PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA E CIÊNCIAS DA SAÚDE
ÁREA DE CONCENTRAÇÃO: NEFROLOGIA

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ASSOCIAÇÃO ENTRE DILATAÇÃO MEDIADA POR FLUXO DA ARTÉRIA BRAQUIAL E MORBIDADE POR PRÉ-ECLÂMPSIA

Porto Alegre
2013

Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS

Programa de Pós-Graduação em Medicina e Ciências da saúde

Área de Concentração em Nefrologia

DISSERTAÇÃO DE MESTRADO

Associação entre Dilatação Mediada por Fluxo da Artéria Braquial e Morbidade por Pré-Eclâmpsia

MATIAS COSTA VIEIRA

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Medicina e Ciências da Saúde da Pontifícia Universidade Católica do Rio Grande do Sul para obtenção do título de Mestre em Medicina e Ciências da Saúde.

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Porto Alegre, RS, Brasil

Janeiro de 2013

DADOS DE CATALOGAÇÃO

V657a Vieira, Matias Costa

Associação entre dilatação mediada por fluxo da artéria braquial e morbidade por pré-eclâmpsia / Matias Costa Vieira. Porto Alegre: PUCRS, 2013.

59 f.: tab.; graf. Inclui artigo científico encaminhado para publicação no periódico International Journal of Cardiology.

Orientadora: Dra. Bartira Ercília Pinheiro da Costa.

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Dissertação (Mestrado) – Pontifícia Universidade Católica do Rio Grande do Sul. Faculdade de Medicina. Pós-Graduação em Medicina e Ciências da Saúde. Área de concentração: Nefrologia.

1. HIPERTENSÃO INDUZIDA PELA GRAVIDEZ. 2. ULTRASSONOGRAFIA DOPPLER. 3. TOXEMIA. 4. GESTAÇÃO. 5. FATORES DE RISCO. 6. ESTUDO DE COORTE. I. Costa, Bartira Ercília Pinheiro da. II. Figueiredo, Carlos Eduardo Poli de. III. Título.

CDD 618.3
CDU 618.3(043.3)
NLM WQ 244

Isabel Merlo Crespo
Bibliotecária CRB 10/1201

FONTES FINANCIADORAS

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES

Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS)

Este trabalho foi desenvolvido na Pontifícia Universidade Católica do Rio Grande do Sul, no Laboratório de Nefrologia do Instituto de Pesquisas Biomédicas e no Ambulatório de Hipertensão na Gestação do Serviço de Obstetrícia do Hospital São Lucas da PUCRS.

DEDICATÓRIA

Ao meu pai, por ser o porto seguro onde as vezes me ancoro;

À minha mãe, pela difícil tarefa que foi concluída com êxito, a minha educação;

À minha irmã Milene Vieira, por ter me ensinado o significado do companheirismo simplesmente com seus gestos;

À Cecília Korb, por alegrar meus dias e me acompanhar nas mais diversas aventuras;

À Patrícia Lago, por me mostrar que não se deve ter medo de encarar a vida;

E aos meus cunhados, Rodrigo Jucá e Daniel Korb, por cuidarem das duas coisas mais preciosas da minha vida, nos momentos de minha ausência.

AGRADECIMENTOS

Às pessoas que acreditaram que eu era capaz e investiram em mim seu tempo ou suas expectativas. Em lembrança a todas, enumero algumas:

Dr Breno José Acauan Filho, Chefe do Serviço de Obstetrícia, responsável por me fazer o convite de trabalhar no Hospital São Lucas;

Dr João Alfredo Píffero Steibel, por ser o colega que eu mais admiro em função de ser justo e de sempre se preocupar com o próximo;

Dr Edson Vieira da Cunha Filho e o Dr Gustavo Steibel, por me mostrarem o caminho que, junto com eles, hoje vou trilhando;

Dra Bartira Ercília Pinheiro da Costa e Dr Carlos Eduardo Poli-de-Figueiredo, meus orientadores, fontes do conhecimento que sempre incentivam os alunos e também os acalmam nos momentos de incertezas.

SUMÁRIO

RESUMO

Objetivos: avaliar a associação entre a Dilatação Mediada por Fluxo (DMF) da artéria braquial e morbidade por pré-eclâmpsia.

Métodos: Foram selecionados sessenta e quatro grávidas com pré-eclâmpsia. A DMF e marcadores de pré-eclâmpsia foram avaliados no no momento do diagnóstico da doença a as pacientes foram seguidas até o parto. As mulheres foram agrupados e comparadas de acordo com os seus desfechos (26 com complicações e 38 sem complicação).

Resultados: A DMF está comprometida em mulheres com pré-eclâmpsia complicada (7,44%; IQR 2,20-13,34%) comparada com aqueles sem complicações (11,80%; IQR 5,36-16,66%) (p = 0,03). O valor de corte de DMF ≤ 4,5% foi associada com aproximadamente quatro vezes mais risco de qualquer complicação (OR 3,79 IC95% 1,23-11,70), semelhante à relação proteína/creatinina > 2,0 (OR 4,50 IC95% 1,21-16,74). Pressão arterial sistólica e diastólica não foram associados com risco de complicação e o ácido úrico teve uma significância limítrofe (OR 3,38, IC95% 0,98-11,72). Além disso, quando as principais complicações (eclâmpsia, síndrome HELLP ou morte fetal) foram selecionadas como um desfecho composto a DMF foi ainda mais baixa (2,84%; IQR 0,00-7,22%) e o valor da DMF ≤ 4,5% foi associado com um acentuado aumento de 15 vezes no risco destes eventos específico (OR 15,55; IC95% 3,55-68,16). Embora a DMF tenha pouca capacidade de predição de quaisquer complicações pela pré-eclâmpsia (AUC = 0,66, IC95% 0,52-0,79), análise da curva ROC mostrou que pode ser um bom marcador de prognóstico para complicações graves (AUC = 0,84, IC95% 0,73-0,96) .

Conclusão: a DMF está associada com morbidade da pré-eclâmpsia, notadamente em mulheres com eclâmpsia, síndrome HELLP ou morte fetal. DMF no momento do diagnóstico da pré-eclâmpsia pode ser usado como marcador prognóstico destes desfechos desfavoráveis.

ABSTRACT

Objectives: to evaluate the association between brachial artery Flow Mediated Dilatation (FMD) and preeclampsia morbidity.

Methods: Sixty-four pregnant women at the diagnosis of preeclampsia were selected. FMD and routine preeclampsia markers were assessed at enrollment and followed until delivery. Women were grouped and compared according to their outcomes (26 developed complications and 38 did not).

Results: Median FMD is impaired in women with complicated preeclampsia (7.44%; IQR 2.20-13.34%) compared to those without complications (11.80%; IQR 5.36-16.66%) (p=0,03). The cutoff value of FMD ≤4.5% was associated with approximately four-fold odds increment of any complication (OR 3.79; IC95% 1.23-11.70), similar to the protein to creatinine ratio >2,0 (OR 4.50; IC95% 1.21-16.74). Systolic and diastolic blood pressure were not associated with risk for complication and uric acid had a borderline significance (OR 3.38; IC95% 0,98-11,72). Moreover, when major complications (eclampsia, HELLP syndrome or stillbirth) were selected as a composite outcome FMD was even lower (2.84%; IQR 0.00–7.22%) and FMD ≤4.5% was associated with a marked 15 fold increased risk for these specific events (OR 15.55; IC95% 3.55-68.16). Although FMD seems to have a weak accuracy to predict any preeclampsia complications (AUC=0.66; IC95% 0.52-0.79), ROC curve analysis showed that it may be a prognostic marker for major complications (AUC=0.84; IC95% 0.73-0.96).

Conclusion: FMD is associated with morbidity of preeclampsia, markedly in women with eclampsia, HELLP syndrome or stillbirth. FMD at preeclampsia diagnostic moment may be used as a prognostic marker of these poor outcomes.

INTRODUÇÃO

Durante a gravidez ocorrem ajustes hemodinâmicos e vasculares significativos, que podem ser representados pela diminuição da resistência vascular periférica, diminuição da pressão sanguínea, aumento do débito cardíaco e subsequente aumento da circulação sistêmica e pulmonar(1-4). Observa-se também, um aumento da atividade vasorrelaxante derivada do endotélio(5). Assim, do ponto de vista hemodinâmico, é fundamental que o endotélio apresente-se funcionante para que uma gravidez desenvolva-se e adapte-se normalmente (6, 7). A pré-eclâmpsia representa uma das manifestações da falha deste mecanismo de adaptação(6).

Segundo dados do Ministério da Saúde observa-se que desde 1998 houve uma mudança no perfil epidemiológico desta doença, que tem se apresentado como a principal causa de complicações na gestação, seguida das hemorragias e infecções(8). Apesar dos esforços de políticas de saúde pública para minimizar os efeitos deletérios da pré-eclâmpsia e suas repercussões de cunho social e econômico, a morbimortalidade associada a esta entidade ainda permanece elevada (9). Isto nos mostra que a prevalência desta doença em nosso meio, assim como suas repercussões, devem ser estudadas.

