Sultan Qaboos University Medical Journal
December 2007 Vol 7, No. 3, p. 227-232
Sultan Qaboos University©
Submitted - 9th April 2007
Accepted - 12th September 2007

Antiphosphatidyl Serine Autoantibodies and Premature Coronary Events

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المضادات الذاتية للفوسفاتيدل سيرين والأزمات القلبية المبكرة

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الملخص: الهدف: دراسة دور المضادات الذاتية للفوسفاتيدل سيرين في حدوث الأزمات القلبية المبكرة لدى مجموعة مختارة من المرضى. **الطريقة:** شملت هذه الدراسة 50 مريضا مصابا بأزمات قلبية و 30 شخصا سليما كمجموعة ضابطة تم جمعهم من مدن الموصل, اربيل, ودهوك/شمال العراق للفترة من آذار/مارس 2004 إلى آذار/مارس 2005. مجموعة المرضى التي كانت أعمارهم دون الخمسين سنة (39.6 ± 5.9 سنة) تتكون من 23 مريضا مصابا باحتشاء عضلة القلب و 27 مريضا مصابا بالذبحة الصدرية . ولم تكن لديهم عوامل خطورة معروفة .أجريت الفحوصات اللازمة لمعرفة وجود المضادات الذاتية للفوسفاتيدل سيرين من نوع ج (IgG) و نوع م (IgM). وذلك باستخدام فحص المقايسة الأنزمية المناعية لقياس كمية المضادات الذاتية للفوسفاتيديل سيرين من نوع ج (IgG) و نوع م (IgM) لدى المشاركين في الدراسة. **النتائج:** شخصت المضادات الذاتية للفوسفاتيدل سيرين (نوع ج -IgG) في 10/50 (20%) من المرضى بينما كانت 1/30 (3.3%) لدى المجموعة الضابطة بفرق معتد إحصائيا (p < 0.05 ;9.1-odds ratio of 3.2 - 95%CI, 1.1). أما المضاد IgM فقد تم الكشف عنه لدى 3/50 (6%) من المرضى فقط دون وجوده لدى المجموعة الضابطة بدون فرق معتد إحصائيا. وكانت تلك الحالات الثلاثة موجبة بالنسبة للنوع ج (IgG) وهذا يعني أن معدل اكتشاف المضادات الذاتية للفوسفاتيدل سيرين نوع IgM هو نفس المعدل لنوع IgG . إضافة لذلك فان هذه المضادات الواصمة وجدت في 12/8 (66.7%) من مرضى الذبحة الصدرية غير المستقرة . وفي 15/2 (13.3%) من مرضى الذبحة الصدرية المستقرة . ولم توجد لدى المرضى المصابين بالجلطة القلبية. **الخلاصة:** تبين هذه الدراسة بان مضادات الفوسفاتيدل سيرين الذاتية نوع IgG ذات أهمية في حدوث الأزمات القلبية المبكرة خاصة عند المصابين بالذبحة القلبية غير المستقرة.

مفتاح الكلمات: مضادات الفوسفاتيدل سيرين الذاتية . المضادات الذاتية . الحوادث . الشريان التاجي.

ABSTRACT ***Objectives:*** To determine whether antiphosphatidyl serine autoantibodies (aPS) are associated with increased risk of occurrence of coronary events in selected patients. ***Methods:*** This study compared 50 patients with coronary events with 30 controls, recruited from the cities of Mosul, Erbil, and Dohuk cities, Northern Iraq, between March 2004 and March 2005. The patient group consisted of 23 individuals with myocardial infarction and 27 with angina. We evaluated the presence of aPS antibodies (IgG and IgM isotypes) by an enzyme-linked immunosorbent assay. The studied cases were less than 50 years of age (mean ± SD, 39.6 ± 5.9) and had no recognizable risk factors. ***Results:*** The frequency of detecting IgG aPS was 10/50 (20%) among patients and 1/30 (3.3%) among controls, with significant difference and with adjusted odds ratio (OR) of 3.2 (95%CI, 1.1-9.1; $p < 0.05$). The IgM aPS frequency was 3/50 (6%) among patients and zero in the controls, with non-significant difference. The three cases were also IgG positive (i.e. the frequency rate for detection of aPS of IgM was the same as for IgG). Moreover, this marker (aPS) was detected in 8/12 (66.7%) of cases with unstable angina, in 2/15 (13.3%) with stable angina, and in none of the cases with myocardial infarction. ***Conclusion:*** IgG aPS autoantibodies are associated with increased risk of coronary events especially angina of unstable subset.

