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Review article:

Thrombophilia abnormalities in recurrent pregnancy loss

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Abstract

Recent evidences associate thrombophilia with adverse pregnancy outcome. Numerous studies confirm Factor V Leiden (FVL) and Prothrombin G20210A (PT G20210A) mutations as important thrombophilia risk factors in Caucasians. However, these mutations are rare in Asians and thrombophilia investigations are therefore considered irrelevant in these patients. Hence, the status of thrombophilia-induced recurrent pregnancy loss (RPL) in Asians is obscure and poorly understood. Four-hundred and two (402) Malaysian RPL-subjects and 160 parous-controls, who are part of the Asian community, were investigated for FVL, PT G20210A, Methylene Tetrahydrofolate Reductase C677T (MTHFR C677T), activated protein C resistance (APCR), protein C (PC), protein S (PS), antithrombin (AT) and antiphospholipid antibodies. One-fifth of the RPL-subjects were identified to have thrombophilia abnormalities. *Acquired* Thrombophilia was more prevalent in Malaysian RPL-subjects compared to the *inherited* form in Caucasians. FVL and PT G20210A mutations were identified in 2.0% of the RPL-subjects, disputing the rarity of these mutations in Asians. The overall findings warrant the need to review thrombophilia investigations and its corresponding management (the use of anticoagulant therapy) in thrombophilia-induced RPL patients of Asian origin.

Key words: Recurrent pregnancy loss, *inherited* and *acquired* thrombophilia, Factor V Leiden, activated protein C resistance.

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Introduction

Normal pregnancy is accompanied by major changes in the coagulation system - increased activities of clotting factors and reduced functions of the naturally-occurring anticoagulants [1-2]. This transient haemostasis changes appear to play the role of a double-edged sword - creating a hypercoagulability state to protect the expectant mother from fatal haemorrhage during delivery, and simultaneously predisposing her to an unfortunate thrombophilia disorder. Thrombophilia is a haemostatic disorder characterized by *inherited* and *acquired* conditions, predisposing patients to increased thrombotic phenomena. In most cases, the patients remain asymptomatic, until a secondary hypercoagulable state like pregnancy, triggers off a sequence of clotting activities at the placental vasculature, resulting in thrombosis with eventual pregnancy loss.

The majority of the studies so far have been on subjects that were predominantly Caucasians. The relatively few studies on Asians were confined to the investigation of individual thrombophilia markers, especially FVL [3-7]. FVL was identified in the early 1993, and even after a lapse of a decade, the status of thrombophilia, in terms of prevalence and clinical management in Asians, is still shrouded by much obscurity. This review documents the relationship between thrombophilia and recurrent pregnancy loss (RPL) in Asian women, resident in peninsular Malaysia (a country within the region of South East Asia).

Pregnancy loss

Challenges

The fertilized egg in the human reproduction system faces numerous challenges before a successful pregnancy is achieved. Between 30% to 50% of all conceptions end in failure to produce a healthy infant, and of the resulting successful pregnancies (≥6 wk of gestation) 10% to 15% of them end in miscarriages [8-10]. A failed progress is a common obstetric problem affecting the lives of over 500,000 women in the United States every year [11].

Miscarriage affects approximately 1% to 3% of couples desirous of starting a family and the risk increases with increasing maternal age [12-14]. However, the majority of continuing pregnancies result in the birth of a healthy child. Surprisingly, women who have one, two or even three first trimester miscarriages will nevertheless go on to have a successful pregnancy. Hence, despite the odds stacked against a successful pregnancy, the human reproduction system is still able to achieve its objective of sustaining a healthy human population.

Definitions

Pregnancy losses have been described by various terms such as stillbirths, miscarriages, missed abortions and blighted ovum. The traditional grouping of pregnancy losses prior to 22 weeks as 'abortion' has been readily used by obstetricians, for reasons best known to them; however, it is a poor term of definition and lacks clinical clarity, promoting much confusion among clinicians and patients. For instance, a patient may not realize that 'spontaneous abortion' is different from 'medical or legal abortion'.

Despite the present widespread use of ultrasound for accurate clinical assessment and diagnosis, there still appears to be no agreed glossary of terms or consensus regarding important gestational milestones. Farquharson *et al*. [15] introduced a revised terminology in an attempt to provide clarity and standardization of its use in literature and in clinical assessment. However, non-compliance of the new terminologies is still the norm today, leaving much to be desired in the standardization of fetal losses.