Apesar da fisiopatologia da pré-eclâmpsia ainda não estar clara, a isquemia placentária tem sido descrita como um fator determinante deste processo(10-13). Estudos em animais mostraram que a redução da perfusão placentária leva a um aumento significativo da pressão arterial e proteinúria(14). A redução no fluxo sanguíneo placentário associa-se a alterações cardiovasculares e renais compatíveis com achados da síndrome de pré-eclâmpsia em humanos, mas os mecanismos que conectam a isquemia placentária às alterações hemodinâmicas ainda precisam ser melhor elucidados (10, 14). Evidências sustentam a hipótese de que sejam responsáveis por este processo algumas citocinas produzidas pela

placenta, como o fator de necrose tumoral alfa, que é uma citocina inflamatória promotora de alterações funcionais e estruturais no endotélio materno(15, 16), desencadeando natriurese e elevação sistêmica da pressão arterial. A associação destes eventos sugere que a disfunção endotelial seja etapa intermediária e crucial deste processo patológico, já que é um evento sistêmico materno desencadeado por alterações focais na placenta.

A disfunção endotelial, ou perda da integridade funcional do endotélio, é definida como desequilíbrio entre produção diminuída de óxido nítrico (NO) e aumentada de fatores contráteis como endotelina. Estudos mostram que a produção de NO está aumentada em gestações normais, proporcionando a vasodilatação necessária na adaptação hemodinâmica da gestação(17). A endotelina, um fator endotelial responsável por vasoconstrição, está aumentada nas pacientes com pré-eclâmpsia(18). Acredita-se que este desbalanço de fatores endoteliais, como o NO e a endotelina, participe no processo da síndrome de pré-eclâmpsia, contribuindo para o aumento da resistência vascular sistêmica e consequente aumento da pressão arterial(19).

A função endotelial tem sido avaliada por uma variedade de métodos invasivos e não-invasivos. Dentre os métodos invasivos, recentemente foi mostrado que, na pré-eclâmpsia, estão diminuídos fatores angiogênicos, como o fator de crescimento vascular endotelial (VEGF) e o fator de crescimento placentário (PlGF), e aumentados fatores antiangiogênicos, como receptor solúvel de VEGF (sFlt-1) e a endoglina solúvel (sEng) (20-22). Estes, assim como outros marcadores ainda em estudo, parecem não ser preditivos e específicos o suficiente para o seu emprego na prática clínica(23-25). Uma das técnicas não-invasivas para avaliação da função endotelial é a análise ultra-sonográfica da artéria braquial. A Dilatação Mediada por Fluxo (DMF) da artéria braquial foi descrita por Anderson e Mark em 1989 e começou a ter aplicação na pesquisa clínica no início dos anos 90(26).

A DMF da artéria braquial é um método atrativo por não ser invasivo e de relativo baixo custo. Este exame busca quantificar a reação vasodilatadora do endotélio em função da

diferença no diâmetro do vaso promovida pelo aumento do fluxo vascular recuperado de um bloqueio. Após uma oclusão vascular sustentada, as células endoteliais reagem ao aumento na tensão de cisalhamento (vetor direcional permanente aplicado à parede do vaso pelo fluxo sanguíneo) promovendo a ativação de vários mecanismos, entre eles a ativação dos canais de potássio, que levam ao aumento da entrada do cálcio intracelular. Este aumento de cálcio nas células endotéliais ativa a enzima óxido nítrico sintetase endotelial que catalisa a liberação de NO a partir de L-arginina. O gás NO se difunde através da membrana plasmática sinalizando a formação de guanosina monofosfato cíclica, promotora do relaxamento do músculo liso vascular subjacente, resultando em vasodilatação com aumento do diâmetro arterial(27-29).

Estudos realizados com DMF na pré-eclâmpsia, têm demonstrado uma evidente disfunção endotelial presente nesta síndrome, em relação à gestação normal. Atualmente tem sido sugerido que esse teste possa ser usado na prenhez para avaliação da função endotelial ou até mesmo na tentativa de predição de toxemia(30-32). Outros trabalhos descreveram, além disso, que em pacientes com quadros mais graves de Síndrome de Pré-eclâmpsia (SPE) os resultados de DMF apresentaram-se piores, porém sua comparação com casos leves da doença não foi realizada possivelmente devido ao pequeno tamanho da amostra estudada(33, 34).

Justificativa

É notória a existência de lesão endotelial como manifestação fisiopatológica na SPE. Esforços têm sido feitos para investigar a resposta endotelial durante a gestação complicada pela síndrome com o objetivo de predizê-lá. Adicionalmente, uma vez a doença estabelecida, torna-se um desafio a identificação das pacientes que serão acometidas por desfechos desfavoráveis. Assim, a avaliação da função endotelial em busca de um marcador prognóstico quando do diagnóstico de SPE é parte justificável deste processo.

Hipótese

Alteração da DMF em gestantes com síndrome de pré-eclâmpsia está associada à gravidade e desfechos perinatais adversos.

OBJETIVOS

Principal

Verificar associação entre a Dilatação Mediada por Fluxo (DMF) da artéria braquial e morbidade na Síndrome de Pré-eclâmpsia.

Secundários

Em gestantes com e sem complicações atribuídas a SPE:

* Comparar a DMF;

* Comparar os níveis pressóricos, acido úrico e proteinúria;

* Avaliar os marcadores de gravidade (pressão arterial sistólica e diastólica, proteinúria de 24h, ácido úrico e relação proteinúria-creatininúria) e a DMF como fatores de risco para complicações pela doença;

* Verificar a capacidade de predição da DMF e dos marcadores da doença para identificar casos de pré-eclâmpsia com complicação;

* Verificar o comportamento da DMF e dos marcadores de gravidade quando selecionados os mais graves desfechos perinatais adversos (morte fetal, eclâmpsia e síndrome HELLP).

PACIENTES E MÉTODOS

Amostra

Foram convidadas a participar do estudo gestantes que estavam internando pelo diagnóstico de Síndrome de Pré-eclâmpsia no Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul (HSL-PUCRS) e consentiram participar do estudo assinando o Termo de Consentimento Livre e Esclarecido aprovado pelo Comitê de Ética em Pesquisa. (Protocolo 368/11).

Critérios de inclusão

Gestantes com diagnóstico de Síndrome de Pré-eclâmpsia(35) no momento da internação no HSL.

Critérios de exclusão

Gestação múltipla, internação em trabalho de parto ou participantes que desistiram de continuar no estudo em qualquer momento do seu acompanhamento.

Desenho do Estudo

Trata-se de um estudo de coorte, no qual as participantes foram agrupadas, de acordo com o desfecho da doença, em *pré-eclâmpsia* (sem complicação) e *pré-eclâmpsia complicada*. Foram considerados como complicação pela pré-eclâmpsia os eventos a seguir: recém-nascido com peso abaixo de 2kg, restrição severa do crescimento fetal (abaixo do percentil 5), escores de APGAR menor que 7 no quinto minuto, idade gestacional ao nascer abaixo de 34 semanas, necessidade de internação em Unidade de Terapia Intensiva (UTI)

neonatal, morte fetal, descolamento prematuro de placenta (DPP), síndrome HELLP, eclâmpsia, necessidade de internação em UTI e morte materna. Para o diagnóstico de pré-eclâmpsia foi considerado pressão arterial ≥140/90 associada à proteinúria de 24h acima de 300mg(36) ou relação proteinúria/creatininúria (P/C) maior que 0,3(37).

As pacientes foram submetidas à medição da DMF no momento da internação, conforme técnica descrita a seguir. No momento da medição da DMF, foram também submetidas à análise Doppler das circulações materno-fetoplacentária (artérias uterinas, umbilicais e cerebral média fetal), assim como foi aferida a estimativa do peso fetal (biometria fetal).

Para identificação de fatores de risco foram usados os seguintes valores de corte: ácido úrico ≥6,0; relação P/C ≥2,0; proteinúria de 24h ≥2.000mg; PAS ≥160mmHg; PAD ≥110mmHg. A DMF ≤4,5 foi considerada como alterada para a estimativa de fator de risco.

Num segundo momento, para melhor caracterizar a associação entre DMF e gravidade da doença, ao final da análise as participantes foram realocadas, selecionando apenas os desfechos mais graves como morte fetal, eclâmpsia e síndrome HELLP, para comparar às demais participantes do estudos.

Tamanho da Amostra

Considerando-se um intervalo de confiança de 5% e um poder de 90%, foi calculado 22 pacientes em cada grupo para detectar diferença de 1 (um) desvio padrão na DMF entre gestantes com pré-eclâmpsia com e sem complicações advindas desta patologia.

Técnica da DMF

Dois ultrassonografistas realizaram as aferições da DMF, sendo obtidas as seguintes variabilidades intraobservador: 6,2% e 6,8%. Para avaliar a variabilidade interobservador o Coeficiente Intra Classe (ICC) estimado em 0,91 foi aceito.

