2005

Assessment of endothelial cell dysfunction in pregnant women with either preeclampsia or preexisting vascular disease by brachial artery ultrasound

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Assessment of Endothelial Cell Dysfunction in Pregnant Women with either Preeclampsia or Preexisting Vascular Disease by Brachial Artery Ultrasound

by

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A Thesis Submitted to the

Yale University School of Medicine

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

By

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MD 2005

Assessment of Endothelial Cell Dysfunction in Pregnant Women with either Preeclampsia or Preexisting Vascular Disease by Brachial Artery Ultrasound.

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The purpose of this research was to investigate vascular dysfunction in pregnant women with either preeclampsia or preexisting vascular disease using non-invasive brachial artery ultrasound.

The first study is a matched case control of 12 preeclamptic vs. 24 normotensive pregnant women. The second study is a prospective cohort of 40 pregnant women with preexisting vascular disease (Diabetes, Chronic Hypertension, Thromboembolic disease, or previous Preeclampsia). This group represents women at high risk for developing preeclampsia.

Main outcome measurements were brachial artery flow mediated vascular dilation (FMVD) and doppler waveform analysis. In the prospective cohort, additional outcome measurements included the development of preeclampsia, the severity of disease and adverse maternal and fetal outcomes.

Brachial Artery flow mediated vascular dilation (FMVD) was significantly reduced in women with preeclampsia (4.5% ± 2.7%) compared with matched controls (9.8% ± 4.0%); $p< 0.002$. The timing of maximum dilation was variable among subjects and represents the best measurement of the difference in FMVD between the two groups. Correlation analysis between the change in Doppler waveform parameters (acceleration, acceleration time, peak systolic velocity and pulsatility index) and change in FMVD identified only peak systolic velocity (PSV) as significant; $p=0.040$. However,

comparison of change in PSV between preeclamptic and normotensive controls was not significant (13.5% ± 22.7%, vs. 20.5% ± 27.8%); p=0.432.

In summary, endothelial function is impaired in women with preeclampsia as well as in women with preexisting vascular disease who are at high risk for developing preeclampsia. These findings support the central role of endothelial dysfunction in the pathogenesis of preeclampsia. Brachial artery sonography is a useful non-invasive method of detecting endothelial dysfunction in pregnant women and may have a role in predicting the development of preeclampsia in high risk groups.

ACKNOWLEDGEMENTS

I would like to thank the Doris Duke Charitable Foundation (DDCF) and the Yale Office of Student Research for the opportunity and funding of my work through the Doris Duke Clinical research grant.

Many people have helped bring this project to fruition and I would like to extend my gratitude to the following individuals:

- Dr. Keith Williams, Hospital of St. Raphael (formerly Department of OB-GYN, Yale University School of Medicine), for his mentorship.
- Dr. Susan Richman, Department of OB-GYN, for her advise and support.
- Dr. Samuel Ramsawak and Dr. Adesh Sirjusingh, Department of OB-GYN, University of West Indies, Trinidad.
- Dr. Maria Small, Dr. Ozan Bahtiyar, Nancy Nickless, RN, Candice Kohn, RN, Maria Jorge, Janice Sanderson; Yale Perinatal Unit, Department of OB-GYN.
- Dr. Ugonna Duru (Brigham/MGH) and Dr. Myriam Fernandes (University of Pennsylvania); formerly Yale School of Medicine.
- Roman Sivion, MBA, for his technical assistance.
- Dr. John Forrest, Dr. Nancy Kim, Donna Corranzo and Cheryl DeFilippo; DDCF at Yale

Last but not least, this work would not have been possible without the strong support of my friends and family.

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INTRODUCTION

A. Preeclampsia: Definition and Dilemmas

Preeclampsia is defined as new onset of hypertension and proteinuria [1] after 20 weeks of gestation (1). It is a common complication of pregnancy, affecting between 3 to 14% of pregnancies worldwide and about 5 to 8% pregnancies in the United States, and is associated with significant maternal and fetal morbidity (2). In a recent review of 335 patients with preeclampsia, 51 (17%) developed thrombocytopenia with 26 (8%) developing the full HELLP syndrome (Hemolysis, Elevated Liver Enzymes and Low Platelets). Twenty-two patients (7.3%) had DIC, with placental abruption occurring in 10 of these patients. Seven patients developed pulmonary edema, 5 developed cerebral complications and 3 developed acute renal failure (3). One of the most feared complications of preeclampsia, is progression to eclampsia, generalized seizures, resulting in very high maternal and fetal mortality. The rates of eclampsia vary significantly depending on the severity of preeclampsia (from 0.5% in mild disease to 2% in severe preeclampsia), use of magnesium prophylaxis and patient demographics (4). Fetal complications of preeclampsia include premature delivery, low birth weight, intra-uterine growth restriction and fetal demise.

Preeclampsia is currently the third leading cause of maternal death in the United States (5). With such a high incidence of severe maternal and fetal complications of preeclampsia it is important to determine early predictors of this disease. Preeclampsia is an evolving dynamic process associated with progressive and generalized vasospasm and

[1] Hypertension is defined as either >140 mmHg systolic or >90 mmHg diastolic. Proteinuria is defined as > 300mg in a 24 hour urine specimen collection and is suggested (but not diagnostic) by a random urine protein of 30mg/dl or 1+ protein on urine dipstick.

inconsistent involvement of organs including kidney, liver, brain and the hematological system. Women at higher risk for developing preeclampsia include primigravidas, women with multiple pregnancies, and women with a strong family history of preeclampsia. Many preexisting medical conditions predispose women to preeclampsia including diabetes, obesity, chronic hypertension, vascular and connective tissue disease, thrombophilia and previous preeclampsia. However, although certain patients are at higher risk for developing preeclampsia, currently there is no way to predict who will go on to have the disease. The pathogenesis of preeclampsia, while extensively studied, is not entirely understood. Without a good understanding of the pathogenesis as well as early predictors of preeclampsia it is difficult to focus on preventive strategies.

B. Preeclampsia: Pathogenesis

The pathogenesis of preeclampsia is yet to be fully understood. It is thought to result from both fetal and maternal factors. In preeclamptic women, there is impairment in trophoblast differentiation and invasion into maternal arteries leading to placental ischemia and release of products causing maternal endothelial cell dysfunction. The primary event that contributes to failed trophoblast differentiation is unknown, but placental ischemia, immunologic factors, and genetic factors are thought to play a role (6). It is unclear if the ischemic placenta is the cause or consequence of abnormal trophoblast invasion. Pre-existing maternal impaired endothelial function is believed to contribute to the pathogenesis of preeclampsia.

C. Endothelial Dysfunction

Regardless of the initiating factors, endothelial cell dysfunction is the final common pathway in the pathogenesis of preeclampsia. The endothelial-dependent response to stress is principally regulated by the release of Nitric Oxide (NO), resulting in dilation. This endothelium-dependent response to stress is called flow-mediated vascular dilation (FMVD). An abnormality in the role of NO has been proposed as the main mechanism underling endothelial dysfunction observed in preeclampsia (7, 8). Recently, it has been shown that the likely mechanism for reduced endothelial NO activity in preeclampsia is reduced action of endothelial NO rather than decrease in production (9). Impaired FMVD has been demonstrated in a number of conditions affecting the endothelium, including atherosclerosis, hypertriglyceridemia, hyperinsulinemia, obesity and a history smoking (10).

Several medical disorders which uniquely promote endothelial cell dysfunction have been associated with a significantly elevated risk of preeclampsia. These include chronic hypertension, diabetes, thrombophila, vascular and connective tissue disease and obesity (11). Endothelial cell changes and resulting dysfunction is thought to occur early in the pregnancy, before the clinical expression of the disease as hypertension and proteinuria. To date, the best in vivo test of endothelial dysfunction has not been identified and applied early in a high-risk population to predict the development of preeclampsia.

D. Brachial Artery Ultrasound to measure Endothelial Dysfunction

Brachial artery ultrasound imaging during reactive hyperemia is a tool used widely for establishing the presence of endothelial dysfunction and has a potential role as a screening tool for coronary artery disease (12, 13). This technique provokes the release of NO, resulting in FMVD that can be quantified using high-frequency ultrasound. Many studies in non-pregnant patients with atherosclerosis and diabetes have identified impaired brachial artery reactivity (defined as a significant reduction in FMVD) which correlated with increasing endothelial cell dysfunction. This non-invasive method of endothelial dysfunction assessment has been shown to be both reproducible and accurate (14). A number of protocols and guidelines exist in the literature describing optimal assessment of endothelial dependent FMVD of the brachial artery (14, 15, 16, 17).

E. Previous Work in Brachial Artery Ultrasound and Pregnant Women

Very few studies have applied brachial artery reactivity as a measure of endothelial dysfunction in pregnant patients. Dorup et al., described the pattern of brachial artery reactivity in normal pregnancy (18). A significant increase in FMVD was noted for all trimesters, reaching the highest value in the third trimester (7.2% ± 2.8% for non-pregnant controls vs. 9.1% ± 4.0% for first trimester, 9.1% ± 3.7% for second trimester and 10.6% ± 4.4% in the third trimester). Savviddou et al., identified a significant reduction in FMVD in 37 insulin requiring diabetic pregnant women at 20 weeks' gestation compared to healthy pregnant controls (6.43% ± 3.66% vs. 9.43% ± 3.69%, $p<0.001$) (19).

There are two studies that specifically examined flow mediated vascular dilatation of the brachial artery in preeclamptic women. In a case-control study of 99 pregnant

women, including IUGR, mild and severe preeclampsia as well as normal controls, Takata et al., noted decrease in FMVD of the brachial artery for both mild and severe preeclamptic group versus controls (2.3% ± 3.7% vs. 1.6% ± 3.8% vs. 5.0% ± 3.2%; $p<0.05$) but not in the IUGR group (5.2% ± 3.1%). However, the protocol used in this study differed from standard protocols for inducing hyperemia and thus the results may not carry external validity (20). Another smaller case-control study by Kuscu et al., demonstrated a significant difference in FMVD between 15 preeclamptic and 11 normotensive women (4.26%, SEM 0.69% vs. 12.18%, SEM 1.97%; $p=0.001$) (21). The authors repeated measurement in the preeclamptic group at 2 and 6 weeks' post-partum and found a gradual increase in FMVD (6.67%, SEM 0.89% at 2 weeks' post-partum and 9.27%, SEM 1.16% at 6 weeks).