Historically, clinicians have defined abortion, which is synonymous with miscarriage, as the spontaneous loss of a pregnancy before the fetus has reached viability. It is caused by the separation of the fetus and placenta from the uterine wall. It includes all pregnancy losses from the time of conception until 20 to 22 weeks of gestation: depending on the country in which it is applied (WHO has defined spontaneous abortion as expulsion of an embryo or fetus weighing 500 g or less, corresponding to about 20 to 22 weeks gestation). Stillbirths refer to the death of a fetus after 24 weeks of pregnancy (The Still-Birth [Definition] Act 1992 - Registration of Births, Deaths and Marriages Act - UK). In the present review, RPL has been defined to include any healthy subject or "patient" with a history of at least three early (<12 weeks gestation) or two late (>12 weeks gestation), consecutive and spontaneous, unexplained fetal loss or the death of a fetus before 22 weeks of gestation.

Pathogenesis

Historically, RPL has been attributed to a wide range of possible causes (*Figure 1*) namely, haemostasis disorders (62%), hormonal imbalances (15%), anatomical abnormalities (10%), chromosomal abnormalities (7%) and unexplained causes (6%) [11,16]. However, different studies give different ranges of occurrences [17-19] causing much uncertainties over the actual prevalence of each causative factor.

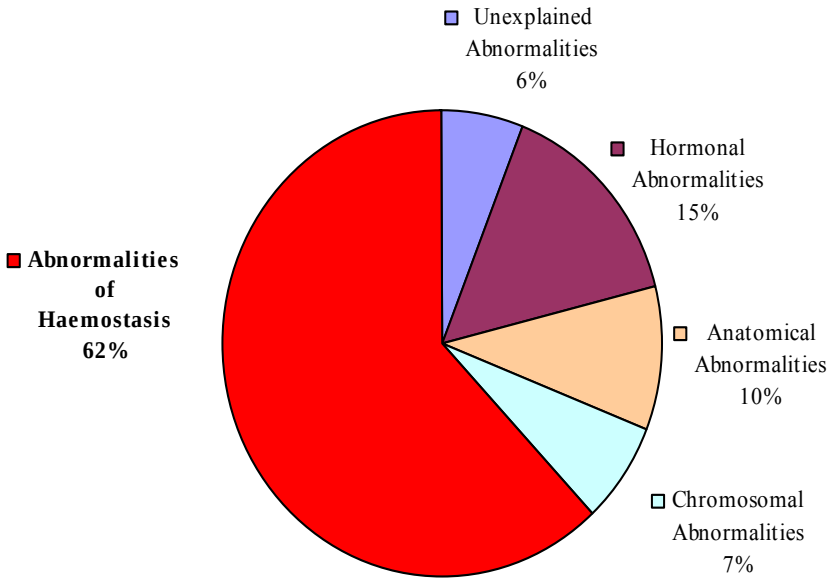

Figure 1: *Causes for recurrent pregnancy loss*

Pregnancy loss due to abnormalities of haemostasis outweigh other recognized causes by at least four- to ten-fold. Its mode of action is probably due to two main physiological mechanisms - hemorrhagic disorders and thrombophilia abnormalities, with the later being more prevalent [11].

Thrombophilia abnormalities

Haemorrhagic Disorders

Haemorrhagic disorders are rare and account for less than 2% [11], and are usually due to deficiencies in clotting Factors XIII, XII, X, IX, VIII, VII, V, II, I and von Willebrand factors. Due to inadequate fibrin formation, the abnormal haemostasis system causes inadequate implantation of the fertilized ovum in the uterus, culminating in pregnancy loss [20-23].

Thrombophilic Abnormalities

All pregnancies undergo significant shift in the equilibrium of the haemostasis mechanism, causing a prothrombotic milieu [24-27]. Hence, it is hypothesized that hypercoagulation at the placental vasculature causes microthrombosis in the placental bed vessels, resulting in placental infarction. This occlusive event compromises the fetomaternal circulatory system, resulting in low placental perfusion with eventual pregnancy loss [11, 25, 28-30].