A DMF mede a diferença percentual do diâmetro da artéria braquial ocorrida após estímulo. Entretanto, ainda é necessário determinar qual o ponto de corte que identifica a disfunção endotelial em população de baixo risco(38); os valores usualmente propostos ficam entre 7 a 10%. Um corte mais baixo que 4,5% foi proposto para identificar risco coronariano(39), este valor também foi demonstrado como determinante de risco cardiovascular após gestações com disfunção placentária(40).

A dilatação da artéria braquial foi mensurada por técnica adaptada de Celemajer (26). O estudo ultrassonográfico bidimensional foi realizado com sonda linear de alta frequência (14 MHz) do aparelho Ultrassonix (Ultrassonix Medical Corporation, Richmond, Canada), através de imagens longitudinais da artéria braquial em um ponto 5 cm proximal à fossa antecubital. As pacientes permaneceram 10 minutos em repouso na posição supina e não tomaram café ou fumaram 8 horas antes do estudo, a temperatura da sala era controlada.

O segmento da artéria braquial com a imagem mais clara entre a parede anterior e posterior do vaso foi identificado para a realização desta medida antes do teste funcional, sendo o modo ultrassonográfico B utilizado para medir o diâmetro do vaso (diâmetro basal artéria braquial). O aumento do diâmetro foi induzido utilizando-se o esfigmomanômetro. O manguito foi insuflado até 20-30 mmHg acima da pressão sistólica (medida antes de iniciar o exame), mantido por 5 minutos, e após desinsuflado para a retirada do sistema de compressão, o que resulta em dilatação reativa da artéria braquial. Sessenta a noventa segundos após desinsuflar o manguito, o diâmetro da artéria braquial foi novamente medido (diâmetro artéria braquial na hiperemia reativa), utilizando, concomitantemente, o Doppler para se identificar a fase diastólica final na artéria braquial (momento em que o calibre do

vaso deve ser medido). Essa técnica foi utilizada com sucesso em estudo prévio neste serviço(33, 41).

A DMF é expressa pela porcentagem de mudança do diâmetro da artéria braquial após estímulo em relação ao diâmetro basal, e calculada conforme a fórmula a seguir:

$$DMF = \left[\frac{\text{diâmetro art. braquial na hiperemia reativa} - \text{diâmetro basal art. braquial}}{\text{Diâmetro basal art. braquial}} \right] \times 100$$

Análise Estatística

A comparação entre os grupos da DMF e dos marcadores de pré-eclâmpsia foi realizada com os testes de Mann Whitney ou Teste *t* de Student, quando apropriado. Para estimar o risco para complicações foi utilizada regressão logística univariada na DMF e nos marcadores de pré-eclâmpsia. A curva ROC (Receiver Operating Characteristic) foi usada para comparar o potencial de predição entre os marcadores. Toda a análise estatística, incluindo a estatística descritiva, foi feita no programa SPSS 17.0 para Windows (StatisticalPackage for the Social Sciences, SPSS Inc, Chicago).

RESULTADOS – ARTIGO ORIGINAL

Anexo 1- Comprovante de submissão do artigo no periódico *International Journal of Cardiology.*

Title – Flow mediated dilatation of brachial artery as marker of preeclampsia morbidity

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Summary

Objectives: to evaluate the association between brachial artery Flow Mediated Dilatation (FMD) and preeclampsia morbidity.

Methods: Sixty-four pregnant women at the diagnosis of preeclampsia were selected. FMD and routine preeclampsia markers were assessed at enrollment and followed until delivery. Women were grouped and compared according to their outcomes (26 developed complications and 38 did not).

Results: Median FMD is impaired in women with complicated preeclampsia (7.44%; IQR 2.20-13.34%) compared to those without complications (11.80%; IQR 5.36-16.66%) (p=0,03). The cutoff value of FMD ≤4.5% was associated with approximately four-fold odds increment of any complication (OR 3.79; IC95% 1.23-11.70), similar to the protein to creatinine ratio >2,0 (OR 4.50; IC95% 1.21-16.74). Systolic and diastolic blood pressure were not associated with risk for complication and uric acid had a borderline significance (OR 3.38; IC95% 0,98-11,72). Moreover, when major complications (eclampsia, HELLP syndrome or stillbirth) were selected as a composite outcome FMD was even lower (2.84%; IQR 0.00–7.22%) and FMD ≤4.5% was associated with a marked 15 fold increased risk for these specific events (OR 15.55; IC95% 3.55-68.16). Although FMD seems to have a weak accuracy to predict any preeclampsia complications (AUC=0.66; IC95% 0.52-0.79), ROC curve analysis showed that it may be a prognostic marker for major complications (AUC=0.84; IC95% 0.73-0.96).

Conclusion: FMD is associated with morbidity of preeclampsia, markedly in women with eclampsia, HELLP syndrome or stillbirth. FMD at preeclampsia diagnostic moment may be used as a prognostic marker of these poor outcomes.

Introduction

Preeclampsia occurs in 2–7% of pregnancies worldwide and is considered a leading cause of maternal and fetal morbidity and mortality(1). It is a hypertensive disorder with proteinuria, occurring after 20 weeks of gestation, which, until now, the only solution is delivery of the fetus and placenta(2, 3). The exact nature of its differences from normal pregnancies remains unclear. Reduced placental perfusion, arising from poor implantation has been proposed as an initiating event in preeclampsia, leading to generalized maternal endothelium dysfunction(4).

The vascular endothelium is a complex organ with paracrine, autocrine and endocrine functions. It secretes numerous factors regulating vascular tone, participates in immune response and interacts with many internal and external stimuli(5). Endothelial dysfunction is thought to play a role in the pathophysiology of preeclampsia and pregnancy has been suggested to be a stress test for women(6). Flow mediated dilatation (FMD) is a noninvasive test capable of identifying endothelial dysfunction(7). It has been employed to investigate the role of vascular alterations in the preeclampsia(8, 9).

Despite the efforts that have been made, it is still difficult to predict preeclampsia(10) and its morbidity(11). Several markers have been proposed to predict preeclampsia and poor outcome but none has reached the necessary standards needed to be clinically useful(12-14). Our hypothesis is that FMD may be useful as a clinical test prognostic marker, specially associated with routine laboratorial exams. The aim of this study is to evaluate the association of endothelial dysfunction, assessed by FMD, with preeclampsia morbidity.

Patients and methods

The study was approved by the Research Ethics Commitee of Pontifícia Universidade Católica do Rio Grande do Sul (Protocol 368/11), and written consent was obtained from all participants before inclusion. This is a cohort study that enrolled women with preeclampsia at the diagnostic moment, accompanied until delivery. Women were grouped according to preeclampsia outcomes dividing into women with or without complications. Preeclampsia was defined as new onset hypertension (blood pressure (BP) above or equal to 140/90 mmHg in two occasion at least 4 hours apart) after the 20^{th} week gestation and proteinuria (300mg in a 24-hour urine collection)(15), or proteinuria to creatininuria ratio (P/C) ≥0,3 on a urine sample(16). Complicated preeclampsia was considered when any of the following occurred: HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), eclampsia, abruptio placentae, mother's Intensive Care Unit admission, maternal death, early prematurity (delivery before 34 weeks gestation), severe growth restriction (percentile<5), newborn lighter than 2kg, APGAR Score <7 in the fifth minute, need of Neonatal Intensive Care Unit admission or stillbirth. Twin pregnancy, women who were in labor on preeclampsia diagnosis and patients who withdraw consent were excluded.

Endothelial function was evaluated by brachial artery FMD, using the protocol adapted from Celermajer(7). FMD was measured in the first day of diagnosis and all patients rested for 10 minutes before baseline evaluation. None smoked cigarettes or drank coffee for at least 8 hours before the ultrasound exam. A 14-hertz linear array transducer (Ultrasonix, Ultrasonix Medical Corporation, Richmond, Canada) was used to identify braquial artery 5 cm above the antecubital fossa. Diameter was measured at the lumen-intima interface in both sides of the artery with electronic caliper. After the baseline measure, a cuff was inflated to 20-30 mmHg above the systolic pressure and maintained for 5 minutes. A post occlusion reading was taken 60-90 seconds after sudden cuff deflation, assumed to be the maximum hyperemia response. FMD is calculated as percentage of reactive hyperemia vasodilatation

[(hyperemia diameter – baseline diameter/baseline diameter) x 100]. This protocol has previously been used in our hospital(17, 18). Ultrasounds were performed by two observers. Intra observer variability was 6.2% and 6.8%. Intra-class coefficient was 0.91 between observers.

