

Full Length Research Paper

Antiphospholipid antibodies in acute cardiac attacks

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Cardiovascular diseases have prevailed all over the world in the last few decades. Mortality and morbidity rates due to myocardial infarction (MI) and angina pectoris (AP) increased and affect younger ages in the present days. There are very few studies that have been carried out to define the prevalence of antiphospholipids (APLs) as a marker to help the people at risk in Saudi Arabia. The objective of our study was to examine the antiphospholipid antibodies levels on plasma including anticardiolipin antibody (ACA) and lupus anticoagulants (LA) among Saudi men living in the metropolis of Riyadh, Kingdom of Saudi Arabia. This study was carried out on a sample of 50 Saudi men, aged less than 45 years, who had angina pectoris (AP) and myocardial infarction (MI) going to Riyadh Medical Complex (RMC) and Al-Iman General Hospital from March, 2006 to January, 2008. They were compared with the control group of forty subjects comprising students and staff of the Riyadh College of Health Sciences. Antiphospholipids antibodies can be detected in patients with autoimmune disorders. The primary antiphospholipid antibody syndrome results from reaction of the immune system. In this study, ACA and LA were tested by Enzyme-linked immunosorbent assay (ELISA) and activated partial thromboplastin time (APTT), respectively. The present results showed a significant increase of ACA in 88% of patients. 16 patients (32%) showed positive LA. Antiphospholipids antibodies were found to be significantly associated with MI and in acute attacks of AP especially in patients showing coronary ischemia or thrombosis. The association of LA and ACA showed marked significant correlation when considered together ($P < 0.05$). More attention should be paid by cardiologist to antiphospholipid syndrome, as it is among the severe and fatal diseases. The rise in APLs is a marker for recurrent stroke risk. Further studies in the area are needed.

Key words: Anti phospholipids, myocardial infarction, angina pectoris, Saudi Arabia.

INTRODUCTION

Antiphospholipids syndrome can be classified as a primary or secondary immunological disease which may be associated with other autoimmune diseases. Antiphospholipids syndrome may be among the causes of vascular thrombosis, pregnancy loss and thrombocytopenia with raised levels of antiphospholipids antibodies (Ravelli and Martini, 2005). Acute heart attacks including AP and MI are diseases engendered by several factors that endangered many young lives (Nevas et al., 2001). APLs had been associated to patients with arterial thrombosis including early coronary artery and cerebrovascular thrombosis (Galli et al., 2003). The antiphospholipids syndrome is also associated with arterial and venous thrombosis. Asherson et al. (2003), Renan et al. (2004) and Ruffiatti et al. (2009) have identified three

primary classes of antibodies associated with the antiphospholipids antibody syndrome. They are Anticardiolipin, Lupus anticoagulant and Beta-2-Glycoprotein 1.

Zanon et al. (2004) have in their work identified that in APS, there exist a variety of cardiac affections including valvular lesions, myocardial dysfunction, MI. In another study by Girardi et al. (2004), APS is also associated with miscarriages, preterm labor, low birth weight and preeclampsia in 20 to 40% of patient. The processes by which the antiphospholipids antibodies cause thrombophilia have not been established. APLs might inhibit phospholipids-dependent blood coagulation.

According to Triplett (2002) and Ruffatti et al. (2009), APLs could negate the usual anticoagulant protein C thus

cause continuous cell activation leading to enhanced thrombin generation (Cervera et al., 2002; Ruiz-Irastorza et al., 2004; Salmon and Girrardi, 2004).

Going by the work of Nomura et al. (2003), Van Wijk et al. (2003) and Giannakopoulos et al. (2007), thrombosis of coronary artery or its branches were solely of platelet origin or platelet-derived microparticles shed from cell membrane of severely damaged, necrotic or apoptotic cells. Previous studies showed that about 3% of all Cardiac Attack Disease (CAD) cases occurred under age of 45 years (Avcin et al., 2008).

APL is identified increasingly as a main cause of vascular thrombosis in patients; catastrophic anti-phospholipids syndrome should take much care and recognition by cardiologists, being one of the fatal disease (Doaa et al., 2010). The present study was carried out to measure and compare levels of APL in AP and MI patients less than 45 years age and admitted to Coronary Care Unit (CCU), with normal subjects.