These studies both demonstrate a significant reduction in FMVD in preeclamptic women signifying impaired vascular reactivity. However, they do not elucidate the timing or duration of endothelial dysfunction. To clarify the role of maternal endothelial dysfunction in preeclampsia, Chambers et al., have examined brachial artery FMVD in women with a history of preeclampsia, at least 3 years postpartum (n=113). Interestingly, there was a significant decrease in FMVD compared with controls and the impairment of endothelial function was more severe in women with recurrent preeclampsia (0.9% ± 4.1% for recurrent group, 2.7% ± 3.5% for single episode group vs. 4.7% ± 4.3% in controls, $p<0.001$) (22). The authors hypothesize that their findings imply that "the bioavailability of endothelial nitric oxide may be reduced in preeclamptic women, even in the nonpregnant state". Their argument is further supported by transient reversal of impairment in FMVD in 15 women with previous preeclampsia, but not in normal

controls, by ascorbic acid, a powerful antioxidant. (2.6% ± 3.3% pre treatment, 5.6% ±3.0% post treatment, p=0.001).

Recently, two studies have demonstrated a significant decrease in FMVD in pregnant women who subsequently develop preeclampsia. The first study, by Savvidou et al., assessed brachial artery reactivity in a cohort of 86 women at 23-25 weeks gestation. FMVD in women who eventually developed preeclampsia (n=10) was significantly reduced (3.58% ± 2.76% vs. 8.59% ± 2.76; p<0.0001) (23). This impairment predated the development or preeclampsia symptoms by at least 10 weeks and the impairment was as great as that observed in non-pregnant patients with cardiovascular risk factors for endothelial dysfunction. The second study, by Takase et al., examined a cohort of 43 pregnant women at high-risk for preeclampsia in the second half of their pregnancy and also demonstrated a significant reduction in FMVD in women who developed preeclampsia, (1.6% ± 1.0% vs. 11.0% ± 4.5%, p<0.05). By additionally examining 14 non-pregnant age-matched controls and obtaining a normal cut-off value of 3%, the authors determined the positive predictive value for development of subsequent preeclampsia is 90% and the negative predictive value is 100% (24).

F. Limitations of previous work

The limited study of brachial artery reactivity in pregnant patients support the hypothesis that endothelial cell dysfunction in preeclampsia is demonstrated by brachial artery FMVD changes. Furthermore, endothelial cell dysfunction can be documented using non-invasive brachial artery ultrasound prior to the onset of preeclamptic symptoms. However, a number of limitations exist among previous work of brachial

artery reactivity in pregnant women. There are few studies using brachial artery ultrasound in pregnant women and, among existing studies, there is variability in protocol as well as outcome measurements of FMVD. The majority of the studies are cross-sectional case-controls with limited outcome data available. Most of the studies assess only brachial artery diameter parameters. The few that measure Doppler waveforms have had inconsistent results. Diabetics have been the only group of patients with preexisting vascular dysfunction examined during pregnancy. There have been no assessments of brachial artery reactivity in asymptomatic patients with specific high risk factors for preeclampsia, including diabetes, chronic hypertension and thromboembolic disease.

HYPOTHESIS AND SPECIFIC AIMS

Our hypothesis is that there are significant differences in flow mediated vascular dilation (FMVD) as well as Doppler waveforms in the brachial artery between preeclamptic and normotensive women and that these differences are present prior to the development of symptomatic preeclampsia.

Primary Specific Aim:

1) To compare vascular changes in the brachial artery between preeclamptic and normotensive women by measuring FMVD, an assessment of endothelial cell dysfunction.

Secondary Specific Aims:

2) To determine the gold-standard for measuring changes in brachial artery diameter among pregnant patients by assessing the time course of the hyperemic response and determining the maximum end-diastolic dilation.
3) To examine the utility of Doppler waveform analysis of the brachial artery and compare the Doppler waveforms and FMVD measurements in preeclamptic and normotensive women.
4) To investigate the role of Brachial Artery Ultrasound in predicting the development of Preeclampsia and to assess the role of maternal vascular dysfunction, as identified by brachial artery ultrasound, in determining pregnancy outcomes.

METHODS

A. Participant Recruitment

I. Study setting and recruitment

The study was conducted at two geographic settings and three hospitals: (1) Yale-New Haven Hospital (YNHH) in New Haven, CT, (2) Mount Hope Maternity and (3) Port of Spain General Hospital in Port of Spain, Trinidad. All three hospitals were affiliated with a University academic center. In Trinidad, we were working in collaboration with the Obstetrics and Gynecology Department of the University of the West Indies.

The study was advertised in the perinatal unit of YNHH as well as the obstetrical clinics of Mount Hope Maternity (see APPENDIX A for advertisement content). To identify potential participants for either the high risk cohort or preeclampsia group at YNHH, we worked closely with Candice Kohn, the Case Coordinator at YNHH, Nancy Nichols, the Diabetes Nurse, as well as attendings, fellows, residents and staff of the high risk service. Potential participants were approached either during their High Risk Clinic appointment or, if inpatient, on the wards. Interested participants were given a brochure describing the study in more detail followed by a five minute explanation of study components, including informed consent. The study time was arranged based on convenience to the patient; either at a later time or directly following recruitment. The study was conducted either at the YNHH Perinatal Unit or directly in the patient room for inpatients.

To recruit potential participants in Trinidad we worked closely with the attendings and residents at the obstetrical clinics and wards of Mount Hope Maternity as well as the inpatient staff at Port of Spain General Hospital. Port of Spain General Hospital has a separate inpatient ward consisting of 8 beds for patients with preeclampsia or gestational hypertension. In the clinics, a separate room was dedicated to the brachial ultrasound study, while on the wards the study was conducted at the bedside of the patient.

Participation in the study was strictly voluntary and under no circumstance interfered with the care of the patient. Participants were allowed to withdraw at any point during or after the study. Participants from YNHH did not receive any compensation for the study while participants from Trinidad received a small baby gift (<$5 US in value) as a token of our appreciation.

The criteria for inclusion in the Case Control Study were the following (1) Gestational Age $\geq$ 20 weeks, and either a (2) confirmed diagnosis of preeclampsia using standard clinical criteria or (3) normotensive, low-risk pregnancy with no previous history of preeclampsia, hypertension, PIH, or diabetes.

For the High-Risk Cohort study we recruited patients with one or more of the following risk factors for endothelial dysfunction and preeclampsia:

(1) Chronic Hypertension – documented blood pressure of $\geq$ 140/90 and prior to pregnancy.

(2) Diabetes – either pre-gestational or requiring gestational diabetics (DMA2). For patients with DMA2, additional risk factors such as obesity were required.

(3) Previous preeclampsia/eclampsia – documented during one of previous pregnancies.

(4) Gestational Hypertension (PIH) – hypertension developing during the current pregnancy without new onset proteinuria.

(5) Thromboembolic disease – Documented DVT or PE during this or prior pregnancy unrelated to trauma, or known thrombophilia with appropriate high risk factors.

All women were taking prenatal vitamins. Nine out of 21 women with chronic or gestational hypertension were taking anti-hypertensive medications at the time of study: lobetalol (5), nifedipine (3), and methyldopa (2). Additional medications in the high risk cohort included albuterol (5), folic acid (2), paroxetene (1) and oxycodone (1). None of the above mentioned medications have known direct effects on endothelial function.

Exclusion Criteria for both of the studies were (1) age $\leq$ 18 (2) timing of smoking < 4 hours prior to the study (3) Multiple pregnancy (4) preexisting kidney disease (5) carpal tunnel syndrome or other arm/hand disabilities and (6) medications that have the potential to interfere with endothelial function, such as nitrates (specifically, Isosorbide Dinitrite or nitroglycerin), antioxidants (Vitamins C and E) or aspirin.

II. HIC approval

HIC protocol approval was obtained prior to the initiation of the study by Dr. Williams on March 3, 2001 (HIC#12188) and amended to include myself as an investigator on August 15, 2003. The protocol was renewed by HIC on March 24, 2004 for 1 year. Approval for conduction of the study in Trinidad was obtained from the Internal Review Board of each of the hospitals and the HIC protocol was reviewed by sponsoring members of each hospital administration.

III. Informed Consent

In accordance with the HIC protocol, all participants of the study at YNHH were given full written disclosure of the project, explaining the procedure, risks, benefits, voluntary participation and withdrawal as well as assurance of confidentiality. They were asked to read and sign two forms (1) Consent for Participation in a Research Project Yale University School of Medicine- Yale-New Haven Hospital (2) Research Authorization (use and disclosure of personal information). The two forms were reviewed with each participant and all questions were answered prior to signature. Participants of the study at Mount Hope Maternity and Port of Spain General hospitals were given oral disclosure of the project in addition to a brief written explanation of the procedure, risks, benefits, confidentiality and voluntary participation. Oral consent was obtained from all participants in accordance with the Internal Review Board of the hospitals as is customary for research participants in non-invasive studies in Trinidad.

IV. Sample Size

Estimations of sample size for our primary aim were calculated using computer software available online[2]. We used values from literature to estimate sample size needed with a 2:1 ratio of controls to preeclamptics for our matched case control study. Assuming a power of 90% and significance at 5% (α=0.05), we would need between 7-10 preeclamptic women (difference in n reflects variation of values from literature) and 14-20 controls.

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[2] UCLA Department of Statistics: *calculators.stat.ucla.edu/powercalc/*
University of Iowa Department of Statistics: *www.virtualstatistician.com*

V. Ethical Considerations

As part of the data collection, we needed to include personal information about each patient such as age, ethnicity, parity, and medical history. Participants were assured the confidentiality of all personal data. Patient status was discussed with medical staff only under strict confidentiality. None of the data or results of the study were shared with the medical staff taking care of the patients. To ensure privacy, codes were substituted for identifiers in all analyses.

The risks to participants in this study were minimal. The brachial artery ultrasound is a non-invasive test. There is mild discomfort experienced during forearm brachial artery occlusion with a blood pressure cuff and all subjects were carefully monitored during the procedure to minimize any discomfort.

Under the condition of the study, we are not able to reveal to the patient or her provider the results of the study until all outcomes are collected. Since currently there are no effective prevention protocols available for Preeclampsia, it would not benefit the patient to know her risk of developing preeclampsia.

B. Ultrasound Assessment of the Brachial Artery

I was responsible for performing all ultrasound measurements and analysis at both YNHH and Trinidad (see section V, technique training for more details).

I. Equipment

All Brachial Artery scans were done using the Logiq book portable ultrasound machine with a 7MHz linear array transducer (both from GE Healthcare). A standard,

manual sphygmomanometer with a regular cuff size was used for forearm occlusion of the brachial artery at the patient's forearm (see Protocol) as well as for blood pressure measurement (using large cuff size when necessary). Data were saved as JPEG, AVI and DICOM files and were transferred from the ultrasound machine to a personal computer. All data and analysis were stored and saved on an IBM laptop personal computer with an encrypted password as well as on separate CD-R's that were kept in a secure cabinet.

