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Antiphospholipid Antibodies in Venous Thromboembolism Patients Younger than 50 Years Old

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ABSTRACT

Background: *Antiphospholipid antibodies are among the most important risk factors of arterial and venous thrombosis. Various studies have demonstrated that these antibodies are seen in patients with deep vein thrombosis (DVT) and pulmonary embolism (PE) more than normal individuals but there are a few studies about prevalence of these antibodies in patients younger than 50 years old with venous thromboembolism (VTE). This study aimed to evaluate these antibodies in this age group.*

Materials and Methods: *This was a case-control study. Fifty patients younger than 50 years old with venous thromboembolism (DVT, PE or both) who were diagnosed according to the standard criteria were compared with 48 subjects in the control group. Subjects in the control group were age and sex matched with patients and had no history of venous thromboembolism. Both groups had no history of malignancy or other chronic diseases. Lupus anti-coagulant and serum anticardiolipin antibodies (IgG and IgM) were measured in both groups. Data were analyzed using SPSS version 11.5 software.*

Results: *Fifty VTE patients younger than 50 years of age enrolled in this study (28 males and 22 females; mean age: 38.14±6.5 yrs). Forty-eight subjects were selected as healthy controls (27 males and 21 females; mean age: 38.35±5.06 yrs). Mann –Whitney test showed a significant difference between serum IgM anticardiolipin antibody levels of VTE patients (8.04 MPL units/ml) and those of healthy subjects (1.85 MPL units/ml) (P=0.001).Also, a significant difference was found between serum IgG anticardiolipin antibody levels of VTE patients (8.29 GPL units/ml) and those of healthy subjects (3.51 GPL units/ml) (P=0.001). In VTE group, 7 patients (M/F = 4/3) had an IgG level >10 GPL units/ml and 6 patients (M/F =2/4) had an IgM level >10 MPL units/ml while none of the healthy subjects had IgG or IgM levels higher than 10 (P_{IgM}=0.015 and P_{IgG}=0.007). Lupus anti-coagulant was positive in four (8%) but negative in all healthy subjects (P=0.04).*

Conclusion: *This study demonstrated that antiphospholipid antibodies were more prevalent in VTE patients younger than 50 years old compared to healthy subjects. Considering the fact that these patients need stronger and longer treatment, it seems necessary to evaluate every VTE patient younger than 50 yrs for antiphospholipid syndrome.* ***(Tanaffos2010; 9(2): 26-32)***

Key words: *Venous thromboembolism, Antiphospholipid antibodies, Lupus anticoagulant*

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INTRODUCTION

Deep Vein Thrombosis (DVT), with an incidence of 1/1000 annually, can lead to pulmonary embolism which is a life threatening condition (1,2). Venous thromboembolism (VTE) is responsible for about 15% of hospital deaths and after cardiovascular diseases and cerebrovascular accidents is the third common vascular disease (3,4). On the other hand, patients with DVT can develop post-thrombotic syndrome, deep vein insufficiency, and damage to lower extremities which in long term would affect their quality of life (4, 5). Therefore, diagnosis and also recognizing the risk factors of DVT and PE are imperative.

Antiphospholipid antibodies (aPLs) are a heterogeneous group of immunoglobulins acting against plasma proteins which are bound to the phospholipids, and are among the acquired sources of venous thrombosis (6, 7). These antibodies can cause coagulopathy and diseases such as myocardial infarction, stroke, central retinal vein or artery occlusion, mesenteric vascular occlusion, DVT, and PE (8) either by adhesion to the cardiolipin in the presence of β2 glycoprotein I (anticardiolipin antibody such as IgM and IgG) or by prolonging the clotting test in the laboratory (lupus anticoagulant, LA) (9). LA is rarely found in normal population. In one study it was estimated that about 0.3% of women in their productive age were positive for LA (10). The prevalence of anticardiolipin antibody (ACA) has been reported to be about 5% in normal population (11). These antibodies increase in autoimmune, lymphoproliferative and infectious diseases (12). Over 20% of patients affected by DVT with or without PE are positive for these antibodies (7). In some studies, one third of patients with positive titer of these antibodies were affected by thromboembolic events (13). The recurrence of thrombosis in these patients is a serious problem which requires prolonged and strong doses of anticoagulant medications to prevent recurrence (7, 14, 15).

