Original article

THROMBOPHILIA AND DAMAGE OF KIDNEY DURING PREGNANCY

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Summary

Objectives: It's known that heritable thrombophilias are a risk factor for the development of obstetrics complications associated to inadequate uterine-placental circulation, as pre-eclampsia/eclampsia, HELLP syndrome, placental abruption and intrauterine growth restriction (IUGR), however it was never investigated the role that they could have in the renal failure associated to such conditions. The purpose of this study is to evaluate if thrombophilia itself that predispose to a possible renal damage or if its occurrence determines a more severe involvement of the kidneys in the course of these obstetric pathologies.
Methods: In the study were enrolled 301 pregnant women, who carried a thrombophilic state, 125 of whom (B group) has had an obstetric complication. In all the women the renal function was assessed taking into consideration proteinuria, creatininaemia and hypalbuminaemia.
Results: Of the three parameters which have been considered as evidence of a severe renal involvement the hypalbuminaemia appears statistically significant compared to the controls. Even creatinaemia is significantly increased in pregnant women with an Anthithrombin deficiency, and increased levels are detected in women with Factor V Leiden.
Conclusions: In obstetric complications associated to thrombophilic state could be a more severe involvement of the kidney.

Key Words: obstetric complications; renal damage in pregnancy; heritable thrombophilias.

Introduction

Women who develop preeclampsia have a higher risk of thromboembolism in later life since there is a correlation between pre-eclampsia and thrombophilia (1,2). An incidence of thrombophilia has been reported in up to 40% of women who have suffered with pre-eclampsia during pregnancy (3). Additionally, thrombophilias are also associated with the development of hypertension and one meta analysis reported an odds ratio (OR) of 2.3 for the development of hypertension in women with Factor V Leiden (4). In addition, intrauterine growth restriction (IUGR) is associated with thrombophilia (5,6), however, this has not been consistently demonstrated in all studies with two extensive prospective studies demonstrating a similar incidence of IUGR in pregnancies from women with or without Factor V Leiden (7). Notwithstanding the association between thrombophilia and IUGR is supported by the observation that fetuses born to women with a history of venous thrombosis have a birthweight which is significantly lower compared at the control population (8). More recently, Pabinger concluded that heritable thrombophilias are a risk factor for the development of obstetric complications as pre-eclampsia, HELLP syndrome, placental abruption and IUGR (9,10). The hypercoagulable state associated with thrombophilias may lead to inadequate uterine-placental circulation: low-pressure intervillous blood flow and subsequent fibrin deposition within the placenta and thus higher rates of infarction (11). Indeed, in early pregnancy, a thrombophilic state may lead to abnormal trophoblastic invasion or defective placental neoangiogenesis can occur. Obstetric complications later in pregnancy are caused by a number of factors of which thrombophilias remain an important risk factor (12-14).
Inherited thrombophilias may include antithrombin III deficiency, mutations in Factor V Leiden methylene tetrahydrofolate reductase (MTHFR) and the prothrombin

gene. Protein C and S are also important causes of thrombophilia and they have an important role in anti coagulation whereby they inactivate factors Va and VIIIa (Table 1) (15).
The aim of our study was to understand the impact of inherited thrombophilias on the development of renal dysfunction. Since the renal dysfunction occurs in hypertensive pathologies including pre-eclampsia, the purpose of this study was to ascertain whether the thrombophilic state could independently result in renal damage during pregnancy.

Table 1. Prevalence rates for congenital thrombophilia in a European population.

Thrombophilic defect	**Prevalence (%)**
Factor V Leiden heterozygous	2-7
Prothrombin G20210A heterozygous	2
Antithrombin defect	0.25-0.55
Proteina C deficiency	0.20-0.33
Proteina S deficiency	0.03-0.13
MTHFR C677T homozygous	10