II. Protocol

The following established protocol was developed from review of current literature as well as a pilot study of non-pregnant subjects (N=10).

1) Patient position

Each patient is placed in a quiet, temperature-controlled room (22-26°C) in either the supine or left lateral position for 10 minutes prior to the ultrasound exam.
A manual sphygmomanometer is placed on the forearm of the patient.

2) Baseline Diameter measurement

The right brachial artery is scanned over a longitudinal section 2-15cm above the elbow to locate the clearest image of the artery lumen. The longitudinal diameter of the brachial artery is measured from high-resolution B-mode image obtained with a 7MHz linear array transducer. The depth and gain controls are set to optimize visualization of the lumen to arterial wall interfaces. A 30 second recording of the brachial artery is obtained to measure baseline diameter.

3) Flow-restriction induction

Flow-mediated dilation is assessed by measuring the post-ischemic changes in the diameter of the brachial artery (the hyperemic state). To create the hyperemic state, the

sphygmomanometer is inflated to a pressure of 250-300mmHg. The pressure is held above 250mmHg for 5 minutes. If the subject experiences discomfort, the pressure is lowered but held at a minimum of >50mmHg above the systolic blood pressure (taken prior to the experiment). After 5 minutes, the blood pressure cuff is rapidly released to zero.

4) Flow-mediated dilation recording

The brachial artery is imaged continually using the color-flow mode from the point of cuff deflation (t=0) to 90 seconds, and again at 120 seconds.

5) Doppler Waveforms

Doppler flow velocity recordings are taken before cuff inflation (baseline) and again at 90 seconds after cuff deflation for duration of at least 3 cardiac cycles. The flow velocity was taken from the center of the brachial artery with the Doppler set parallel to artery lumen.

III. Image storage and measurements of vessel diameter

All images were recorded and stored on both the ultrasound machine HD and CD-Rom for later analysis. Data were saved as JPEG, AVI and DICOM files. Images were saved as a single folder with the assigned subject identification number and were not linked to any identifying data. Measurements were taken at a later point, with the investigator blinded to subject identification.

Measurements of the vessel diameter were taken from the leading edge of the anterior wall to the leading edge of the posterior wall of the brachial artery at the end of diastole (See Figure 1). Each measurement was recorded as the mean of 3 different points along the length of at least 0.8cm of lumen wall. The baseline (resting) vessel

diameter was calculated as a mean of two measurements between 0-30 seconds of recording. The measurements of flow-mediated hyperemic response were calculated between 10 and 90 seconds at 15 second intervals and again at 120 seconds for a total of 7 post-cuff release measurements.

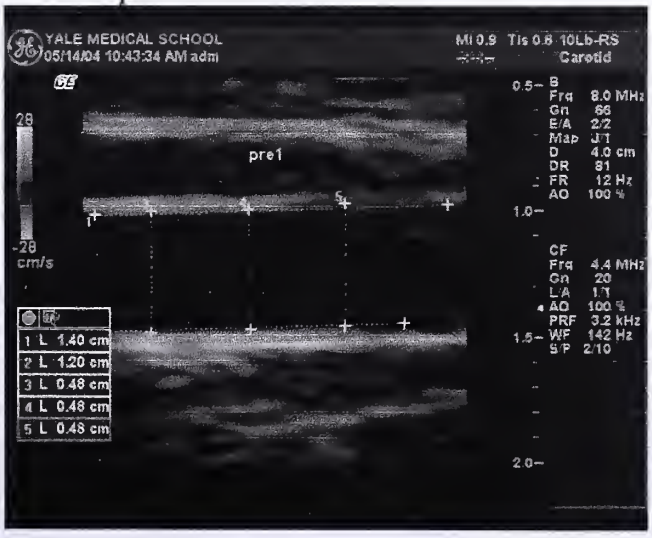
Changes in diameter at each time point were calculated as percentage change relative to the resting diameter (%FMVD):

$$\textbf{\% FMVD}_{\textbf{at x seconds}} = \frac{\text{(Vessel diameter after cuff deflation} - \text{Baseline diameter)}}{\text{Baseline diameter}} \times 100$$

The timing of Maximum FMVD (Tmax) was determined from the seven measurements between 10 and 120 seconds. If maximum FMVD was equal at two different time points, the average time between the two time points was assigned. (ex: FMVD=8.5% at both 50 seconds and 70 seconds; FMVDmax recorded as 8.5% at Tmax = 60 seconds.)

FIGURE 1. Sample measurement of Flow Mediated Vascular Dilation (FMVD)

A. Example of Baseline diameter measurement



Sample Calculation of FMVD:

Baseline Diameter=0.48 cm

Diameter at 40 seconds = 0.50 cm

$$\%\ \text{FMVD} = \frac{(0.50-0.48)}{0.48} \times 100$$

$$= \mathbf{4.2\%}$$

B. Example of hyperemic response at 40 seconds.

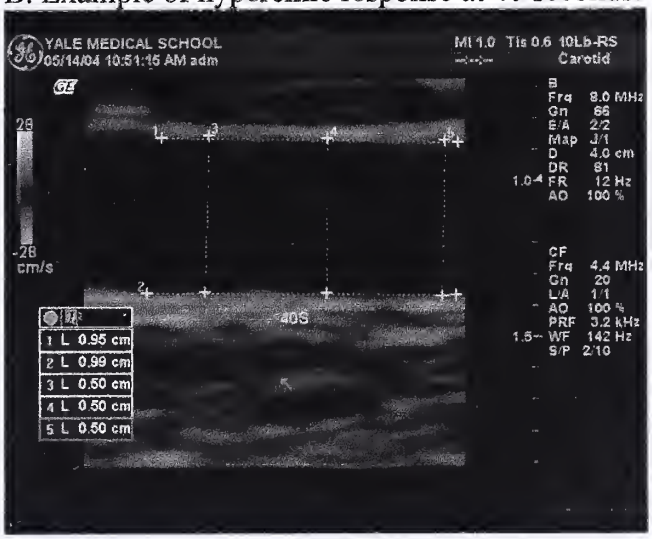


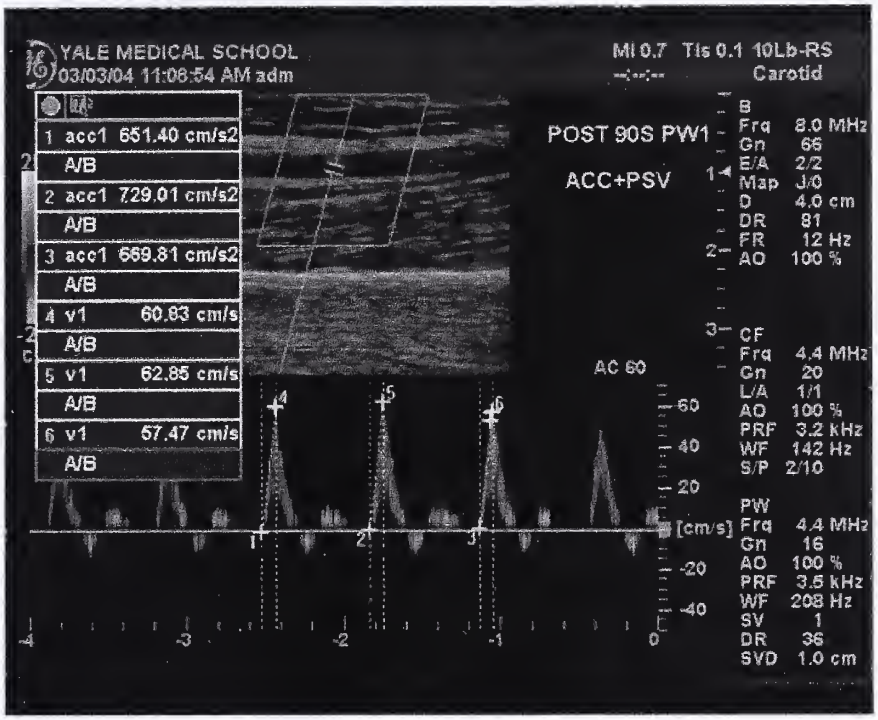
Fig 1. A sample measurement of brachial artery diameter at (A) baseline and (B) 40 seconds post cuff deflation. Each measurement is recorded as the mean of 3 different points along the length of the lumen wall. Calculation of the Flow Mediated Vascular Dilation (FMVD) is illustrated on the right.

IV. Doppler Waveforms

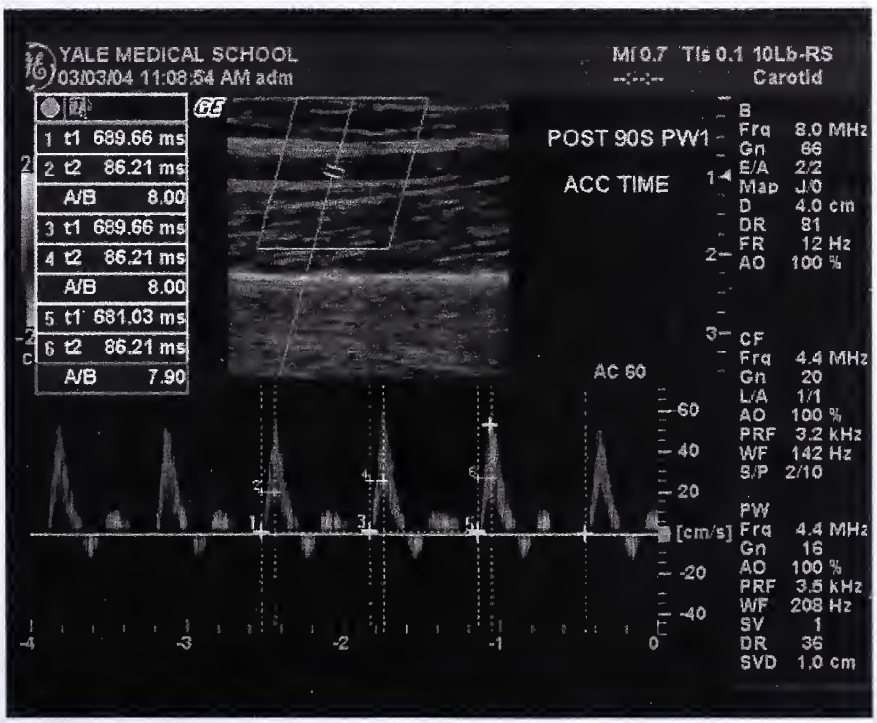
Doppler waveforms were analyzed at baseline and 90 seconds after cuff deflation. We assessed four Doppler parameters: Peak Systolic Velocity (PSV), Acceleration, Percent Acceleration Time (%AT), and Pulsatility Index (PI). Percent Acceleration Time is the time taken to achieve peak systolic velocity from end diastole. Pulsatility Index is defined as (systolic-diastolic velocity)/mean velocity. All values were taken as the average of 3 cardiac cycles. (See Figure 2).