Considering the fact that these antibodies are associated with increased incidence and recurrence of thrombosis, it seems rational to check these antibodies in patients with thromboembolism. Although several studies have assessed the possible causes of increased coagulation in patients younger than 50 years with venous thromboembolism, only a few studies have focused on the prevalence of antiphospholipid antibodies in this age group. In this study, we aimed to evaluate the prevalence of these antibodies in patients younger than 50 years old suffering from venous thromboembolism.

MATERIALS AND METHODS

This was a case-control study aiming to measure anticardiolipin antibodies and lupus anticoagulant in patients younger than 50 years old affected by venous thromboembolism. The patients were hospitalized at Shahid Sadoughi Hospital in Yazd from summer 2007 to spring 2008. Patients younger than 50 years old with a diagnosis of venous thromboembolism (DVT, PE or both) who had no history of malignancy or chronic diseases were assigned to the case group. Due to the confounding effect of age on serum level of antiphospholipid antibodies, older patients were excluded from the study. DVT was diagnosed based on clinical features and positive Doppler sonography for thrombophlebitis. Diagnosis of PE was made based on the presence of Wells criteria for clinical probability of PE (in the range of moderate to high) along with high probability of PE in the lung perfusion scan or confirmation of PE in the chest spiral CT scan. Patients with clinical and para-clinical features of both DVT and PE were allocated to the combined group. Patients with unconfirmed paraclinical findings were excluded from the study. Other exclusion criteria were collagen-vascular diseases such as SLE, IV drug abusers and cancer patients. Controls were matched to the cases in terms of age and sex. They expressed no history of VTE or any other fundamental diseases and were selected from blood donors in Yazd transfusion center.

Fifty subjects were in the case group and 48 in the control group. Required data were collected using a questionnaire containing demographic information, clinical signs and symptoms, past medical history, family history of venous thromboembolism, physical examination findings, imaging findings, and serology results. The questionnaire was filled out and blood sample was taken after explaining the research project to patients and obtaining their consent. In order to assess lupus anticoagulant, 2.5 ml of blood was transferred to a tube containing trisodium citrate (3.2%). Using a specific kit (ORG, Germany) antiphospholipid antibodies were measured via ELISA method. The kit, as a quantification immunoassay kit, was utilized to determine the presence of auto antibodies of class IgG and IgM including: anticardiolipin, phosphatidyl serine, phosphatidyl inositol, and phosphatidic acid. Adhesion of antiphospholipid antibodies depends on the existence of β2GPI co-factor. In the aforementioned kit, a mixture of above antibodies with human β2GPI exists. This kit appears to have no cross reaction with anti-DNA or syphilis antibodies.

The normal range of anticardiolipin antibodies (both IgG and IgM) has reported to be less than 10 GPL/ml. Therefore, amounts higher than 10 were considered positive in this study. The first blood sample was taken 36-48 hours after the diagnosis of venous thromboembolism. If the sample was positive for anticardiolipin antibodies or LA, another sample was taken 8-10 weeks later. Patients with two positive blood samples were considered positive for mentioned antibodies. Data analysis was performed using SPSS version 11.5 software, t-test and Chi-square test.

RESULTS

Cases were 22 (44%) women and 28 (56%) men with a mean age of 38.14 ± 6.5 years. The control group consisted of 48 subjects including 21 (43.7%) women and 27 (56.3%) men with a mean age of 38.35±5.06 years. Fourteen patients (28%) presented with DVT, 23 (46%) with PE, and 13 (26%) with DVT+PE. Whilst, none of the subjects in the control group showed anticardiolipin antibody levels higher than 10 GPLunits/ml, 13 patients had anticardiolipin antibody levels higher than 10 GPLunits/ml (Table 1). Regarding anticardiolipin antibodies, the average serum IgM concentration was 8.04 MPL Units/ml and 1.85 MPL Units/ml in cases and controls, respectively (P= 0.001). The mean serum IgM concentration was 3 in the case group and 1.05 in controls .The mean serum IgG was 8.29 GPL Units/ml and 3.51 GPL Units/ml in case and control groups, respectively (P=0.001) (Table 2). The median of serum IgG was 3.1 and 1.7 in case and control groups, respectively. In the case group, 7 patients (M/F = 4/3) (14%) had an IgG level >10, 5 patients (10%) had an IgG level between 10-20 and 2 (4%) higher than 20. Six patients (M/F =2/4) (12%) had an IgM level >10 among which, 5 (10%) had IgM between 10-20 and one (2%) above 20. Among different subgroups of patients with venous thromboembolism, IgG more than 10 was seen in 14.29% of patients with DVT, 13.05% of patients with PE, and 15.39% of patients with DVT+PE. IgM concentration higher than 10 was seen in 14.29% of patients with DVT, 13.05% of patients with PE, and 7.7% of patients with DVT+PE. However, none of the subjects in the control group had IgM or IgG above 10 [P(IgG)=0.007, P(IgM)=0.015] (Table 3). Lupus anticoagulant was positive in 4 (8%) VTE patients but negative in all healthy subjects (P=0.04).