Thrombosis may also interfere with the initial formation of the spiral artery-intervillous circulation [31-32]. In early pregnancy, the placental and uterine vascular system is comparatively smaller and thus possesses a greater propensity towards partial or total thrombotic events. Histological examinations from pregnancies complicated by thrombophilia reveal increased fibrin clot deposition, suggesting thrombosis as the underlying etiology. Hence, pregnant women have a five-fold increased risk for venous thrombosis when compared with similarly age-matched non-pregnant women. Moreover, with simultaneous occurrence of thrombophilia, pregnant women face an added increased risk for thrombosis and fetal loss [24-25, 31,33].

Middeldorp [34] however, disputes the hypothesis of hypercoagulability at the placental vasculature as the pathophysiological mechanism linking thrombophilia with RPL. The author is of the opinion that it is not biologically plausible for a thrombotic mechanism to play a role prior to 10 to 12 weeks of gestation, as it is only then that the placenta vasculature develops. Hence, hypercoagulability is unlikely to be the *sole* mechanism by which thrombophilia increases the risk of pregnancy failure. It is therefore the opinion of most researchers that thrombophilia is a complex disease arising from the combined interactions of inherited and acquired risk factors (Table 1).

Table 1: Acquired and Inherited Thrombophilia Risk Factors

Thrombophilia	*Inherited*	*Acquired*	Other Possibilities
Common Markers	▪ Factor V Leiden ▪ Prothrombin G20210A ▪ MTHFR C677T	Antiphospholipid Antibodies: ▪ Lupus anticoagulant ▪ Anti-Cardiolipin ▪ Anti-β2-Glycoprotein I ▪ Anti-Prothrombin ▪ Anti-Annexin V ▪ Protein C/S/AT deficiency ▪ "Non-specific" abnormalities	▪ Oral contraceptives ▪ Hormone replacement therapy ▪ VTE ▪ Malignancies ▪ Surgery ▪ Major trauma ▪ Prolonged immobilization
Rare Markers	▪ Antithrombin deficiency ▪ Protein C deficiency ▪ Protein S deficiency	▪ Elevated Factors VIII, IX, XI ▪ High vWF ▪ Dysfibrinogenemia	▪ Age >50 yrs ▪ Males

Inherited Thrombophilia

The *inherited* thrombophilia markers consist of FVL, PT G20210A and MTHFR C677T mutations, deficiencies in AT, PC and PS, and APCR.

Factor V Leiden Mutation

The evaluation of *inherited* thrombophilia prior to 1993 was limited to PC, PS and AT deficiencies, which together were found in less than 10% of patients with VTE [35]. The laboratory approach to thrombophilia testing changed in 1993, when Dahlback and colleagues [36] described a new and very common familial thrombophilia, hereditary resistance to activated PC (APC). It involved the production of an abnormal clotting Factor V molecule. A single G to A missense mutation at nucleotide 1691 of the Factor V gene results in an amino acid substitution of arginine for glutamine at position 506 of

the Factor V procoagulant molecule [37]. Bertina *et al*. [38] described the Factor V mutation and named it after the Dutch city, Leiden. The normal arginine at position 506 is one of the three sites where APC normally cleaves and inactivates the procoagulant Factor Va. The substitution of arginine to glutamine renders the Va molecule partially resistant to the anticoagulant action of APC (*Figure 2*), and is thus inactivated at an approximately ten-fold slower rate than the normal Factor Va molecule, resulting in increased thrombin generation with hypercoagulation. The mutated Va molecule remains active in the presence of APC and promotes thrombosis, resulting in a lifelong hypercoagulable state. Since a hypercoagulable state is present, patients present with either venous or arterial thrombosis or recurrent miscarriage syndrome [39]. Unlike many of the other *inherited* thrombophilias, this disorder is not evenly distributed among different ethnic groups, being much more common in people of European descent.

Zammiti et al [40] in their study on 348 patients with RPL and 203 parous controls identified FVL in 19.4% of the patients and 5.5% in the controls. He concluded that FVL posed a significant and independent risk for pregnancy loss from 8 weeks onwards. Similarly, Onderoglu *et al*. [41] investigated the prevalence of FVL in 101 patients with recurrent miscarriages and affirmed that women with recurrent fetal miscarriage had a high frequency of this genetic mutation. Likewise, numerous similar studies have confirmed the close association of FVL with early RPL, and being responsible for 9.0% - 48% of the recurrent miscarriages [42-46].

