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Markers of insulin resistance and sedentary lifestyle are predictors of preeclampsia in women with adverse obstetric results

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Abstract

Some thrombophilias and severe preeclampsia may increase the risk for preterm deliveries and fetal death due to placental insufficiency. Our objective was to evaluate clinical and laboratory data as predictors of preeclampsia in a population of mothers with 3rd trimester fetal losses or preterm deliveries. In a longitudinal retrospective study, 54 consecutive women (age range: 16 to 39 years) with normotensive pregnancies were compared to 79 consecutive women with preeclampsia (age range: 16 to 43 years). Weight accrual rate (WAR) was arbitrarily defined as weight gain from age 18 years to the beginning of pregnancy divided by elapsed years. Independent predictors of preeclampsia were past history of oligomenorrhea, WAR >0.8 kg/year, pre-pregnancy or 1st trimester triglyceridemia >150 mg/dL, and elevated acanthosis nigricans in the neck. In a multivariate logistic regression model, two or more predictors conferred an odds ratio of 15 (95%CI [5.9-37]; $P < 0.001$) to develop preeclampsia (85% specificity, 73% sensitivity, c-statistic of 81 ± 4%; $P < 0.0001$). Clinical markers related to insulin resistance and sedentary lifestyle are strong independent predictors of preeclampsia in mothers with 3rd trimester fetal losses or preterm deliveries due to placental insufficiency. Women at risk for preeclampsia in this particular population might benefit from measures focused on overcoming insulin resistance.

Key words: Preeclampsia; Acanthosis nigricans; Weight accrual rate; Triglycerides; Oligoamenorrhea

Introduction

Placentas from thrombophilic women whose normotensive pregnancies are complicated by placental insufficiency share histopathological findings with pregnancies complicated by preeclampsia, such as infarcts and thrombosis (1). This pro-thrombotic metabolic profile in preeclampsia has been commonly associated with a sedentary lifestyle and pre-pregnancy complaints of irregular menses (2). Furthermore, mothers with preeclampsia may remain hypertensive after delivery, with a potential increase in lifelong risk for adverse cardiovascular outcomes (3). The aim of the present study was to analyze clinical and laboratory data regarding pregnancies that ended with fetal losses or preterm deliveries related to placental insufficiency as predictors of preeclampsia.

Patients and Methods

Study design

From January 2008 to December 2010, a cohort of 133 consecutive women referred to Unidade Materno-Fetal de Alto Risco, Hospital Federal dos Servidores do Estado, Rio de Janeiro, with either a preterm delivery or a 3rd trimester fetal loss as a consequence of placental insufficiency were enrolled in a longitudinal, retrospective and observational study. Subjects comprised 54 normotensive mothers with a diagnosis of thrombophilia or with a systemic thrombotic event before pregnancy or during the puerperium (NM, 175 pregnancies, 179 offspring) and 79 mothers with preeclampsia (PM, 238 pregnancies, 243 offspring). The study protocol was approved by the Ethics Committee of Hospital Federal dos Servidores do Estado (registration #000377) on September 14, 2009.

The NM group was selected on the basis of all of the following criteria: i) women with normotensive pregnancies; ii) death of a morphologically normal fetus beyond 28 weeks of gestation or a premature delivery; iii) extensive placental thrombosis; iv) thrombophilia or any documented episode

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of systemic thrombosis before pregnancy. Thrombophilia screening included factor V Leiden, prothrombin G20210A polymorphism, antithrombin III, functional protein C, free protein S assessed far from pregnancy, puerperium or infectious episodes, lupus anticoagulant, and anticardiolipin antibodies IgM and IgG. Whenever positive, antiphospholipid antibodies were repeated after a 12-week interval. Anticardiolipin antibody cut-off was ≥40 GPL or MPL. Homozygosis or double heterozygosis of methylene tetrahydrofolate reductase was not considered to be relevant because folate supplementation is part of obstetrical routine.

The PM group was selected on the basis of the presence of all of the following criteria: i) women with severe preeclampsia (see definition below); ii) death of a morphologically normal fetus beyond 28 weeks of gestation or a premature delivery.

Exclusion criteria for NM and PM were any of the following: i) diabetes; ii) untreated anatomical causes responsible for either fetal loss or preterm delivery; iii) illicit drug addiction; iv) medications that could alter metabolic parameters or induce fibrinolysis such as metformin or full-dose heparin use during pregnancy. Cut-off at 28 gestational weeks was arbitrarily defined to exclude patients with notches in uterine artery Doppler scan in the NM group.