FIGURE 2: Example of Doppler Waveform Data

A. Calculation of Acceleration and PSV at 90 seconds



B. Calculation of Acceleration Time at 90 seconds.



C. Calculation of Pulsatility Index at 90 seconds

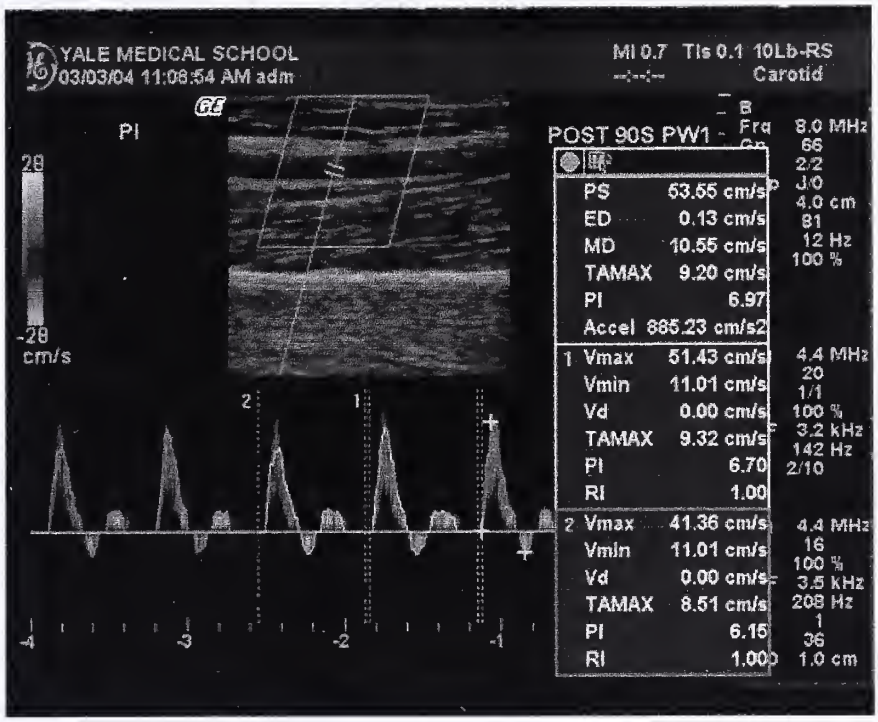


Fig 2. Illustration of Doppler Measurement of (A) Peak Systolic Velocity, PSV, and Acceleration, (B) Acceleration Time, and (C) Pulsatility Index, PI, at 90 seconds post cuff deflation. Doppler waveform calculations were obtained from both automatic (C) and manual (A and B) caliper measurements and values were taken as an average of 3 cardiac cycles.

V. Technique training

Intensive training in Brachial Artery ultrasound technique was obtained under the supervision of Dr. Keith Williams prior to scanning any study participants. One month was spent learning the technique, becoming comfortable with the ultrasound machine, properly recording data and subsequently doing accurate measurements. During this time, approximately 30 ultrasounds were performed on non-pregnant volunteers (friends

and staff of the YNHH perinatal unit). Finally, 10 trial ultrasounds were done on patient volunteers in both the perinatal unit and the ward.

C. Data Collection

Subject data were collected at the time of Ultrasound directly from patients and confirmed with subsequent chart review. Lab data were gathered from patient charts. No personal identifiers were recorded on the data collection sheets and patients identified by study number and initials only. Patient names and assigned study numbers were stored in a separate password encrypted file. To view the data collection sheets please refer to APPENDIX B.

D. Measured Outcomes

The primary outcome is the development of preeclampsia defined as 1) Blood pressure increase of >140 mmHg systolic or >90 mmHg diastolic after 20 weeks of pregnancy and 2) Proteinuria, defined as 0.3g in a 24hr urine collection or 0.30mg/dl in at least 2 random urine specimens. Charts were reviewed for official diagnosis of preeclampsia among the participants enrolled in the study. Secondary outcome measures include development of early onset or severe preeclampsia, HELLP syndrome, Eclampsia (the presence of seizures or coma), placental abruption and gestational hypertension. Fetal and neonatal outcomes include intra-uterine growth restriction (IUGR), small for gestational age (SGA), fetal demise, apgar scores and neonatal intensive care unit (NICU) admission.

E. Statistical Analysis

All data were expressed as mean ± S.D. and 95% confidence interval, when appropriate. Student's t-test was used for comparisons between two groups and ANOVA was used for comparisons among three or more groups. Pearson correlation was measured using the bivariate correlation model. Sample size calculations are described in the participant recruitment section. All other statistical analysis of the data was done using SPSS, Version 12.0 statistical package.

True-positive (TP) was defined as abnormal test results in individuals with disease (preeclampsia, adverse pregnancy outcomes); false-positive (FP) as abnormal test results in individuals without disease; true-negative (TN) as normal test results in individuals without disease; false-negative (FN) as normal test results in individuals with disease. Positive Predictive Value (or PPV) was calculated according to the formula of TP/(TP+FP) and Negative Predictive Value (or NPV) was calculated as TN/(TN+FN).

Interobserver agreement was validated by having two investigators, both blinded to identity of the subjects, measure the diameters and calculate 15 separate FMVD measurements on randomly chosen subjects. The intraclass correlation coefficient was 0.948 [CI 0.847-0.982] for N=15, representing a high level of agreement. In other words, all values fell within <6% difference between two observers (See Figure 3).

Figure 3. Interobserver Percent difference

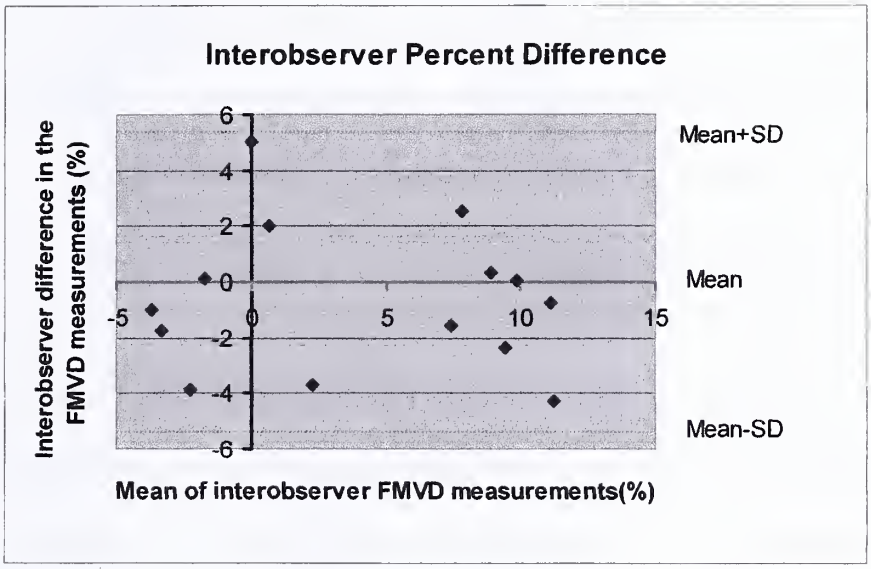


Fig. 3 Interobserver agreement demonstrated by examining the percent difference from the mean measurement between two observers for 15 measurements of FMVD. The intraclass correlation coefficient was 0.948, confirming a high level of agreement between two observers.

RESULTS

A. STUDY OVERVIEW: Clinical Characteristics and Demographic Data

A total of 87 women were enrolled in the study from both YNHH and Trinidad (See Table 1). The combined group included 12 Preeclamptic women, 30 Normotensive controls and 40 women with pre-existing vascular dysfunction who were at high risk for developing preeclampsia. Twelve women from YNHH cohort were enrolled at < 20 weeks gestational age (minimum=15.2 weeks). Six of the 12 women had a repeat scan at >24 weeks'. In comparison with subjects enrolled from YNHH, women from Trinidad were of higher gestational age, lower BMI, more likely to be nulliparous, and were less likely to have a history of tobacco use (see Table 2). Each group was a representative sample of the community clinic population.

TABLE 1. Total number of women enrolled, total with complete scans and clinical characteristics of women from YNHH, Trinidad and Combined groups.

Study patients	YNHH	Trinidad	Combined
Total number enrolled	50	37	87
Number excluded from analysis [A]	4	1	5
Total with complete scan analysis	**46**	**36**	**82**
• Normotensive Controls	10	20	30
• Preeclamptic	4	8	12
• High Risk: Vascular Dysfunction [B]	32	8	40

[A] Reasons for exclusion: 2 incomplete scans (less than 5 minutes or 200mmHg cuff occlusion), 1 incomplete consent, 1 equipment malfunction, 1 patient carrying twins)

[B] Women considered to be at high risk for developing preeclampsia had one or more preexisting diagnosis; pre-gestational diabetes, insulin-requiring gestational diabetes (DMA2), chronic hypertension, gestational hypertension (PIH), previous preeclampsia, thromboembolic disease.

TABLE 2. Demographic and Clinical Characteristics of women from YNHH and Trinidad groups.

Characteristic Mean		YNHH N=50	TRINIDAD N=37	P Value
Maternal Age (mean ± S.D.)		29.5 ± 6.1	27.7 ± 6.1	0.29
Gestational Age (mean ± S.D.)		26.7 ± 6.8	30.1 ± 5.8	*0.003*
Gravida (mean ± S.D.)		3.88 ± 3.9	2.9 ± 2.2	*0.019*
Nulliparous (n)		*6 (12%)*	*12 (32%)*	
BMI (mean ± S.D.)		29.4 ± 9.0	24.4 ± 6.1	*0.015*
				TOTALS
Ethnicity (n) [A]	**Black**	18	23	41 (47%)
	Hispanic	16	0	16 (18%)
	White	12	0	12 (14%)
	East Indian	0	10	10 (11%)
	Other	4	4	8 (9%)
Smoking Status (n) [B]	**Pre Pregnancy**	13 (26%)	1	14 (16%)
	Current	6 (12%)	2	8 (9%)

[A] Ethnicity as self identified by patients. East Indian refers to participants of South East Asian origin

[B] Current smokers were asked the time of their last cigarette and none of the subjects in the study smoked <4 hours prior to time of experiment.