However, there are also studies that dispute the thrombotic effect of FVL. One study on 91 patients with adverse pregnancy outcome, concluded that FVL (12.1% vs 18.7%; P = 0.304) did not play a role in causing adverse pregnancy outcomes [47]. Gonen *et al*. [48] likewise in a similar study on 37 women with unexplained third trimester stillbirths did not find any association between unexplained third-trimester intrauterine fetal deaths and *inherited* thrombophilia. Pauer *et al*. [49] compared the prevalence of FVL between 84 women with a history of two or more fetal losses (64 of first and 20 of second trimester) and 87 controls and found that 10.7% of patients and 9.2% of controls were carriers of FVL. Similarly, Kutteh *et al*. [50], Dizon-Townson *et al*. [51] and Roque *et al.* [52] showed the absence of any association between thrombophilia and RPL.

Prothrombin G20210A Mutation

The discovery of FVL was followed a few years later in 1996, with the discovery of another genetic mutation, PT G20210A. Prothrombin, the precursor of thrombin in the coagulation mechanism, undergoes mutation involving a G to A transition at position 20210 of the 3'untranslated prothrombin gene (G20210A). Carriers of the mutation have about 30% higher plasma levels of prothrombin than the non-carriers [53] and thus have the potential to form thrombin easily [54] - setting a patho-physiologic environment suitable for thrombosis. Brenner *et al*. [55] showed PT G20210A mutation increased the risk of recurrent miscarriage by 2.2-fold. Rey *et al.* [45] in their meta-analysis of 31 studies also affirmed the association of PT G20210A with early recurrent (OR 2.56, 95% CI 1.04-6.29) and late non-recurrent (OR 2.30, 95% CI 1.09-4.87) fetal loss. Likewise, other studies too by Kovalevsky *et al*. [56], Beretta *et al*. [57] and Sehirali *et al*. [58] also confirmed the pathological effect of PT G20210A with RPL.

Just as in FVL mutation, there were also studies that dispute the thrombogenic effect of the PT G20210A gene. A study on 146 patients with $\geq$ 3 consecutive pregnancy losses and 99 age-matched controls showed the absence of a close association between PT G20210A and RPL (2.74% vs 4.04%) [59]. Similarly, Kutteh *et al*. [50] and Zahed et al. [47] concluded that PT G20210A did not play a significant role in causing adverse pregnancy outcome.

Methylene Tetrahydrofolate Reductase C677T Mutation

Homocysteine is an amino acid metabolite of methionine. A mutation at the nucleotide 677 from C to T in the methylene tetrahydrofolate reductase gene results in the production of an abnormal thermolabile enzyme, MTHFR C677T. This mutation causes approximately a 50% reduction in the activity of the enzyme MTHFR resulting in mild to moderate hyperhomocysteinaemia. While the patho-physiologic influence of homocystine on haemostasis is poorly characterized, it is clear that hyperhomocystinemia is associated with arterial and venous thrombosis. Brenner, [60] showed that women with MTHFR C677T had a two-fold enhanced risk for recurrent miscarriage. A similar study conducted by Lissak *et al*. [61] on 41 patients with unexplained recurrent and spontaneous abortions reported an increased prevalence of recurrent early fetal loss among patients with the MTHFR C677T. Likewise, Raziel *et al*. [62] found MTHFR C677T to be more common in patients with RPL than in controls. Overwhelmingly, there appears to be a close association between MTHFR C677T mutation and RPL in the Caucasians.

However, an investigation on 80 recurrent miscarriage patients and 100 controls suggested the absence of any significant relationship between MTHFR C677T and recurrent miscarriages (8% vs 15%, P = 0.134, OR 0.4, 95% CI: 0.1-1.2) [63]. Similarly, a study on 91 women did not show any significant relationship between MTHFR C677T and pregnancy losses (53.8% vs 6.9%, p=0.130) [47]. Similarly, Wramsby *et al.* [64] in his investigation

on 84 women with three or more consecutive miscarriages and Rey *et al.* [45] in his meta-analysis of 31 studies showed the absence of any relationship between MTHFR C677T and recurrent miscarriages [45]. In fact, some studies have even reported a decreased risk of miscarriage in women with *inherited* thrombophilia [65], whereas one study reported that multiple genetic thrombophilic mutations in either partner seem to increase the risk of miscarriage in subsequent pregnancies [66]. Apparently, there is still much confusion and uncertainty over the thrombogenic effect of the three mutations namely, FVL, PT G20210A and MTHFR C677T.