Maternal information was retrieved from patient interview and review of medical records. Data were obtained from last pregnancy that ended at ≥28 weeks of gestation either with preterm delivery or fetal death as a consequence of placental insufficiency.

Placental insufficiency was characterized by the presence of either an excess placental thrombosis/infarction at postpartum histopathological assessment or intrauterine growth restriction combined to oligohydramnios (fetal urinary tract abnormalities were excluded).

Definitions

Some pregnant women are young and, as a consequence, sedentary lifestyle might not yet have been necessarily translated into increased body mass index (BMI). Furthermore, we know from experience that women who gain weight over time tend to be more sedentary. Thus, we have created an index to express this weight accrual rate (WAR) as an alternative. WAR is defined as weight at the beginning of pregnancy minus weight at the age of 18, divided by the number of elapsed years. For example, a 23-year-old mother weighing 60 kg at the beginning of pregnancy and 50 kg when she was 18, gained 10 kg in 5 years and her WAR is 10:5 = 2.

Preeclampsia was considered to be severe if one or more of the following criteria were present: i) sustained systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg after the 20th week of gestation and proteinuria ≥300 mg/24 h; ii) sustained systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg after the 20th week of gestation and proteinuria ≥5 g/24 h; iii) sustained systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg after the 20th week of gestation, proteinuria ≥300 mg/24 h and multisystemic involvement, such as: pulmonary edema, oliguria, abnormal liver enzymes with persistent epigastric or right upper-quadrant pain, platelet count <100,000/mm^3, or persistent and severe central nervous system symptoms, such as seizures, altered mental status, headaches, blurred vision, or blindness. Eclampsia was defined as preeclampsia with seizures and HELLP syndrome as microangiopathic hemolysis, elevated liver enzymes and platelet count <100,000/mm^3 (4).

Statistical analysis

Continuous variables are reported as means ± SD and discrete variables as ratio or percent, and were compared by the two-tailed Student *t*-test or chi-square (Fisher exact) test in univariate analysis, as appropriate. Continuous variables had their optimal cut-off values calculated by receiver operating characteristic (ROC) curve analysis. Significant variables in univariate analysis were entered into a stepwise multivariate logistic regression model to identify independent predictors of preeclampsia. In the model, covariant variables were identified by regression analysis and were retained according to the Wald test significance. Significant variables in the logistic model were equally weighed to 1 and were entered into a predictive model, which accounted for the sum of the weight of each variable. A bootstrap re-sampling procedure using multivariate logistic regression modeling was employed to validate the present score. The alpha error level was set to 0.05.

Results

Placental insufficiency was confirmed by histopathology for 120 mothers, whereas the remaining 13 mothers had fetal demise associated with intrauterine growth restriction and oligohydramnios, without ultrasound evidence of fetal urinary tract malformation. Mean maternal age did not differ between groups: 29 ± 5 years for NM (range: 16-39 years), and 31 ± 7 years for PM (range: 16-43 years) (P = 0.3). Three NM and 1 PM were smokers (≥1 pack/day) and smoked throughout pregnancy (P = 0.64). Thrombotic tendency was higher in the NM group than in the PM group. While 25 NM had deep venous thrombosis (DVT) pre-pregnancy or during the puerperium and 1 had a stroke (48%), 13 PM had a DVT pre-pregnancy or during the puerperium, 7 had a stroke and 1 had both a DVT and a stroke pre-pregnancy (27%; P = 0.02). The prevalence of antiphospholipid syndrome was similar in NM and PM (9 and 8%, respectively; P = 0.98). In NM, 9 had protein S deficiency, 6 had protein C deficiency, 5 had antithrombin III deficiency, 2 were heterozygous for factor V Leiden, 2 were heterozygous for factor II G20210A, and 1 had essential thrombocythemia.

Preeclampsia occurred 126 times, complicated by eclampsia in 9 and by HELLP syndrome in 10, complete or

not: 45 of 79 PM (57%) had had only one episode of preeclampsia, and 53% of them were nulliparous; 34 of 79 PM (43%) had had recurrent preeclampsia or eclampsia, 85% of them with the same partner, 9% with a different partner, 6% with either a different or the same partner. In the PM group, 63 of 79 women (80%) were resting and avoiding any kind of exercise when preeclampsia was eventually detected. Table 1 summarizes the obstetric outcomes.

