INTRODUCTION

The antiphospholipid syndrome (APS) is an acquired thrombophilic disease, characterized by the occurrence of thrombosis and/or pregnancy related morbidity associated with anti-phospholipid (APL) antibodies ***(Agar et al., 2010).***

Recurrent pregnancy loss is a main issue for women's health, with 3 or more successive losses affecting 1% to 2% of women at reproductive age and 2 or more successive losses affecting approximately 5% ***(Clark et al., 2010).***

The antiphospholipid syndrome (APS) is defined by both clinical and laboratory criteria. Laboratory criteria include the presence of positive lupus anticoagulant and/or medium to high titers of anticardiolipin, or anti β2GPI ***(Tebo et al., 2008).***

A phospholipid-binding protein, $ß_2$GPI has been identified, and antibodies to this antigen can also be demonstrated in patients with APS. Anti $ß_2$GPI antibodies that associate with the APS clinical phenotype are predominantly of IgG isotype ***(Giannakopoulos et al., 2009).***

Although the original clinical and laboratory criteria for the identification of APS patients were published in 1999, in the so-called Sapporo criteria, and the updated Sapporo

APS Classification Criteria is commonly used for APS diagnosis, in which one clinical and one laboratory criterion are required for diagnosis, the diagnosis of this syndrome remains challenging ***(Devreese and Hoylaerts, 2010).***

Despite international efforts to standardize laboratory testing for antiphospholipid antibodies, significant variation in the performance of antiphospholipid antibodies assays remains a critical problem **(Devreese, 2014).** Large interlaboratory variation in anticardiolipin antibody testing has been amply documented ***(Perches et al., 2009).***

Agreement among commercially available kits is also poor. It is not surprising that the prevalence of antiphospholipid antibodies varies from center to center. In turn, this contributes to the controversies in our current understanding of antiphospholipid syndrome ***(Perches et al., 2009).***

LA testing guidelines which are published on 2009 & solid phase assays testing guidelines that are issued on 2014 should be adopted by all laboratories in order to minimize the interlaboratory variations ***(Devreese et al., 2014).***

AIM OF THE THESIS

To study the clinical utility of using β_2GPI enzyme-linked immunosorbent assay, in conjunction with the established lupus anticoagulant assays and anticardiolipin enzyme-linked immunosorbent assay, for diagnosing patients with repeated pregnancy loss, and correlate its titers with repeated pregnancy loss.

I- ANTIPHOSPHOLIPID SYNDROME

Historical background of APS:

Antiphospholipid Syndrome (APS) first began to be defined as an entity in the 1950s with the recognition of 2 unusual laboratory phenomena: false-positive syphilis tests and the occurrence of a non specific coagulation inhibitor ***(Devreese and Hoylaerts, 2010).***

APL already had been detected earlier in the 20th century by immunological assays used in the diagnosis of syphilis. In 1907, Wasserman et al. developed a complement fixation assay for syphilis, using as an antigen (which they named reagin) the phospholipids (PLs) derived from saline liver extracts of fetuses with congenital syphilis ***(Devreese and Hoylaerts, 2010).***

In 1941, Pangborn demonstrated that reagin was an anionic PL, and it was renamed cardiolipin because from then on it was isolated from bovine heart muscle. As serological tests for syphilis were used increasingly for the screening of large numbers of patients, it became evident that a subpopulation of individuals with positive serological tests for syphilis showed none of the clinical symptoms of the disease ***(Lim, 2013).***

Moore and Mohr, in 1952, were among the first to identify such individuals and found that these transiently false-positive tests were associated with several other infectious diseases besides syphilis. Persistently false-positive tests were also associated with an increased risk for future development of systemic lupus erythematosus (SLE) and several related autoimmune diseases ***(Devreese and Hoylaerts, 2010).***

Nearly concurrently, Conley and Hartmann observed an acquired circulating inhibitor that prolonged the coagulation time in vitro in 2 patients with SLE. Seven years later, Loeliger described the presence of a factor in some plasma that prolonged the clotting time, even when the plasma was diluted with normal pooled plasma, and proposed prothrombin as a possible candidate ***(Lim, 2013).***

In 1963 Bowie et al first described the association of this anticoagulant with thrombosis instead of bleeding. The association of the anticoagulant with thrombosis and intrauterine death was subsequently described by other investigators ***(Devreese and Hoylaerts, 2010).***

It was not until 1972, however, that Feinstein and Rapaport introduced the term “lupus anticoagulants” (LA) for an inhibitor directed against coagulation cascade PLs.