Protein C, S and Antithrombin Deficiency

PC and PS are vitamin-K-dependent proteins. PC, a glycoprotein, is activated by the thrombin-thrombomodulin complex into its activated form, APC (*Figure 2*). APC is a potent anticoagulant and it selectively and proteolytically degrades activated Factor V and Factor VIII, thereby reducing thrombin generation and the corresponding risk of thrombosis. This reaction requires the presence of the cofactor PS [67]. Any mutational event leading to decreased levels or decreased activity of PC and PS would tilt the haemostasis system in favour of a hypercoagulable state with subsequent increased risk towards thrombosis.

AT is an essential inhibitor of thrombin, activated clotting Factors X, IX, X, XI, XII, plasmin, kallikrein and PC and PS. The outcome is a decrease in the production of thrombin. In addition to regulating the levels of activated clotting factors, AT also exerts its anticoagulant function through its activity with heparin. Any mutation that leads to decreased levels of AT, or decreased ability to interact with the activated factors or heparin, will result in an increased risk towards thrombosis.

Rey and others [45] in their meta-analysis consisting of 31 studies confirmed the association of PS deficiency with RPL (OR 14.72, 95% CI 0.99-218.01) and late non-recurrent fetal loss (OR 7.39, 95% CI 1.28-42.63). However, Rey *et al*. [45] excluded PC and AT deficiencies as thrombophilia risk factors for pregnancy loss. The prevalence of AT, PC and PS deficiencies in the *inherited* form is extremely rare.

Activated Protein C Resistance

Abnormalities in the PC pathway are manifested by poor anticoagulant activity of the plasma, resulting in prothrombotic conditions [38]. The common causative factors are abnormal functional levels of PC and PS activities and abnormal Factor V molecules – FVL, Factor V Cambridge [68], Factor V Hong Kong and Factor V HR2 haplotype [69-70]. The abnormal activated Factor V molecules are resistant to inactivation by APC, resulting in the production of higher levels of thrombin and a higher likelihood for thrombotic events.

The PC pathway plays an important anticoagulation role by down regulating a hypercoagulable state in haemostasis (*Figure 2*). APC proteolytically inactivates the activated clotting Factors V (Va) and VIII (VIIIa) [71-72]. The natural anticoagulant activity APC is initiated with the binding of the active procoagulant thrombin to thrombomodulin (TM). The TM-thrombin complex, a potent activator of PC, activates PC to APC, a serine protease. APC then rapidly inactivates the phospholipid-bound activated forms of coagulation Factors Va and VIIIa. The loss of Va and VIIIa activities causes a subsequent reduction in the synthesis of new thrombin, thus down-regulating a hypercoagulable state. The APC in turn is slowly neutralized by three inhibitors, PC inhibitor, trypsin inhibitor and α_2-macroglobulin.

More recently, elevated plasma Factor VIII levels have been shown to confer up to a seven-fold increased risk for VTE [73]. Since Factor VIII is inactivated by APC, high Factor VIII levels may lead to APCR [74]. Thus, Factor V genetics and Factor VIII levels seem to be closely linked to APCR.

Lindqvist *et al*. [75] in a prospective study on 2480 patients concluded that patients with APCR showed an increase prevalence of second trimester fetal loss (7.3% vs. 2.7%, $p = 0.01$). Similarly, Rey *et al*. [45] and Rosendorff & Dorfman [76] concluded that APCR was the most common cause of *inherited* thrombosis accounting for 40% to 50% of cases.

Factor V Leiden and Prothrombin G20210A mutations are rare in the Asian Population

The FVL and PT G20210A mutations are important thrombophilia markers that warrant investigations in patients with thrombotic events. Rees *et al*. [77] in his analysis of 3380 chromosomes from 24 populations concluded that the FVL is relatively common among individuals of Caucasian descent - identifiable in 2% to 15% of the general population. Rosen *et al*. [78] in his study on 175 *Israeli-Arab* men and 26 females aged 19-68 years reported a high frequency of 24.3% for FVL in the *Israeli-Arab* population [78]. This is almost five-fold the prevalence in the *Israeli-Jewish* population (5.1%, $p<0.001$) and is the highest frequency of FVL (heterozygotes) in any ethnic group reported to date.