PM women showed a higher prevalence of clinical and laboratory markers related to insulin resistance than NM, such as oligomenorrhea (60 and 27%, respectively; P < 0.001), higher BMI at the beginning of pregnancy (28 ± 7 and 24 ± 4 kg/m^2, respectively; P < 0.001), higher WAR (1.6 ± 1.4 and 0.6 ± 0.8 kg/year, respectively; P < 0.001), higher pre-pregnancy or 1st trimester triglyceridemia (131 ± 56 and 109 ± 49 mg/dL, respectively; P = 0.02), and elevated acanthosis nigricans in the neck (62 and 12%, respectively; P < 0.001). No difference was found in pre-pregnancy or 1st trimester plasma LDL or HDL levels between NM (103 ± 33 and 60 ± 13 mg/dL) and PM (97 ± 32 and 60 ± 18 mg/dL, respectively; P = 0.31 and P = 0.99). At least one episode of acute bacterial, viral or fungal infection during the month that preceded 3rd trimester fetal demise or premature delivery was observed in 17 NM (17/77, 22%) and in 32 PM (32/128, 25%; P = 0.89). While hypertension was not a characteristic in the NM group, in PM, 11 of 79 were hypertensive prior to the pregnancy under study (0 and 14%, respectively; P = 0.003). After a pregnancy complicated by severe preeclampsia, 28 of the 79 PM women remained hypertensive at least 3 months after delivery, as opposed to none in the NM group (35 and 0%, respectively; P < 0.001). Essential hypertension and type 2 diabetes were more frequent in parents of PM (46 and 16%) than in parents of NM (28 and 5%, respectively; P = 0.002 and P = 0.001). Table 2 summarizes the maternal characteristics.

In univariate analysis, ROC curve analysis of WAR showed an optimal cut-off value at >0.8 kg/year, with 70% sensitivity and 74% specificity (OR = 7; 95%CI [3-15]; P < 0.001; c-statistic = 0.74 ± 0.05; P < 0.001).

We carried out a stepwise logistic regression analysis to identify independent predictive variables for preeclampsia in this population of mothers with pregnancies that ended with fetal losses or preterm deliveries related to placental insufficiency. The model was appropriately adjusted to regression data (model significance test: P < 0.001; residual significance test: P = 0.9). Independent variables for preeclampsia were: i) oligomenorrhea (Wald test: χ^2 = 6.7; P = 0.006); ii) WAR >0.8 kg/year (Wald test: χ^2 = 15; P = 0.001); iii) pre-pregnancy or 1st trimester triglyceridemia

Table 1. Obstetric outcomes of the patients studied.

Variables	NM (N = 53)	PM (N = 79)
Offspring	176 (100%)	243 (100%)
Spontaneous embryonic/fetal death, 1st trimester	37 (21%)	42 (17%)
Spontaneous fetal death, 2nd trimester	35 (20%)	42 (17%)
Spontaneous fetal death, 3rd trimester	27 (15%)	36 (15%)
Preterm deliveries	50 (29%)	92 (38%)*
Full-term deliveries	27 (15%)	31 (13%)

Data are reported as number with percent in parentheses. NM = normotensive mothers; PM = mothers with preeclampsia. *P < 0.05 compared to NM (chi-square or Fisher exact tests when appropriate).

Table 2. Maternal characteristics.

Variables	NM (N = 53)	PM (N = 79)
Irregular menses or amenorrhea	27%	60%*
Managed to get pregnant before one year	71%	48%*
Body mass index (kg/m^2)	24 ± 4	28 ± 7$^+$
Weight accrual rate (kg/year)	0.6 ± 0.8	1.6 ± 1.4$^+$
Afro-descendants	42%	44%
Elevated acanthosis nigricans in the neck	12%	62%$^+$
Triglycerides, 1st trimester or pre-pregnancy (mg/dL)	109 ± 49	131 ± 56$^+$
Infection in the month that preceded 3rd trimester fetal death or preterm delivery	22%	25%
Essential hypertension in the maternal parents	28%	46%*
Type 2 diabetes in the maternal parents	5%	16%*
Antiphospholipid syndrome	9%	8%

NM = normotensive mothers; PM = mothers with preeclampsia; weight accrual rate = weight at the beginning of pregnancy minus weight at the age of 18 divided by the number of elapsed years. *P < 0.05 compared to NM (chi-square or Fisher exact tests when appropriate); $^+$P < 0.05 compared to NM (two-tailed Student *t*-test).

