# LABORATORY AND CLINICAL SIGNIFICANCE OF RARE ANTIPHOSPHOLIPID ANTIBODIES IN PREECLAMPSIA PATIENTS: A PRELIMINARY STUDY

BY

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Dissertation Submitted In Partial Fulfillment of

The Requirements For The Degree Of

Masters Of Pathology (Haematology)



UNIVERSITI SAINS MALAYSIA

2015

# ACKNOWLEDGEMENT

All praises to Allah s.w.t. the most Merciful and the most Beneficent.

Firstly, I would like to express my thankfulness to Allah s.w.t. for giving me the strength and ability to complete this research project.

With great pleasure, I would like to acknowledge Dr Wan Haslindawani Wan Mahmood, my supervisor and lecturer of Haematology Department, PPSP for her continuous supervision, ideas and support. I am also grateful to Assoc. Prof. Dr Wan Zaidah Abdullah, my co-supervisor and also lecturer of Haematology Department for her brightful ideas, support and encouragement.

I also wish to thank Dr Indhira Subbiah, Haematopathologist in HSAJB, Dr Mohd Haslan bin Hassan, O&G specialist in HSIJB and Dr Ahmad Amir bin Ismail, lecturer of O&G department PPSP as my co-supervisors. Without their helps, this study would be very difficult to accomplish. Many thanks to all staffs of Haematology Department HUSM especially En. Khairul Putra for their invaluable contribution.

I also would like to express my highest gratitude to my husband, Wan Mohd Fatihilkamal bin Wan Mohd Ridzwan for his love, sacrifices and assistance throughout my study. My thanks also goes to my respected parents, Hj Abdullah bin Ab Kadir and Hjh Zaini bt Abd Ghani and my children for their understanding and unconditional support to finish this study.

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| | |
|---|---|
| Abs | Antibodies |
| aCL | Anticardiolipin antibody |
| antiAnV | Antiannexine V antibody |
| APA | Antiphospholipid antibody |
| aPC | Antiphosphatidyl choline antibody |
| aPE | Antiphosphatidyl ethanolamine antibody |
| aPG | Antiphosphatidyl glycerol antibody |
| aPI | Antiphosphatidyl inositol antibody |
| ApoER | Apolipoprotein E receptor |
| APS | Antiphospholipid syndrome |
| aPS | Antiphosphatidyl serine antibody |
| aPS/PT | Antiphosphatidyl serine/prothrombin complex |
| aPT | Antiprothrombin |
| aPTT | Activated partial thromboplastin time |
| $\beta_2$GPI | Beta$_2$ glycoprotein I |
| DBP | Diastolic blood pressure |
| ELISA | Enzyme linked immunosorbent assay |
| eNOS | Endothelial nitric oxide synthase |
| FBC | Full blood count |
| GPL | IgG phospholipid unit |
| Hb | Haemoglobin |
| HEEC | Human endometrial endothelial cell |
| HSAJB | Hospital Sultanah Aminah Johor Bahru |
| HSIJB | Hospital Sultan Ismail Johor Bahru |
| LA | Lupus anticoagulant |
| LDL | Low density lipoprotein |
| MPL | IgM phospholipid unit |
| MPs | Metalloproteinases |
| NO | Nitric oxide |
| PE | Preeclampsia |
| PI | Phosphatidyl inositol |
| Plt | Platelet |
| PT | Prothrombin time |
| SBP | Systolic blood pressure |
| sEng | Soluble endoglin |
| sFlt1 | Soluble fms-like tyrosine 1 |
| TGF | Transforming growth factor |
| TWBC | Total white blood cell |

# ABSTRAK

Preeclampsia (PE) terus menyebabkan kematian dan morbiditi dalam kehamilan. Antibodi antifosfolipid (APA) diketahui sebagai pencetus proinflamatori dan mekanisme terjadinya trombosis. Hubungan antara PE dan APA mula disyaki disebabkan oleh kadar PE yang tinggi pada wanita yang menghidapi sindrom antifosfolipid (APS). Ia tidak diketahui sama ada jenis APA yang jarang ini terdapat dalam pesakit PE terutamanya dalam populasi kami. Tujuan kajian ini adalah untuk mengesan antibodi antiannexin V (antiAnV) dan antiphosphatidyl inositol (aPI) dalam pesakit PE.

Kajian ini telah dijalankan di HSIJB dan HUSM dari Januari 2013 hingga Disember 2014. Lapan puluh empat sampel telah dikumpulkan dengan bilangan pesakit PE dan bukan PE adalah sama dan dianalisa untuk ujian kiraan sel darah (FBC), ujian pembekuan darah, profil biokimia dan antiAnV dan aPI. Antibodi ini dianalisa dengan menggunakan kaedah ELISA.

AntiAnV IgG telah dikesan dalam satu kes PE tahap bahaya dalam kehamilan kembar semasa tempoh mengandung dan selepas bersalin (lima bulan). Tiada antibodi lain yang dikesan. Kes ini didiagnos dengan PE yang bermula awal dan kelahiran pramatang pada 33 minggu kehamilan kerana tahap PE yang bahaya. Ujian aPTT beliau menunjukkan masa untuk penghasilan darah beku yang singkat (19 saat) selaras dengan keputusan kami yang purata aPTT menunjukkan perbezaan yang ketara antara PE yang sederhana dan teruk ($p$ = 0.007).

APA yang jarang ini tidak dikesan dalam pesakit PE dan bukan PE yang lain. Walau bagaimanapun, terdapat perbezaan yang ketara dalam nilai IgM aPI antara PE dan bukan PE. Pertalian dengan APS tidak dapat dipastikan kerana ia adalah jenis IgM. Walau bagaimanapun, kehadiran IgM dalam kehamilan walaupun sebagai antibodi sementara adalah penting dari segi klinikal kerana ini berkaitan dengan kekalutan sistem imun dalam tempoh kehamilan dan boleh menjadi pencetus kepada PE.