Table 1. Comparison of the percentage of antiphospholipid IgM and IgG in cases and controls.

Antibodies Group	IgG Antiphospholipid GPL Units/ml		IgM Antiphospholipid MPL Units/ml	
	Positive (>10)	Negative (<10)	Positive (>10)	Negative (<10)
Patients	7 14%	43 86%	6 12%	44 88%
Controls	0 0%	48 100%	0 0%	48 100%
Fisher's exact test	P=0.01		P=0.02	

Table 2. The mean levels of antiphospholipid antibodies of IgG and IgM in cases and controls

Groups Antibodies	Patients		Controls		t-test
	Mean	SD	Mean	SD	
IgG (GPL) Units/ml	8.29	2.32	3.51	2.45	P=0.001 T= 12.2
IgM (MPL) Units/ml	8.04	2.05	1.85	1.99	P= 0.001 T= 23.2

One GPL or MPL unit/ml is defined as the binding activity of 1 mg/ml of IgG or IgM standard serum respectively.

Table 3. The prevalence of IgG and IgM antibodies in different groups of VTE.

Groups	IgG (GPL Units/ml)		IgM (MPL Units/ml)	
	Negative No.(%)	Positive No.(%)	Negative No.(%)	Positive No.(%)
DVT	12 (85.71)	2 (14.29)	12 (85.71)	2 (14.29)
PE	20 (86.95)	3 (13.05)	20 (86.95)	3 (13.05)
DVT+ PE	11(84.61)	2 (15.39)	12 (92.30)	1 (7.7)
Total	43 (86)	7 (14)	44 (88)	6 (12)

DVT: Deep Vein Thrombosis, PE: Pulmonary Embolism.

DISCUSSION

In our study, levels of IgM and IgG antiphospholipid antibodies were found to be higher in patients with venous thromboembolism in comparison with healthy subjects. This is in agreement with studies of Ishikura and Margaglione (16, 17). The rate of positive or high titers (titer above 10 units) of IgM and IgG antiphospholipid antibodies in our study was 12% and 14% respectively; whereas, these rates in Ishikura study were 18.8% and 20.8% respectively. In Margaglione study, the level of IgG antiphospholipid antibody in patients with DVT/PE was reported to be 14.3%. However, the titers of these antibodies were different in different groups of VTE patients. In fact, in Margaglione study, they found that 9.8% of DVT patients and 7.9% of PE patients had high titers of antiphospholipids. In our study, 15.39% of patients with DVT/PE had high titers of antibodies in comparison to 14.29% of patients with DVT alone. According to these studies, the more extensive the thrombosis, the higher would be the level of antibodies.

Palomo and co-workers studied 226 patients with thrombosis out of which, 78 were identified as venous thrombosis and 30 of them were under 50 years old. In this group, 30.8% of patients were positive for anticardiolipin and 3.9% for anti β2 GPI. In their study, all patients with positive anticardiolipin, had titers higher than 40 units (high titers) (9).

De Godoy et al. examined 60 patients with DVT and found that 56% of them were positive for anticardiolipin antibody among which 31.6% had titers as high as 10-15 units and 25% had titers higher than 15 units. They also observed that the rate of recurrence of thrombotic events was much higher among patients with higher titers (18).

In a study by Kahwa and his colleagues on Jamaican young women, they reported that 16 of 50 under study patients affected by venous thromboembolism had high titers of antiphospholipid antibodies (32%) while just 25 subjects of 148 controls (16%) were positive for these antibodies (19). Garcia and his co-workers evaluated these antibodies in 100 Spanish patients younger than 50 years old who were hospitalized due to traumatic thromboemboli (with no history of neoplastic or chronic diseases) and found positive titers in 19% of these patients (20). In our study; however, 26% of patients were positive for anticardiolipin out of which 6% had titers higher than 20 units. The difference in levels of antibodies between our study and some other studies may be due to epidemiological profile or the kit type used.

Also, some studies have been performed on the relationship between aPL antibodies and thrombosis in Iran. Owlia et al. measured antiphospholipid antibodies in younger patients (under 50 years old) with stroke and showed statistically significant difference in mean IgM concentrations between the cases and controls. They concluded that antiphospholipid antibodies should be measured in every middle-aged patient with storke (21).