Key words: Antiphosphatidyl Serine; Autoantibodies; Events, coronary.

Advances in Knowledge

Recently, it was found that antiphosphatidyl serine (aPS) antibodies detection correlated more specifically with APS than anti-cardiolipin (aCL) antibodies in APS. Since, cardiolipin is present in intracellular membranes and the dose not becomes exposed to coagulation proteins in vivo, it was hypothesized that tests for antibodies against antiphosphatidyl serine, which is normally present in the inner leaflet of the plasma membrane, may be more

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relevant pathophysiologically in this syndrome. The phosphatidyl serine is also exposed on syncytialized cells, on apoptotic cells and on activated platelets and their antibodies interfere more with anticoagulant cascade.

The aPS, the anionic non-cardiolipin phospholipid, have recently reported to be associated with an increased risk of stroke in young patients and positive association was reported among ischaemic stroke patients. Therefore, the risk associated with APLAs in APS may be underestimated if the testing of these autoantibodies is not included in the list of APLAs used currently. Consequently, the aPS IgG isotype showed to carry an independent risk factor associated with ischaemic events especially among cases with unstable angina. On the other hand, many clinical events related to APS were observed to be positive for this marker alone, in the absence of other markers.

Application to patient care

It is generally assumed that an association does exist between APLAs and ischaemic coronary events. This assumption is based mainly upon the results obtained from different studies. The evidence supporting this association needs to be strong enough since the presence of these autoantibodies influences the management approaches of such patients. Furthermore, the clinicians who diagnose such cases should realize that these cases are prone to recurrences and long-term anticoagulants need to be prescribed.

Therefore, the recent trend of including APLAs tests in cases with premature ischaemic events may totally change the therapeutic options from those used in traditional ischaemic cases. The diagnosis of APS attributed to detection of aPS autoantibodies or other APLAs may justify the need for more prolonged or even lifelong management to prevent recurrences.

The antiphospholipid syndrome (APS) is an autoimmune disease characterized by existence of antiphospholipid antibodies (APLAs). This syndrome is characterized by susceptibility to vascular thromboembolism and fetal loss.[1] The lupus anticoagulant (LA) and aCL antibodies are associated with clinical events related to APS with varying severities.[2] Anti- β 2- glycoprotein I (aβ-2-GPI) had been identified in patients with APS and found to play an important role in the pathogenesis of this syndrome.[3]

The APLAs (LA, aCL, and aβ-2-GPI) have been studied frequently and reported to be associated with an increased risk of thromboembolic phenomena such as ischaemic stroke.[2, 4-6] The aPS antibodies have also been reported to be prevalent in adult stroke patients.[7] There has been no study assessing the strength of their association with coronary events in the general population. The aim of this study was to determine the prevalence of aPS in a highly selected group of patients with coronary events, who were below 50 years of age and without any conventional risk factors and had not been diagnosed as systemic lupus erythematosus (SLE) cases. Moreover, we compared the results of aPS obtained in this study to those of aCL autoantibodies from a previous study performed on the same group of patients.

METHODS

Individuals in this study were a subset of those who participated in a PhD study disclosing the role of APLAs in patients with cardio-cerebrovascular diseases in the region.[8] This study was carried out on 50 patients suffering from different coronary events and 30 healthy individuals, recruited from the cities of Mosul, Erbil, and Dohuk in Northern Iraq, during the period March 2004 and March 2005. The cases were selected to be under 50 years of age and having no recognizable risk factors including hypertension (systolic blood pressure > 140 mm Hg and/or diastolic of > 90 mm Hg), diabetes mellitus (fasting blood glucose of ≥ 126 mg/dl and/or 2-hours post-prandial glucose of ≥ 140 mg/dl), dyslipidemia (total cholesterol level of > 240 mg/dl and HDL of < 35 mg/dl), non-smoking (current or previous), and lastly not overweight (body mass index of less than 25). The cases were not previously diagnosed as SLE. The purpose of the study was discussed in detail with the participants of both groups and obligatory ethical agreement was obtained from the volunteer cases accepted for inclusion in this study.