Dari kajian ini, kelaziman APA yang jarang ini pada pesakit PE adalah rendah (2.4%). Walau bagaimanapun, kajian yang lebih besar diperlukan bagi data yang lebih tepat dan lebih banyak APA yang diuji untuk perkaitan yang lebih baik dan meningkatkan pemahaman tentang penyakit ini. Komplikasi klinikal yang lain perlu diambilkira dan dikaji untuk membantu dalam perawatan pesakit PE dengan lebih baik supaya morbiditi dan mortaliti dapat dikurangkan. Dalam kes PE tahap bahaya, ujian aPTT yang singkat adalah suatu keputusan yang penting dan perlu diterokai terutama pada pesakit PE yang dikesan positif APA.

# ABSTRACT

Preeclampsia (PE) continues to cause mortality and morbidity in pregnancy. Antiphospholipid antibody (APA) is known to induce proinflammatory and prothrombotic mechanisms. Relationship between PE and APA was first suspected due to high rate of PE in women with antiphospholipid syndrome (APS). It is not known whether rare types of APA are present in PE patients especially in our populations. The aim of our study is to detect antiannexin V (antiAnV) and antiphosphatidyl inositol (aPI) antibody in PE patients.

A cross sectional study was done at HSIJB and HUSM from January 2013 to December 2014. Eighty four samples were collected with equal number of PE and non PE and analyzed for FBC, coagulation, biochemical profiles and antiAnV and aPI. These antibodies were analyzed using ELISA method.

AntiAnV IgG was persistently detected in one case of severe PE associated with twin pregnancy during pregnancy and 5 months postnatal period. No other antibodies were detected. This positive case was diagnosed with early onset PE and had preterm delivery at 33 weeks gestation due to severe PE. Her activated Partial Prothrombin Time (aPTT) was shortened (19 sec) in keeping with our finding of significant different aPTT between mild and severe PE ($p = 0.007$).

APA was not detected in other PE patients and non PE. However, there was a significant different of aPI IgM concentration between PE and non PE. The association with APS is uncertain as it is IgM type. However, the presence of IgM in pregnancy

even as a transient antibody could be of clinical significance as this is related to immune derangement during pregnancy period and may lead to PE development.

From this study, the prevalence of rare types of APA in PE patients is low (2.4%). However, larger studies are needed for more accurate data and to include more APA for better correlation and understanding of the disease. Other clinical outcomes need to be evaluated in order to provide better management of PE so that the maternal and fetal morbidity and mortality can be minimised. Shortened aPTT in severe PE is a significant finding and need to be explored especially in PE patients with positive for APA.

# 1.0 INTRODUCTION

Antiphospholipid syndrome (APS) is an acquired autoimmune disorder characterized by thrombosis (could be from venous, arterial or/and microvascular), recurrent fetal loss or placental dysfunction in association with persistent elevation of antiphospholipid antibodies (APA) at least 12 weeks apart. The diagnosis of APS is made when at least one clinical criteria and one laboratory criteria is fulfilled. APS can occur in pregnant women as well as in non pregnant individuals.

APA are antibodies that directed against phospholipid antigen or protein antigen that binds to phospholipid (Giannakopoulos *et al.*, 2007b). Most common APA are lupus anticoagulant (LA) and anticardiolipin antibodies (aCL). However there are many other APA such as anti-$\beta_2$-glycoprotein I (anti-$\beta_2$GPI), antiphosphatidyl serine (aPS), antiprothrombin, anti-annexin V (anti-AnV), antiphosphatidyl inositol (aPI), antiphosphatidyl glycerol (aPG) and antiphosphatidyl ethanolamine (aPE). The rare types of APA which will be tested in this study are antiAnV and aPI. Limited studies are available on these rare APA in the literatures and so far no report among the Malaysian population regarding these rare APA.

Preeclampsia (PE) is a clinical syndrome which consists of new onset hypertension and proteinuria after $20^{th}$ weeks of gestational period. It affects 3-5% of pregnant women worldwide and causes significant mortality and morbidity (Young *et al.*, 2010a). PE is a progressive disease. There are different pathophysiologies to explain PE such as

microthrombosis, vascular, endothelial inflammation or imbalance of angiogenic and antiangiogenic placental factors.

The diagnosis of PE is made after 20 weeks of gestation when previously normotensive women presented with high blood pressure (systolic blood pressure (SBP) ≥ 140mmHg or diastolic blood pressure (DBP) ≥ 90mmHg) measured twice 6 hours apart and proteinuria ≥ 0.5g in 24 hours. Early onset of PE is defined as the detection of PE before 34 weeks of gestation where as late onset of PE is defined as the detection of PE at or after 34 weeks of gestation (Geller *et al.*, 2004; Young *et al.*, 2010b).

Recent studies had revealed that APA induced proinflammatory as well as prothrombotic mechanisms mainly in recurrent miscarriage and fetal loss, so the similar mechanisms could be assumed in APA induced PE (Ferrer-Oliveras *et al.*, 2012). Relationship between PE and APA was first suspected due to high rate of PE in women with APS. They usually present early in gestation and associated with severe PE.

The outcomes of the pregnancy and fetus of PE mothers can be devastating as PE can cause placental insufficiency and leads to miscarriage, preterm delivery, low birth weight, fetal distress or stillbirth. It is estimated that 10% of pregnant women with recurrent pregnancy loss were positive to APA such as aCL and LA (Atterbury *et al.*, 1997). First trimester abortions have been reported in almost 50% of untreated pregnancies in women with LA and/or medium to high levels of IgG aCL (D Ware *et al.*, 1992). Women with positive APA and a prior fetal death had a pregnancy loss rate greater than 95% in subsequent untreated pregnancies (D Ware *et al.*, 1992).

