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Dear Sir,

Thrombophilia describes the inherited and acquired disorders of the haemostatic mechanism which predisposes to thrombosis (1). Though occasional pregnancy loss is a common condition, the majority of the losses are unrecognized, and their cause remains unidentified (2). A clear etiology is yet to be identified in about 50%-80% of the cases (2, 3). However, new evidence now implicates inherited thrombophilia as increased risk for many recurrent fetal losses (RFL) (4–6). Factor V Leiden (FVL) (4, 5, 7, 8) and prothrombin (PT-G20210A) gene mutations (7, 9) appear to be the most common ones, followed closely by protein C (PC), protein S (PS) and antithrombin deficiencies (10). In a meta-analysis of 31 studies from 1975 to 2002, Rey et al. (7) and Pai-das et al. (11) showed overwhelming evidences linking RFL to inherited thrombophilia. RFL is classically defined as three or more spontaneous fetal losses before the 20th week of gestation (12). Although RFL is rare, it is a major health problem affecting 0.5% to 1.0% of pregnant women in Western countries. All these findings were predominantly from patients with a Caucasian background, and confined mainly to Europe, the Americas and the Mediterranean regions. There are no significant data on patients of Asian origin, especially on patients indigenous to Southeast Asia.

It is also an established consensus from Western countries that FVL is unheard of and PT G20210A mutation is virtually non-existent in the Asian population (13–15). The acceptance of this Western-derived conclusion has invariably discouraged the local clinicians from screening patients for these mutations – with consequential failure to treat high-risk patients with established guidelines in anticoagulant therapy (11, 16).

The present study was designed to determine the prevalence of inherited thrombophilia markers (ITM) in Malaysian women with RFL and in particular to verify the non-existence of FVL and PT G20210A mutations in patients of Asian origin. The findings would invariably open a new chapter in the diagnosis and thrombo-prophylaxis of Malaysian women with RFL.

The study was on Malaysian women selected from the three main ethnic groups – Malay, Chinese and Indian. Only subjects who had given written consent were enrolled in this study. Group I consisted of patients from the Maternity Hospital Kuala Lumpur. Selection criteria were: age between 18 and 45 years; clinical history of at least three RFL, no living children; absence of any chromosomal abnormalities, hormonal imbalances and anatomic defects; no congenital or acquired hemorrhagic disorders; free of any known pathologies such as hypertension, diabetes mellitus and thyroid diseases; non-smoking. Group II consisted of female controls with the following criteria: age between 18 and 45 years; married with at least one living child; free from any clinical pathology; regular blood donors and/or staff at the National Blood Centre, Kuala Lumpur.
FVL and PT G20210A mutations were determined using the real-time polymerase chain reaction principle. Genomic DNA was isolated from frozen 3.2% citrated whole blood using a standard commercial mammalian genomic DNA extraction kit (GenElute, Sigma, USA). A fluorescence-based multiplex test kit (Agen, Australia) identified the mutations with the real-time cycler (Rotorgene 2000 Corbett Research). The melting curves gave results as wild-type, homozygous and heterozygous mutations.

Plasma-free PS activity was determined using a commercial enzyme-linked immunosorbent assay (Imulone Free Protein S ELISA, American Diagnostica, USA). Free PS concentrations were determined against a curve prepared from a frozen pool of citrated normal plasma and standardized against the Secondary Standard for Coagulation/International Society on Thrombosis and Haemostasis (SSC/ISTH) preparation. The normal range was 50–150% and was consistent with the normal range published in the literature and reported by other commercially available assays. PC activity was quantitatively measured in citrated plasma using the chromogenic substrate S-2366 (Chromogenix-Coamatic Protein C). A micro-plate methodology was used, and results were read off at 405 nm using a spectrophotometer. The established normal range was 70–140% activity. Plasma antithrombin activity was determined using a thrombin-specific chromogenic substrate (Accucolor Antithrombin, Sigma Diagnostics, USA) on the Amelung Coagulation Analyzer (Amelung, Austria). The established normal range was 70–140% activity. All samples outside the established normal range on two occasions were considered as abnormal.