However, none of the 1600 chromosomes from Southeast Asia, Africa and Australasia showed the presence of FVL [77]. Hence, investigators [77] were of the opinion that this partly explained the rarity of thromboembolic diseases in the Asian population. Similar studies by Bontempo *et al*. [39] and Ro *et al*. [79] showed the absence of

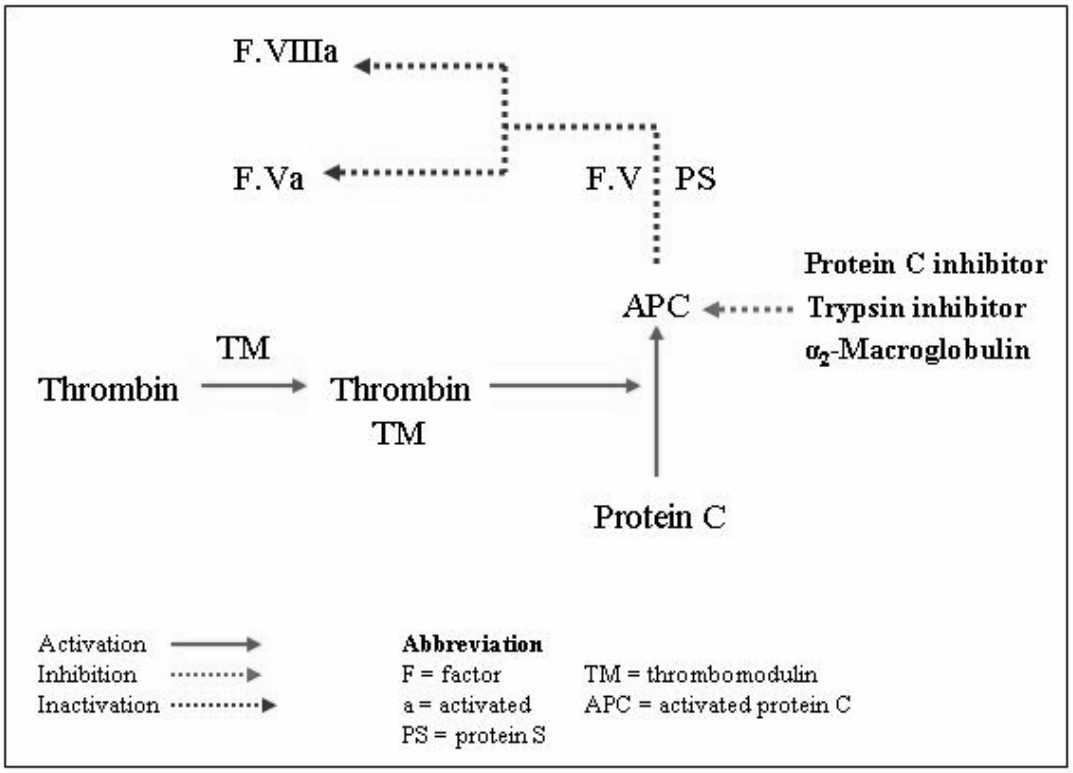

Figure 2: The Protein C Pathway

FVL in Asians. Kobashi *et al.* [5] studied 83 *Japanese* women with recurrent spontaneous abortions and 174 controls. They concluded that neither FVL nor MTHFR C677T mutations were associated with recurrent abortions in the *Japanese* population. Thus, overwhelming evidences are supportive of the absence of FVL and PT G20210A in patients with Asian ancestries and therefore thrombophilia investigations was irrelevant in these patients.

Acquired Thrombophilia

The most important *acquired* thrombophilia responsible for RPL is the antiphospholipid syndrome (APLS). APLS consists of a complex spectrum of autoimmune antibodies that are directed against phospholipid binding plasma proteins. They include the predominant lupus anticoagulant (LA) and the anti-cardiolipin (aCL) antibodies, followed

by a host of other lesser common ones such as anti-prothrombin, anti-annexin V, anti-β2-glycoprotein I, anti-ethanolamine, anti-choline, anti-phosphatidyl inositol, anti-phosphatidyl serine and anti-phosphatidic acid. The prevalence of APLS in women with recurrent miscarriage is 15%-20% [80], and is clearly the most common prothrombotic risk factor leading to fetal wastage and recurrent miscarriage [81-83]. Many clinicians consider APLS as the most common prothrombotic disorder among both *inherited* and *acquired* thrombophilia disorders [11,48, 84-85].