Material and Methods

From September 2004 until May 2008, women with inherited thrombophilias and who were pregnant were enrolled in this study, from the Department of Obstetrics and Gynecology.
Patients were divided into two groups: A (control women), and B, [women who presented one of the above obstetric pathologies correlated to inadequate women with feto-placental abnormalities such as pre-eclampsia, Haemolysis-Elevated liver enzymes-Low Platelets (HELLP) syndrome, gestational hypertension, IUGR, intrauterine death, abruptio placentae, CID and preterm delivery].
The criteria for the inclusion of patients in B group were:
- HELLP syndrome: defined as severe pre-eclampsia complicated by hemolysis (serum lactate dehydrogenase >600 IU/L, serum bilirubin >1.2 mg/dL); increased serum aspartate aminotransferase concentrations (70 IU/L); and thrombocytopenia (platelet count <100 000/mm3) (11);
- Fetal growth restriction (IUGR): defined as a weight at birth < 10° percentile (11);
- Abruptio placentae: was diagnosed clinically when vaginal bleeding and uterine tenderness was present, and confirmed by placental examination;
- Intrauterine death: defined as the stillbirth of a fetus after the 24th week of pregnancy (11);
- Gestational hypertension: increased systolic blood pressure >30 mmHg and/or diastolic pressure >15 mmHg compared to the average values taken before the 20th week or the presence of values equal or above 140/90 mmHg taken at least 4 hours away one from the other (16);
- Preterm delivery: defined as the birth before the 37th week of gestation;
- Disseminated intravascular coagulation (CID): defined on Levi M et all criteria (17).
The criteria for the diagnosis of preeclampsia follows the recommendations of the *Consensus Report of American Working Group on High Blood Pressure in Pregnancy* and of German Society of Gynecology and Obstetrics (16):
- Increased systolic arterial pressure >30 mmHg and/or diastolic pressure >15 mmHg compared to the average values taken before the 20th week or the presence of values equal or above 140/90 mmHg taken at least 4 hours away one from the other;
- Proteinuria, defined as the presence of at least 0,3g of protein in urine of the 24 hours or 30 mg/dl with a stick (a + in commercial kits) using random samples of the urine, 4 hours one from the other;
- Onset of symptoms after the 20th week of gestation, and regression of the symptom within 6 weeks from the deliver.
Women excluded from the study included those with a fetus affected by congenital anomalies, or those women with chronic hypertension, diabetes mellitus and renal dysfunction prior to pregnancy.
All women in the study provided peripheral blood samples. Women with more than one thrombophilia were excluded from the study. All patients with the MTHFR mutation (both homozygotes and heterozygotes) or with homocystinaemia (higher than 12 mol/l) were advised to take folate supplementation daily until delivery (18), in agreement with the internal protocol of our department.
The patients enrolled in the study provided written informed consent to participate in the study.

Laboratory analysis for thrombotic factors

To study the mutations such as MTHFR (A1298C and C677T), Factor V Leiden, PAI-1 and the mutation of G20210A of prothrombin, a commercial kit was used, following the manufacturer instructions.
The technique used was inverse hybridization: here, DNA was isolated from EDTA blood samples after cellular lysis obtained using GenXtract resine, following kit instructions. DNA was simultaneous amplified using Taq polimerase enzime, the amplification mix undergoing 35 cycles inside the thermocycler (using GeneAmp PCR System 2400 by Perkin Elmer, Milano, Italy). The amplified fragment was then hybridized with different allele-specific biotin conjugated probes. The subsequent streptavidin conjugat facilitated the diagnosis of mutations.
The determination of plasmatic homocysteine was undertaken using a commercial kit (Axis Homocysteine EIA, Axis-Shield Diagnostic Ltd, The Technology Park, and Dundee DD2 1Xa, UK) as per manufacturer's instruction. The normal range of reference is between 5 and 12 mmol/l and the sensitivity is 1 mmol/l. In all patients total Antithrombin III (ELISA; Diagnostica Stago; Asnieres, Francia) and protein S were also measured (19,20).
The altered resistance to C-reactive protein was determined using plasma deprived of Factor V (Coated activated protein C resistance; Chromogenix; Goteborg, Sweden) (21).

Quantitative determination of proteinuria

Urine samples were collected and stored at 4 degrees.

