

Prevalence of antiphospholipid auto antibodies in patients with thrombosis

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Abstract:

Background: *Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by elevated levels of antiphospholipids antibodies (aPL), recurrent venous thrombosis or arterial occlusive events, and fetal losses because of hyper coaguability state associated with it.*

Objective: *To determine the prevalence of antiphospholipid autoantibodies among patients with thrombosis at age less than 50 years old attending military teaching hospital.*

Materials and Methods: *A cross-sectional hospital based study was conducted at military teaching hospital during 2013. A total of 48 patients with thrombosis at different sites of the body in addition to 12 healthy individuals were enrolled in this study. Serum specimens were collected and tested for activated partial thromboplastin time (APTT) by cephaloplastin time, prothrombin time by high sensitivity prothrombin time reagent, platelets count using sysmex machine, and antiphospholipids autoantibodies screening test for IgG /IgM by ELISA.*

Results: *48 patients with thrombosis, 16 of them (30%) of them have recurrent thrombosis, (17%) of them disclosed positivity of APL IgM autoantibodies, half of them showed positivity for both IgM and IgG autoantibodies.*

8% of patients group have prolonged PT and high INR results, 17% of patients group showed prolonged PTT results, and 17% of patients group have a high PLTs count.

The statistical analysis showed that there was no significant difference between patients group and control group in PT, PTT, INR, PLTs count, and APL screening tests.

Conclusion: *Classification of patients with APS at high or low risk for thrombosis is an essential step towards achieving the goal of preventing the occurrence of thrombosis in asymptomatic APS carriers*

Key words: Phospholipid, auto antibodies, Thrombosis

Thrombosis is a physiological process of highly important during injury upon vessel bleeding which must be stopped, and thus this process is life-saving. Under normal circumstances, several factors prevent unnecessary thrombosis (such as natural anti-coagulants). Thrombosis within vessels which supply blood to the brain, heart, kidneys or any other vital organ might be disastrous. There are many factors which may increase the risk of thrombosis by increasing the hypercoagulability (blood tendency to coagulate and form thrombosis) status of the blood (Martinelli *et al.*, 2010). Antiphospholipid syndrome (APS) is one of the leading causes of acquired hypercoagulability, and is characterized by formation of thrombosis within vessels.

APS means thrombosis in the presence of antiphospholipids autoantibodies (aPL), and aPL are found in higher frequency in several manifestations attributed to blood hypercoagulability of deep vein thrombosis, stroke, myocardial infarction, and recurrent abortion (Sherer and Shoenfeld, 2004).

Hypercoagulability is caused by, for example, genetic deficiencies or autoimmune disorders. Recent studies indicated that neutrophils play a pivotal role in deep venous thrombosis, mediating numerous pro-thrombotic actions (Fuchs *et al.*, 2010; Brill *et al.*, 2011; Borissoff, *et al.*, 2011).

Autoimmune diseases (AID) are the outcome of abnormal activity of the immune system against self-molecules. Due to various reasons including genetic factors and environmental factors like infectious agents, the activity of the immune system is impaired, and part of it is directed against the self. There are many AID, some are rare but others are very frequent (Sherer and Shoenfeld, 2004).

The antiphospholipid antibody syndrome (APS or APLS or), also often called Hughes syndrome, is an autoimmune, hypercoagulable state caused by antibodies against cell-membrane phospholipids (Petri, 2011) and is one of the thrombosis causes due to hypercoagulability state associated with it.

The syndrome occurs due to the production of auto antibodies against self phospholipid (aPL), a cell membrane substance. These autoantibodies are a group of organ nonspecific autoantibodies that bind to anionic phospholipids and provokes thrombosis formation in both arteries and veins and cause a clinical disorder characterized by recurrent arterial and venous thrombotic events, fatal losses, thrombocytopenia, neurological symptoms, livedo reticularis, and haemolytic anaemia (Cuadrado *et al.*,1997; Navarrete *et al.*,2000; Sherer and Shoenfeld, 2004).