Demographic, clinical and laboratorial data were collected in the first day of admission. Systolic blood pressure (SBP), diastolic blood pressure (DBP), uric acid, 24 hour proteinuria and P/C ratio were considered as preeclampsia markers, care was defined by the assistant obstetrician. As a routine others laboratory tests were performed once or twice a week for expectant management of preeclampsia, according to disease severity. The most altered levels of platelets, Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and creatinine were recorded for severity assessment and comparisons. Fetal Doppler Ultrassound was also repeated in the follow up of patients according to clinical needs, but this data was only used for diagnosis of intra uterine growth restriction, oligohydramnio and abnormal fetal circulation.

For risk assessment of preeclampsia markers, altered cut off values were assumed to be: uric acid ≥6.0mg/dL(19); P/C ratio ≥2.0; 24h proteinuria ≥2g; SBP≥160mmHg or DBP≥110mmHg(15). FMD was considered of risk for complications when equal or less than 4.5%, a cutoff referred as able to predict coronary events(20) and cardiovascular events after pregnancy with placental disease(21).

To better evaluate FMD association with morbidity, a composite of major outcomes (ie. stillbirth, eclampsia or HELLP syndrome) was selected from complicated preeclampsia group, to compare their FMD with all other subjects.

Sample size was calculated to detect a difference of one standard deviation in the FMD (90% power and alfa 0.05), estimating at least 22 subjects by group. Comparisons between groups of FMD and preeclampsia markers were made using Mann Whitney or Student's *t* test, for non-parametric and parametric data, respectively. To estimate risk for

preeclampsia complications univariate logistic regression was used in FMD and preeclampsia markers. The receiver operating characteristic curve (ROC) was plotted to calculate area under the curve (AUC) of FMD for complicated preeclampsia and also for major outcomes. Statistical analysis was made on SPSS 17.0 for Windows (Statistical Package for the Social Sciences, SPSS Inc, Chicago).

Results

The study included 66 women with preeclampsia and two were excluded, one twin pregnancy and one that delivered in another city. The remaining 64 subjects were analyzed, 26 developed complicated preeclampsia and 38 had preeclampsia without associated morbidities. Clinical characteristics are summarized in Table 1. There were no significant differences between groups for maternal age, chronic hypertension, cigarette smoke and parity. The indexes of fetal birth weight, and gestational age at preeclampsia diagnosis and at birth were significantly lower in the preeclampsia group.

Laboratory and Doppler ultrassound variables are shown in Table 1. Higher levels of AST, ALT, LDH and creatinine were observed in complicated preeclampsia group and platelets counts were lower in this group. Obstetric ultrasound Doppler identified higher resistance index (RI) of uterine artery and umbilical artery in preeclampsia with complications. RI of median cerebral artery was similar in both groups.

FMD in complicated preeclampsia group was 7.44% (IQR 2.20-13.34%) while in preeclampsia group was 11.80% (IQR 5.36-16.66%) (P=0.03) as shown in Figure 1. Comparisons from preeclampsia markers are described in Table 2. P/C Ratio, 24h proteinuria, uric acid, SBP and DBP were all significantly increased in complicated preeclampsia. When evaluating risk assessment of markers (Table 3), FMD≤4.5% was associated with a risk of 3.79 (IC 95% 1.23-11.70) in the Odds Ratio (OR) for complicated preeclampsia. P/C≥2.0 also registered an increased risk for complications with an OR of 4.50 (IC 95% 1.21-16.74).

Logistic regression of 24 hours proteinuria could not be calculated statistically because none of women on solely preeclampsia group had proteinuria above 2g. The finding that all pregnant with 24 hours proteinuria higher than 2g developed complications suggests the high risk it represents (p=0.008 at Fisher Exact Test). Uric acid ≥6.0 increased risk for complications with a borderline significance (OR 3.38; IC95% 0.98-11.72). SBP≥160 and DBP≥110 were not associated with a risk of complicated preeclampsia. FMD was also plotted in a ROC to evaluate its potential to predict morbidities at the moment that preeclampsia was diagnosed (Figure 2). AUC of FMD was 0.66 (IC95% 0.52-0.79) for any complication.

Finally, subjects were regrouped according to major outcomes (HELLP syndrome, eclampsia or stillbirth) and 13 women fit these criteria. FMD was 2.84% (IQR 0.00–7.22%) in this sub-group compared to 11.90% (IQR 5.56-16.62%) of all other women (p<0,001) (Figure 3). For major outcomes, Odds Ratio of FMD≤4.5 was 15.55 (IC95% 3.55-68.16) and AUC was 0.84 (IC95% 0.73-0.96) (Figure 2).

Discussion

Endothelial dysfunction evaluated through FMD is known to be present in preeclampsia patients(9, 17, 22-25), but the association with severity of the disease has been poorly investigated. The present study demonstrates that decreased FMD may be linked directly to morbidity of preeclampsia. These results are in agreement with a study that evaluated preeclampsia and severe preeclampsia, showing worst endothelial function on the severe group, even though the authors haven't made a statistical comparison(24). Also, by comparing with the usual markers of the disease that we use in clinical practice, it seems FMD has similar strength as a risk factor and prognostic marker.

Endothelial dysfunction is thought to be one of the mechanisms involving preeclampsia manifestations (hypertension and proteinuria). While in normal pregnancy FMD increases through pregnancy(26), in women with preeclampsia it is reduced(22). Also, FMD

has been shown to be altered even before the onset of the disease(23, 24). We tested its association with disease severity, supported by the theory of impaired nitric oxide bioavailability in preeclampsia(27, 28). Our results have shown that FMD is impaired in complicated preeclampsia and it was reassured by the fact that in women with the worst outcomes it was remarkably lower. Furthermore, FMD of equal or less than 4.5% seems to be a reliable cutoff point associated with a four-fold increment of complications and a 15 fold increased risk of major adverse outcomes. It is interesting to remember that low cutoff point should only be used in a set of patients with known impaired endothelial function, data is lacking to determine cutoff point for low risk population(29). Despite the fact that our study was not meant to determine prediction power, a preliminary analysis of the ROC curve suggests that FMD might be a weak prognostic marker for preeclampsia complications, since AUC were relatively small. When used to predict major outcomes AUC increases markedly (AUC=0.84; IC95% 0.73-0.96).

When expectant management is suggested, close supervision of mother and fetus is crucial, since it is impossible to predict preeclampsia clinical course and its deterioration can occur rapidly(11). As much as we know, there is no trial showing an evidence-based protocol for managing these patients, especially in severe cases. Usually management includes hospitalization, BP monitoring, maternal evaluation by symptoms and laboratorial tests, and fetal surveillance through Doppler ultrasound, amniotic fluid index or fetal non-stress test(30).

Serial 24 hours collected urine for protein quantification has been proposed in the past to identify patients with poor diagnosis. Our results go along other observational studies where patients with increased proteinuria had a worst outcome(31). However, further studies had shown that proteinuria alone, even above 10g/24h, should not indicate pregnancy interruption because expectant management of these cases significantly prolongs pregnancies and maternal complications does not increase(32, 33). It is suggested that once the threshold

of 300 mg/24 hours has been exceeded serial proteinuria evaluation should not be used for making clinical decisions(12). Its use may improve costs and interventions without proved benefits. As P/C ratio has been compared to 24 hours proteinuria(34, 35), we can infer that its uses should be advised for screening or diagnosing preeclampsia(36). Furthermore, both may not be used alone for decision making in expectant management of preeclampsia.

When considering blood pressure, the threshold of 160mmHg for SBP and 105 or 110mmHg for DPB determine a remarkable risk for cerebral hemorrhage or seizures(15, 37). The use of antihypertensive therapy does not affect the course of preeclampsia because the pathogenic process is placental under perfusion, due to abnormal trophoblastic invasion, leading to endothelial dysfunction. Despite the lack of potential to change disease course, medical treatment is usually started when blood pressure reach those values with the aim of preventing maternal stroke(15). Although we observed an increased blood pressure in complicated preeclampsia group, it was not a determinant of complications. Probably there was no association because blood pressure may predict only maternal complications and our outcomes were mainly perinatal morbidity. We believe that by increasing the sample an impact in those less frequent events could appear.

Hyperuricemia is often seen in preeclampsia, but its role is not clear. Although uric acid has failed to predict preeclampsia or complications in two systematic reviews(38, 39), recent studies has shown it identifies women at increased risk of adverse maternal and fetal outcome(19, 40, 41). Our results have shown that uric acid higher than 6,0mg/dL increases risk of preeclampsia complications with borderline statistical significance (OR 3.38; IC95% 0.98-11.72).

Preeclampsia has many clinical presentations, it is a multisystemic disease that affects organs in different proportion, as illustrated by cases of preeclampsia without proteinuria or hypertension. FMD of brachial artery evaluates only one site, brachial artery endothelium, and sometimes it might not reflect the severity of whole disease. The variety of damage that

can be caused by preeclampsia may be a limitation for this method to predict the disease and complications.

