The placenta is a unique organ with dual blood circulation, functioning throughout foetal development. Placental trophoblasts express and produce coagulation components, participating not only in haemostasis but also in placental vascular development and differentiation.\(^1\)

For pregnancy to proceed normally the placenta must be allowed to develop and grow appropriately so that an adequate blood supply is available to support and promote the growth of the developing foetus. Failure of the normal uterine physiological changes to occur and the development of intra-placental pathology

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**Background & objectives:** Acquired and genetic thrombotic conditions, both organ and non organ specific, are associated with increased foetal wastage. This study was carried out to examine the placenta from women with abnormal pregnancies and a history of unexplained foetal loss, and to associate with maternal thrombophilia status.

**Methods:** Placentas from eight women with history of unexplained foetal loss were analyzed for histopathological characteristics. All the women were simultaneously screened for the common acquired and genetic thrombophilia markers i.e., lupus anticoagulants (LA), IgG / IgM antibodies for anticardiolipin (ACA), β2 glycoprotein I (β2GPI) and annexin V, protein C (PC), protein S (PS), antithrombin III (AT III), factor V Leiden (FVL) mutation, prothrombin (PT) gene G20210A, methylene tetrahydrofolate reductase (MTHFR) C 677T, endothelial protein C receptor (EPCR) 23 bp insertion and plasminogen activator inhibitor (PAI-1 4G/5G) polymorphisms.

**Results:** Six of eight women were positive for one or more thrombophilia markers. The placenta in all the cases except one, showed the characteristic features of infarct fibrin deposition and calcification. Among two women who were negative for thrombophilia, one showed clear evidence of thrombus in the placental sections while the other did not show any characteristic infarcts in the placental sections.

**Interpretation & conclusion:** Our findings showed that the histopathological examination of the placentas confirmed thrombophilia as the aetiological cause of thrombosis in 6 of the 8 women. The presence of thrombus in a negative thrombophilia woman suggests yet unidentified thrombophilia markers or probably non-haemostatic factors causing thrombosis.
will ensure placental insufficiency and the features that accompany are failing placenta, i.e., intra-uterine growth retardation (IUGR), pre-term delivery, pre-eclampsia, and toxemia of pregnancy. Thrombotic lesions of the placenta are a common feature in women with recurrent foetal loss or with adverse pregnancy outcomes. However, the aetiology of foetal loss or other associated adverse conditions of the pregnancy is unknown. It is believed that these are associated with abnormal placental vasculature and haemostatic disturbances leading to inadequate maternal foetal circulation.

The known thrombotic nature of the placental lesions and the risk associated with both acquired and genetic thrombophilias strongly suggest a cause-effect relationship between thrombophilias and severe obstetric complications. The evidence of thrombosis or infarcts in the placental sections suggests a haemostatic abnormality with or without association with thrombophilia. In the present study, the histopathological analysis has been done in placenta from eight women and subsequently compared to their thrombophilia status to objectively confirm the effect of thrombophilia on the placental vasculature.

Material & Methods

The study was carried out between July 2004 and June 2007 at the Institute of Immunohaematology, (IIH), Mumbai, in collaboration with the Department of Histopathology, KEM Hospital, Mumbai. Placenta from eight consecutive women having history of unexplained foetal loss were collected at the time of delivery for histopathological evaluation. These women had either second or third trimester pregnancy losses or both. The selection of these placenta from eight women has been done based on the following criteria: It was randomly selected and the number was restricted by the large number of sections which were to be taken and processed for each placenta so that important pathological changes are not missed. The conventional causes of pregnancy loss i.e., hormonal (T3, T4, TSH, 17 α-hydroxyprogesterone, estradiol, estriol, β hCG), immunological (autoimmune markers – ANA, ds DNA, rheumatoid factor) and karyotyping were excluded in all women. The placental tissues were fixed with 10 per cent formalin immediately after delivery and following dehydration were embedded in paraffin immediately. Microtome sections were subsequently prepared and fixed on the slides using egg albumin. The slides were then stained with hematoxylin and eosin and mounted with DPX mountant for histopathological analysis under an optical microscope (Olympus BX50 - SP-350). The study was approved by the Ethics Committees of both the IIH and KEM Hospital.

Screening for thrombophilia: Ten ml of blood was collected from each women by venipuncture into 3.18 per cent trisodium citrate (1:9 anticoagulant to blood) and EDTA tubes. Plasma samples were stored in -70° C until analysed. The cell pellet was preserved at -20° C for DNA extraction. The samples were collected 6 months after the miscarriage. As the natural inhibitors of blood coagulation i.e., protein C, S and AT do not get normalized immediately after the delivery or a miscarriage. The general practice is to wait for a minimum of 6 months before one collects for these tests.

