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Intervention of Low-Molecular-Weight Heparin in M2/ANXA5 Haplotype Carriers: A Case Report in Pregnancy

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Abstract

Hereditary thrombophilia is viewed as a possible factor for repeated pregnancy loss (RPL), resulting in the onset of thrombotic events at the placenta that lead to adverse pregnancy outcome. The M2/ANXA5 haplotype is recognized as a third RPL susceptibility gene, RPRGL3, OMIM entry 614391, RPRGL1 is the factor V Leiden mutation (OMIM entry 614389), and RPRGL2 is the prothrombin mutation 20210G>A (OMIM entry 614390). An intervention with low molecular weight heparin (LMWH) could be a successful anticoagulant treatment for M2/ANXA5 carriers with RPL, rendering healthy pregnancy outcome. This is a case report of a heterozygous M2 carrier couple (both parents) with RPL history, who underwent the combination of aspirin and LMWH treatment and successfully delivered a live healthy singleton baby. Perinatal genotyping identified the newborn as M2 homozygote. Thus, in accordance with clinical trials addressing M2 carrier status, anticoagulation treatment might well be a remedy for recurrent miscarriage, supported with clinical evidence from this particular case. To address the general relevance of such treatment, a properly powered clinical trial will be instrumental that offers clear advantage if conducted with Malay RPL couples as target population.

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Introduction

Repeated pregnancy loss (RPL) is defined as the loss of two or more consecutive pregnancies by the American College of Obstetrics and Gynecology [1]. RPL is a complex and multifactorial obstetric problem with polygenic background involving a variety of genetic, physiological, and environmental factors [2]. It could affect 5% to 8% of Malay couples trying to conceive [3]. Hereditary thrombophilia has been reported to be possibly etiologic for idiopathic RPL [4], and impeded placental perfusion was proposed as a reason for adverse pregnancy outcomes. The two most common thrombotic risk lesions, factor V Leiden (G1691A, R506Q, rs6025) and prothrombin (PTm) G20210A (rs1799963) have been well studied in European populations [5,6]. A decade ago, a constellation of 4 single-nucleotide polymorphisms (SNPs), c._467G>A, rs112782763; c._448A>C, rs28717001; c._422T>C, rs28651243; and c._373G>A, rs113588187 in the proximal core promoter region of the annexin A5 (ANXA5) gene, transmitted as haplotype and termed M2/ANXA5, was reported, which represents another hereditary predisposition to thrombophilia-related RPL [7]. First indication for the biological role of M2/ANXA5 came through the reduced expression of a reporter gene in a cell line model when compared to the expression level of the normal wild type allele [7]. Subsequently, reduced expression through the M2 haplotype was proven "*ex vivo*" on mRNA [8,9] and protein level [10] in placental chorion. Generally, reported M2 carrier rates in European and Asian (Japanese) control populations ranged from 11% to 17% [11-15]. Recent studies of the Malaysian Malay population reported the carrier rate of M2/ANXA5 to be as high as 42.2% [3,16]. When estimating the association between M2/ANXA5 and RPL, the odds ratios were from 1.5 to 2 when comparing to the general population of German [7,12,13], UK [11], Bulgarian [12], and Malaysian [3,16] ethnic backgrounds; accordingly, elevated odds ratios (1.8 to 3.0) were documented when comparing to parous controls (negative history for infertility or miscarriage) in German [7,12,13], UK [11], Italian [15], Japanese [14] and Malaysian [3] cohorts. In accordance with the proposed physiological role of M2/ANXA5 in embryonic anticoagulation several studies showed similar paternal risk in RPL couples [3,11-13,16].

RPL is an aggravating medical condition that has an adverse psychosocial impact on women

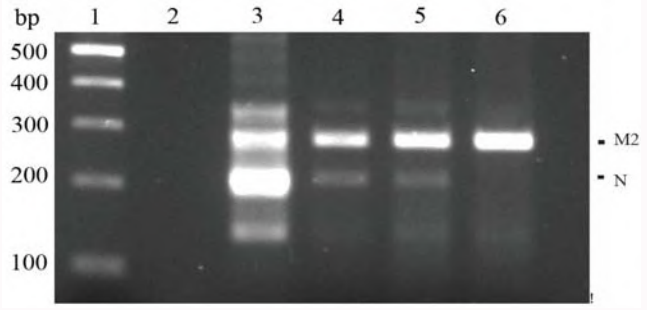

Figure 1: Allele-specific (AS)-PCR for detection of the M2/ANXA5 haplotype (3). Lane 1: DNA ladder; Lane 2: negative control; Lane 3: positive control, M2/ANXA5 heterozygous sample; Lane 4: maternal DNA amplification; Lane 5: paternal DNA amplification; Lane 6: newborn DNA amplification. The amplicon size of 276 bp is indicative for the M2 allele, whereas a 210 bp amplicon diagnoses the 'normal' allele (N).

and on their families, resulting in depression or anxiety for the next pregnancy [17], therefore different therapeutics options have been evaluated and discussed. The most popular antithrombotics treatment options for managing RPL are low-dose of aspirin, unfractionated heparin and low-molecular-weight heparin (LMWH), either prescribed alone or in a combination. Heparin acts like the natural anticoagulant antithrombin; whereas aspirin inhibits platelet aggregation. The application of heparin can be somewhat burdening as the pregnant woman needs to inject subcutaneously daily or twice-daily for a duration of 18.5 weeks [18].