FMD of brachial artery is decreased in complicated preeclampsia in comparison with preeclampsia without complications. Severe endothelial dysfunction, measured by FMD test, is associated with higher risk of poor outcomes and may predict complications of the disease. Preeclampsia etiology and pathophysiology is not fully understood and probably this is the reason a specific predictor and prognostic marker haven't been found. Our findings support the evidence of endothelial dysfunction as a key mechanism for preeclampsia complications and also that FMD may be a prognostic marker.

Acknowledments: MCV, MH had support from CAPES. Poli-de-Figueiredo is a CNPq researcher.

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Table 1 – Clinical characteristics, laboratory and Doppler exam.

	PE (n= 38)	**Complicated PE** (n= 26)	**P**
Age (years)†	27.5 ±6.4	26.2 ±7.1	0.41
Chronic hypertension n(%)*	10 (26.3)	10 (38.5)	0.29
Cigarette smoke n(%)*	4 (10.5)	6 (23.1)	0.49
Nulliparity n(%)*	16 (42.1)	13 (50.0)	0.61
GA diagnosis (weeks)†	35.6 ±1.9	29.4 ±4.4	<0,001
GA at birth (weeks)†	37.5 ±1.5	31.0 ±4.4	<0.001
Birth weight (g)†	3,037 ±546	1,482 ±870	<0.001
AST (units/L)‡	21 (17-26)	40 (24-109)	<0.001
ALT (units/L)‡	19 (14-24)	36 (23-101)	<0.001
LDH (units/L)‡	467 (405-558)	718 (546-1,190)	<0.001
Creatinine (mg/dL)†	0.71 ±0.15	0.89 ±0.28	0.02
Platelets (x1000/mcL)†	219 ±65	169 ±68	0.005
Uterine artery RI†	0.50 ±0.10	0.68 ±0.18	<0.001
Umbilical artery RI†	0.57 ±0.08	0.70 ±0.12	<0.001
Median cerebral artery RI†	0.81 ±0.08	0.79 ±0.07	0.47

GA-Gestational age; AST-Aspartate aminotransferase; ALT-Alanine aminotransferase; LDH-Lactate dehydrogenase; RI-Resistance index.
† Mean ± Standard deviation and Student t Test; ‡ Median (IQR 25-75) and Mann Whitney Test; * Percentual and Qui-square Test.

Table 2 – Comparison of preeclampsia markers between groups.

	PE (n= 38)	**Complicated PE** (n= 26)	**P**
FMD (%) ‡	11.80 (5.36-16.66)	7.44 (2.20-13.34)	0.034
Proteinuria 24h (mg) ‡	365 (295-474)	673 (471-2,614)	0.001
P/C Ratio ‡	0.49 (0.27-0.71)	0.67 (0.36-4.67)	0.038
Uric Acid (mg/dL) †	4.5 ±1.2	5.3 ±1.4	0.013
SBP (mmHg) †	156 ±17	167 ±18	0.012
DBP (mmHg) †	98 ±12	104 ±13	0.045

FMD-Flow Mediated Dilatation; P/C Ratio-Proteinuria/Creatininuria Ratio; SBP-Systolic Blood Pressure; DBP-Diastolic Blood Pressure.
† Mean ± Standard deviation and Student t Test; ‡ Median (IQR 25-75) and Mann Whitney Test.

Table 3 – Risk assessment of preeclampsia markers for complications.

	PE (n= 38)	**Complicated PE** (n= 26)	**OR (IC95%)**
FMD ≤4.5%	7 (18.4%)	12 (46.2%)	3.79 (1.23-11.70)
Proteinuria 24h ≥2g*†	0 (0%)	5 (22.7%)	-
P/C ratio ≥2.0	4 (10.5%)	9 (34.6%)	4.50 (1.21-16.74)
Uric acid ≥6.0mg/dL	5 (13.5%)	9 (34.6%)	3.38 (0.98-11.72)
SBP ≥160mmHg	18 (47.4%)	18 (69.2%)	2.50 (0.87-7.13)
DBP ≥110mmHg	11 (28.9%)	10 (38.4%)	1.53 (0.53-4.41)

FMD-Flow Mediated Dilatation; P/C Ratio-Proteinuria/Creatininuria Ratio; SBP-Systolic Blood Pressure; DBP-Diastolic Blood Pressure.
* 5 patients in PE and 4 in complicated PE group didn't collect 24 hour proteinuria; † Fisher exact test of 0.008.

Figure 1. Comparison of FMD between groups.

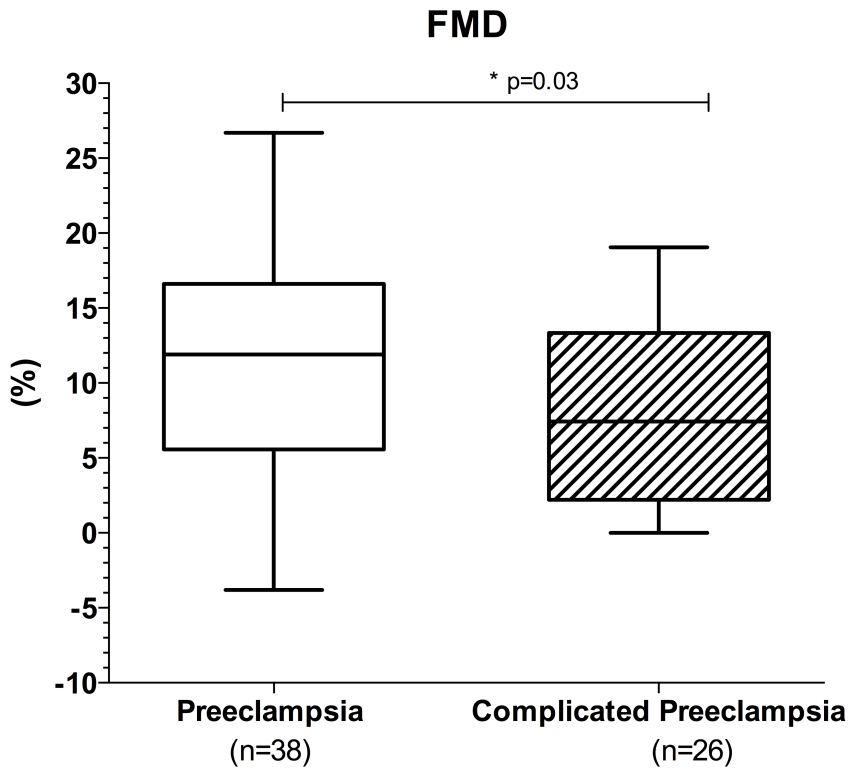
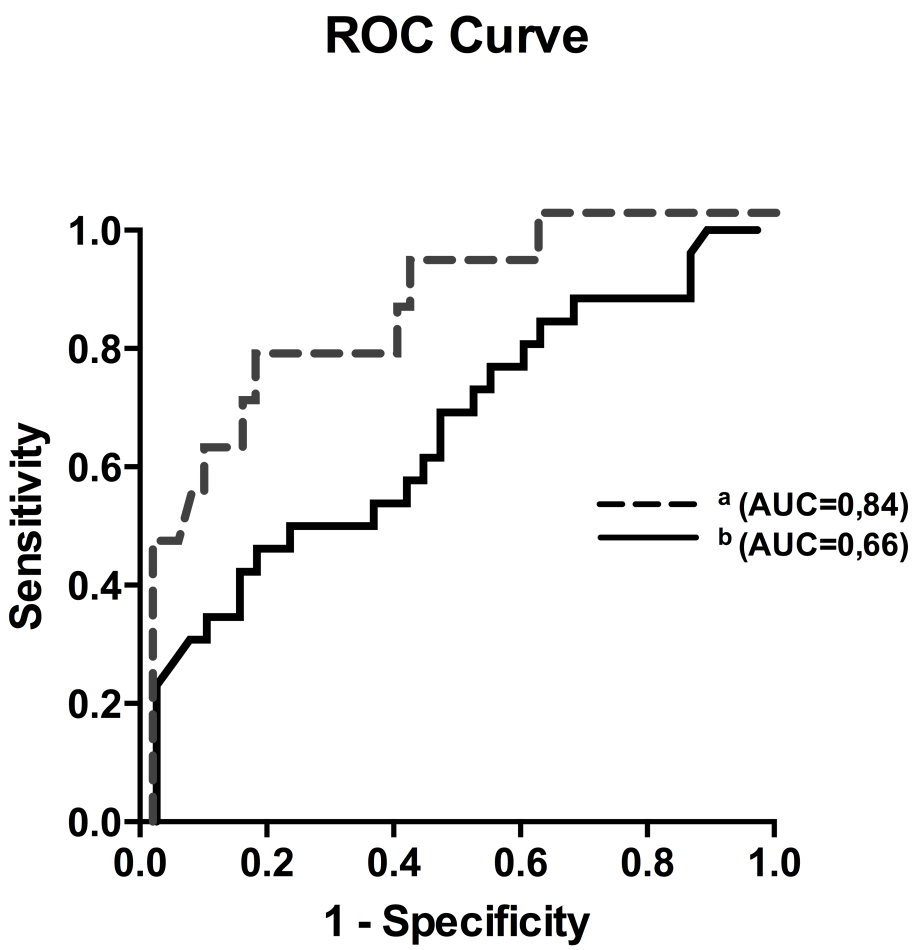