The age range of the studied cases was 29-49 years (mean ± SD, 39.6 ± 5.9) with premature coronary occlusive and ischaemia diseases. Twenty-three patients had myocardial infarction (MI), 15 stable angina (SA) and 12 unstable angina (UA). These cases were diag-

Table 1: ***The frequencies of the different antiphospholipid autoantibodies in patients with coronary events.***

Test \ Groups	Patients No. (%)	Control No. (%)	OR	95% CI	*P* value*
aPS (GPL units)					
< 10	40 (80)	29 (96.7)	3.2	1.1 – 9.1	*P* > 0.05
> 10	10 (20)	1 (3.3)			
aPS (MPL units)					
< 10	47 (94)	30 (100)	3.8	1.7 – 24.6	NS
> 10	3 (6)	0 (0.0)			
aPS of IgG and/or IgM	10 (20)	1 (3.3)	3.2	1.1 – 9.1	*P* > 0.05

* Chi-square test

NS: Not significant

OR: Odds ratio

95% CI: Confidence interval

nosed by a cardiac specialist depending on the acute clinical presentation, ECG changes, elevated serum cardiac marker such as creatine phosphokinase (CPK), exercise study, and ECHO study. The age range of the control group was 26-48 years (mean ± SD, 36 ± 6.5), with no history of any thromboembolic manifestations and without recognizable risk factors such as smoking, hypertension, diabetes mellitus, hyperlipidemia, or overweight. The individuals were recruited from the Central Blood Bank, Infertility Unit, Outpatient clinics, and volunteers.

A blood sample of 10 ml was obtained from each participant and tested. A second blood sample taken after 6 weeks or more was collected from APLAs positive cases. Enzyme-immunoassay kits (Orgentic Diagnostika from Mainz, Germany) were used for the detection of IgG or IgM aPS by indirect solid phase enzyme immunoassay (ELISA) and the tests performed in the Postgraduate Study Laboratory of Dohuk Medical College. The IgG and IgM isotype results were assessed in IgG phospholipid (GPL) and IgM phospholipid (MPL) units, with one unit equal to 1μg/ml of IgG or IgM. They were considered positive if the titer was > 10 GPL units and > 10 MPL units for both isotypes, as suggested by the ELISA kit manufacturer.

A significant relative risk factor was considered when the OR was > 1. The proportions were compared using the Chi-square test. The 95% CI of proportions were calculated according to the binomial distribution.

RESULTS

Table 1 demonstrates the prevalence of IgG and IgM aPS autoantibodies in the studied cases and controls. A persistence positive IgG aPS titer, in both samples, was present in 20% of patients (10/50) and 3.3% of controls (1/30), with significant difference and OR of 3.2 (95%CI, 1.1 - 9.1; $p > 0.05$). Moreover, a positive IgM a PS titer was detected in 6% of patients (3/50) in initially tested samples and in 4% (2/50) in second tested samples and in none of the controls, with non-significant difference. These IgM aPS positive cases were also IgG positive (i.e. the frequency rate for aPS of any isotype were the same as for IgG). Eight out of the 10 IgG aPS positive cases had unstable angina, while, two IgG aPS positive cases had stable angina. However, none of the studied cases with a history of MI were revealed to be positive for any isotype of aPS antibodies.

The mean titers of IgG isotype was 64.1μg/ml among patients with UA and 12.9μg/ml in patients with SA. However, the mean titer of IgM aPS in the 2 persistence positive cases was 29μg/ml with UA.

In comparison, the frequency of persistent IgG aCL with a concentration of < 30 GPL units was detected in 14% of cases (7/50), with significant difference and OR of 9.8 (95%CI, 7.8 - 76.4; $p > 0.01$), while none of the control showed such a concentration.

The results of transthoracic echocardiographic findings of the 10 aPS positive cases are summarized in Table 2. Normal results were reported in 40% of patients (4/10), two with SA and another two with UA.