Occasionally, pregnancy-induced PS deficiency and elevated levels of Factor VIII activity can precipitate thrombotic episodes. Other *acquired* thrombophilia risk factors include transient increased levels of von Willebrand factor, clotting Factors IX and XI, hidden malignancies, prolonged immobilization, surgeries and major traumas.

Thrombophilia in Asians

No comprehensive studies on the prevalence of thrombophilia in Asian women with fetal loss have been carried out. The closest was that by Vora *et al.* [86] who evaluated the prevalence of aPL antibodies in Indian women with fetal loss. They reported the prevalence of LA and ACA in 23.2% of their patients as against 1% in the controls. Kobashi *et al.* [5] evaluated the prevalence of only 2 thrombophilia markers - MTHFR C677T and FVL - on forty-five Japanese patients with unexplained RPL and concluded the absence of any close association of these 2 markers with RPL in their patients. There were other similar studies carried out on Asian women with RPL but the thrombophilia parameters covered were only 1 or 2 thrombophilia markers (Anti-Annexin V – Matsubayashi *et al.* [87]; PT G20210A – Akimoto *et al.* [88]; FVL –

Hashimoto *et al.* [3]; ACL antibodies – Higashino *et al.* [89]; FVL – Ko *et al.* [4]).

Thrombophilia in Malaysian women

A comprehensive thrombophilia study on Malaysian RPL-women (402 patients vs 160 parous controls) by Thiruchelvam *et al.* [90] produced some significant findings, one of which was the documentation of a greater prevalence of *acquired* thrombophilia in Malaysian RPL-women compared to the *inherited* form in the Caucasian population. More than one-quarter (27.4%) of the "healthy" Malaysian RPL-subjects had thrombophilia. Another interesting finding was the detection of the "non-specific" abnormalities in Malaysian subjects with abnormal APCR. This group of subjects, despite having an abnormal APCR, showed normal activity levels for PS, PC and F.VIII:C and were negative for the FVL mutation and aPL antibodies. There had been no other studies documenting the presence of such "non-specific" antibodies or inhibitors. A recent study on recurrent pregnancy loss-subjects confirmed that the true cause for

acquired APCR still remained unknown [91]. An in depth study needs to be conducted to decipher the biological role of this "new" thrombophilia marker.

Another outcome of the studies by Thiruchelvam *et al.* [90] was the identification of FVL and PT G20210A mutations in 2.0% of the RPL-subjects, thereby disputing the findings of numerous studies, that both these mutations were non-existent in patients of Asian ancestries.

Thrombophilia profiling

A complete thrombophilia profile, as carried out by most Western investigators, includes both *inherited* and *acquired* thrombophilia markers. Thrombophilia investigations on Malaysian RPL-subjects produced some results that were in sharp contrast to that seen in the Caucasian RPL-subjects. For instance, *acquired* APCR was much more prevalent in Malaysian RPL-subjects (90.8%) compared to a prevalence of only 5.0% in the Caucasians. As for *inherited* APCR, the prevalence was only 9.2% in Malaysian RPL-subjects but it was found to be significantly high of 90% - 95% in the Caucasians.

Based on their results, Thiruchelvam *et al.* [90] proposed a "Thrombophilia Profile" appropriate for subjects of Malaysian origin (*Table 3*). The thrombophilia markers, PT G20210A mutation and AT deficiency have been excluded from the thrombophilia profile because of their extreme low prevalence in the Malaysian population. Anti-annexin V has also been excluded because of its greater prevalence in the controls compared to the RPL-subjects. For MTHFR C677T, the authors were of the opinion that the results need to be validated with a greater subject number before a decision could be taken on its significance as a thrombophilia marker in Malaysians with thromboembolic disorders

Table 3: Thrombophilia Markers and Methodologies

Heritance	Thrombophilia Markers	Methodology
Inherited	Factor V Leiden mutation	RT-PCR
	Activated Protein C Resistance	Clot-base
	Protein C deficiency	Clot-base
	Protein S deficiency	Clot-base
	Coagulation Profile	Clot-base
Acquired	Lupus anticoagulants	Clot-base
	Antiphospholipid Antibodies:	
	Anti-Prothrombin	ELISA
	Anti-Cardiolipin	ELISA
	Anti-β2-Glycoprotein I	ELISA

Management

The pre-existing skewed haemostatic imbalance in pregnancy further increases the risk of thrombosis in women with thrombophilias. Treatment is therefore in the form of anticoagulant therapy [92-94]. There are various treatment options, the choice of the therapy depending on the causative factors responsible for the pregnancy loss.