B. Specific Aims 1 and 2: Case Control Summary Data

To compare FMVD in preeclamptic vs. normotensive women we selected 12 women diagnosed with preeclampsia (4 from YNHH and 8 from Trinidad) and matched each to two normotensive controls. Patients were matched 2:1 for gestational age, maternal age and ethnicity. Table 3 shows the clinical and demographic characteristics of women in the Case Control study. Note that in addition to the matching criteria, there was no significant difference in the BMI, the baseline brachial artery diameter and

smoking status of the women. Based on comparison of these characteristics we were assured that we had a well matched, diverse case-control sample.

TABLE 3. Characteristics of Controls and Preeclamptic Women

Characteristic		Controls (N=24)	Preeclamptics (N=12)	P Value
Gestational age weeks (mean ± S.D.)		30.7 ± 5.6	31.7 ± 4.7	0.58
Maternal age years (mean ± S.D.)		28.0 ± 6.9	28.3 ± 5.2	0.93
Gravida (mean ± S.D.)		2.9 ± 1.8	2.9 ± 2.6	0.95
Nulliparous (n)		7 (29%)	4 (33%)	
BMI		23.4 ± 5.1	26.3 ± 9.1	0.22
Baseline BA diameter (mean ± S.D)		3.65 ± 0.40	3.89 ± 0.52	0.143
Systolic BP (mean ± S.D, mm Hg) [A]		104 ± 14	156 ± 13	***<0.0001***
Diastolic BP (mean ± S.D, mm Hg) [A]		59 ± 11	91 ± 10	***<0.0001***
Smokers (n) [B]		2 (8%)	1 (8%)	
Ethnicity(n) [C]	***Black***	12 (50%)	7 (58%)	
	White	4 (17%)	2 (17%)	
	East Indian	6 (25%)	2 (17%)	
	Hispanic	1	0	
	Other	1	1	

Cases and controls were matched for gestational age, maternal age and ethnicity.

BA=Brachial artery, diameter measured in millimeters

[A] Blood pressure measurements taken prior to brachial artery scan.

[B] Current smokers were asked the time of their last cigarette and none of the subjects in the study smoked <4 hours prior to time of experiment.

[C] Ethnicity as self identified by patients. East Indian refers to participants of South East Asian origin

Table 4 reveals a comparison of FMVD between preeclamptic women and normotensive controls measured at 7 time points post cuff deflation. FMVD was significantly reduced in women with preeclampsia compared with matched controls at each time point after 15seconds. Individual maximum end-diastolic flow mediated vascular dilation (FMVD max) represents the best measurement of the difference in FMVD between the two groups (4.5% ± 2.7% in preeclamptics vs. 9.8% ± 4.0% in controls; $p < 0.0002$). There is a non-significant trend towards an earlier maximum FMVD in the preeclamptic group (51 ± 14 seconds) vs. controls (61 ± 26 seconds); $p=0.211$.

The time course for mean FMVD in hyperemic response in the preeclamptic and control groups is illustrated in Figure 4. The mean for FMVD does not rise above 3% in the preeclamptic group and is below 0 (representing vessel constriction) up to 30 seconds post cuff deflation and again at 120 seconds, at the end of the hyperemic response. In the control group, we have a normal rise in FMVD after 15 seconds, reaching the peak value of 7.1% at the 60-75 second interval. From Table 4 and Figure 4, it is also evident that we have a gradual rise to peak mean value between 30 and 75 seconds with wide intervals in standard deviation. Looking at individual maximum FMVD values again offers the best measurement of brachial artery hyperemic response, both within and between groups.

TABLE 4. Comparison of mean Flow Mediated Vascular Dilation (FMVD) measured at 7 time points post cuff deflation in Preeclamptic and Control group

Time (s = seconds)		N [A]		Mean (%)± SD	P value
FMVD1	0-15s	Control	23	-0.8 ± 6.5	*0.434*
		Preeclamptic	10	-2.6 ± 4.6	
FMVD2	15-30s	Control	24	4.7 ± 4.6	***0.008***
		Preeclamptic	11	-0.05 ± 4.5	
FMVD3	30-45s	Control	23	6.6 ± 4.6	***0.012***
		Preeclamptic	11	2.2 ± 4.1	
FMVD4	45-60s	Control	22	6.4 ± 4.6	***0.044***
		Preeclamptic	11	2.7 ± 4.9	
FMVD5	60-75s	Control	24	7.1 ± 5.8	***0.005***
		Preeclamptic	12	1.7 ± 3.1	
FMVD6	75-90s	Control	24	4.8 ± 5.4	***0.023***
		Preeclamptic	12	0.7 ± 3.4	
FMVD7	120s +	Control	22	3.1 ± 4.6	***0.002***
		Preeclamptic	12	-1.8 ± 2.5	
FMVD Max [B]		Control	24	9.8 ± 4.0	***0.00018*** [C]
		Preeclamptic	12	4.5 ± 2.7	
Time Max (s)		Control	24	61 ± 26	*0.211*
		Preeclamptic	12	51 ± 14	

[A] Numbers do not always correspond to 24/12 because a clear image of the lumen was not available during the 15 second interval for one or more of the subjects. All subjects had at least 5 available scan intervals.

[B] FMVD Max corresponds to maximum FMVD for each patient and Time Max is the time point for FMVD Max

[C] Equal variances assumed. If equal variances not assumed $p < 0.0001$

FIGURE 4. Time course of FMVD in Controls vs. Preeclamptics

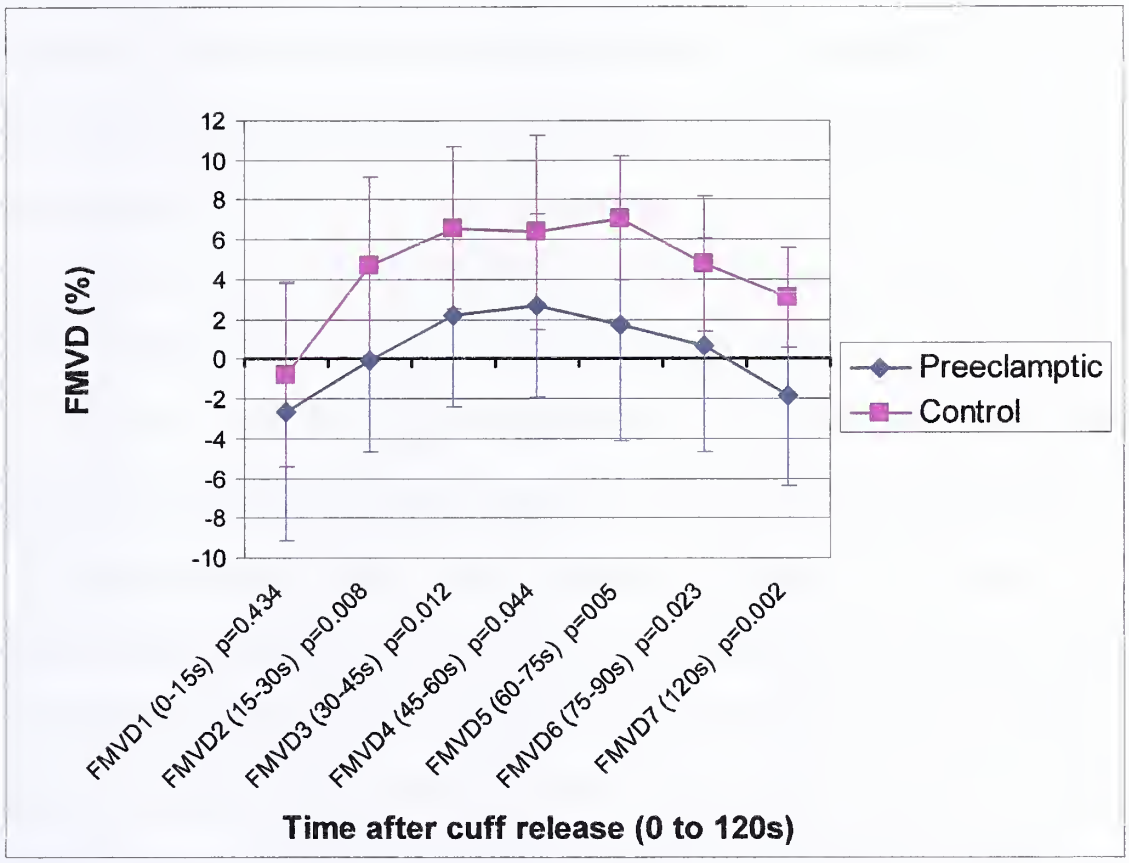


Fig. 4 Timing of reactive hyperemia in Control and Preeclamptic group. FMVD 1-7 represent percent flow-mediated vascular dilation values at each time point measurement from 0 to 120 seconds (s) post cuff release. P values are noted next to individual time points. Error bars represent standard deviation; squares and diamonds represent means of control and preeclamptic group, respectively.

One concern for combining preeclamptic patients from YNHH and Trinidad is that the combined results for FMVD might mask individual differences between the two groups. While diagnostic criteria for preeclampsia are equivalent, management of preeclampsia is different. Preeclamptic patients in Trinidad are typically not started on

Magnesium Sulfate for seizure prophylaxis and labor is not usually induced pre-term unless there are severe complications of preeclampsia. None of the preeclamptics from Trinidad in our study were treated with Magnesium Sulfate, while all four from YNHH were started on Magnesium Sulfate prior to the study. We compared FMVDmax in preeclamptics from YNHH vs. Trinidad to assess for possible differences between the two groups. There was no statistical difference in FMVDmax between the two groups (3.3% ± 2.6% for YNHH vs. 5.1% ± 2.7% for Trinidad, p=0.308).

Figure 5 illustrates the distribution of FMVD in Preeclamptic and Control groups. While we have a highly significant difference in the means of the two groups (4.5% ± 2.7% in preeclamptics vs. 9.8% ± 4.0% in controls, $p < 0.0002$), a cut-off value to distinguish the two groups could not be set. Several women in the control group outside of the 95% CI had values close to the mean of the preeclamptic group and one of the preeclamptic women had max FMVD of >10%. As this was not a longitudinal study, one possible explanation for the overlap is that a percentage of women in the Control group eventually developed preeclampsia. Since this was a low-risk population, this number is not likely to exceed 10% or 2.4 of the 24 controls and would not significantly alter our results.