Screening coagulation tests i.e., prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) were performed using commercial reagents (Organon Teknika, Durham, USA). Any sample showing >5 sec prolonged clotting time (APTT) than the control sample, was analysed for the presence of lupus anticoagulants (LAs). Fibrinogen was measured by clotting assays using commercial kits (Diagnostica Stago, Asniers, France).

Tests of antiphospholipid antibodies (APA): The plasma was mixed with normal pool plasma (NPP). Kaolin clotting time (KCT), dilute Russels Viper Venom time (DRVVT) were performed as described earlier using commercial reagents (Dade Behring, Germany). IgG/IgM antibodies for anticardiolipin (ACA), β-2 glycoprotein-1 (β2GP1), annexin V were measured by ELISA using commercial kits (Varelisa, Freburg, Germany). All the results were reconfirmed with a fresh sample after a minimum duration of 4 months.

Tests for inherited thrombophilia: Protein C (PC) and protein S (PS) were measured by ELISA using commercial kits (Diagnostic Stago, Asniers, France). Antithrombin III (ATIII) was measured by chromogenic assays using commercial reagents (Diagnostica Stago, Asniers, France). DNA was extracted from citrated cell pellet using standard methods.

Factor V Leiden (FVL) mutation was identified by PCR amplification (Applied Biosystems, CA, USA) of 220 bp fragment using MnI 1 enzyme for restriction digestion. The prothrombin (PT) gene G20210A polymorphism was identified by HindIII cleavage of 322 bp PCR amplified product. The C677 T polymorphism
of methylene tetrahydrofolate reductase (MTHFR) was detected using Hinf 1 cleavage of 175 bp PCR product while endothelial protein C receptor (EPCR) 23 bp insertion was detected by PCR amplification without any further restriction digestion. Plasminogen activator inhibitor-1 (PAI-1 4G/5G) polymorphism was detected by allele specific PCR amplification using two sets of primers as described. In brief, the PCR protocols were as follows: For FVL, 30 cycles of 94°C for 30 sec, 55°C for 40 sec and extension at 72°C for 1 min; for MTHFR and PT, 30 cycles of 94°C for 1 min, annealing at 66°C for 1 min and extension of 1 min at 66°C ; for EPCR, 30 cycles of 94°C at 1 min, annealing at 55°C for 1 min and an extension of 70 °C for 3 min; for PAI-1 4G/5G, 30 cycles of 94°C for 1 min, annealing at 65°C for 45 sec, extension at 72°C for 75 sec.

Results

The histological features in the placental tissue sections and their association with maternal thrombophilic status was shown in the Table.

Except two cases in all remaining cases more then one thrombophilic risk factor was observed. Three women were homozygous for PAI-1 4G/4G polymorphism while one was homozygous for MTHFR T677T polymorphism. While two were positive for APA. Of the two women in whom thrombophilia was not detected, one had low platelet count, a history of portal venous occlusion, and clear evidence of thrombosis in the histological analysis of placental sections while in another women neither thrombophilia nor thrombus in placental sections was observed. A large area of subchorionic haemorrhage was seen in the sections.

The primary pathology observed in all the placentas was thrombosis. Placental infarction, decidual vessel thrombosis, chronic villitis and excessive perivillous fibrin deposition were seen in a few sections. Multifocal uteroplacental thrombosis was also a significant observation in some of the placental sections (Figs 1-5).

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Patient ID /Age (yr)</th>
<th>No. of abortions/ IUFD*</th>
<th>Histological features</th>
<th>Maternal clinical features</th>
<th>Maternal thrombophilia status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CY/ 29</td>
<td>2/2</td>
<td>Villi with stromal fibrosis, a few foci of calcification, chronic ischaemia, organized thrombus stem vessels</td>
<td>Retro-placental clot</td>
<td>β2GP1 35MPL-U/ml Anti-annexin V 25MPL-U/ml Fibrinogen gene polymorphism Arg/Lys</td>
</tr>
<tr>
<td>2</td>
<td>AD/ 27</td>
<td>0/1</td>
<td>Premature villi, focal haemorrhage, vessels showing recent thrombosis.</td>
<td>Portal vein thrombosis</td>
<td>Not detected</td>
</tr>
<tr>
<td>3</td>
<td>PG /28</td>
<td>0/3</td>
<td>Villi shows stromal fibrosis, chronic ischaemia, infarct, placental insufficiency, thrombosis in vessel.</td>
<td>PIH</td>
<td>Anti annexin V 22GPL-U/ml</td>
</tr>
<tr>
<td>4</td>
<td>SJ / 23</td>
<td>0/3</td>
<td>Intervillous haemorrhage, infarct, foetal stems arteries show organized thrombus.</td>
<td>PIH</td>
<td>PAI-1 4G/4G Homozygous MTHFR C677T heterozygous, PAI-1 4G/4G Homozygous MTHFR T677T homozygous</td>
</tr>
<tr>
<td>5</td>
<td>MS / 29</td>
<td>0/4</td>
<td>Mild perivillous fibrin deposit, Mild neutrophilic infiltrate, immature villi with retroplacental haemorrhage, No thrombus.</td>
<td>Excessive bleeding</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>CT /22</td>
<td>0/2</td>
<td>Membrane shows normal amnion and chorion, large area of subchorionic haemorrhage.</td>
<td>-</td>
<td>Not detected</td>
</tr>
<tr>
<td>7</td>
<td>UN / 31</td>
<td>5/0</td>
<td>Areas of infarct, foci of extramedullary haematopoiesis, polymorph infiltrate suggestive of ischaemic changes with possible chorioamnionitis.</td>
<td>Doppler suggestive of mild resistive flow in left uterine artery</td>
<td>MTHFR T677T Homozygous</td>
</tr>
<tr>
<td>8</td>
<td>NP /22</td>
<td>0/1</td>
<td>Calcification, infarct</td>
<td>USG suggestive of absence of foetal movement</td>
<td>PAI-1 4G/4G Homozygous</td>
</tr>
</tbody>
</table>