MATERIALS AND METHODS

The present study was undertaken at Al-Iman General Hospital (AIGH) and Riyadh Medical Complex (RMC), Riyadh, KSA during the period of March, 2006 to January, 2008. Two groups had been established:

1. Group A Table 1: The patients group; 50 male patients from AIGH and RMC with the age ranging from (20 to 44 years). The selection of patients of AP and MI had the following criteria: (a) Age < 45 years at the initial stage of AP and MI; (b) Complete absence of hypertension, Diabetes and obesity; (c) Confirmed past history of MI in accordance with American Heart Association Guidelines (Lockshin, 2006). Samples from the patients were collected from 8 to 12 weeks after admission to the hospital.
2. Group B Table 2: Negative control group, 40 students and staff-members of Riyadh College of Health Sciences (RCHS), with the age ranging from (24 to 42 years) having no history of MI or AP.

10 ml of venous blood sample was collected in vacutainers containing 0.109 sodium citrate as anticoagulant. The samples were centrifuged at $3500 \times g$ at 4°C for 15 min, then recentrifuge at $2500 \times g$ at 4°C for 15 min to minimize residual platelets at less than $10 \times 10^9/\text{L}$ and then stored at -20°C in aliquots of 300 μL . Of all the patients, none of them was on oral anticoagulants. The serum was collected from patients and control in vacutainers tube without anticoagulant frozen at -20°C for testing for ACA. Standard diluted sera samples of 1:100 was used and added to a coated microplates well with reference standards, then washed twice and incubated with anti-human horseradish peroxidase (HRP) and a substrate at room temperature (20 to 25°C). A color appears after 15 min; ELISA reader at 450 nm was used to measure IgG ACA. A record of IgG ACA equal or above 12 IgG phospholipid (GPL units)/L and IgM ACA above 7 IgM phospholipid (MPL units)/L was taken as significant value.

The condition for positive cases of LA is considered when there is extended coagulation time in stage I or persistent prolongation of time in stage II after adding equal volume of normal plasma. The case can be positive also if there is normal activated partial thromboplastin time reversal due to inhibition of anticoagulant effect on addition of excess phospholipid reagent. Positive cases for LA and ACA were confirmed using both liquid phase coagulation assays and solid phase ELISA assays. APL were positive if it was \geq

12 GPL/units (Miyakis et al., 2006).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) 11 was used for all statistical analysis. Student t-test was applied to compare any quantitative data while the chi-square test was used for qualitative variables. $P < 0.05$ was taken to indicate statistical significance.

RESULTS

The Tables 1 and 2 show the number of subjects, age, risk factors, APL and LA titer for the two groups A and B, respectively. Forty patients from group A (80%) had suffered more than one MI episode. 6 of Group A were presented with unstable angina and silent MI. The remaining 4 suffered from severe chest pain. In the control group B, ACA was within the average levels ≤ 10 GPL units/L (Table 2).

In Table 2, smoking was the commonest risk factor in the Group (80%), followed by hyperlipidemia 20% and positive family history (16%). APLs were detected in 44 patients > 12 GPL units/L, which is considered positive (88%) for ACA. 16 patients were positive for LA (32%). The peak value for ACA was 32 GPL units/L for 10 patients (20%). Also, the high level of isotope was IgG in all the patients with only six patients as an exception. It is important to note that there was no patient who suffered from thrombocytopenia. The study has also shown that APLs had no connection with age, frequency of occurrence or the sheer intensity of AP and MI. On the whole, the availability of ACA and LA indicate considerable relationship to the occurrence of MI and AP ($P = 0.04$), although one is bound to get a different result when we examine them separately.

DISCUSSION

We need to know that ACA and LA are two distinct entities. ACAs had apparent linkage to lamellar Phospholipids in a bilayer composition, LAs on the other hand show potent tendency to hexagonal phospholipids, and it is quite normal to identify LAs utilizing lupus sensitive activated partial thromboplastin time (APTT) The cut-off levels of IgG and IgM for ACA can be found in the guidelines stipulated by the American Association of Clinical Pathologists. It defines negative result as < 5 GPL units. More than or equal 12 GPL are stated as low-positive results; 15-18 GPL units are classified as medium level. $80 > \text{GPL}$ units are grouped as high levels (Miyakis et al 2006) and (Lockshin 2008). In another study Low ACA levels were reported on subjects with frequent miscarriage (Girardi et al 2004)

In the present study, 30 patients had ACA levels 20 to 32 GPL units/L and the peak value was 32 GPL units/L (Table 1). The results has tailed with our hypothesis that

Table 1. Profile of the patients group (Group A).