All samples were used within 24 hours. Samples were centrifuged before analysis. The sample was pre-incubated in an alkaline solution containing sodium hydroxide: 530 mmol/l; EDTA-Na: 74 mmol/l. and then Benzethonium chloride as per standard protocol. The reference values for proteinuria were as follows:
Reference values:
- Randomized: <120 mg/l
- In the 24 hours: <150 mg/l

Quantitative determination of albuminuria

Samples were analysed using the immunoturbidometry method which permits the quantitative determination of albumin. Samples were incubated with antibodies anti - albumin (sheep polyclonal antibodies anti human albumin; swab TRIS: 100mmol/l at pH 7.2). The antibodies anti-albumin react with the samples, forming antibodies-antigens complexes, which, after agglutination, are measured turbidimetrically.
The reference values used were:
- Adults second urine of the morning: <20mg albumin/g creatinine.
- Adults urine in the 24 hours: <20 mg/l or <30 mg/24h.
For the test have been used automatic analyzers of clinical chemistry by Roche/Hitachi.

Statistic analysis

For comparison between the study and control groups, the Mann-Whitney non-parametric test and where appropriate Student's t-test was used.

Results

In total, 301 pregnant women who were heterozygotes for an inherited thrombophilia were enrolled in this study. Group A included 176 women with an uneventful pregnancy whilst group B had 125 pregnant women with obstetric complications. The characteristics of the study groups are shown in Table 2. The mean/median gestation at delivery was 39±2 in group A vs 35±4 in group B; (p=0.045) and this difference was statistically significantly. Birthweight was significantly lower (3325g ± 398g vs 2560g ± 256g) from pregnancies in group B and again this result was statistically significant (p=0.034). The numbers and frequencies of obstetric complications are shown in Table 3.
As for the purpose of the study, i.e. the evaluation of renal function in the obstetric pathologies and its possible correlation to the thrombophilic state, proteinuria is constant in control group (all 0), therefore, instead of a compared test between the two groups, we proceed analyzing the distribution rates in the pathological group.
As the main aim of the study was to evaluate renal dysfunction in women with inherited thrombophilias during pregnancy, we found higher levels of proteinuria in group B compared to the control group. In group B, as expected, we outlined extreme rates of the parameter, and the distribution was observed as follows: mean proteinuria 0,72 mg/dl; SD 1,77 mg/dl.
Table 4 shows differences between two groups in terms of albuminaemia, azotemia and creatinine observed as mean, SD and Min-Max value. Only the value of albuminaemia was significantly different when analyzed with non parametric test -Mann-Whitney- (54,88g/L ±3,99 vs 42,49g/L ±12,84; p<0.01).
A comparison of factors demonstrating altered renal function in women with thrombophilic factors in pregnant women with a complicated gravidic outcome, points out that the deficit of ATIII is positively correlated to creatininaemia > 60 mg/dl (p=0.031) and there is a tendential association between Factor V Leiden and creatininaemia >60 mg/dl.

Table 2. Characteristics of study population.

	Group A (n° 176)	***Group B (n° 125)***	***P-values***
Age	33.2 ± 5.1	34.7 ± 4.1	0.124
BMI (Body Mass Index)	24.4 ± 3.1	25.0 ± 3.3	0.235
Gestational age at delivery (Weeks)	39 ± 2	35 ± 4	0.045
Birthweight (g)	3325 ± 398	2560 ± 256	0.034

Table 3. Pregnancy outcome of study population.

OUTCOME	***N.***	***FREQUENCY (%)***
Normal pregnancy	*176*	*58.8*
Intrauterine death	*6*	*1.64*
Gestational hypertension	*26*	*8.22*
Pre-eclampsia	*9*	*2.30*
HELLP Syndrome	*15*	*4.60*
Intrauterine growth restriction (IUGR)	*41*	*12.82*
Placental abruption	*17*	*5.26*
Disseminated intravascular coagulation (CID)	*3*	*0.65*
Preterm delivery	*8*	*2.43*

Table 4. Thrombophilic factors and parameters of the renal function in pregnant women with a complicated gravidic outcome.