>150 mg/dL (Wald test: χ^2 = 4.8; P = 0.04); iv) elevated acanthosis nigricans in the neck (Wald test: χ^2 = 5.7; P = 0.001).

Each significant variable was arbitrarily assigned a unit weight. A score was built by summing up individual weights, ranging from 0 to 4. The optimal cut-off value of preeclampsia score, assessed by the ROC curve, was ≥2, with 73% sensitivity and 85% specificity (c-statistic = 0.81 ± 0.04; P < 0.001), OR = 15 (95%CI [5.9-37]; P < 0.001; Figure 1).

We carried out 1000 bootstrap uniform re-sampling procedures to validate the final logistic model. The average model coefficient for each independent predictor was oligomenorrhea = 2.7 ± 1.5 (P < 0.001); WAR >0.8 kg/year = 1.7 ± 1.6 (P < 0.001); pre-pregnancy or 1st trimester triglyceridemia >150 mg/dL = 1.8 ± 1.9 (P < 0.001), and elevated acanthosis nigricans in the neck = 2.0 ± 1.8 (P < 0.001). In bootstrap frequency distribution analysis of the final logistic model, oligomenorrhea, WAR >0.8 kg/year, pre-pregnancy or 1st trimester triglyceridemia >150 mg/dL and elevated acanthosis nigricans in the neck independently predicted preeclampsia in 99.4, 99.9, 99.4, and 99.2% of cases, respectively.

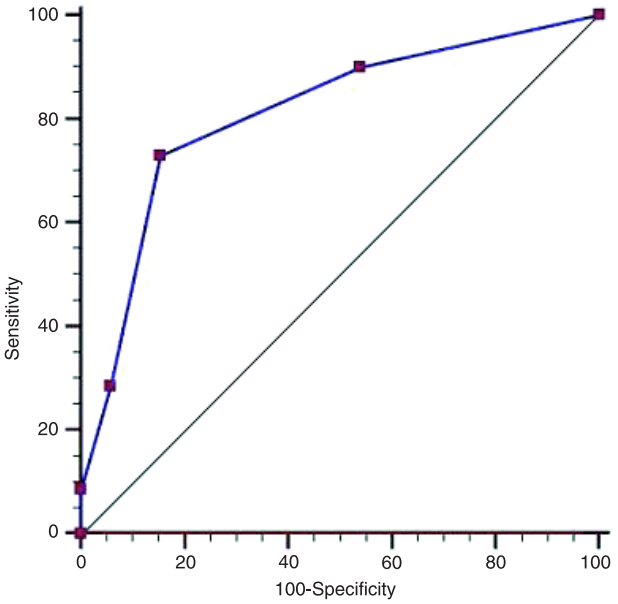

Figure 1. Receiver operating characteristics (ROC) curve analysis of preeclampsia score as a predictor of preeclampsia. The optimal cut-off value of the score was ≥2, yielding 73% sensitivity and 85% specificity (c-statistic = 0.81 ± 0.04; P < 0.001). Hazard ratio = 15 (95%CI [5.9-37]; P < 0.001).

Discussion

In this retrospective observational study, we analyzed clinical, anthropometric and metabolic markers in a population of women with fetal losses and preterm delivery due to placental insufficiency divided into two groups in order to assess independent predictors for preeclampsia. Oligomenorrhea, WAR >0.8 kg/year (from 18 years of age to the beginning of pregnancy), pre-pregnancy or 1st trimester triglyceridemia >150 mg/dL and elevated acanthosis nigricans in the neck were independent predictors of preeclampsia. The presence of at least two of four makers showed 81% total predictive accuracy.

In another cohort study of 295 thrombophilic patients regularly assisted by one of the authors at Hospital Federal dos Servidores do Estado, Rio de Janeiro, it was found that the prevalence of both factor V Leiden and prothrombin G20210A polymorphisms was less than 5% (Hoirisch-Clapauch S, unpublished data). In the present study, the prevalence of factor V Leiden and prothrombin G20210A polymorphism in NM was similar to this previous observation. In PM, thrombophilia screening was limited to anticardiolipin and lupus anticoagulant antibodies.