Figure 5. Distribution of Max FMVD in Controls and Preeclamptics

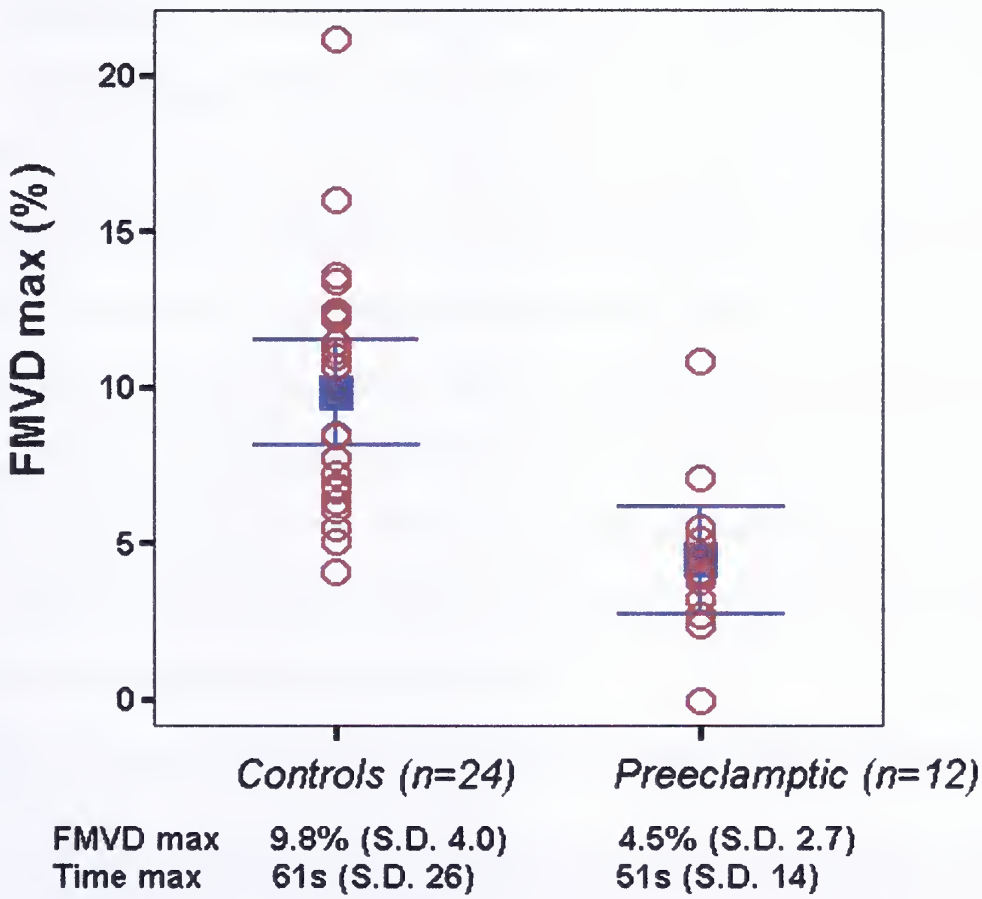


Fig. 5 Distribution of maximum Flow Mediated Vascular Dilation (FMVD max) values in Control and Preeclamptic groups. Individual values are marked as open circles. Bars show 95% Confidence Interval (CI) of the mean. The corresponding time for FMVD max in each group is noted as Time max, in seconds (s).

C. Specific Aim 3: Waveform Analysis. Is there a correlation?

We measured four separate Doppler Ultrasound parameters; Acceleration (Acc), Peak Systolic Velocity (PSV), percent time spent in Acceleration Phase (%AT) and Pulsatility Index (PI). All Doppler waveforms were done before brachial artery occlusion and at 90 seconds post cuff deflation and percentage change from baseline in value was calculated for each measurement (Δ). Baseline waveform parameters, prior to brachial artery occlusion, were no different between the preeclamptic group and normotensive controls (See Table 5). Analysis of percentage change in doppler waveforms at 90 seconds from baseline in preeclamptic women vs. normotensive controls is shown in Table 6. We used all 4 waveform characteristics in the analysis (Δ PSV, Δ Acc, Δ %AT, Δ PI) but found no statistical difference between preeclamptic and normotensive controls at 90 seconds. In contrast, FMVD at both 75-90 seconds and at 120 seconds remained significant between the two groups (see Table 4).

TABLE 5. Brachial artery Doppler waveform parameters before occlusion in normotensive controls and preeclamptics.

Waveform	Class	N [A]	Mean	Std. Deviation	P value
PSV (cm/s)	Normal	20	101.53	26.8	0.422
	Preeclamptic	12	93.99	24.7	
Acc (cm/s^2)	Normal	21	1105	282	0.905
	Preeclamptic	11	1091	329	
%AT (%)	Normal	21	13.91	2.5	0.675
	Preeclamptic	12	13.42	3.5	
PI	Normal	14	4.19	1.8	0.757
	Preeclamptic	9	4.67	4.3	

Δ PSV = change in peak systolic velocity, Δ Acc = change in acceleration, Δ %AT = change in percent acceleration time, Δ PI = change in Pulsatility Index. [A] N represents the number of subjects with available measurements.

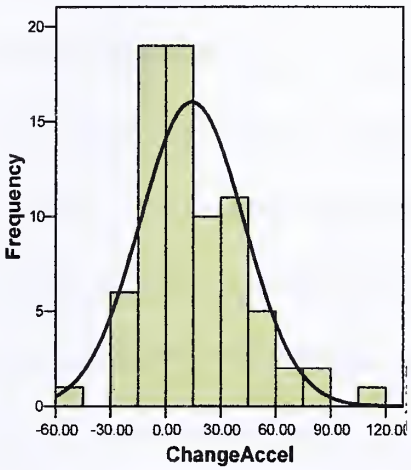
TABLE 5. Percentage change in waveform parameters in normotensive controls and preeclamptics at 90 seconds after cuff release.

Waveform	Class	N [A]	Mean	Std. Deviation	P value
Δ PSV	Normal	20	13.46	22.70	0.432
(%)	Preeclamptic	12	20.64	27.75	
Δ Acc	Normal	20	6.92	26.55	0.638
(%)	Preeclamptic	11	12.37	36.45	
Δ % AT	Normal	20	-2.49	26.77	0.580
(%)	Preeclamptic	12	2.43	18.60	
Δ PI	Normal	11	-8.35	31.11	0.307
(%)	Preeclamptic	9	18.86	70.66	

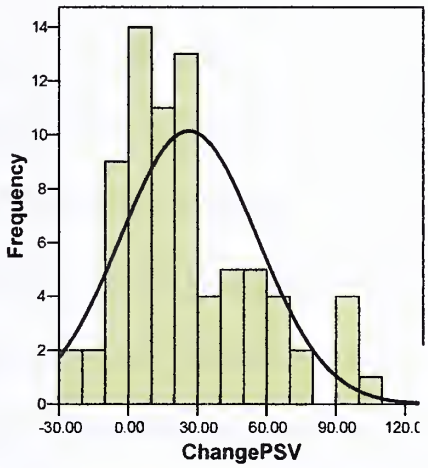
All measurements were taken at 90 seconds post cuff release and percentage change was calculated in reference to baseline values for each parameter. Δ PSV = change in peak systolic velocity, Δ Acc = change in acceleration, Δ % AT = change in percent acceleration time, Δ PI = change in Pulsatility Index. [A] N represents the number of subjects with available measurements.

To verify the normality of value distribution we examined each waveform parameter in 75 subjects with available waveform analysis data. Measurements of Acceleration, PSV and %AT were available for 75 of the subjects and measurements of PI for 60 of the subjects. The mean, standard deviation (S.D.) and distribution of values for each waveform characteristic are shown in Figure 6. It can be seen from the histograms that there is a large variation around each mean and that the S.D. is greater than the mean for each of the four waveform characteristics. Overall, there was minimum change from baseline for both %AT and PI (<10% increase) and only a moderate change for Acceleration (<15%). However, with a wide distribution around the mean, a large number of cases with change of 50% or more were identified.

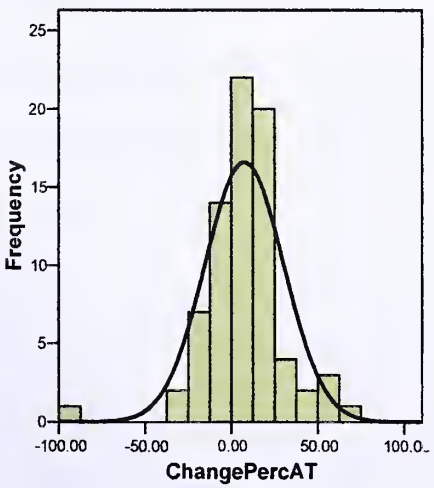
FIGURE 6: Frequency Histograms showing distribution of values for each of the Waveform Characteristics: (A) Change in Acceleration, (B) Change in PSV, (C) Change in Percent Acceleration, and (D) Change in PI



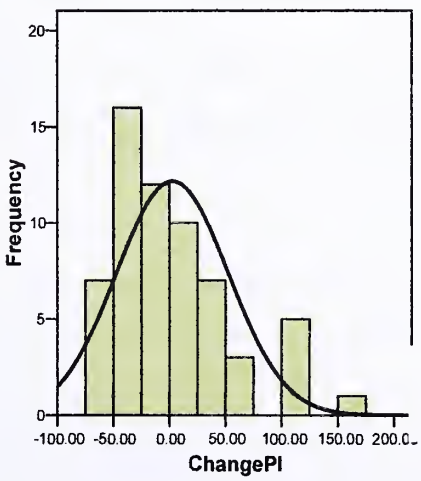
6A. Change in Acceleration (ΔAccel): ***Mean=14.3 ± 28.4% (N=75)***



6B. Change in Peak Systolic Velocity (ΔPSV): ***Mean=26.2 ± 29.9% (N=75)***



6C. Change in Percent of Acceleration Time (ΔAT): ***Mean=7.4 ± 22.9% (N=75)***



6D. Change in Pulsatility Index (ΔPI): ***Mean = 2.4 ± 50.0% (N=60)***

Next, we were interested to see if there was a correlation between FMVD and any of the Doppler waveform characteristics among the 75 subjects. Table 6 illustrates correlation analysis for each waveform characteristic (Δ Acc, Δ PSV, Δ %AT, Δ PI) as compared to FMVD. In our correlation analysis we used values for both FMVD6 (from 75-90 seconds, mean=86±4 seconds), FMVD7 (at 120 seconds) as well as the average of FMVD6/FMVD7 (mean = 103 ± 4 seconds). Although we could not obtain the exact value for FMVD at time of Doppler waveform measurement (The ultrasound machine did not allow both Doppler waveform and flow assessments to be done simultaneously), the average of FMVD6/ FMVD7 likely represents the closest approximation since Doppler was measured 90 seconds after cuff deflation and it took approximately 10 seconds to obtain correct Doppler measurements. It can be seen from the correlation analysis in Table 7 that only ΔPSV significantly correlated with both FMVD7 and average of FMVD6/FMVD7 (pierson coefficient 0.223, 0.239, $\alpha = 0.04$, respectively). To determine how well ΔPSV predicted FMVD we used a Scatter plot with linear regression through the origin to determine the best fit equation (See Figure 7). Based on the Scatter plot and the pierson coefficient values it is evident that ΔPSV at 90 seconds is not strongly correlated with FMVD and cannot be used as an accurate independent predictor of FMVD.

