, three IgG and IgM) and reported significant relation between presence of aCL and retinal vasculitis (23). In 1993, Zouboulis et al. detected that 14 of 30 patients with BD (46.7%) were positive for aCL, significantly IgM class, the presence of which were associated with cutaneous vasculitis such as erythema nodosum (24). In Korea, Ji et al. reported that 40% of patients with BD had aCL, mainly IgM class (25), and Park et al. reported 28.6%, mainly IgG class (26). Our prevalence of aCL was 25.5%, and mainly IgG class were detected. The reason for disparity between these studies remains unclear. It cannot be explained by clinical or ethnic differences in the groups with BD studies. This discrepancy might rather result from different technical approaches in the measurement of aCL. Harris et al. found that the assay methods affected the results of ELISA test for the detection of aCL and the use of phosphate buffered saline (PBS) alone, PBS/Tween, or 0.3% gelatin/PBS as diluent were not valid (8). The need of evaluation and standardization of aCL test were suggested. Many researchers have used PBS/Tween as diluents, which might be a reason of disparity in aCL results.

In this study, the presence of aCL did not show any relation with either clinical activity or clinical features including erythema nodosum, and retinal vasculitis. The levels of serum aCL in the patients with BD were all low positive and significantly lower than those in SLE. IgM only or low titer aCL are considered clinically less important than high titer IgG aCL (27). The search for lupus anticoagulant was negative in 69 patients with BD (28). Moreover, we could not find any patient with BD who had aGPI, in contrast 9 patients with SLE (64.3%) were positive for aGPI. aGPI are more specific marker of thrombosis than aCL in the patients with SLE (29, 30). Although we could not confirm any correlation between aGPI and vascular complication in patients with SLE, these results suggest that aCL in patients with BD are authentic aCL unlike those in patients with SLE and their presence may not be related with vascular complications in BD.

REFERENCES

1. Forastiero RR, Martinuzzo ME, Kordich LC, Carreras LO. *Reactivity to β_2 glycoprotein I clearly differentiates anticardiolipin antibodies from antiphospholipid syndrome and syphilis. Thromb Haemost 1996; 75: 717-20.*
2. McNally T, Purdy G, Mackie IJ, Machin SJ, Isenberg DA. *The use of an anti-β_2-glycoprotein-I assay for discrimination between anticardiolipin antibodies associated with infection and increased risk of thrombosis. Br J Haematol 1995; 91: 471-3.*
3. Pengo V, Biasiolo A, Fior MG. *Autoimmune antiphospholipid antibodies are directed against a cryptic epitope expressed when β_2-glycoprotein I is bound to a suitable surface. Thromb Haemost 1995; 73: 29-34.*
4. Horkko S, Miller E, Dudl E, Reaven P, Curtiss LK, Zvaifler NJ, et al. *Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids. Recognition of cardiolipin by monoclonal antibodies to epitopes of oxidized low density lipoprotein. J Clin Invest 1996; 98: 815-25.*
5. Koike T, Tsutsumi A, Ichikawa K, Matsuura E. *Antigenic specificity of the "anticardiolipin" antibodies. Blood 1995; 85: 2277-80.*
6. International Study Group for Behcet's Disease. *Criteria for diagnosis of Behcet's disease. Lancet 1990; 335: 1078-80.*
7. Lee ES, Lee YD, Bang D, Lee SH, Lee S. *A study of interleukin 2 activity in Behcet's syndrome. Ann Dermatol 1991; 5: 5-11.*
8. Harris EN, Gharavi AE, Patel SP, Hughes GRV. *Evaluation of the anticardiolipin test: report of an international workshop held 4 April 1986. Clin Exp Immunol 1987; 68: 215-22.*
9. Finazzi G, Brancaccio V, Moia M, Ciaverella N, Mazzucconi MG, Schinco PC, Ruggeri M, Pogliani EM, Gamba G, Rossi E, Baudo F, Manotti C, D'Angelo A, Palareti G, De Stefano V, Berrettini M, Barbui T. *Natural history and risk factors for thrombosis in 360 patients with antiphospholipid antibodies: a four-year prospective study from the Italian Registry. Am J Med 1996; 100: 530-6.*
10. Erickson E, Najmey S, Keil LB, El-Kadi HS, DeBari VA. *Reference calibrators for IgG antibodies to β_2-glycoprotein I: preparation, properties and availability to investigator. Clin Chem 1996; 42: 1-2.*
11. McNeil HP, Chesterman CN, Krilis SA. *Immunology and clinical importance of aniphospholipid antibodies. Adv Immunol 1991; 49: 193-280.*
12. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. *Antiphospholipid antibodies are directed against complex antigen that includes a lipid-binding inhibitor of coagulation: β_2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 1990; 87: 4120-4.*
13. Galli M, Comfurius P, Barbui T, Zwaal RFA, Bevers EM. *Anticoagulant activity of β_2 glycoprotein I is potentiated by a distinct subgroup of anticardiolipin antibodies. Thromb Haemost 1992; 68: 297-300.*
14. Oosting JD, Derksen RHWM, Entjes HTI, Bouma BN, de Groot PG. *Lupus anticoagulant activity is frequently dependent on the presence of β_2-glycoprotein I. Thromb Haemost 1992; 67: 499-502.*
15. Roubey RA, Pratt CW, Buyon JP, Winfield JB. *Lupus anticoagulant activity of autoimmune antiphospholipid antibodies is dependent upon β_2-glycoprotein I. J Clin Invest 1992; 90: 1100-4.*
16. Galli M, Comfurius P, Maassen C, Hemker HC, de Baets MH, van Breda-Vriesman PJ, Barbui T, Zwaal RF, Bevers EM. *Anticardiolipin antibodies (ACA) directed not to cardiolipin but*