There has been a general controversy in the thromboprophylaxis with LMWH in RPL. Several clinical studies suggested that LWMH increases live birth rates [19-21], however, others generated indifferent outcomes [22-24]. Regarding the possibility of selected intervention in M2 carriers, the EThIGII and the CARE Fertility Group clinical trials delivered information of positive LMWH effect on live birth rates [25,26]. This is a report of a clinical case, where anticoagulant intervention was implemented successfully in a M2/ANXA5 carrier couple.

Patients and Methods

A married couple had a history of four consequent pregnancy losses and had previously volunteered for M2/ANXA5 haplotype screening. They did not have any successful pregnancies. The miscarriages occurred between 6 to 20 weeks of gestation, within a span of 3 years. The couple had participated in a study approved by the Human Ethics Research Committee of the University of Science Malaysia (USMKK/PPP/JEPeM (245.3)) [2] and by the Malaysian National Medical Research Register (NMRR), ID: NMRR-11-1044-9519. Informed consent for M2 genotyping was obtained from both partners.

Standard histopathology examination of previous miscarriages confirmed conceptus without molar tissue that would be indicative of gestational trophoblastic disease. The wife was pre-screened for potential causes that would lead to RPL [27]. Uterine anomalies, endocrinological dysfunctions (polycystic ovary syndrome) according to the Rotterdam criteria [28] and thyroidal dysfunctions, if anamnestic were excluded, as well as fetal and parental chromosomal disorders (numerical aberrations). Inherited thrombophilia (FVL, PTm) and deficiencies in anti-thrombotic factors (protein C, protein S, factor XII, antithrombin III) were ruled out. The result of Antiphospholipid (APS) screening was negative. Genotyping of DNA extracted from peripheral blood was performed by allelic specific PCR (AS-PCR) for the M2/ANXA5 haplotype as described [3], with some modifications.

The woman presented again in 2016 in her 5th pregnancy at 6 weeks of gestation with a threatened miscarriage. At the time of pregnancy, the woman and her husband were 29 and 32 years old respectively. Both were of Malay origin that was verified to not have intermarriage across three generations.

Ultrasound examination revealed a viable fetus with a fetal heart rate of 154 bpm. Treatment with oral Aspirin 75 mg daily, subcutaneous fondaparinux 2.5 mg daily and oral dydrogesterone 10 mg twice daily was initiated. Dydrogesterone was stopped at 14 weeks of gestation. Aspirin and Ffondaparinux were continued for the rest of the pregnancy. The woman was monitored closely throughout the pregnancy and repeated growth scans and blood tests (full blood count and clotting profile) were normal throughout.

She was scheduled for an elective Caesarean section at 38 weeks to avoid any intrapartum complications. Aspirin was stopped one week prior to the operation, and fondaparinux was stopped the night before the operation, to reduce the risk of intraoperative bleeding.

The newborn's blood was collected for M2/ANXA5 genotyping at four days of age.

Results and Discussion

The Caesarean section was uneventful and a healthy baby boy weighing 3.1 kg was delivered. The mother was discharged in good general health on the third postoperative day. Both partners were found heterozygous M2 carriers (Figure 1). This genotyping information together with a history of 4 idiopathic miscarriages motivated the physician to try anticoagulant therapy. Therefore, anticoagulant treatment with low-dose aspirin (LDA) and a low-molecular-weight heparin (LMWH) was initiated upon detection of fetal heartbeat at 6 weeks gestation and continued until delivery. The combination of LDA and LMWH has been previously shown to be more effective than LMWH alone in treating obstetric anti-phospholipid syndrome (OAPS) women with prior fetal loss [29] and in secondary prevention of placental vascular complications [30]. Since M2/ANXA5 (RPRGL3) has been previously recognized as a predisposition factor for obstetric placental complications [15], the LDA plus LMWH treatment was chosen as the better, potential risk minimizing option. This treatment protocol resulted in healthy singleton operative delivery. Fondaparinux was chosen as the LMWH because of its once daily dosing and its synthetic, not animal-derived origin. The offspring was found to be a homozygous M2 carrier (Figure 1). Diagnostics of the M2/ANXA5 haplotype (RPRGL3), appears highly relevant in repeated pregnancy loss (RPL) subjects of Malay origin since carriers could benefit from LMWH treatment [25,26]. M2 homozygotes occur in 3% to 5% of the RPL Malay population on a background of a high carrier rate in 52% to 53% of couples [3,16]. The proposed physiological role of M2/ANXA5 in embryonic anticoagulation [11-13] suggests that couples, where one or both parents are M2 carriers should equally benefit from antithrombotic therapy. The present case of M2 homozygous offspring born without complications following such therapy supports the notion of successful intervention, implying that reduced ANXA5 levels in chorion can be successfully supplemented with anticoagulants [31]. This in accordance with the recently proposed biomarker role for M2 in ANXA5, a precision medicine approach that appears highly

relevant for the Malay population [30]. In addition, a LMWH clinical trial that may be conducted in Malaysian RPL couples offers a clear advantage of a lower number of participants required to reach the necessary statistical power [25].

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