Post partum complication is rare however it has been observed in the post partum period up to 5 weeks post delivery. The complications include pericardial and pleural effusions, subclavian thrombosis, pulmonary embolus, thrombocytopenia, pulmonary hypertension and cardiomyopathy (Atterbury *et al.*, 1997).

The aim of this study was to investigate the presence of rare APA (antiAnV and aPI) in PE patient and compared with normal control subjects including the outcomes of the pregnancy. These antibodies were chosen because till date no similar study had been done in Malaysia and neighbouring countries. These two antibodies are not favourable to researchers however, a few studies showed a significant increased of these antibodies in patient with recurrent fetal loss (D'Ippolito *et al.*, 2014). PE could be induced by APA as explained by the pathophysiological process in many cellular models and hence we could expect the possible contribution of these antibodies to the development of PE and its complications. Hence the clinical significance of these rare antibodies among PE patients could be explored.

## 2.1 ANTIPHOSPHOLIPID SYNDROME

### 2.1.1 INTRODUCTION

APS is an acquired autoimmune disorder firstly described in 1983 characterized by the presence of APA with at least one clinical symptom either vascular thrombosis or obstetric manifestation such as repeated miscarriage. APS can be primary or secondary (when it is associated with other systemic disease mainly systemic lupus erythematosus), which affects 1 to 20 in every 100,000 women, depending on the ethnic origin. The population prevalence of primary APS is uncertain. It is estimated to affect about 0.5% of the population. The male: female ratio for primary disease is 1:3.5 and 1:7 for secondary APS. The mean age at diagnosis is 35 years, where the incident in children is very rare (D'Ippolito *et al.*, 2014).

Many studies have been conducted to further understanding of APS. Recent studies had established the two distinctions of APS which are obstetric and vascular based on observation that i) patients can display either vascular thrombosis with no pregnancy complications or, alternatively, obstetric manifestations alone with no vascular symptoms and ii) the coexistence of both thrombosis and miscarriage only affects about 2.5–5% of APS pregnancies. This is because the mechanisms involved are different between obstetric and vascular APS (D'Ippolito *et al.*, 2014).

Diagnosis of APS is made based on the presence of clinical criteria of thrombus formation with detection of APA. The antibodies in this disorder are directed against protein antigens that bind to anionic phospholipids, such as $\beta_2$-glycoprotein I ($\beta_2$GPI) and prothrombin or directed against anionic phospholipid per se such as cardiolipin and

phosphatidyl serine. Thus there are many types of antiphospholipid antibodies which have shown to have significant role in promoting thrombus formation in this disorder. However, to date only three antibodies (lupus anticoagulant, anticardiolipin and anti $\beta_2$-glycoprotein I antibody) are included in the laboratory criteria to diagnose APS and they are widely tested in the major hospital laboratories. Table 2.1 shows the criteria to diagnose APS in current setting.

Table 2.1 Antiphospholipid Syndrome: clinical criteria and laboratory criteria (D'Ippolito *et al.*, 2014)

| Clinical criteria | Laboratory criteria |
|---|---|
| **1. Vascular thrombosis**<br>One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.<br><br>**2. Pregnancy morbidity**<br>(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or<br>(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of:<br>(i) eclampsia or severe preeclampsia, or<br>(ii) recognized features of placental insufficiency, or<br>(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded. | 1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart.<br><br>2. Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or > the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized enzyme-linked immunosorbent assay (ELISA).<br><br>3. Anti $\beta_2$-glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma (in titer > the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.<br><br>*GPL and MPL is IgG and IgM phospholipid unit |

International classification criteria for this syndrome were proposed in 1998 in Sapporo (Japan) and updated in 2006 in Sydney (Australia). At least one clinical manifestation together with positive laboratory tests is required to fulfil the classification criteria.

Beyond classical criteria, "non-criteria" clinical and laboratory manifestations should be mentioned. Non-criteria clinical findings include: heart valve disease; livedo reticularis; thrombocytopenia; nephropathy; neurological manifestations. Non-criteria laboratory findings include: IgG or IgM aCL or anti $\beta_2$GPI levels in the range of 20 to 39 GPL or MPL units; IgA aCL and IgA anti $\beta_2$GPI; aPS and aPE; antiprothrombin antibodies (aPT) and antibodies to the phosphatidyl serine–prothrombin complex (aPS/PT).

It is not known whether the above mentioned conditions may "predate" the development of APS and may be regarded as "pre-APS" manifestations. Until such information has been well established they cannot be included in the diagnostic criteria because of potential loss of specificity in recognizing APS, which might lead to unwarranted treatments (D'Ippolito *et al.*, 2014).

## 2.1.2 PATHOGENESIS OF ANTIPHOSPHOLIPID SYNDROME

Phospholipids are an important component of the cell membranes. They form a lipid bilayer in which their hydrophillic (attracted to water) head areas arranged to face the aqueous cytosol and the extracellular fluid, while their hydrophobic (repelled by water) tail areas faced away from the cytosol and extracellular fluid. The lipid bilayer is semi-permeable, meaning that only certain molecules are allowed to diffuse across the membrane. There are a few major phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylglycerol (cardiolipin is made of two phosphatidylglycerol and mainly exclusively found in inner mitochondrial membrane).

*Giannakopoulos et al, 2007a*, unveiled a few hypotheses regarding pathogenesis of APS when they did studies on mechanism that predispose to thrombosis in APS patient. The dominant antigenic targets in APS are $\beta_2$GPI and prothrombin. Antigenic targets other than $\beta_2$GPI and prothrombin have been identified in APS patients, including tissue plasminogen activator (tPA), plasmin, annexin 5 and thrombin.