No of Subjects	Age (years)	Smoking	Hyperlipedemia	FH	Low protein C	Obesity	LA	APL titer (GPL units/L)
Group 1 (n=4)	20-24	+	-	+	-	-	-	ACA 12
Group 2 (n=4)	24- 28	+	-	+	-	-	-	ACA 20
Group 3 (n=16)	28- 32	+	-	-	+	-	-	ACA 23
Group 4 (n=6)	32-36	+	-	-	+	-	+	ACA < 10
Group 5 (n=10)	36-40	+	-	-	-	+	+	ACA 32
Group 6 (n=10)	40-44	-	+	-	-	-	-	ACA 12

ACA = anticardiolipin antibodies in GPL unit/L, LA = lupus anticoagulants, FH = positive family history.

Table 2. The control Group B.

No of subjects	Age (years)	Risk factors	APL titer (GPL units/L)
2	20-24	Smoking + obesity	10
10	24-28	Hyperlipedemia	5 <
8	28-32	---	5 <
14	32-34	Smoking	5 <
4	34-38	Family history	5 <
2	38-42	Obesity	5 <

ACA = anticardiolipin antibodies in GPL unit/L, LA = lupus anticoagulants, FH = positive family history.

the patients in KSA have moderate level of ACA compared to the western countries (Nomura et al., 2003; Lockshin, 2006). Low ACA levels were reported in another study on subjects with frequent abortions (Girardi et al., 2004). The current study has been carried out on patients less than 45 years of age, given the fact that they are less prone to atherosclerosis. A previous study by Graham (2009) had reported 60 to 90% concordance for ACA and LA; however in the present study, 16 cases (32%) showed presence of both ACA and LA (Table 1). We therefore are of the view that samples must be tested for both ACA and LA. Elevated ACAs were present in 20 cases

representing 40%, with the range of 20 to 23 GPL units/L and 10 cases representing (20%) with ACA 32 GPL Units/L (Table 1). These results were in accordance with the study that showed ACAs elevation in 22% of survivors of MI, aged less than 50 years (Graham, 2009).

The high level-positive results > 80 GPL units/L as described in different studies had not been found in the present study. The high peak value of ACA was 32 GPL units/L. The high level-positive figure of ACA seemed to be seen in older patients with atherosclerosis and rheumatic disorders (Asherson et al., 2001). A prospective study revealed that raised levels of ACA at 40 years of

age correlated positively with the incidence of AP and MI, and mortality related to MI 10 to 20 years later (Heilmann et al., 2008). More than 20% of young (< 45 years) survivors of acute MI carry ACA and in those surviving, 61% of the patients with persistent antibodies experienced later another thromboembolic episode (Carl et al., 2008).

A limitation of this study was its retrospective orientation and small number of subjects (50) enrolled. Extensive studies are required on patients with AP and MI in Saudi Arabia. There seemed to be no clear linkage between ACA and MI in patients within these age groups.

The role of LA in arterial thrombosis is generally acknowledged, however the part played by ACA is still debatable (Pierangeli et al., 2000). This is in agreement with our observation that the prevalence of both LA and ACA assumed statistical significance ($p \leq 0.05$) only when considered together and not in disparate form.

Conclusion

This study was conducted on AP and MI Saudi patients below 45 years. The patients group includes 50 patients (Table 1) and 40 normal controls subjects (Table 2) without any other associated disease. APLs had been elevated in 88% of patients, although that did not coincide with the constant recurrence of MI. The high level of APLs did not also relate with the MI intensity. A clear detection of APLs could apparently, give a lead in explaining the patients' risk of having arterial and venous thrombosis. It could also assist in guiding the therapeutic administration of AP and MI patients. Further research on the prevalence of APLs among the Saudi populace is indeed, needed. The study, if carried out with considerable number of controls and patients, would show the role of APLs in the pathogenesis of MI.

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