		Mean	*Std. Deviation*	*Std. Error*	*Minimum*	*Maximum*
ALBUMINAEMIA* g/L	Group A	54,88	3,99	1,26	50,00	60,60
	Group B	42,49	12,84	2,80	24,00	59,10
(* Mann-Whitney p < 0.01)	**Total**	**46,48**	**12,22**	**2,19**	**24,00**	**60,60**
AZOTEMIA	Group A	25,50	5,58	1,77	17,00	33,00
mg/dL	Group B	26,00	11,33	2,47	13,00	59,00
	Total	**25,84**	**9,74**	**1,75**	**13,00**	**59,00**
CREATININE mg/dL	Group A	0,59	0,16	0,03	0,40	0,90
	Group B	0,69	0,19	0,06	0,50	0,90
	Total	**0,62**	**0,17**	**0,03**	**0,40**	**0,90**

Discussion

Renal dysfunction may follow many obstetric complications such as pre-eclampsia. Interestingly, the presence and relative contribution for thrombophilias in this pathology has never formally been assessed.

Normally, in pregnancy, renal adaptations may occur. A strategy to improve renal perfusion occurs through increased cardiac output which leads to an increased renal flow (of 80%) compared to the pre-pregnancy and as a result glomerular renal filtration (GFR) increases. Reduced GFR leads to lower serum creatinine, azotemia and urea when not pregnant (22). It is well recognized that even in the face of high proteinuria real function as measured by serum creatinine, is within normal parameter (22). Here we demonstrate a rise in serum creatinine (> 60 mg/dl) in women with inherited thrombophilias in the presence of obstetric complications. This rise was more pronounced when associated with ATIII deficiency. This may explain more severe renal involvement in women who have an underlying thrombophilia and develop obstetric pathologies.

Possible prognostic indicators of the severity of renal dysfunction in pre-eclampsia are proteinuria, elevated creatinine and hypoalbuminaemia. One systematic review has examined how the proteinuria can predict maternal and fetal complication and concluded that high levels of proteinuria lead to a negative maternal-fetal prognostic index (particularly there is a significant increase of fetal mortality when proteinuria is >5g/dl) (23). The degree of renal failure however was not addressed in that review (23) but rather it was demonstrated that in pre-eclampsia, the proteinuria is caused by an interstitial inflammatory process, leading to progressive deterioration, making women prone to developing subsequent hypertension or chronic renal disease (24,25). Generally, even when there is a high proteinuria, the levels of renal functionality, particularly creatinine, are still normal (26), in our study it was demonstrated a significant augment of creatinine (> 60 mg/dl) in thrombophilic women in the presence of obstetric pathologies, particularly when associated to a deficit of ATIII, which can be the evidence of a more severe involvement of the kidney in course of obstetric complications, with an underling thrombophilia.

Hypoalbuminaemia is deemed an important parameter of the severity of the pathologies associated to the inadequate feto-placental circulation, as the result of multiorgan hypoperfusion and the diffuse endothelial damage associated to it. Placental hypoxia stimulates the release of vasoactive substances in the blood which have an effect on the heart. The consequent rise in perfusion pressure leads to fluid shifts into the interstitial fluid resulting in edema and favoring hypovolemia. The reduction in intravascular volume further reduces organ perfusion, leads to catecholamine release with concomitant reductions perfusion at renal and hepatic levels. Hepatic hypoperfusion determines the decrease in albumin production with consequent hypoalbuminaemia and fall of oncotic pressure which further facilitates fluid shifts and worsening edema (27).

Hypoalbuminaemia, both alone and in the context of a nephrotic syndrome is a marker of clinical severity in many conditions including pre-eclampsia and it outlines and important renal involvement (28). It has previously been demonstrated that in course of pre-eclampsia hypoalbuminaemia and the development of a nephrotic syndrome may cause more renal damage than the hypertensive disease alone. This suggest that hypoalbuminaemia is a negative prognostic sign and is marker for severe systemic dysfunction.

Our study demonstrated significant hypoalbuminaemia in women affected by thrombophilia and poor obstetric outcome, which could imply in the presence of thrombophilia a more severe involvement of the kidney and a more severe renal damage in the course of the afore mentioned obstetric pathologies. The underlying pathophysiology is the underlying hypercoagulable state, which leads to the release of vasoactive substances with consequent renal and hepatic hypoperfusion, and hypoalbuminaemia.

This data presented although interesting needs a follow up study to investigate and compare adverse obstetric outcomes in non thrombophilic women. In additions, we hope to substantiate whether the high serum creatinine and hypoalbuminaemia can be used as prognostic factors of the severity of renal involvement in pre-eclampsia.

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