### 2.1.2(a) Complement

The possible role of complement activation in APS pathogenesis is suggested by the demonstration of increased complement activation products in the plasma of patients with APS who have had a cerebral ischemic event, which may be an important

mediator in thrombosis pathogenesis, compared with patients suffering from non-APS–related cerebral ischemia (Giannakopoulos *et al.*, 2007a).

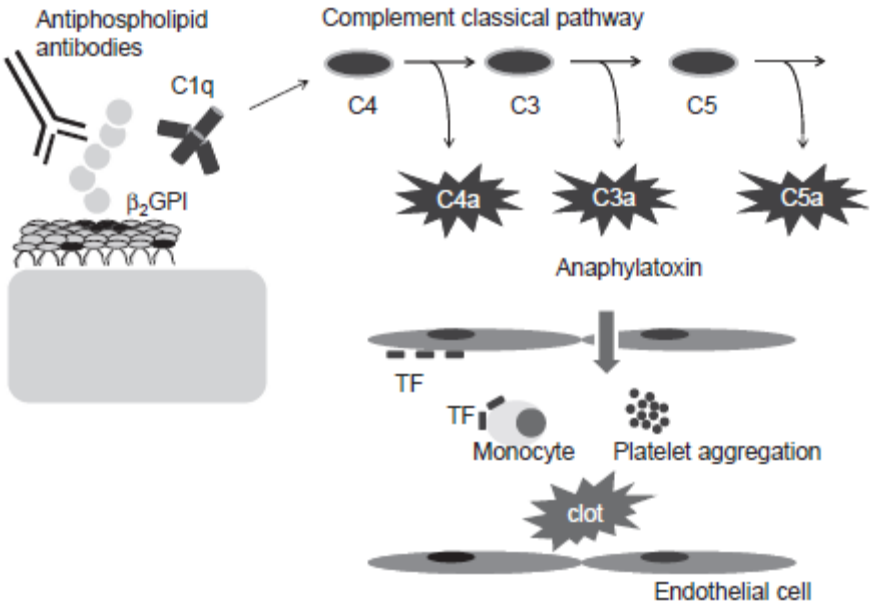



Figure 2. 1 Pathogenic mechanism of complement activation in antiphospholipid syndrome (adapted from Pathophysiology of thrombosis and pregnancy morbidity in the antiphospholipid syndrome, Oku et al, 2012)

### 2.1.2(b) Dysregulated activation of platelets, endothelial cells, and monocytes by the anti $\beta_2$GPI Ab/$\beta_2$GPI complex bound to the cell surface

Anti $\beta_2$GPI Ab/$\beta_2$GPI complex has to initially form on the exposed PS before interacting with specific platelet receptors to potentiate activation of the platelet leading to thrombosis. $AnV_2$ and Toll-like receptors (TLR) family also interact with anti $\beta_2$GPI Ab/$\beta_2$GPI complex on the endothelial surface to mediate activation.

Antibodies that bind directly towards $AnV_2$ without $\beta_2$GPI have also been shown to associate with the thrombotic manifestations seen in APS patients. They are able to directly activate nonstimulated endothelial cells, same to the effect of the anti $\beta_2$GPI Ab/$\beta_2$GPI complex. However, it is not clear why anti $\beta_2$GPI Ab/$\beta_2$GPI complex itself cannot induce activation without priming factor in vivo.

Anti $\beta_2$GPI Ab/$\beta_2$GPI complex also can activate monocytes to express tissue factors and release various pro-inflammatory cytokines leading to thrombosis. However, the relevant cell surface receptor has not yet been identified (Giannakopoulos *et al.*, 2007a).

**2.1.2(c) Platelet receptors**

Apolipoprotein E receptor 2 (ApoER2) the only receptor of the low-density lipoprotein (LDL) family described on platelets, mediates a role in the activation of platelets. This receptor was able to coprecipitate with dimerized $\beta_2$GPI, providing evidence for a direct interaction between $\beta_2$GPI and the receptor (Giannakopoulos *et al.*, 2007a).

**2.1.2(d) Endothelial cell receptor**

*Zhang et al, 2005,* demonstrated that $\beta_2$GPI binding to AnV enabled anti $\beta_2$GPI antibodies (Abs) to activate endothelium, inducing the expression of a procoagulant phenotype. *Meroni et al, 1998,* also noted that anti $\beta_2$GPI Abs, in the presence of $\beta_2$GPI, are able to activate the nuclear factor-κB (NF-κB) pathway in endothelial cells,

leading to the expression of a procoagulant and proinflammatory phenotypes (Giannakopoulos *et al.*, 2007a).

*Giannakopoulos et al, 2007a*, also suggested prothrombotic mechanism based on the disruption of the interaction between the anticoagulant factors and the PS surface as well as disruption in fibrinolysis.

### 2.1.3 PATHOPHYSIOLOGY OF OBSTETRIC ANTIPHOSPHOLIPID SYNDROME

Most common manifestation of APS in pregnancy is preeclampsia, eclampsia followed by abruptio placenta. It is suggested there is possibility of negative effect of APA towards placentation. Normal process requires invasion of trophoblasts into maternal endometrial tissues and new vessels formation in decidualized endometrium. The trophoblasts will invade up to inner one third of myometrium and the uterine vasculature where they will secrete metalloproteinases (MPs), a proteolytic enzyme capable to degrade extracellular matrix component. Anti $\beta_2$GPI Abs can adhere to both trophoblasts and human endometrial endothelial cells (HEEC) lead to functional damage and poor obstetric outcome.

*Yamamoto et al, 1996*, suggested that one of the causes of preeclampsia is the production of APA that binds to placental villous membrane antigens. Three types of APAs were tested in this study; aCL, aPS and antiphosphatidyl choline antibody (aPC).