Table 2: ***The echocardiographic findings in antiphosphatidyl serine autoantibodies positive patients with coronary events***

Finding (s)	No. (10)	%
Normal	4	40
Hypokinetic ventricles	4	40
Valvular abnormality	2*	20

* One case with mitral valve prolapse and the other with aortic valve insufficiency.

Abnormal findings were recorded in the remaining 60% (6/10), 4 in the form of hypokinetic ventricles (all were with UA) and three cases as heart valves abnormalities with UA.

DISCUSSION

The association of APLAs with coronary artery diseases has been shown in several studies but remains controversial.[8, 9, 10] The mechanisms through which these APLAs can induce pathological changes and tissue necrosis in MI or initiating atherosclerotic changes are debatable.[9] The association between cardiac events especially MI and IgG aCL has been suggested in other studies.[10, 11] Billi et al.[12] reported that in post-infarction patients elevated IgG aCL antibodies are an independent risk factor for recurrent cardiac events and patients with elevated IgG aCL and low IgM aCL antibodies have the highest risk.

The aPS antibodies, members of the non-cardiolipin APLAs, have been shown to be associated with increased stroke risk in SLE patients with a variety of APLAs to non-cardiolipin antigens.[13] Another study by Toschi et al.[14] of stroke and/or transient ischaemic attack patients, but with unselected patients, demonstrated also a positive correlation. Although these autoantibodies have been associated with stroke events related to APS, their prevalence in coronary events among patients without conventional risk factors is unknown.

In this study, although the number of the studied cases were relatively small, a positive association was demonstrated between IgG aPS in patients with coronary events especially those with UA. On the other hand, three of our patients who were IgG aPS positive were negative for IgG aCL, i.e. 6% of the studied patients would have been characterized as APLAs negative if only an aCL assay were used.

Moreover, the estimated mean titer of IgG aPS was higher in patients with UA (64.1μg/ml) compared with cases with SA (12.9μg/ml). This finding could be attributed to the more intensified immune process going on in patients with UA than in SA events related to APS. The absence of aPS autoantibodies in patients with MI may signal to the weak immune inducer effect of the tissue necrosis characterizing these events.[9]

An echocardiographic study of aPS positive cases revealed that 60% (6/10) have abnormal cardiac findings in form of hypokinetic ventricles and valvular infection and all cases were sufferings from UA. One case was with mitral valve prolapse and one with aortic insufficiency. Niaz and Butany's study in 1998[15] revealed that 35% of patients with primary APS had valvular abnormalities, while Bouillanne et al., in 1996 [16] reported that about 20% of cardiac patients with valvular heart disease had evidence for APLAs compared with about 10% of matched control subjects. Deposits of APLAs such aCL antibodies and of complement components are common in the affected valves of patients with primary or secondary APS.[17] Another study of patients with SLE and primary APS did not find any relationship between increased aCL antibodies and valvular abnormalities.[18]

A small number of studies investigated patients with cardiac events and their correlation with APS. Some of them studied cases with MI and tested for aCL antibodies only [10, 11, 19] while others studied the relationship between APLAs and atherosclerosis in ischaemic heart disease using the aβ 2-GPI antibodies tests only.[20, 21, 22] Over the last decade, many studies have been performed and suggest that aβ 2-GPI plays a corner stone role in the pathological consequences [23, 24], but studies of aPS and coronary events were lacking,

The mechanisms by which these antibodies may influence thrombogenicity are incompletely understood, but these have recently been reviewed comprehensively.[14, 25] These mechanisms may include the stimulation of platelet aggregation, the recognition of vascular endothelial cells and the disruption of the annexin-V shield that forms a carpet protecting anionic phospholipids on endothelial cell surfaces from participating in coagulation reactions.[1, 14, 25] This last proposed mechanism may be applicable to the role of aPS specifically because aPS have been shown to remove annexin-V from and facilitate binding of prothrombin to cell surfaces.[26]

CONCLUSION

The results of the current study demonstrate an increased risk and positive association between IgG aPS and coronary events of angina subsets among cases with UA. Moreover, our results revealed that testing for this antibody, along with other APLAs, may identify more cases related to APS. This, therefore, justifyies the routine use of aPS as a serologic marker in the general population in suspected coronary events.

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