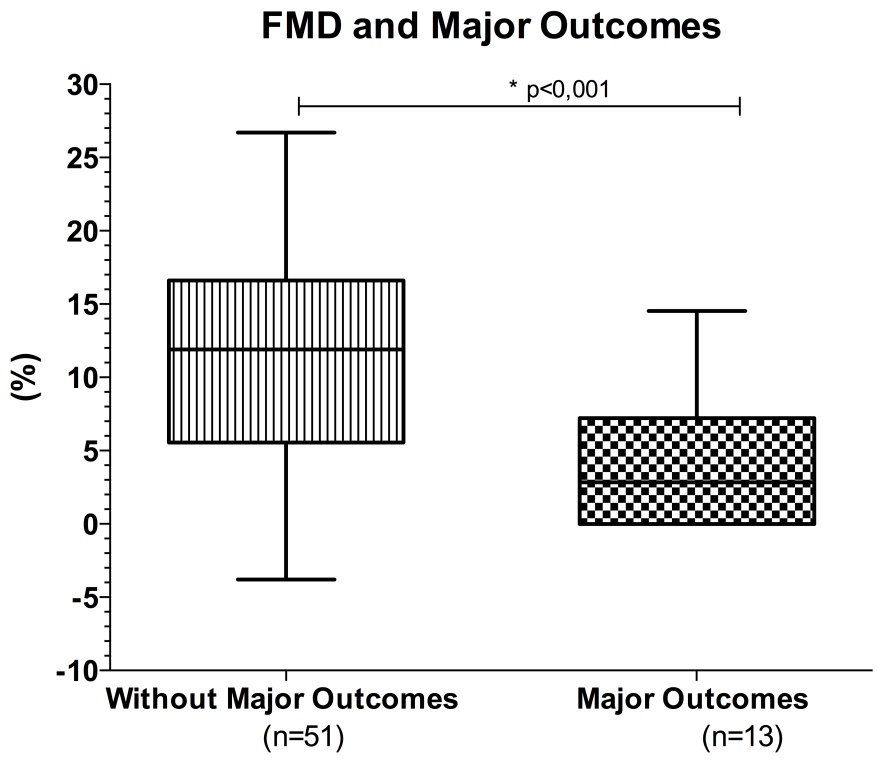
Figure 2. FMD as predictor of complicated preeclampsia and composite major outcome.



[a] Estimated prediction of FMD for complicated preeclampsia.

[b] Estimated prediction of FMD for composite major outcomes (HELLP syndrome, eclampsia or stillbirth).

Figure 3. Comparison of FMD between preeclamptics with and without composite major outcomes (HELLP syndrome, eclampsia or stillbirth).



CONSIDERAÇÕES FINAIS

O programa de Pós-graduação em Medicina e Ciências da Saúde – FAMED / PUCRS não exige um formato específico para a apresentação da dissertação de mestrado. Atualmente, tem sido a opção da maioria dos alunos e orientadores que o artigo completo, no formato de publicação, seja incluído na dissertação de mestrado. Optamos por assim fazer pois acreditamos que, além de trazer todas as análises de valor, o artigo *per se* é um dos resultados desta dissertação de mestrado. Tendo em vista que durante o aprendizado, além do conhecimento técnico adquirido, o aluno também amadurece os seus valores pessoais, neste momento optamos por abrir espaço para um breve relato da história deste trabalho e do local onde ele foi desenvolvido, bem como de seu(s) autor(es).

O Grupo de Pesquisa de Nefrologia da Faculdade de Medicina da Pontifícia Universidade Católica do Rio Grande do Sul (FAMED-PUCRS) é uma unidade de pesquisa de destaque no Hospital São Lucas da PUCRS (HSL-PUCRS) e vem desenvolvendo projetos sobre hipertensão na gestação desde 1993. Esta linha de pesquisa permitiu o aperfeiçoamento de diversos obstetras, entre eles muitos que participaram de minha formação de especialista, como Prof. Dr. João Alfredo Píffero Steibel, Prof. Dr. Breno José Acauan Filho, Prof. Dr. Plínio Vicente Medaglia filho e a Prof. Dra. Letícia Germany de Paula. Em 2008, através da dissertação de mestrado do Dr Edson Vieira da Cunha Filho, foi proposto o início, no Laboratório de Nefrologia, de pesquisas em ultrassonografia utilizando a dilatação mediada por fluxo da artéria braquial em gestantes com pré-eclâmpsia.

Neste mesmo ano de 2008, eu recém terminara minha formação como médico e vinha buscar no HLS espaço para minha formação de especialista. Ao passo que me aprimorava como ginecologista e obstetra, descobri meu gosto pelo ambiente acadêmico. Já certo do meu

desejo pela pós-graduação fui tendo a oportunidade de conhecer a possibilidade de aperfeiçoamento no programa de Pós Graduação em Medicina e Ciências da Saúde (PPGMCS) da FAMED-PUCRS, porém tive de aguardar o término da minha especialização para ir em busca desde objetivo. No final de 2010, quando recebi o convite para integrar o Serviço de Obstetrícia do HSL passei a ter a oportunidade de utilizar sua estrutura e seus pacientes na minha formação e então, no ano seguinte, me engajei neste projeto de mestrado em conjunto com o grupo de pesquisa de nefrologia onde fui gentilmente acolhido pela Prof. Dra Bartira Ercília Pinheiro da Costa e pelo Prof. Dr Carlos Eduardo Poli de Figueiredo.

Em paralelo, durante o ano de 2010 e 2011 busquei formação em ultrassonografia em ginecologia e obstetrícia. Acredito que a ecografia hoje é um alicerce no acompanhamento da gestação uma vez que nosso paciente, o feto, encontra-se escondido no ventre materno. Além de ampliar meu universo de atuação como médico e de qualificar o meu atendimento às gestantes, a formação de ultrassonografista foi chave para permitir a execução deste projeto.

Apesar do meu gosto pelo ensino e pela pesquisa, durante o andamento desde projeto tive momentos de incerteza quanto a minha aptidão para o cargo de professor e pesquisador. Aproveito para registrar aqui o presente que me foi dado pelos alunos da Faculdade de Medicina e que servirá de estímulo para que prossiga buscando o caminho do aperfeiçoamento após este projeto. No ano de 2012, a turma de formandos da FAMED-PUCRS me escolheu para figurar entre os homenageados na sua colação de grau. Já feliz por ter sido lembrado, fiquei realizado por ouvir destes que o empenho e também a qualidade no ensino justificavam a homenagem. Saibam os alunos que meu empenho surge do desejo deles pelo aprendizado, e que carregarei este momento em minha memória eternamente.

Durante a execução deste projeto tive a oportunidade de participar de outros trabalhos dentro e fora do Laboratório de Nefrologia. Considero que aulas possuem um caráter teórico em sua maioria, e apesar da sua extrema importância neste programa de pós-graduação, acredito que o contato com a prática, representada neste caso pela elaboração de artigos

científicos, deve estar na bagagem do aluno previamente à elaboração de sua dissertação. A convite da Prof. Dra. Bartira, participei do trabalho "Nitric oxide and Preeclampsia" no qual aperfeiçoei minhas habilidades de redação e aprofundei meu conhecimento sobre o endotélio e a via do óxido nítrico. Este trabalho será submetido a revista *Hypertension in Pregnancy* e segue como Anexo 2 para apreciação do leitor por tratar-se do mesmo tema desta dissertação, a Pré-eclâmpsia. Além disso, em parceria do Serviço de Obstetrícia com o Serviço de Neurologia do HSL, participei da confecção do artigo "Amyotrophic lateral sclerosis and riluzole use during pregnancy: A case report" publicado na revista *Amyotrophic Lateral Sclerosis* em 2012 (DOI: 10.3109/17482968.2012.673171).

A perspectiva do encerramento deste trabalho trouxe, nos últimos meses, a busca por novos caminhos e a criação de novos planos. O título de mestre com frequência é adquirido por um caminho árduo, porém para aqueles que o fazem com satisfação o Doutorado parece um caminho natural a ser trilhado. Este parece ser o meu caminho. Aproveitando-me do exemplo dos meus mestres e ouvindo os dizeres do Dr Poli-de-Figueiredo, acredito que a vivência em uma realidade diferente, em outro sistema de saúde e com outras regras, parece ter valor inestimável para ampliar os horizontes de um pesquisador e para permitir conhecimento de novas técnicas por parte de um médico. Ademais, devo ao Dr Poli-de-Figueiredo a apresentação ao Dr Dharmintra Pasupathy, Sênior Lecturer do King's College London, com quem estou em contato para desenvolver um projeto de Doutorado.