APAs were able to:

(i) Inhibit syncytiotrophoblast differentiation, as shown by the reduced secretion of human chorionic gonadotrophin (hCG);

(ii) Impair trophoblast invasiveness in an in vitro Matrigel assay. This effect is well correlated with a significant inhibition of expression/activity of MPs;

(iii) Affect the trophoblast expression of integrins and cadherins. These represent adhesion molecules, whose expression is regulated during the process of trophoblast adhesion and invasion into maternal tissues. In particular it was found that APAs decrease alpha 1 integrin and vascular endothelial (VE)-cadherin and up-regulate alpha 5 integrin and epithelial (E)-cadherin.

(iv) Block endometrial angiogenesis both in vitro and in vivo, by inhibiting the HEEC tube formation and the production of specific factors up-regulated during angiogenesis, such as vascular endothelial growth factor (VEGF). Studies on the involvement of $\beta_2$GPI in the angiogenesis showed that, in contrast to the previously reported anti-angiogenic properties of $\beta_2$GPI, a cleaved form of $\beta_2$GPI is able to block the activity of angiostatin, a well known inhibitor of angiogenesis. The effect of anti $\beta_2$GPI antibodies on endometrial angiogenesis: whether the observed inhibitory effect is due to an imbalance between the intact and the cleaved form of $\beta_2$GPI still remains to be explored (D'Ippolito *et al.*, 2014).

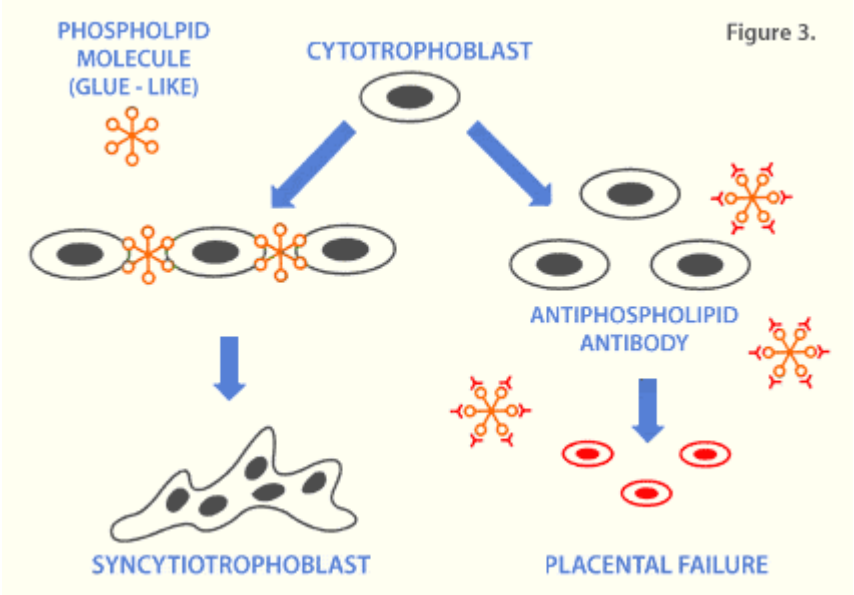
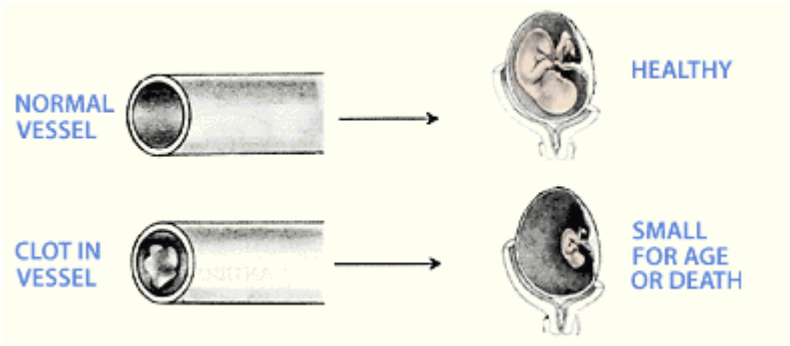
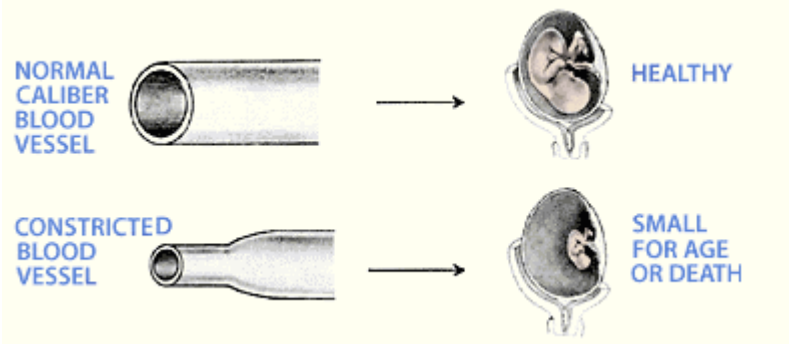



Figure 2.2 Effect of APA on placenta (adapted from Miscarriages can be Prevented, Reproductive Immunology Associates)




Antiphospholipid antibodies can also cause blood vessels to constrict, causing decreased blood flow throughout the circulatory system.




The combination of blood clots and constricted blood vessels may impair blood supply to the fetus and placenta resulting in complete fetal demise or growth retardation.

Figure 2.3 Fetal outcome in abnormal blood vessels (adapted from Miscarriages can be Prevented, Reproductive Immunology Associates)

### 2.1.3(a) Antiannexin V

AnV is a placental anticoagulant protein expressed on the surface of syncytiotrophoblast. It plays an important role as thrombomodulatory and contributes to the fluidity of the maternal circulation through the intervillous surface. AntiAnV IgG antibodies have been reported with higher frequency in women with unexplained fetal loss compared to women with normal pregnancy suggesting that these antibodies may represent a risk factor for fetal loss.