The antiphospholipid antibodies have been detected in approximately one-third of the patients with SLE. High anticardiolipin antibodies titres, lupus anticoagulant (LAC) and especially anti-beta 2GPI antibodies are important predictors of APS clinical manifestations in SLE patients. A rare form of this disease is the catastrophic APS, in which there is rapid organ dysfunction and failure (Asherson *et al.*, 2003).

Patients with APS are usually between 20 and 60 years of age and affect women more than men (Mak and Saunders, 2004).

APS is characterized by various clinical manifestations, and it is the cause of a significant number of thrombotic episodes and recurrent abortions.

The prevalence of a PL among patients having venous thrombosis (usually involving deep veins of the thigh and legs) is between 5–30%. Finding of aPL possess a risk for first event of venous thrombosis, recurrent events and death .Moreover the presence of aPL is a risk factor for myocardial infarction (heart attack) (Sherer and Shoenfeld, 2004).

Most AID has a genetic background, but this hereditary component is not as obvious as diseases which are transferred from a parent to his children in half of quarter of cases .The genetic predisposition for APS is partially explained by markers called human leukocyte antigens (HLA). Some of these HLA molecules are related to aPL: HLA type DR4, DR7, DRw53, and DQB1*0302 are associated with the presence of aCL (Sherer and Shoenfeld, 2004).

The exact cause of this disease is unknown, but abnormal activation of the coagulation system is occurring. The autoantibodies were directed against prothrombin activator complex glycoproteins (such as B2-glycoprotein I or prothrombin) that can associate with anionic phospholipids once they are exposed on the cell surface. Such exposure might occur following damage to the endothelial layer (Mak and Saunders, 2004).

According to the Sapporo criteria for the diagnosis of the APAS, which are adopted by the American College of Rheumatology, diagnosis with APS requires at least one clinical and one laboratory manifestation (Wilson *et al.*, 1999; Sherer and Shoenfeld, 2004; Miyakis *et al.*, 2006).

PATIENTS AND METHODS:

Patients: This study was cross-sectional hospital based study conducted at the Military Teaching Hospital during November

2012-March 2013. A total of 48 patients with thrombosis at different sites of the body, diagnosed by specialists, in addition to 12 healthy individuals as control were recruited in the study. All the study participants' were with age equal or less than 50 years old. Verbal informed consents were obtained from all participating individuals. The data were obtained through direct interview with the study group in a pre-designed questionnaire include information about participant's age, sex, residence, occupation and risk factors associated with the disease such as family history, in addition to the history of the disease were recorded.

Methods: Eight ml blood samples were collected from each patient and each control individual and distributed into 3 containers as 2.5ml in container contain 0.27ml of sodium citrate anticoagulant for PT and PTT test, and 2.5 ml in container contain K3-EDTA anticoagulant for PLTs count. The remained 3 ml were placed in plain container for APL test.

All participants were investigated for Platelets count by sysmex machine, partial thromboplastin time (APTT) by cephaloplastin reagent using ellagic acid as an activator, Prothrombin Time (PT) by High sensitivity prothrombin Time (PT-HS) reagent. And for antiphospholipids screening IgG/IgM antibodies by Indirect Enzyme Linked Immune Sorbent Assay (ELISA).

Statistical analysis:

The data was analysed statistically using SPSS software (Statistical Package for Social Sciences). Correlation coefficient test was used to compare the different groups. P value ≤ 0.05 was considered as significant.

Results:

More than half of the patients 28(58%) were in age group (38-47), while only 4(8%) were in age group(18-27). The overall

history of the disease among the patients extended for up to 12 years. Twelve of the patients (25%) have history of less than a year while 4 (8%) have a history of 12 years.