Ao final do presente estudo encerra-se um ciclo, de onde levo 3 trabalhos para publicação, o conhecimento sobre ensino e redação científica e as amizades de meus colegas e professores. Certamente um novo ciclo se iniciará, e hoje eu o encaro com a mesma inquietante e curiosidade que uma criança deseja seu presente ainda sem saber o que tem dentro.

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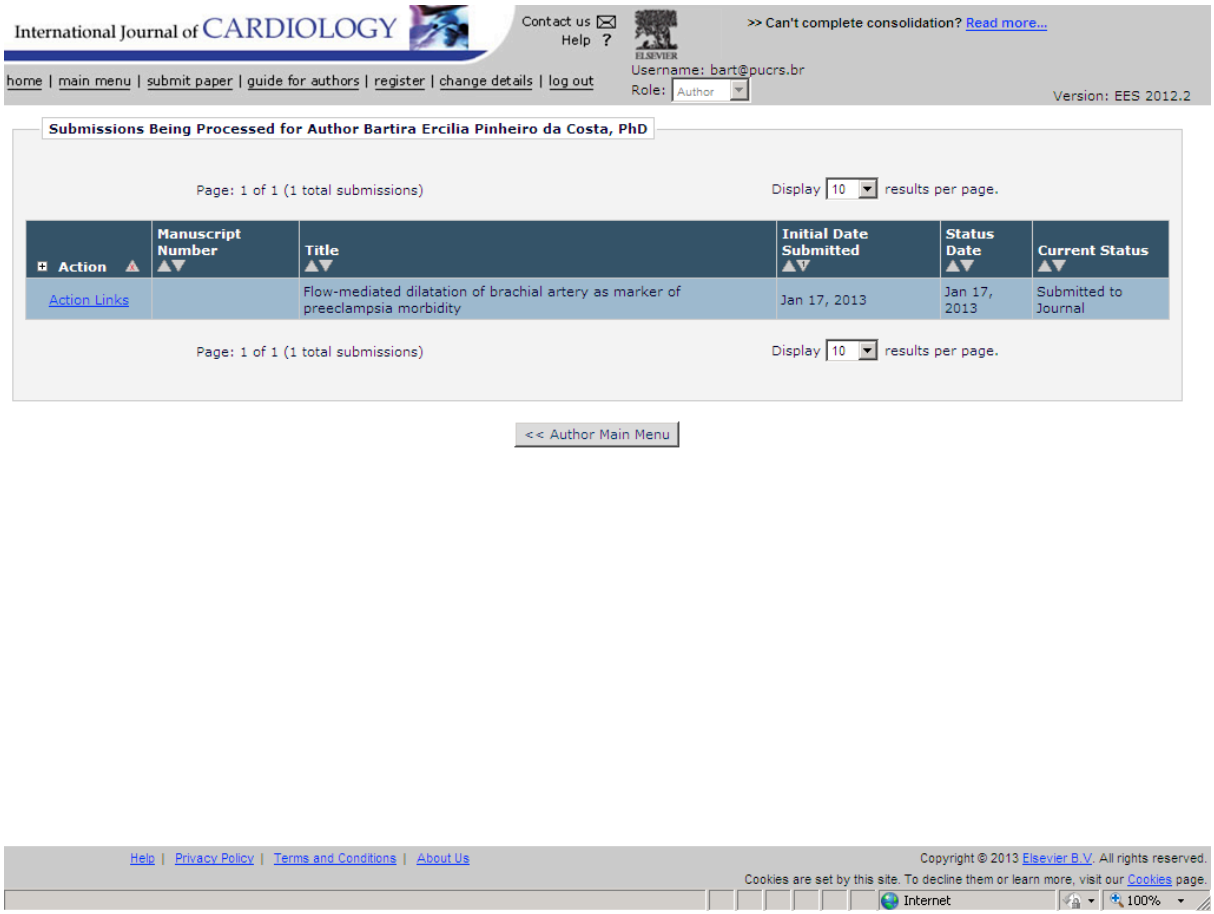
Anexo 1



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Anexo 2

Title: Nitric Oxide and Preeclampsia

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Summary

Objectives: To compare plasma levels of nitric oxide in primigravidae women, both with and without preeclampsia, as well as to assess its association with the clinical parameters of the disease.

Methods: 105 women were studied and were divided into preeclampsia group (n=40) and normotensive group (n=66). Nitrites, nitrates and NOx (total nitric oxide content) were measured in the plasma and clinical and laboratorial data were also recorded for comparison.

Results: The preeclampsia group presented a significant increase in levels of nitrates and NOx ($p=0.01$ and $p=0.01$, respectively) in comparison to the control group (Figure 1). Uric acid has shown a moderate correlation with the nitrates ($r=0.38$; $p<0.001$) and NOx ($r=0.38$; $p<0.001$). Nitrates and NOx also had a significant but weak correlation with other factors, such as, gestational age ($r=0.21$; $p=0.02$ and $r=0.20$; $p=0.03$), systolic arterial pressure ($r=0.22$; $p=0.01$ and $r=0.22$; $p=0.02$), diastolic arterial pressure ($r=0.23$; $p=0.01$ and $r=0.23$; $p=0.01$) and creatinine ($r=0.22$; $p=0.03$ and $r=0.21$; $p=0.03$).

Conclusion: An increase in nitric oxide was observed in women with preeclampsia. A failure in the mechanism of action, dependent on cyclic GMP, may justify this finding.

Introduction

Preeclampsia is one of the most important complications of pregnancy affecting between 2-7% worldwide(1). Little is known about the pathophysiology of this disease, but it is believed to be associated with the sequence of inadequate trophoblast invasion, low placental perfusion, placental ischemia, oxidative stress, and the consequent imbalance in the factors derived from the placenta(2). These factors play a key role in the induction of systemic endothelial dysfunction(2), which is considered to be the underlying cause of the clinical manifestations of preeclampsia, such as hypertension and proteinuria(3).

Nitric oxide (NO) is a potent endogenous vasodilator that also acts on the inhibition of platelet aggregation and adherence to the endothelium. It is synthesized by the nitric oxide synthase enzyme (NOS), that catalyses the degradation of the L-arginine amino acid. The endothelial isoform of NOS (eNOS) can be detected in the placenta and is found in the endothelium of the umbilical cord, chorionic plate and vessels of the villi(4). NO produced in the endothelium causes relaxation of the muscle layer of the blood vessels by means of dependent cyclic GMP (cGMP)(5). Among the various biological functions of NO is its active participation in the hemodynamic adaptation to pregnancy, and it appears to play an important role in the pathogenesis of placental dysfunction(6, 7).

Various metabolites that influence the NO pathway have already been studied, such as homocysteine, asymmetric dimethylarginine (ADMA), endothelin, superoxide dismutase (SOD) and arginase(4, 8-10). However, the relationship between NO and preeclampsia is not clear yet. Several conflicting studies have reported increased levels of NO not only in women with healthy pregnancies, but also in those suffering from preeclampsia, while others have found decreased levels(8, 9, 11, 12). The aim of this study is to compare plasma levels of NO in primigravidae women, both with and without preeclampsia, as well as to assess its

association with the clinical parameters of the disease.

Materials and Methods

This study was approved by the Ethics Committee of the Sao Lucas Hospital, Pontifical Catholic University of Rio Grande do Sul (PUCRS), and informed, written consent was obtained from each participant before inclusion. The sample group consisted of nulliparous pregnant women, carrying a single fetus, with no prior history of chronic disease and with a diagnosis of preeclampsia, according to the criteria set out by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (BP ≥ 140/90 measured on 2 separate occasions at least 4h apart and associated with urinary proteinuria ≥ 300mg/dL in 24h)(13). The control group included low risk nulliparous pregnant women in their third trimester. All women taking part in this study were pregnant for the first time. Those women developing other conditions during their pregnancy were excluded.

Demographic data was collected from all study participants, along with 5mL of peripheral blood. Plasma samples were stored at –80^{o}C and deproteinized by incubation with ethanol (3:1) at O^{o}C for 30 minutes. It was then centrifuged at 14,000 rotations per minute and the supernatant removed for analysis.

The measurement of nitrite and nitrate levels was performed using a Sievers *Nitric Oxide Analyzer280* (Colorado, USA) in accordance with manufacturer recommendations. Detection of the reaction product was made by chemiluminescence. Nitrite levels were determined using reduction with potassium iodide (5:1) in acid pH. The procedure for measurement of nitrate levels employed the same system, however, the reduction agent used was vanadium chloride (8%) diluted in hydrochloric acid (1M) at 94^{o}C. The concentrations used to produce the calibration curve were: 0.01; 0.05; 0.1, 0.5; 1.0; 1.5 and 2.0 uM of NO_2, with a correlation between the points of 0.9891 and a nitrate curve of r= 0.9977. Calculation

of the plasma concentration of nitrite and nitrates was made via comparison with standard solutions of these. The mean coefficient of variation between the duplicated samples was 8.2 and 3.2 for the compound measurements under analysis. Finally, the mean of the duplicates was multiplied by three in order to correct for the dilution of the samples in the deproteinization procedure. The results are presented as nanomolar (nM) units of concentration. Total NO content (sum of nitrites and nitrates) are represented as NOx.