A significant elevation of antiAnV has been reported in women with recurrent fetal loss via a study done by *Sater et al, 2011*, compared to controls. However consecutive study failed to demonstrate association between antiAnV and the prediction of miscarriage thus another factor is proposed to contribute to recurrent fetal loss. A study done by Rand *et al*, 2010, showed there is increased prevalence of antiAnV in women with obstetric APS compared to controls.

### 2.1.3(b) Antiphosphatidyl inositol

Phosphatidyl inositol (PI) is a type of anionic phospholipid and the involvement of aPI in the pathophysiology of APS and PE is still debated. Very little study had been done in relating this antibody with APS. When this antibody is reported, it is usually associated with aCL or aPS. However, there is a report of aPI as the only APA present in six young patients with cerebral ischemia of undetermined cause (McIntyre *et al.*, 2003).

### 2.1.4 PREGNANCY OUTCOMES IN APS

APS in pregnancy can give rise to serious adverse event to the mothers as well as the neonates. The clinical outcomes of APS in pregnancy include recurrent abortion, intrauterine fetal death, intrauterine growth retardation (IUGR), maternal arterial and venous thrombosis and PE (Munday and Jones, 1993). Hence, correct and early diagnosis is important for the maternal and fetal wellbeing. Many studies had been done and some are still going on for better understanding of the disease and better management of the patients.

## 2.2 PREECLAMPSIA

### 2.2.1 INTRODUCTION

Preeclampsia is a systemic syndrome which can affect morbidity and mortality of a pregnant mother as well as the fetus. It is diagnosed after 20 weeks of gestation when the patient presented with persistent hypertension on two occasions and proteinuria. Study had suggested of imbalance of placental angiogenic and antiangiogenic factor as a pathogenesis for preeclampsia. Delivery of the placenta is the only known treatment suggestive of placental aetiology (Voto *et al.*, 1999; Steegers *et al.*, 2010; Young *et al.*, 2010b; Ahmed, 2011).

### 2.2.2 PATHOGENESIS OF PREECLAMPSIA

PE is thought to be due to inadequate invasion of placental cytotrophoblast with endothelial dysfunction. Study had shown excess of antiangiogenic factors soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) were released by the placenta leading to widespread endothelial dysfunction resulting in hypertension, proteinuria and other systemic manifestation of APS (Young *et al.*, 2010a).

In normal pregnancy, cytotrophoblast will invade the myometrial artery for adequate placental perfusion. In PE, the cytotrophoblast is unable to invade myometrial artery effectively due to alteration in angiogenic pathway (upregulation of sFlt1 and increased Transforming Growth Factor (TGF-β) and alteration in expression of VEGF, placental growth factor and VEGF Receptor-1 (Young *et al.*, 2010a).

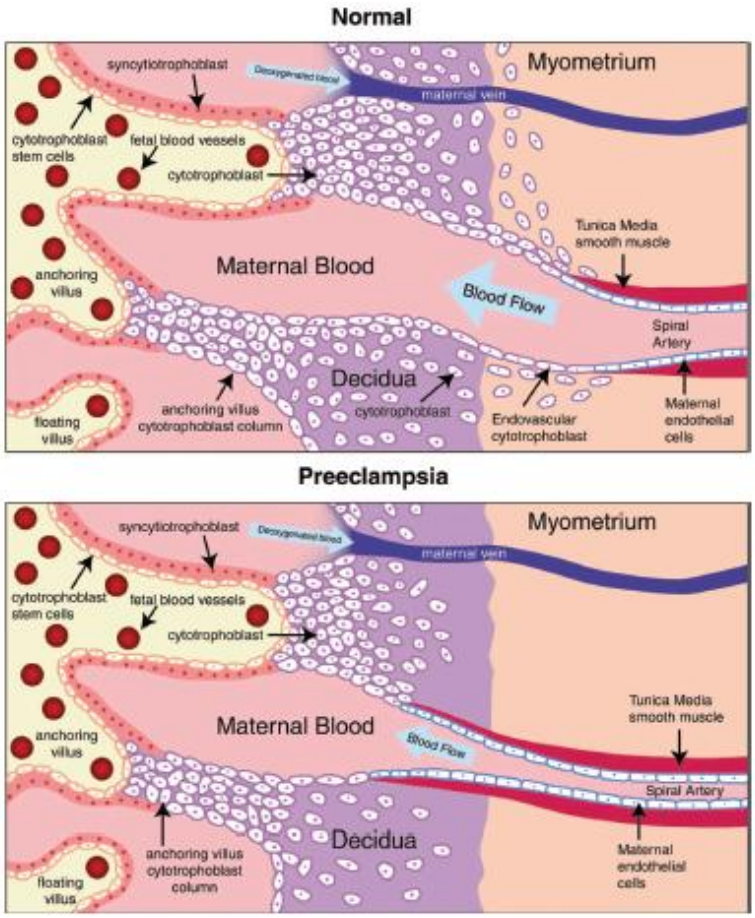



Figure 2.4 Placentation in normal pregnancy and in PE (adapted from Journal of American Heart Association, Powe *et al*, 2011)