Antiphospholipid Syndrome

Almost two decades following the discovery of a close association between aCL antibodies and fetal death and

after approximately 4200 publications on APLS and poor pregnancy outcomes, we still lack scientific and evidence-based rules for the treatment of APLS. This failure is due in part to a lack of well-designed prospective studies and in part to the clinical complexity of the syndrome, which is in fact is a two disease syndrome – primary and secondary APLS. Although it has been well established that APLS is a leading cause of miscarriage and maternal/fetal morbidity [46, 93, 95-97], there are still considerable disagreements regarding the mechanisms of actions of aPL antibodies in these patients. Presently the pathophysiology of APLS in women with RPL has been attributed to thrombotic events occurring at the uteroplacental vasculature [98-100] and impairment of trophoblast cell maturation and defective placentation [91, 101].

Treatment options used for improving pregnancy outcome in APLS-pregnant women are two prong – one directed towards suppressing the immune system (prednisone, intravenous immunoglobulin and progestational agents) and the other inhibiting thrombosis (low-dose aspirin and heparin). As the preferred treatment, for otherwise healthy women with obstetric APLS, most authorities now advocate the use of low-dose aspirin (80-100 mg/day) together with prophylactic low molecular weight heparin

(LMWH) [93, 102-105]. Empson *et al.*[106] in a recent meta-analysis supported the combined use of unfractionated heparin (UFH) and aspirin, resulting in the reduction of pregnancy loss by 54%. A preference for LMWH comes, despite higher costs compared to UFH, from the comfort to the patient with once daily injection and reduced risks for heparin-induced thrombocytopenia and osteoporosis [107]. An added advantage is the inability of heparin to cross the fetus-placental barrier and induce a teratogenic effect and/or cause bleeding in the fetus. Many independent studies have highlighted that acetylsalicylic acid (aspirin) when administered at low doses (50-150 mg) is a safe drug to be used during pregnancy. It is usually started before conception, following a positive pregnancy test. Heparin too, is usually started with a positive pregnancy test, or when fetal cardiac activity is demonstrated [92]. More recently, El-Hafeg *et al.* [108] achieved 100% live birth rate with plasmapheresis and low dose prednisone therapy. They advocated the use of plasmapheresis in patients with failed first line of treatment using aspirin and/or heparin.

Non-Antiphospholipid Thrombophilia Abnormalities

For subjects with non-aPL antibody abnormalities such as AT deficiency, FVL or PT G20210A and APCR, therapeutic heparin (LMWH or UFH) throughout pregnancy is advocated [109]. However, recent reports that are more recent are of the opinion that there should not be any firm recommendations, at least for the time being, because of too few studies having been conducted on the use of anticoagulants and women without the antiphospholipid syndrome [110-111].

Conclusion

The association between thrombophilia (*inherited* and *acquired*) and RPL is still shrouded by many uncertainties. Is there truly a direct relationship between thrombophilia and failed pregnancies? Although laboratory tests to identify thrombophilia are presently available in a reasonably uniform manner in developed countries spanning Europe and the Americas, they are yet to establish a "gold-standard" test that could identify with confidence the pregnancy that is at high risk of miscarriage.

The association between thrombophilia and miscarriage has been demonstrated in the majority of studies concerning FVL and PT G20210A but not so clearly in those with PC, AT deficiencies and MTHFR C677T mutation. In the Caucasian patient-population, it is *inherited* thrombophilia that is prevalent, however in the Asian population it is the *acquired* form. Despite numerous studies from the West documenting the absence of FVL and PT G20210A in Asians, recent studies conducted locally show the prevalence of both these mutations in RPL-patients. Studies conducted worldwide are many; however, the results obtained are not always homogeneous, even with different studies carried out in the same geographical area.

To overcome this void in thrombophilia investigations, future clinical studies need to include both genetic and phenotypic assessments of risk factors. There should be a shift in emphasis from the usual restrictive concept of looking for a single dominant cause for a prothrombotic tendency towards the concept of multiple risk factors. A healthy collaborative study between obstetrician and hematologist in cases with thrombophilic etiology could be the key to a successful pregnancy outcome. The 21st century would indeed pose considerable challenge to the clinicians in the pathology of maternal-fetal medicine.

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Thrombophilia abnormalities