In another study conducted by saadatnia et al. in Isfahan, of 117 young patients (<45 years old) with ischemic stroke, 7 men and 16 women (23 patients, 19.6%) had increased levels of IgG type aPL antibodies. Increased titers of IgM and IgG were found in 19 (82.6%) and 6 (26%) patients for aPL antibodies and in 15 (83.3%) and 8 (44.4%) cases for aCL alone (22).

Kazemi et al. reported antiphospholipid antibodies in 47(15.66%) cases out of 300 patients with a history of thrombosis. Twenty-nine patients had low titers and 18 patients had high titers of antiphospholipid antibodies (23). These studies also showed results similar to those of our study and we conclude that aPL antibodies are risk factors for thrombosis in the lungs and other sites.

Lupus anticoagulant in our study, was positive in 8% of patients with venous thromboembolism which was significantly higher than the control group (P=0.04). Likewise, in a study by Schulmans et al. 3-17% of patients had positive lupus anticoagulant (14). In Ishikura study, 38.6% of patients affected by thromboembolism had positive lupus anticoagulant and hence the authors designated this antibody as the most significant risk factor for DVT/PE (16). However, Ginsberg et al. in their study on 65 patients with VTE (mean age of 55 years) measured lupus anticoagulant antibody and found that 24% of these patients had positive titer of this antibody (in comparison with 18% of patients without VTE). They found significant relationship between LA and VTE (24).

De Groot and co-workers evaluated 472 patients with DVT with a mean age of 45 years and reported 3.1% positive LA in comparison with 0.9% positive in normal individuals (25). In addition, in a study by Windyga and Simioni, LA became positive in 7.7% and 8.5% of cases respectively whilst none of the studies found positive LA in controls (26, 27). It seems that lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies and the difference between VTE patients and healthy controls is higher for lupus anticoagulants than for anticardiolipin antibodies. A systematic review showed that the detection of lupus anticoagulants and, possibly, IgG anticardiolipin antibodies at medium or high titers helps to identify patients at risk for thrombosis. This study showed that the risk of lupus anticoagulants was independent of the site and type of thrombosis, the presence of systemic lupus erythematosus, or the coagulation tests employed to detect them (13, 28). Also, some clinical trials have shown the superiority of the lupus anticoagulant and anti-beta2 glycoprotein-I antibodies assays over anticardiolipin determination for the identification of APS patients at risk for thromboembolic complications.

The results of our study indicated that the presence of antiphospholipid antibodies in patients younger than 50 years old who were suffering from venous thromboembolism were more prevalent than in normal individuals. Since pervious studies are in consistent with our findings, it is recommended that these antibodies be regularly assessed in young patients with venous thromboembolism especially in patients younger than 50 years old.

One of the limitations of this study was the long period of time to choose patients carefully and eliminate those with unconfirmed diagnosis. Moreover, small sample size was another limitation of this study .Another study with a larger sample size is required to evaluate other laboratory markers of antiphospholipid antibodies in this group of patients to institute stronger recommendations.