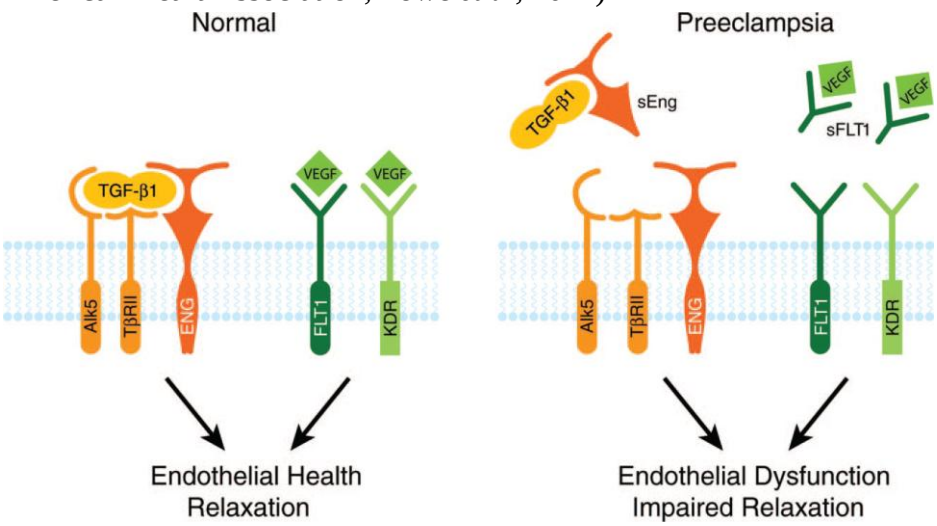



Figure 2.5 sFlt1 and sEng cause endothelial dysfunction by antagonizing VEGF and TGF-β1 signalling (adapted from Journal of American Heart Association, Powe *et al*, 2011)

Other contributory factors to PE are placental hypoxia, immune factors due to paternal antigen, renin-angiotensin-aldosterone (RAA) pathway which there is increase sensitivity towards angiotensin II leads to vasoconstrictive effect, alteration in placental enzymes for example cathecol-o-methyltransferase enzyme deficiency which can lead to placental hypoxia and oxidative stress/placental debris which increase in superoxide and reduce antioxidant capacity (Powe *et al.*, 2011).

Nitric oxide (NO) is important for angiogenesis. In PE, endothelial dysfunction is due to loss of endothelial nitric oxide synthase (eNOS) activity leads to production of superoxide instead of NO. This will cause widespread endothelial damage resulting in hypertension and proteinuria (Ahmed, 2011). Hemeoxygenase-1 (HO-1) enzymes also offer cytoprotection against tissue and cellular injury and protective against ischaemia-reperfusion injury. Study reported that loss of HO activity leads to placental damage.

AnV antigen is present on trophoblast cells. Anti-AnV Abs can induce apoptosis and reduce trophoblast gonadotrophin secretion which can cause defective in placentation (Di Simone *et al.*, 2001). This eventually can manifest as clinical symptoms such as PE.

Antiphosphatidyl inositol is rarely of interest of most studies. However, study showed that the level of this antibody is increased in cases like recurrent fetal loss (Ulcova-Gallova, 2012). Assuming the pathophysiology is due to abnormal placentation same as in PE, this study will help further in understanding the significance of this antibody in PE patient.

### 2.2.3 CLINICAL OUTCOMES OF PREECLAMPSIA

Preeclampsia is one of the leading causes of maternal and neonatal morbidity and mortality. Maternal impacts on PE includes impairment of the renal function, or complicated with seizure (eclampsia); or development of HELLP syndrome (hemolysis, elevated liver enzymes and low platelet) or the worst outcome is mortality. This condition is reversible with the delivery of placenta (Voto *et al.*, 1999; Steegers *et al.*, 2010; Young *et al.*, 2010a; Ahmed, 2011; Powe *et al.*, 2011).

Placental abruption, ascites, hepatic infarction, hepatic rupture, pulmonary edema and intra-abdominal bleeding are all severe manifestation of PE. PE targeted endothelium as its pathogenesis hence, resulting in systemic effect affecting organ such as liver, kidney and brain. Stroke and cerebral haemorrhage are examples of cerebrovascular accidents which becomes major cause of eclampsia-related maternal death (Powe *et al.*, 2011).