Because the majority of patients with LA do not have SLE, the term is clearly a misnomer but has been retained for historical reasons ***(Garg and Deodhar, 2012).***

In 1983, Harris and coworkers developed a radioimmunoassay for the detection of anticardiolipin (ACL) antibodies, and 2 years later, they developed the first quantitative ELISA .Using this assay on a large population of SLE patients, they showed that the subgroup with increased ACL antibodies had a higher incidence of thrombosis and pregnancy morbidity. This led to the first description of the so-called anticardiolipin syndrome, also referred to as the antiphospholipid syndrome by the same investigators ***(Devreese and Hoylaerts, 2010).***

One of the most surprising findings in the early 1990s was that the ACL antibody activity in most APS patients depends on the presence of a protein cofactor. Three independent groups showed that so-called ACL antibodies were not directed against cardiolipin per se, but against a cofactor, a plasma apolipoprotein known as β2-glycoprotein I (β2GPI), a 50-kDa single-chain polypeptide glycoprotein that binds to anionic PLs. There are five homologous motifs of 60 amino acids (Sushi domains). (Fig. 1) ***(Mack worth-Young, 2004).*** The structure of domain V deviates from the standard fold of the four other domains and forms the putative PL-

binding site giving the "fishhook" appearance ***(Bouma et al., 1999).***

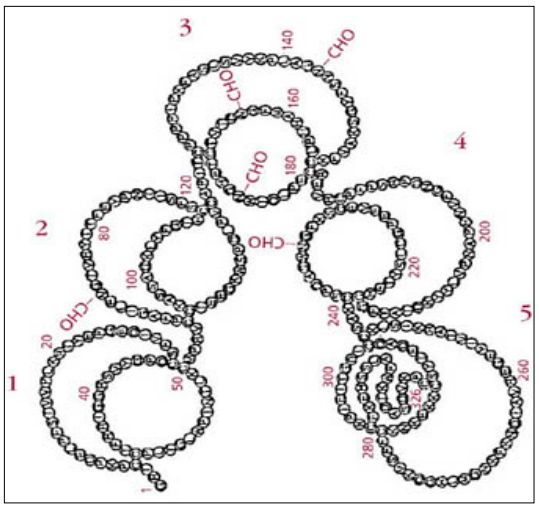

Figure 1: ß2GPI structure. Sushi domains are numbered 1 – 5 *(Mack worth-Young, 2004).*

However, antibodies to $ß_2$GpI are generally of low affinity, and it has been proposed that the binding of anionic PL, as CL, to $ß_2$GpI enhances this affinity through the revealing of a cryptic epitope on the $ß_2$GpI molecule (Fig. 2)**.** Furthermore, there is evidence for the binding of APL to different domains of $ß_2$GpI, although binding to domain I may predominate ***(Giles et al., 2003).***

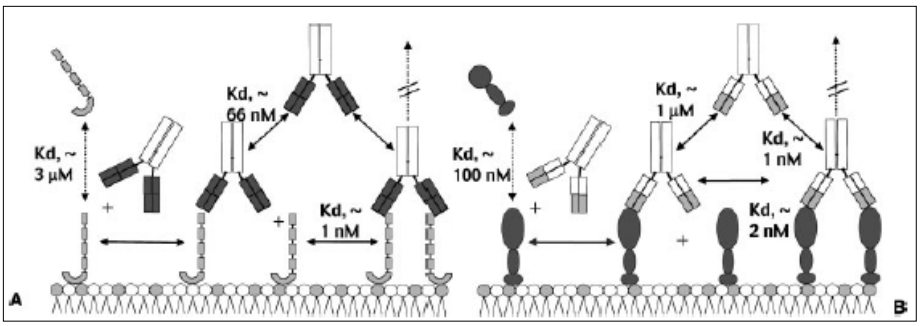

Figure 2: Anti-prothrombin antibodies (A) and anti-ß2GpI (B) antibodies form stable trimolecular complexes with the respective antigens at the anionic (PL) surface *(Giles et al., 2003).*