The results showed that 12 (25%) of thrombotic patients are hypertensive. While 4 patients (8%) have other diseases (cancer, diabetes) and the majority of the patients 32(67%) have no other disease. The study found that 16 (33%) of the patients showed recurrent thrombosis while 32 (67%) were not.

Table 1: Co-infections and Recurrent Thrombosis in thrombotic Patients

Diseases	Hypertension	Recurrent Thrombosis	Other Diseases	No other Diseases
No. of Patients	12	32	4	32
Percent	25%	67%	8%	67%

4 (8%) of the patients group illustrate high PT and INR results and 44 (92%) showed normal results, while all control group showed normal PT and PTT results, there was no significant difference between the patients and control groups (p value = 0.3).

8 (17%) of patients group have high PTT results, and 40 (83%) show normal results ,while all control group showed normal PTT results, there was no significant difference between the patients and control groups (p value = 0.13).

4 (8%) of the patients group showed high PLTs count while 44 (92%) showed normal results. While all control group showed normal PLTs count .No significant difference between the patients and control groups found (p = 0.3).

Table 2: Results of PT, PTT, INR, and PLTs count in Thrombotic Patients

Tests	PT	PTT	INR	PLTs count
High	4(8%)	8(17%)	4(8%)	4(8%)
Normal	44(92%)	40(83%)	44(92%)	44(92%)

P value=0.3 for PT, INR, and PLTs count. P value=0.13 for PTT.

The overall prevalence of antiphospholipids antibodies in patients group was 17% (8 of 48). The results showed that 8 (17%) of the patients group give positive results for APL, 8 (100%) of them were positive for IgM ,and 4 (50%) were positive for both IgM and IgG, while 40 (83%) were negative for APL test . All control group showed negative results in APL test. The results also showed no significant difference between the patients and control groups ($p=0.1$).

Table 3: Antiphospholipids autoantibodies test results in Thrombotic Patients

Tests	IgM	IgG
Positive	8(17%)	4(8%)
Negative	40(83%)	44(92%)

P value=0.1.

DISCUSSION:

This study shows the prevalence of an antiphospholipid antibody in the study patients was 17% (8 of 48). According to the Sapporo criteria for the diagnosis of the APS, which are adopted by the American College of Rheumatology, the isolated detection of antiphospholipid antibodies in the studied patient's serum is not enough to make the diagnosis of APS. The diagnosis of APS to be made, the patient must have at least one clinical criterion and one laboratory criterion for APS. The laboratory criteria include the detection of anicardiolipin antibodies in high titer or lupus anticoagulant activity in the patient's serum on at least two occasions for twelve weeks apart (Wilson *et al.*, 1999; Sherer and Shoenfeld, 2004; Miyakis *et al.*, 2006).

About 25 % of the study patients have a history of hypertension disease before their affected by thrombosis , which is risk factor that can cause the thrombosis due to high venous stasis associated with it (Martinelli , 2010).

About 8% of the patients group have history of cancer before their affected with thrombosis and this may be the cause of thrombosis because the cancer and malignancies are risk factors for thrombosis, also treatment of cancer (radiation ,chemotherapy) often cause additional hypercoaguability (Martinelli , 2010).

The present study found that there was negative correlation between the IgG and PTT which agree with Jacob *et al* (1998) who found that IgG from patients with antiphospholipid syndrome reduce the annexin-V on non-cellular phospholipid coated surfaces and accelerate coagulation with phospholipids used for standard clinical coagulation assays.

There is a positive correlation between the age and the APL and this agreed with Miykais (2006), who reported that the APL found mainly in patients with increasing age.

The PT, PTT, and INR appeared normal in the majority of study patients and this may be due anticoagulant therapy which used to monitoring the activity of coagulation factors and to prevent recurrent thrombosis or immobilization of thrombus (Soff, 2012).

More than third (33%) of the patients showed recurrent thrombosis although they show negative results in antiphospholipids screening test and this may be due to inappropriate anticoagulant therapy or absence of self-monitoring (Heneghan, 2012).

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