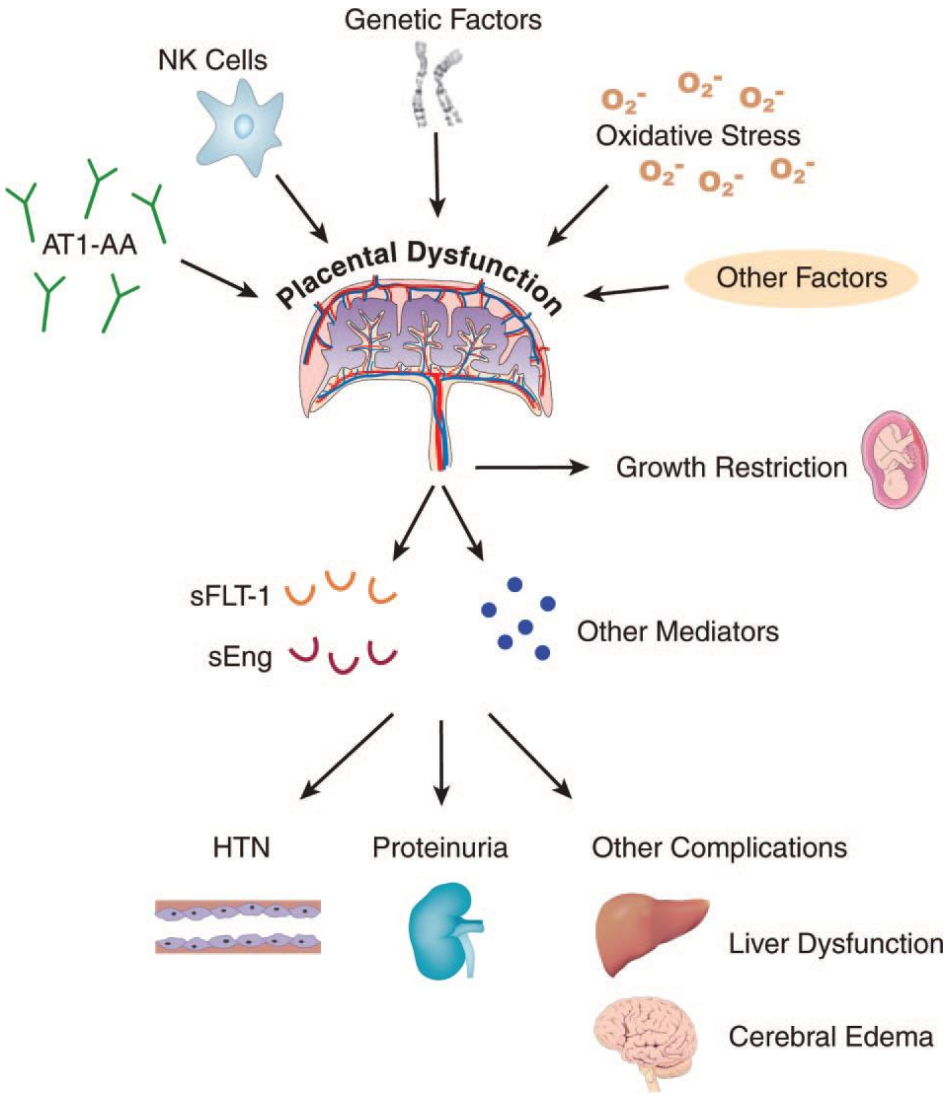



Figure 2.6 Summary of pathogenesis of PE (adapted from the journal American Heart Association, Powe *et al*, 2011)

Fetal outcomes include IUGR, low birth weight, preterm delivery, neonatal intraventricular haemorrhage, respiratory distress syndrome and the worst case is fetal death (Buchbinder *et al.*, 2002). As the pathophysiology is due to abnormal placentation, it will impair the supply of the oxygen and the nutrients from the mother to the fetus for normal fetal development. In this study, I will only see the weight of the baby in view of the limitation I had.

## 2.3 ANTIPHOSPHOLIPID SYNDROME IN PREECLAMPSIA PATIENT

Obstetric APS is a combination of thromboembolic events, APA and obstetric complications (Galli *et al.*, 2003). It can be divided into primary and secondary in which the secondary cause is usually due to underlying autoimmune disease such as Systemic Lupus Erythematosus (SLE). There are studies that suggested association between APS with PE (Munday and Jones, 1993; Ahmed, 2011; D'Ippolito *et al.*, 2014; de Jesus *et al.*, 2014).

One of the causes of PE is due to the production of APA that binds to placental villous membrane antigen and can cause placental villous dysfunction and abnormal fetal growth (Yamamoto *et al.*, 1996). Apart from the similar pathophysiology between these two entity, the clinical outcomes to the mother as well as the fetus also almost the same.

To date, there is not a single test meets the standard to predict PE. However, combination of ultrasound Doppler for uterine artery, placental thickness and homogeneity, serum pregnancy-related plasma protein A, serum free placental growth factor, body mass index, presence of nulliparity or previous PE can give high specificity and sensitivity in predicting early PE (Steegers *et al.*, 2010)

This study is targeting the rare APA, anti-AnV and aPI, to observe association with PE and fetal weight as the clinical outcome.

# 3.0 OBJECTIVES OF THE STUDY

## 3.1 GENERAL OBJECTIVE

To study the laboratory and clinical significance of rare APA (antiAnV and aPI) in pregnant women with PE and normal pregnant women in Hospital Universiti Sains Malaysia (HUSM) and Hospital Sultan Ismail, Johor Bahru (HSIJB).

## 3.2 SPECIFIC OBJECTIVES

1. To determine the prevalence of rare APA in PE patients in HUSM and HSIJB.
2. To determine the proportion of the presence of APA among PE and normal pregnant women in HUSM and HSIJB during pregnancy and 2 months post partum.
3. To compare the proportion of the presence of rare APA among different groups of PE in HUSM and HSIJB.
4. To study the frequency of clinical complication and abnormal haematological parameters among PE patients.

# 4.0 MATERIALS AND METHOD

## 4.1 Study design

This comparative cross sectional study was conducted in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan (HUSM) and Hospital Sultan Ismail Johor Bahru (HSIJB) over two years period from January 2013 until December 2014. Protocol for this study was approved by Medical Research and Ethics Committee, Ministry of Health Malaysia and School of Medicine Science Research and Ethical Committee. All subjects gave written consent (see Appendix).

## 4.2 Sampling method

### 4.2.1 Sources population for cases

The source population of the subjects was all pregnant women with PE and attended Obstetrics and Gynaecology (O&G) department in HSIJB and HUSM from January 2013 until December 2014.

### 4.2.2 Sampling frame for cases

The sampling frame was those with PE and fulfilled the inclusion and exclusion criteria.

### 4.2.3 Inclusion criteria for cases

The inclusion criteria were antenatal mothers who were diagnosed to have PE (after $20^{th}$ weeks of gestation). The patients must be previously normotensive women presented with high blood pressure (systolic blood pressure (SBP) ≥ 140mmHg or diastolic blood pressure (DBP) ≥ 90mmHg measured twice (6 hours apart) associated with proteinuria ≥ 0.5g in 24 hours. Severe preeclampsia is defined as SBP ≥ 170 mmHg or DBP ≥ 110 mmHg (acute hypertensive crisis in pregnancy) on two occasions, with proteinuria of 1 g/day or DBP ≥ 100 mmHg on two occasions, with significant proteinuria (1+ on dipstick), with two or more signs or symptoms of imminent eclampsia, which include:

a. severe headache

b. visual disturbance

c. epigastric pain and/or vomiting

d. clonus

e. papilloedema

f. liver tenderness

g. platelet count below 100,000 x $10^9$/l

h. abnormal liver enzymes

i. HELLP syndrome (haemolysis, elevated liver enzymes, low platelets)

j. intrauterine growth restriction (IUGR)

k. pulmonary oedema and / or congestive cardiac failure