The observation that many ACL are directed to an epitope on B2GPI led to the development of the anti-β2GPI antibody immunoassay. Anti β2GPI antibodies are strongly associated with thrombosis and other features of the APS ***(Sikara et al., 2010).***

It is debatable whether current data are sufficient to claim that the mere presence of anti-β2GPI antibodies is a risk factor for pregnancy complications or improves the diagnosis of obstetric APS ***(Sikara et al., 2010).***

Further research uncovered that not all ACL antibodies were β2GPI dependent and that the in vitro anticoagulant effect of LA-positive ACL antibodies depended on the presence of β2GPI only in a subset of patients. β2GPI-dependant antibodies were found only in patients with autoimmune diseases, whereas patients with infectious

diseases had β2GPI-independent Ab. Subsequently, other groups also reported that β2GPI was needed as a cofactor for LA activity. In other patients, the LA activity was shown to be dependent on the presence of prothrombin ***(Sikara et al., 2010).***

A number of other autoantibodies have been reported in patients with APS, including antibodies to annexin V, high and low molecular weight kininogens or, less frequently, prekallikrein and factor XI. Some data suggesting that autoantibodies could be directed against components of protein C pathway; which includes protein C, protein S, and thrombomoduline ***(Sikara et al., 2010)*.**

The association of such antibodies with APS and their clinical significance is far from being known and that these tests are far from being standardized. Therefore, their application should be restricted to research rather than to routine diagnostic use ***(Tan et al., 2013).***

Epidemiology of APS:

Antiphospholipid syndrome (APS) is an important thrombophilic condition because it is of high prevalence and is associated with considerable morbidity and mortality ***(Gómez-Puerta and Cervera 2014).***

The prevalence of APL in healthy obstetric population is difficult to be determined since APL have been implicated in pregnancy morbidity. However, in two studies with large number of healthy pregnant women, APL were identified in 0.7 and 5.3% respectively. It has been reported that APL are detected in 11-29% of women with preeclampsia **(Sikara et al., 2010).** The diagnosis of secondary APS clearly leads to a three fold increase of miscarriages, especially after the 20th week of gestation ***(Grand and Voto, 2010).***

Clinical Presentation of APS:

The central feature of APS is thrombosis that may be venous or arterial causing various systemic presentations. Non-thrombotic manifestations are also evident ***(Sikara et al., 2010)***.

1- Venous Thrombosis:

Venous thrombosis constitutes 59% of thrombi occurring in APS ***(Sammaritano, 2005).***

DVT in the lower extremities is the most common manifestation of APS, occurring in 29% to 55% of patients during follow-up of less than 6 years, with as many patients having pulmonary emboli ***(Alarcón et al., 1992).*** Venous thrombosis of upper extremities, intracranial veins, inferior and superior vena cava, hepatic veins (Budd-Chiari

syndrome), portal vein, renal vein, and retinal vein has also been described ***(Farmer-Boatwright and Roubery, 2009)*.**

Thrombosis may occur spontaneously or in the presence of a predisposing factor, such as vascular stasis, trauma, surgery, and oral contraceptive use ***(Lim, 2013).*** In some patients a predisposing factor could be genetic, such as heterozygosity for factor V Leiden polymorphism ***(Valenzuela et al., 2010).***

2- Arterial Thrombosis:

Arterial thrombosis is a main clinical feature of APS, although it occurs less frequently than venous, constituting 28% of thrombi occurring in APS. The most common site of arterial thrombosis is the cerebral circulation, up to 50% of cases and usually in the form of stroke or transient ischemic attack ***(Sikara et al., 2010).***

3- Obstetric Complications:

A- Pregnancy loss:

The risk of pregnancy loss in women with APL antibodies is greatest from the 10th week of gestation onward (fetal period). This is in contrast to pregnancy loss in the general population, which is most frequent during the first 9 weeks of gestation ***(Grand and Voto, 2010).***