IUFD, intrauterine foetal death; PIH, pregnancy induced hypertension; USG, ultrasonography
Fig. 1. Placental tissue showing thrombus in foetal stem vessel. (HE stain 100x). The arrow indicates areas of infarct in the placental sections.

Fig. 2. Placental tissue showing infarcted villi with coagulative necrosis. (Avascular villi) (HE stain 400x). The arrow indicates areas of infarct in the placental sections.

Fig. 3. Placental tissue showing many areas of calcification. (HE stain 400x). The arrow indicates areas of infarct in the placental sections.

Fig. 4. Normal third trimester villi with syncitial knots and dystrophic calcification. (HE stain 400x). The arrow indicates areas of infarct in the placental sections.

Fig. 5. Organized thrombus in foetal stem vessel. (HE stain 100x). The arrow indicates areas of infarct in the placental sections.

Discussion

The reports of histomorphological changes in placenta in women with recurrent pregnancy loss in association with thrombophilia are relatively recent\textsuperscript{14-30} and all of them broadly show non vascularisation of villi, multiple vessel thrombosis, infarction, fibrosis and decidual vasculopathy. Our findings are in line with these findings. The studies show that 60-73 per cent of the abnormal histopathological placentas were positive for thrombophilia.

The primary pathology observed in the present study was thrombosis. Some of the other pathological features observed were placental infarction, decidual vessel thrombosis, chronic villitis and excessive perivillous fibrin deposition. Multifocal utero-placental thrombosis was also a significant feature in patients with thrombophilia.

Many physiological changes occur in the uterine wall during pregnancy in order to allow the foetus to grow. These include dilation and enormous growth of the spiral arteries, destruction of the spiral artery endothelium and decidual invasion by trophoblast\textsuperscript{31}. Only if an adequate blood supply is provided by the
maternal blood vessels, normal placental growth and development will continue. In case of thrombophilia related foetal loss, where there is a compromised blood supply, it will not be surprising that the placenta exhibits significant pathological changes.

The contribution of the foetal genotype in determining pregnancy outcome demands further investigation. The placenta receives two arterial supplies (one maternal and one foetal). Placental thrombosis occurs if any one of the vascular supplies is compromised. It has been reported that placental infarction was significantly more when foetus carried the FVL allele compared with normal factor V genotype. Abruption, fibrosis and hyper-vascularity of villi were the common histological features observed in patients. Of the eight placentas analysed histologically in the present study, in six cases maternal thrombophilia could be attributed to foetal loss. Of these six, two were positive for APA while four were positive for inherited thrombophilia.

One woman who had focal haemorrhage and vessels showing recent thrombosis was negative for all the thrombophilic markers studied and another who showed subchorionic haemorrhage was negative for all the thrombophilic markers studied. Thus, there could be more thrombophilia markers, yet to be identified, or more markers are added to the testing panel, we may get an answer for the cause of recurrent foetal loss.

Histology of placenta from miscarriages gives a fair idea as to the cause of pregnancy failure, though the precise pathogenesis of the thrombotic diathesis associated with thrombophilia still remains unknown. Analyzing previous placentas could have been helpful as the same pathological process is repeated in subsequent pregnancies, making it possible to decide a therapeutic regime for individual patients. It is well known that the thrombosis is due to a complex interaction between the inherited, acquired and circumstantial risk factors. The thrombosis was objectively confirmed in majority of the placentas tested and who were also positive for thrombophilia. Except APA we have not analysed other acquired risk factors for thrombosis. A comprehensive analysis of all these risk factors will probably provide answers to all women with unexplained foetal loss.

References


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