REFERENCES

1. Binder B, Lackner HK, Salmhofer W, Hofmann-Wellenhof R. Risk factors for deep vein thrombosis in women aged 18 to 50: a retrospective analysis. ***Dermatol Surg*** 2009; 35 (3): 451- 6.
2. Madhusudhana S, Moore A, Moormeier JA. Current issues in the diagnosis and management of deep vein thrombosis. ***Mo Med*** 2009; 106 (1): 43- 8; quiz 48-9.
3. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ 3rd. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. ***Arch Intern Med*** 1998; 158 (6): 585- 93.
4. Wakefield TW, Caprini J, Comerota AJ. Thromboembolic diseases. ***Curr Probl Surg*** 2008; 45 (12): 844- 99.
5. Callam MJ, Harper DR, Dale JJ, Ruckley CV. Chronic ulcer of the leg: clinical history. ***Br Med J (Clin Res Ed)*** 1987; 294 (6584): 1389- 91.
6. Bick RL, Ancypa D. The antiphospholipid and thrombosis (APL-T) syndromes. Clinical and laboratory correlates. ***Clin Lab Med*** 1995; 15 (1): 63- 84.
7. Farmer-Boatwright MK, Roubey RA. Venous thrombosis in the antiphospholipid syndrome. ***Arterioscler Thromb Vasc Biol*** 2009; 29 (3): 321- 5.
8. Kinuya K, Kakuda K, Matano S, Sato S, Sugimoto T, Asakura H, et al. Prevalence of deep venous thrombosis in the lower limbs and the pelvis and pulmonary embolism in patients with positive antiphospholipid antibodies. ***Ann Nucl Med*** 2001; 15 (6): 495- 7.
9. Palomo I, Pereira J, Alarcón M, Vásquez M, Pinochet C, Vélez MT, et al. Prevalence and isotype distribution of antiphospholipid antibodies in unselected Chilean patients with venous and arterial thrombosis. ***Clin Rheumatol*** 2004; 23 (2): 129- 33.
10. Lockwood CJ, Romero R, Feinberg RF, Clyne LP, Coster B, Hobbins JC. The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. ***Am J Obstet Gynecol*** 1989; 161 (2): 369- 73.
11. Petri M. Epidemiology of the antiphospholipid antibody syndrome. ***J Autoimmun*** 2000; 15 (2): 145- 51.
12. Dutta TK, Venugopal V. Venous thromboembolism: the intricacies. ***J Postgrad Med*** 2009; 55 (1): 55- 64.
13. Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. ***Blood*** 2003; 101 (5): 1827- 32.
14. Schulman S, Svenungsson E, Granqvist S. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. ***Am J Med*** 1998; 104 (4): 332- 8.
15. Derksen RH, de Groot PG, Kater L, Nieuwenhuis HK. Patients with antiphospholipid antibodies and venous thrombosis should receive long term anticoagulant treatment. ***Ann Rheum Dis*** 1993; 52 (9): 689- 92.
16. 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 (4): 338- 42.

17. Margaglione M, Brancaccio V, De Lucia D, Martinelli I, Ciampa A, Grandone E, et al. Inherited thrombophilic risk factors and venous thromboembolism: distinct role in peripheral deep venous thrombosis and pulmonary embolism. ***Chest*** 2000; 118 (5): 1405- 11.
18. de Godoy JM, de Godoy MF, Braile DM. Recurrent thrombosis in patients with deep vein thrombosis and/or venous thromboembolism associated with anticardiolipin antibodies. ***Angiology*** 2006; 57 (1): 79- 83.
19. Kahwa EK, Sargeant LA, McFarlane-Anderson N, Smikle MF, Forrester T, Wilks RJ. Anticardiolipin antibodies and risk of thromboembolic disease in young Jamaican women. ***J Cardiovasc Risk*** 2001; 8 (6): 349- 54.
20. Garcia G, Achouh L, Cobarzan D, Fichet D, Humbert M. Severe venous thromboembolic disease in Churg-Strauss syndrome. ***Allergy*** 2005; 60 (3): 409- 10.
21. Owlia MB, Hekmati-moghaddam SH, Fallah F, Dehghani Z.Antiphospholipid syndrome and stroke in patients under 50 years of age. ***J Indian Rheumatol Assoc*** 2005:13 140-2.
22. Saadatnia M, Zare M, Haghighi S, Tajmirriahi M, Hovsepian S. High frequency of IgM antiphospholipid antibodies in young Iranian patients with stroke. ***Neurosciences*** 2007; 12; 124- 6.
23. Kazemi A, Hajmoosa H, Razavi SM, Shashaani T, Jazebi SM. A Study of natural occuring antiquagulant and antiphospholipid antibodies in patients with history of thrombosis in Tehran. ***IUJM*** 2002: 9, 266-72.
24. Ginsberg JS, Wells PS, Brill-Edwards P, Donovan D, Moffatt K, Johnston M, et al. Antiphospholipid antibodies and venous thromboembolism. ***Blood*** 1995; 86 (10): 3685-91.
25. de Groot PG, Lutters B, Derksen RH, Lisman T, Meijers JC, Rosendaal FR. Lupus anticoagulants and the risk of a first episode of deep venous thrombosis. ***J Thromb Haemost*** 2005; 3 (9): 1993- 7.
26. Windyga J. Antiphospholipid antibodies as risk factor for venous thromboembolism. ***Pol Arch Med Wewn*** 2002; 108 (5): 1065- 70.
27. Simioni P, Prandoni P, Zanon E, Saracino MA, Scudeller A, Villalta S, et al. Deep venous thrombosis and lupus anticoagulant. A case-control study. ***Thromb Haemost*** 1996; 76 (2): 187- 9.
28. Zawilska K. Antiphospholipid antibodies--risk factors for venous thromboembolism and arterial thrombosis. ***Pol Arch Med Wewn*** 2007; 117 Suppl: 41- 5.