to a plasma protein cofactor. Lancet 1990; 335: 1544-7.

17. Pierangeli SS, Harris EN, Davis SA, DeLorenzo G. *β_2-glycoprotein I (β_2-GPI) enhances cardiolipin binding activity but is not the antigen for phospholipid antibodies. Br J Haematol 1992; 82: 565-70.*
18. Wagenknecht DR, McIntyre JA. *Changes in β_2-glycoprotein I antigenicity induced by phospholipid binding. Thromb Haemost 1993; 69: 361-5.*
19. Arvieux J, Roussel B, Jacob MC, Colomb MG. *Measurement of anti-phospholipid antibodies by ELISA using β_2-glycoprotein I as an antigen. J Immunol Methods 1991; 143: 223-9.*
20. Chamley LW, McKay EJ, Pattison NS. *Cofactor dependent and cofactor independent anticardiolipin antibodies. Thromb Res 1991; 61: 291-9.*
21. Roubey RA, Winfield JB. *Certain lupus anticoagulants recognize neo-antigens of phospholipid bound β_2-glycoprotein I. Thromb Haemost 1993; 69: 1011.*
22. Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T. *Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. J Immunol 1992; 148: 3885-91.*
23. Hull RG, Harris EN, Gharavi AE, Tincani A, Asherson RA, Valesini G, Denman AM, Froude G, Hughes GR. *Anticardiolipin antibodies: occurrence in Behcet's syndrome. Ann Rheum Dis 1984; 43: 746-8.*
24. Zouboulis CC, Puttner P, Tebbe B, Orfanos CE. *Anticardiolipin antibodies in Admantiades-Behcet's disease. Br J Dermatol 1993; 128: 281-4.*
25. Ji HG, Bang DS, Lee SN, Cha YS. *Anticardiolipin antibodies in patients with Behcet's disease. Ann Dermatol 1991; 32: 326-9.*
26. Park DJ, Hong OJ, Kim SH, Park SY, Kim HY, Kim DJ. *Anticardiolipin antibody measured by ELISA in Behcet's disease. Korean J Int Med 1989; 37: 205-9.*
27. Silver RM, Porter TF, van Leeuween I, Jeng G, Scott JR, Branch DW. *Anticardiolipin antibodies: clinical consequences of "low titers". Obstet Gynecol 1996; 87: 494-500.*
28. Bang DS, Ji HG, Choi YS, Lee SN. *Absence of lupus anticoagulants in Behcet's disease. Yonsei Med J 1991; 32: 326-9.*
29. Pengo V, Biasiolo A, Brocco T, Tonetto S, Ruffatti A. *Autoantibodies to phospholipid-binding plasma proteins in patients with thrombosis and phospholipid-reactive antibodies. Thromb Haemost 1996; 75: 721-4.*
30. Swadzba J, de Clerck LS, Stevens WJ, Bridts CH, van Cotthem KA, Musial J, Jankowski M, Szczeklik A. *Anticardiolipin, anti-β_2-glycoprotein I, antithrombin antibodies, and lupus anticoagulant in patients with systemic lupus erythematosus with a history of thrombosis. J Rheumatol 1997; 24: 1710-5.*