Table 1 summarizes the prevalence of abnormal ITM in the three ethnic groups of the Malaysian population. Our investigation on 402 patients showed that 13.2% (53 from 402) of the patients had at least one inherited thrombophilia abnormality. The control group of 160 women was free of any thrombophilia abnormalities.

The most common inherited thrombophilia abnormality was PS deficiency, which occurred with a prevalence of 8.5% (34 in 402 patients). PC deficiency was found in 2.2% (9 in 402) of the patients. Antithrombin activity was normal in all the patients.

FVL mutation (heterozygous) was identified in eight (2.0%) patients – three Malays and five Indians. PT G20210A mutation (heterozygous) was detected in two (0.5%) patients – an ethnic Malay and Indian patient. No mutations were detected in the Chinese patients.

This recent finding, a first of its kind in the Asian region, reports the prevalence of FVL and PT G20210A mutations in the three ethnic groups of the Malaysian population. The presence of these mutations in the ethnic groups – Malay and Indian – clearly disputes all previous documented evidences from Western countries on the non-occurrences of FVL and PT G20210A mutations among patients of Asian origin. However, the absence of these mutations in the Chinese ethnic group still stands. As the study was carried out on a small sample population, there is a need to verify the prevalence of the mutations on a larger sample number.

It is the opinion of many studies that women with adverse pregnancy outcomes should be tested for genetic markers of thrombophilia (4–6, 10). As these complications tend to recur in subsequent pregnancies, the identification of thrombophilia markers in these patients is necessary. It offers an opportunity to reduce the risk of recurrence with prophylactic anticoagulant therapy (16).

In light of the present findings, it is imperative that Malaysian women with RFL should now be screened for FVL and PT G20210A mutations. Together with the readily available treatment option – anticoagulant therapy, Malaysian women can now hope for a better pregnancy outcome.

Table 1: Inherited thrombophilia markers in the three ethnic groups (Malay, Chinese, Indian).

<table>
<thead>
<tr>
<th>Type of inherited thrombophilia</th>
<th>Malay (Pat = 287; Con = 82)</th>
<th>Chinese (Pat = 33; Con = 66)</th>
<th>Indian (Pat = 82; Con = 12)</th>
<th>Total (Pat = 402; Con = 160)</th>
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<tbody>
<tr>
<td>Abnormal inherited thrombophilia markers identified in patients and controls</td>
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<tr>
<td>FVL n (%)</td>
<td>Pat 3 (1.1)</td>
<td>0 (0)</td>
<td>5 (6.1)</td>
<td>8 (2.0)</td>
</tr>
<tr>
<td></td>
<td>Con 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PT-G20210A n (%)</td>
<td>Pat 1 (0.4)</td>
<td>0 (0)</td>
<td>1 (1.2)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Con 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PC deficiency n (%)</td>
<td>Pat 7 (2.4)</td>
<td>1 (3.0)</td>
<td>1 (1.2)</td>
<td>9 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Con 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>PS deficiency n (%)</td>
<td>Pat 26 (9.1)</td>
<td>4 (12.1)</td>
<td>4 (4.9)</td>
<td>34 (8.5)</td>
</tr>
<tr>
<td></td>
<td>Con 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>AT deficiency n (%)</td>
<td>Pat 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Con 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Abnormal thrombophilia n (%)</td>
<td>Pat 37 (12.9)</td>
<td>5 (15.2)</td>
<td>11 (13.4)</td>
<td>53 (13.2)</td>
</tr>
<tr>
<td></td>
<td>Con 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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</table>

Pat, patient; Con, control; n, number; FVL, Factor V Leiden mutation; PT-G20210A, Prothrombin G20210A mutation; PC, protein C; PS, protein S; AT, Antithrombin; %, percentage.
In conclusion, 13.2% of Malaysian patients with RFL have at least one abnormal inherited thrombophilia marker. FVL and PT G20210A mutations invariably do occur in patients of Asian origin – Malay and Indian ethnic groups – with a prevalence of 2.0 and 0.5%, respectively.

Acknowledgements
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References