TABLE 7. Correlation between Doppler Waveforms and FMVD in 75 brachial artery scans.

Time post cuff release and mean FMVD	Waveform parameter	N	Pierson coefficient (p)	Alpha (2-tailed)
FMVD6 ***(t.ave=86± 4 seconds)*** [B]	Δ PSV	75	0.227	0.0503
	Δ Acc	75	0.143	0.222
Mean=4.31% ± 5.2%	Δ % AT	75	0.121	0.861
	Δ PI	60 [A]	-0.170	0.195
FMVD7 ***(t=120 seconds)***	Δ PSV	75	***0.223***	***0.0441***
	Δ Acc	75	0.043	0.716
Mean =1.59% ± 4.5%	Δ % AT	75	0.134	0.250
	Δ PI	60 [A]	-0.235	0.071
Average of FMVD6/FMVD7 ***(t.ave=103 ± 4seconds)*** [B]	Δ PSV	75	***0.239***	***0.0401***
	Δ Acc	75	0.097	0.412
Mean = 2.99% ± 4.5%	Δ % AT	75	0.074	0.532
	Δ PI	60 [A]	-0.214	0.104

[A] N = 60 for PI, 15 scans did not have Pulsatility Index available.

[B] t.ave = average time during the specified period for all scans. i.e., t.ave for scans done between 75 and 90 seconds was 86± 4 seconds and t.ave for scans between 75 and 120 seconds was 103 ± 4seconds.

Δ PSV = change in peak systolic velocity, Δ Acc = change in acceleration, Δ %AT = change in percent acceleration time, Δ PI = change in Pulsatility Index.

FIGURE 7. Scatter plot of Mean FMVD6/FMVD7 vs. ΔPSV

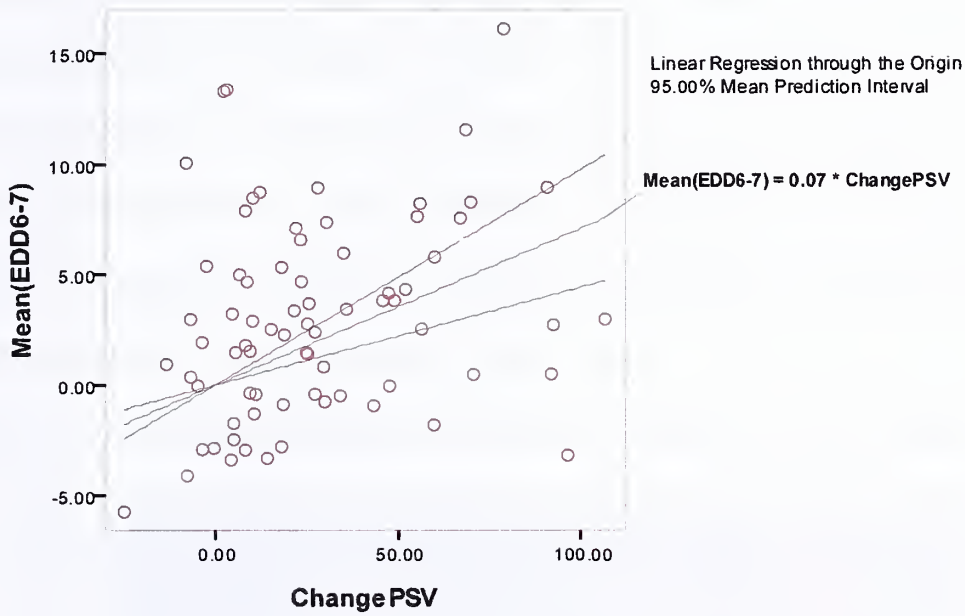


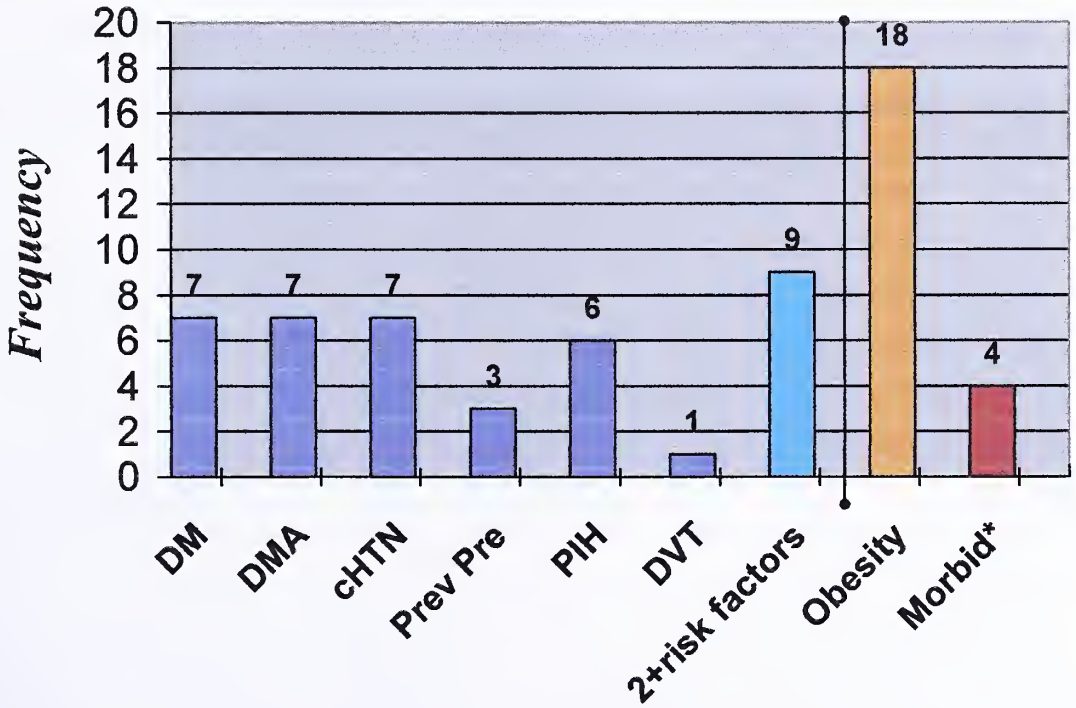
Fig. 7 Graphic representation of the correlation between change in Peak Systolic Velocity (PSV) at 90 seconds and FMVD between 75 and 120 seconds (average t =103 ± 4 seconds). Pierson Correlation=0.239, α=0.04.

D. Specific Aim 4. High Risk Cohort: Preliminary data from YNHH

The clinical characteristics of women in the high risk cohort (N=40) for developing preeclampsia are summarized in Figure 8. Insulin requiring gestational diabetics (DMA2) were included in this group if they had additional risk factors for developing preeclampsia (obesity, advanced maternal age > 35 (AMA), grandmultipara) or had early very elevated glucose values indicating that they were likely previously undiagnosed pre-gestational diabetics. Nine of the women in the high risk cohort had multiple risk factors for preeclampsia and a complete tally of their risk factors is included

in Figure 8. Obesity (defined as pre-gestational BMI ≥30), while a known high risk factor for both vascular dysfunction and preeclampsia, was not included in the selection criteria for the high risk cohort. While many women in the high risk cohort were also obese they did not carry this diagnosis in the chart and were referred to the high risk clinic for other reasons. We did calculate the BMI of all participating subjects and 45% of our high risk cohort women were obese with a 10% prevalence of morbid obesity (defined as pre-gestational BMI ≥ 40). In contrast, only 13% of normotensive controls (low risk) had BMI ≥ 30 with no morbidly obese women.

FIGURE 8. Total Subjects in High Risk Cohort by Risk Factors and Obesity



LEGEND	2+ Risk Factors:
DM = pre-gestational diabetes	
DMA = gestational diabetes (DMA2)	3 HTN/PIH
cHTN = chronic hypertension	2 DMA/cHTN
Prev Pre = previous preeclampsia	1 DMA/PIH
PIH = gestational hypertension	1 DM/Prev Pre
DVT = DVT during this pregnancy	1 DM/HTN
Obesity = defined as BMI ≥30	1 DM/cHTN/PrevPre
Morbid = morbid obesity, BMI ≥40	

At the time of analysis, outcome data were available for 28 of the 32 women in the High Risk Cohort at YNHH. Six out of 28 women developed preeclampsia (21%) and two women developed gestational hypertension. Severe complications of preeclampsia occurred in 3 of the 6 women (see Table 8). These included HELLP syndrome, eclampsia, and abruption leading to fetal demise at 29 weeks. In addition to preeclampsia other adverse outcomes such as gestational hypertension, abruption and intrauterine growth restriction (IUGR) are likely associated with vascular dysfunction. Table 9 includes a complete list of adverse pregnancy outcomes in our cohort that were likely related to vascular dysfunction. Eleven out of 28 women, or 39% of the cohort, had adverse outcomes during their pregnancy. Note that 3 women (11%) suffered fetal demise due to either severe preeclampsia, abruption or early gestational hypertension.

TABLE 8. Clinical Characteristics and Outcome of High Risk Women who Developed Preeclampsia

High Risk Class	GA at study [A]	GA at delivery	Outcome
Pre-gestational DM, Previous Preeclampsia	15 and 24 wks	29 wks	Severe preeclampsia, abruption, IUFD
Gestational DM	32 wks	41 wks	Preeclampsia
Pre-gestational DM	18 and 34 wks	37 wks	Preeclampsia
Pre-gestational DM	18 and 32 wks	35 wks	Severe preeclampsia and HELLP
Chronic Hypertension	19 and 34 wks	37 wks	Post-partum Preeclampsia
Previous Preeclampsia	24 wks	36 wks	PIH, eclampsia

[A] GA = gestational age as written in patients' chart. All scans done under 20 weeks were repeated at ≥ 24 weeks.