The variables were expressed as the mean ± standard deviation. 105 patients were required to detect a difference of 4uM with a standard deviation of 8uM (power of 95% and a=0.05). Student's t-test or c^2 were used to evaluate the difference between the groups, and where appropriate, Pearson's correlation coefficient was used for analysis of the correlations. A value of $P<0.05$ was considered to be statistically significant. Statistical analysis was performed using the software SPSS 17.0 for Windows (Statistical Package for the Social Sciences, SPSS Inc, Chicago).

Results

The maternal and neonatal characteristics of both groups are shown in Table 1, 40 women in preeclampsia group and 66 in normotensive group. The group with preeclampsia had a significant increase in systolic and diastolic arterial blood pressure, as well as having lower birth weight babies, placental weight and gestational age at birth. Increased levels of uric acid and creatinine were also evident in these women (Table 2).

The preeclampsia group presented a significant increase in levels of nitrates and NOx ($p=0.01$ and $p=0.01$, respectively) in comparison to the control group (Figure 1). Uric acid has shown a moderate correlation with the nitrates ($r=0.38$; $p<0.001$) and NOx ($r=0.38$; $p<0.001$). Other factors, such as, gestational age ($r=0.21$; $p=0.02$ and $r=0.20$; $p=0.03$), systolic arterial pressure ($r=0.22$; $p=0.01$ and $r=0.22$; $p=0.02$), diastolic arterial pressure ($r=0.23$; $p=0.01$ and

r=0.23; p=0.01) and creatinine (r=0.22; p=0.03 and r=0.21; p=0.03) presented a weak correlation with the nitrates and NOx.

Discussion

The relationship between NO and preeclampsia remains contradictory, and various changes in levels of NO have already been described (8, 9, 11, 12, 14-16). In this study, increases in NO serum concentrations were observed in patients with preeclampsia, when compared to the healthy pregnant control group. This finding is similar to results from previously undertaken studies involving pregnancy with and without preeclampsia(14-16). One of the reasons for such discrepancies in values can be attributed to the variety of different methods that have been used for its measurement(17). Nonetheless, it is believed that preeclampsia is associated with alterations at some point along the NO formation pathway or even in its mechanism of action(18).

Serum levels of L-arginine are lower in preeclampsia(19), however, an increase in the transport of L-arginine has been recorded not only during healthy pregnancy(20), but also in those with this condition(21). This suggests that despite the lower availability of the circulating substrate in preeclampsia, the provision within the cell is sufficient for demand. There are also reports of the presence of L-arginine analogues, such as ADMA(10, 22) and N^{ω}-nitro-L-arginine methyl ester (L-NAME)(23), that compete for the eNOS enzyme in these patients and which, once catalyzed, will not produce NO. Additionally, the NOS is also inhibited by other competitors such as N^{ω}-mono methyl-L-arginine (L-NMMA) and N^{ω}-methyl-L-arginine (L-NMA)(24). Sandrim *et al.* found that the formation of NO in preeclampsia is inversely related to serum levels of anti-angiogenic factors(25). Data related to the polymorphism of eNOS are also conflicting in preeclampsia. Shaik *et al.* observed an association of the disease with the genetic condition(26), in contrast to Chen *et al.* who showed them to be independent conditions(27).

It has been described, in relation to the L-arginine-NO pathway, that cGMP is raised in the plasma of women with preeclampsia(28), with this being evidence that the relaxing function dependent on this nucleotide is not being effective. There are reports of urinary cGMP quantities being decreased in patients with preeclampsia, as compared with healthy pregnancies(29, 30). In this sense, it has been suggested that endogenous enzymes that break down cGMP are activated in this syndrome, such as our previous study reporting raised maternal serum phosphodiesterase activities in preeclampsia compared to normotensive controls(31).This also supports the experimental findings of Wareing *et al.* that showed an improvement in the vascular function of blood vessels in women with preeclampsia treated with phosphodiesterase inhibitors(32).

Put together, these studies suggest that the alteration lies in the mechanism of action of NO and not in the lack of its production. This fact helps us to understand how an increase in its levels can co-exist with vasoconstriction in preeclampsia. Several research studies, experimental tests and in humans, have investigated the role of this metabolic pathway in preeclampsia(33-35). Studies using phosphodiesterase inhibitors on rats have shown improvements in arterial pressure, proteinuria and birth weight, as well as a reduction in fetal mortality(33, 34). In human studies, improvements were observed in the capacity to relax of blood vessels after immersion in a solution of PDE inhibitors, these vessels having been removed from the myometrium after cesarean section in patients with preeclampsia or fetal growth restriction(36, 37). However, to date, only one clinical trial has been conducted using a PDE inhibitor on pregnant women with preeclampsia. It was observed that the use of Sildenafil from first diagnosis of PE does not significantly increase the time period between diagnosis of the disease and the subsequent birth(35).

A significant production of superoxide in preeclampsia takes place, resulting from the inflammatory response associated with this disease(11, 38, 39). It is known that NO participates in the inflammatory response through its production induced by macrophages

(iNOS)(12). However, increased quantities of NO in a medium with superoxide results in the production of peroxynitrite, which is cytotoxic to the endothelium(40). The superoxide reacts quicker with the NO than with the SOD, inactivating the superoxide(41). This suggests that the increase of NO found in this study may be harmful to pregnant women and can contribute to preeclampsia

On the other hand, some studies have found a decrease in the levels of NO in preeclampsia(8, 9, 11, 12), suggesting a change in its production as a cause(42). Dietary supplementation with L-arginine and antioxidants during pregnancy was tested on women at high risk of preeclampsia, with a decrease in the incidence of the disease being noted(43). However, a previous study evaluating pregnant with chronic hypertension showed no benefit from this(44). This indicates a need for a better understanding of the pathophysiology of preeclampsia in order to verify whether disturbances in the production of NO play a relevant role.

Finally, the fact that preeclampsia is a multifactorial disease with many forms of presentation should be considered. The difficulty in understanding the pathophysiology of this disease may be caused by the existence of more than one mechanism, which could explain the differences in the relationship between NO levels and preeclampsia that have been reported. A failure in the mechanism of action, dependent on cyclic GMP, may be a reason for the increase in NO found in women with preeclampsia.

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Table 1 – Demographic and clinical characteristics.

Variables		Normotensive **Pregnancy (n:66)**	**Preeclampsia (n:40)**	**P**
Age (years)		22.7 ±5.6	21.5 ±5.3	0.277
Ethnicity [n (%)]	White Mixed Black	52 (78.8) 10 (15.2) 4 (6.1)	27 (67.5) 5 (12.5) 8 (20.0)	0.090
Patient Weight (kg)		70.7 ±10.2	75.7 ±13.2	**0.048**
Hist. UTI [n (%)]	Yes No	37 (56.1) 29 (43.9)	16 (40.0) 24 (60.0)	0.160
FH hypertension [n (%)]	Yes No	36 (55.4) 29 (44.6)	19 (48.7) 20 (51.3)	0.548
GA at inclusion		34 ±3.5	35±3.0	0.080
Systolic AP		107±12.6	158±17.7	**<0.001**
Diastolic AP		66±8.5	102±10.1	**<0.001**
Nb weight (g)		3275±482	2400±781	**<0.001**
Nb Gender [n (%)]	Fem Mal	35 (53) 31 (47)	23 (57.5) 17 (42.5)	0.691
Apgar Score 1^{O} min		8.1±1.4	7.7±2.0	0.278
Apgar Score 5^{O} min		9.2±0.6	9.1±0.9	0.361
Placental weight (g)		640±122	499±150	**<0.001**
GA at delivery(weeks) *		39.3±1.8	36.4±3.2	**<0.001**

Hist. UTI: History of urinary tract infection; FH: Family history; GA: Gestational age; Nb: Newborn; Fem: Female; Mal: Male.
mean ± deviation; ** Student's *t*-test for independent samples; *** chi-square; **** Fisher's exact test.

Table 2 – Laboratory Data

Variables*	**Healthy Pregnancy (N=66)**	**Preeclampsia (n=40)**	**P****
Hematocrit (%)	35±2.9 (n=66)	35±3.2	0.199
Hemoglobin (g/dL)	11.8±1.0 (n=66)	11.5±1.2	0.077
Creatinine (mg/dL)	0.6±0.1 (n=51)	0.7±0.1	**<0.001**
Uric Acid (mg/dL)	3.7±1.1 (n=57)	5.9±1.5	**<0.001**
Proteinuria (g/24 hours)		3.78±3.6	

* mean ± standard deviation; ** Student's *t*–test for independent samples.

Figure 1 - Nitrites, nitrates and NOx (means in ηM; in primigravidae patients with Normotensive pregnancies (n=66) and with Preeclampsia (n=40). * p=0,01