The two greatest risk factors for fetal loss are high titer IgG ACL and a history of previous fetal loss. These patients have up to 80% risk of recurrent pregnancy loss ***(Sikara et al., 2010).***

B- Other maternal obstetric complications:

In addition to pregnancy losses, associated maternal obstetric complications include pre-eclampsia/eclampsia and HELLP syndrome. Arterial or venous thrombosis and other APL -related complications such as severe thrombocytopenia may also occur. Prevalence of LA in preeclampsia was found to be 19% and in IUGR 22% ***(Grand and Voto, 2010).***

Preterm delivery is the strongest risk factor for adverse neonatal outcome ***(Meroni et al., 2011).***

4- Neurologic Manifestations:

The CNS involvement is co-responsible for high morbidity & mortality of the syndrome, with strokes & transient ischemic attack being the most common manifestation ***(Sikara et al., 2010)***.

5- Cardiac Manifestations:

Valvular heart disease, similar to (Libman-Sacks) endocarditis of SLE, is the most common form of APL -related cardiac involvement. Prevalence is estimated at 32 to 82% of APL patients ***(Sikara et al., 2010)***.

Histological examination of involved valves shows deposits of IgG ACL, complement, and fibrin; inflammation is not prominent ***(Sammaritano, 2005).***

6- Renal Manifestations:

Thrombosis may develop at any location within the renal vasculature, including the renal artery, intrarenal arterioles, glomerular capillaries, and renal veins. Acute thrombosis at the level of capillaries, arterioles, and venules may give an impression of hemolytic-uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), or other thrombotic angiopathy. Thrombotic procedures in APS could be chronic, causing slow but progressive organ dysfunction ***(Meroni et al., 2011).***

7- Pulmonary Manifestations:

In addition to pulmonary embolism, patients with APS may have spontaneous thrombosis in pulmonary vessels. APL antibodies have also been described in pulmonary hypertension and in non-inflammatory pulmonary vasculopathy. Pulmonary manifestations such as dsypnea and respiratory distress syndrome are common in patients with catastrophic APS ***(Sikara et al., 2010)***.

8- Gastrointestinal Manifestations:

Most symptoms are secondary to arterial or venous thrombosis. These include Budd-Chiari syndrome, intestinal ischemia and infarction, colonic ulceration, esophageal necrosis and perforation, hepatic infarction, acalculous cholecystitis with gall bladder necrosis, and mesenteric and portal vein thrombosis ***(Meroni et al., 2011).***

9- Hematological Manifestations:

Hematological manifestations include thrombo-cytopenia, Coomb's positive hemolytic anemia, Evans syndrome and microangiopathy. Thrombocytopenia, defined by platelet count less than $100x10^{9}/L^{-1}$, is found in approximately 20% of patients with primary APS and more than 40% of patients with secondary APS in SLE. The prevalence of autoimmune hemolytic anemia in APS patients was calculated at 6.6% to 9.7%. On the other hand, studies confirm the presence of APL in patients with autoimmune hemolytic anemia at a rate ranging from 55 to 72% ***(Sikara et al., 2010)***.

The bleeding complications from APS are extremely rare; nevertheless, they can occur. Severe bleeding secondary to acquired hypoprothrombinemia has been described. This is suspected in patients with prolonged bleeding time, and specific assays for prothrombin should be obtained for the diagnosis ***(Gezer, 2003)***.

10- Cutaneous Manifestations:

Skin events in APS vary both in form and severity, with livedo reticularis and leg ulcers being the commonest. Livedo reticularis is caused by stagnation of blood in dilated superficial capillary venules and its prevalence in APS is calculated at 24%, according to the study of Cervera et al. This lesion represents the first manifestation of the syndrome in17-40% of patients ***(Sikara et al., 2010)***.

11- Avascular Necrosis (AVN) of the Bone:

AVN may be increased in APL-positive patients independent of corticosteroid use: 73% of SLE patients with AVN were APL -positive and asymptomatic AVN on MRI was identified in 20% of primary APS patients ***(Tektonidou et al., 2003).***