Lack of exercise favors weight accumulation and impairment of glucose metabolism. A sedentary lifestyle by itself favors insulin resistance (5). Elevated acanthosis nigricans in the neck and hypertriglyceridemia are clinical manifestations related to insulin resistance (6). Furthermore, basic and clinical evidence has linked insulin resistance to impaired fibrinolysis through increased plasminogen activator inhibitor-I (PAI-I) production (7,8). During normal pregnancy, elastic fibers in the walls of the placental spiral arteries are progressively destroyed by matrix metalloproteinases in order to provide a low resistance placental circulation that ensures appropriate fetal growth. Since plasmin is required to activate metalloproteinases (9,10), PAI-I-related hypofibrinolysis prevents elastin destruction.

Physiological insulin resistance of pregnancy may be intensified by lack of exercise, stimulating pancreatic β-cells to increase insulin and proinsulin production (11). Proinsulin stimulates PAI-I production in both hepatocytes and stromal cells of adipose tissue (12). Therefore, one could expect that a sedentary lifestyle would favor preeclampsia through a hypofibrinolysis-related mechanism that increases the resistance of placental vessels. It is noteworthy that 80% of PM women in this population were resting and avoiding any kind of exercise when preeclampsia occurred.

A higher recurrence rate of severe preeclampsia than previously reported was observed in the present study (13). The reason for this is not apparent. It was our observation that, after a preeclampsia-related fetal demise, patients were often advised to rest as much as possible, a condition that was associated with 80% of preeclampsia recurrence versus 42% when the mother was not resting in bed (OR = 5.5; 95%CI [1-30]; P = 0.04). Therefore, we speculate that bed rest might have contributed to the recurrence of preeclampsia. Although overall neonatal weight was similar in the NM and PM groups (1533 ± 735 and 1564 ± 742 g, respectively; P = 0.8), intrauterine growth restriction was

more frequent in NM (42%) than PM (34%; P < 0.01).

Irregular menses or amenorrhea may also be a result of hypofibrinolysis particularly related to sedentary lifestyle (2). Metalloproteinases mediate tissue remodeling in the ovary during follicular development, ovulation, formation and regression of corpus luteum, and follicular atresia (14). As these metalloproteinases are activated by plasmin, hypofibrinolysis prevents adequate remodeling. In addition, it has been shown that ovulation damages the ovarian surface and a blood clot is formed. If one of the components of the blood clot (the fibrin net) is not destroyed by fibrinolysis it will contribute to the abnormal anatomy of the ovary. The resulting polycystic ovaries prevent ovulation and are related to oligoamenorrhea (15). Oligomenorrhea was quite common in PM, and it is interesting to note that exercises can cure polycystic ovaries (16). It should be stressed that 48% of PM compared to 71% of NM (women P = 0.01) managed to get pregnant before 1 year of having sexual intercourse on a regular basis without contraception (Table 2).

Preeclampsia has been shown to have a seasonal pattern worldwide. In countries with marked seasonal variations, either extremely cold winters or unbearably hot summers, pregnant women are less likely to walk around (17-20). The hypothesis that exercise could prevent preeclampsia has been confirmed in animal models (21).

Some limitations of the present study should be addressed. In this retrospective longitudinal study, we focused on investigating preeclampsia predictors in a population of mothers with fetal losses and preterm delivery due to placental insufficiency. Therefore, extrapolation of the present data to a general population awaits confirmation. At Hospital Federal dos Servidores do Estado, Rio de Janeiro, plasma insulin and C-peptide assessments are not part of obstetric routine and, thus, homeostatic model assessment and other indexes were not readily available to characterize insulin resistance. As an alternative, we employed available laboratory data on serum triglyceride as a surrogate marker of insulin resistance. The newly created variable defined as WAR intended to provide a measurement of weight gain rate from 18 years of age up to the beginning of the current pregnancy. According to the rationale of this variable, it was postulated that the shorter the time spent in activities requiring energy expenditure the greater the weight gain. Thus, the WAR variable was employed as an alternative index linked to sedentary lifestyle before pregnancy. Although WAR was validated by bootstrap as an independent predictor of preeclampsia in this population, the present postulate needs to be confirmed.

Oligomenorrhea, WAR >0.8 kg/year, pre-pregnancy or 1st trimester triglyceridemia >150 mg/dL, and elevated acanthosis nigricans in the neck are independent predictors of preeclampsia in a population of mothers with fetal losses and preterm delivery due to placental insufficiency. Insulin resistance mechanisms seem to play a key role in the development of preeclampsia.

We suggest that the current preeclampsia index might identify women at risk for preeclampsia who might benefit from exercises and other therapeutic approaches particularly directed at overcoming insulin resistance.

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