TABLE 9. List of Adverse Pregnancy Outcomes [A]

Maternal Morbidity	Maternal and Fetal Morbidity
• 3 Preeclampsia	• 1 Preeclampsia +abruption + IUFD
• 1 Preeclampsia/HELLP	• 1 PIH + IUFD
• 1 PIH/Eclampsia	• 1 abruption + IUFD
• 1 abruption/hemorrhage	• 1 PIH + IUGR
• 1 new onset PIH	

[A] Outcomes unrelated in pathogenesis to underlying vascular dysfunction were not included in analysis (i.e. chorioamnionitis, PPROM, PTL)
IUFD = intrauterine fetal demise. IUGR = intrauterine growth restriction

Table 10 shows maximum FMVD based on pregnancy outcome among the 28 women in the High Risk cohort. Although the mean in the group that developed preeclampsia was reduced, it was not significantly lower compared to the women who remained normotensive (6.2% ± 5.2% vs. 10.1% ± 5.6%, p=0.152). When the group of women who had an adverse pregnancy outcome was compared to the group with a favorable pregnancy outcome, the difference in FMVD was highly significant (4.5% ± 4.2% vs. 11.4% ± 4.9%, p < 0.001, See Table 10). Figure 9 illustrates the distribution of maximum FMVD in the adverse and favorable outcome groups. A cut off point of <5% in FMVD was selected for the normal FMVD cutoff value (represented by the dotted blue line) in Figure 9. This cut off is based on previous values in the literature indicating high likelihood of endothelial dysfunction.

TABLE 10. FMVD max by Pregnancy Outcome

Outcome group	N	FMVD max (%)		P value	95% CI
		Mean	± S.D.		
Normotensive [A]	20	10.06	5.55	*Compared with normotensive*:	
Preeclamptic	6	6.18	5.24	*0.152*	[-1.74, 9.51]
Favorable Outcome	17	11.39	4.89	*Compared with favorable:*	
Poor Outcome	11	4.52	4.23	***<0.001***	[3.27, 10.47]

[A] Two women with new onset gestational hypertension (PIH) were excluded from analysis.

FIGURE 9. Distribution of FMVDmax based on pregnancy outcomes

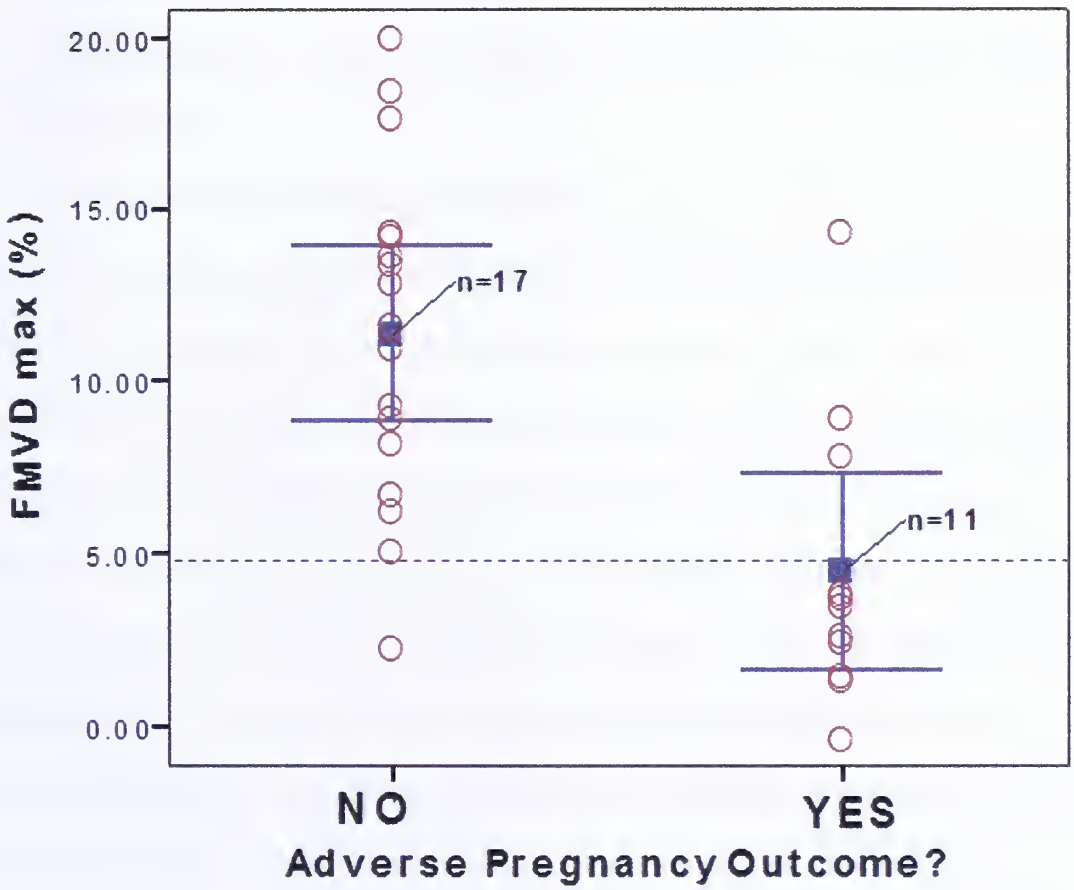


Fig 9. Distribution of maximum Flow Mediated Vascular Dilation (FMVD max) in the high risk cohort based on adverse pregnancy outcome. Individual values are marked as open circles. Bars show 95% Confidence Interval (CI) of the mean. Dotted Blue line represents the selected cut-off point of <5% FMVD.

DISCUSSION

Endothelial dysfunction is recognized to play a central role in the pathogenesis of preeclampsia. We have shown that Brachial Artery Ultrasound is a non-invasive, reproducible method for measuring endothelial dysfunction in pregnant women with preeclampsia and may have utility in predicting the future development of preeclampsia in high risk patients.

We have demonstrated a significant reduction in flow mediated vascular dilation (FMVD) in women with preeclampsia compared with matched controls ($4.5\% \pm 2.7\%$ vs. $9.8\% \pm 4.0\%$; $p < 0.002$). This reduction is consistent with several other studies in the literature (20, 21). However, existing studies using brachial artery ultrasound in pregnant women do not use a standardized protocol for inducing brachial artery hyperemia and have variable criteria for measuring FMVD. While all studies demonstrate a reduction in the preeclamptic group, the mean in reduction is variable. Some of the variation is likely due to differences in population and severity of preeclampsia. However, without standardized technique and outcome measurements, it is difficult to discern the external validity of the studies.

There needs to be a gold-standard for measuring FMVD as well as for the protocol for inducing brachial artery hyperemia. From the review of cardiovascular literature using Brachial Artery Ultrasound we have concluded that it is necessary to maintain forearm cuff occlusion at $\geq$ 250mmHg (or at least 50mmHg $\geq$ systolic pressure) for 5 minutes (10, 12, 13, 14, 15). These requirements are outlined in the guidelines set

by the international brachial artery reactivity task force (16) and should be followed in all future studies involving pregnant women.

Standards for optimal measurement of FMVD are more difficult to determine due to a change in pattern of hyperemic response of the brachial artery during pregnancy. It has been shown in both in-vitro and in-vivo studies that FMVD is enhanced in normal pregnancy (18, 25). Measurement of FMVD in the non-pregnant subject is typically taken at 60 seconds post cuff release and represents the maximum hyperemic response (10, 12, 13, 14, 15). However, by examining the hyperemic response at 15 second intervals from 0 to 90 seconds and at 120 seconds, we have shown that there is a wide variation in the timing of maximum FMVD (61 ± 26 seconds) for normotensive pregnant women. Additionally we noted a trend towards earlier timing of maximum FMVD in preeclamptic women (51 ± 14 seconds; p=0.211). Takata et al., have also found a significant decrease in recovery time for hyperemic response in preeclamptics (13). Thus, care should be exercised in the interpretation of FMVD in pregnant women as the timing of maximum end-diastolic dilation may vary from patient to patient and subgroups with vascular dysfunction (such as preeclamptics). We conclude that individual maximum end-diastolic flow mediated dilation (FMVD max) represents the optimal measurement of FMVD in pregnant women.

Overall, our case-control study was superior to previous studies in the literature based on several key aspects including optimal study design, protocol and outcome measurements. Firstly, preeclamptics were matched 2:1 to normotensive controls based on gestational age, maternal age, and ethnicity and had similar BMI, rate of smoking, and baseline brachial artery diameter. Secondly, we had a diverse ethnic sample of

population from two unique settings, New Haven and Port of Spain, Trinidad. The diversity of our sample maximizes the external validity of our study. Thirdly, we followed a strict protocol for induction of brachial artery hyperemia that has been extensively validated in the cardiovascular literature. Lastly, we used individual maximum FMVD for outcome measurement, which represents the optimal measurement of FMVD in pregnant women.

In addition to examining FMVD, we also looked at changes in Doppler waveform parameters (peak systolic velocity, acceleration, percent acceleration time, and pulsatility index). Correlation between FMVD and change in Doppler waveforms identified only the change in Peak Systolic velocity (Δ PSV) as significant ($p=0.040$). We did not find a difference in Δ PSV between preeclamptic and normotensive controls. As Doppler measurements were taken at 90 seconds, they did not correspond to timing of maximum hyperemic response.

Several investigators have examined various Doppler waveform parameters with conflicting results. Chambers et al. found no change in PSV between the previously preeclamptic and normotensive group (22). Takata et al. measured PI at maximum FMVD and noted a decrease in PI among women with severe Preeclampsia (20). In contrast, using a different protocol for inducing hyperemia, Veille et al., have demonstrated increase in PI in women who developed Preeclampsia (26). Most recently, Williams and Kocer showed a significant increase in PI as well as percent acceleration time (%AT) in preeclamptic women (27). Each of the studies varied in respect to protocol for inducing hyperemia as well as the time of Doppler measurement. Thus, it is difficult to draw any conclusions from the existing literature. Waveform parameters were

previously measured by Williams and Kocer at 30-45 seconds after cuff deflation. However, since our primary aim was to examine the time course of FMVD and we could not simultaneously measure waveform parameters and vessel diameter, we only calculated changes in Doppler waveforms at 90 seconds, after the peak of the hyperemic response. In future assessments of utility of Doppler ultrasound of the brachial artery hyperemic response, Doppler measurements should be appropriately measured earlier (prior to 60 seconds), corresponding to the peak of flow mediated vascular dilation.

In our cohort study, we set out to predict Preeclampsia based on FMVD reduction. We enrolled a total of 40 pregnant women in our high risk cohort (32 from YNHH and 8 from Trinidad). Pregnancy outcomes were available for 28 of YNHH patients at the time of analysis. Outcomes from Trinidad have not been received. Six of the 28 women developed preeclampsia (21%), an expected rate among the high-risk population in our cohort. Based on the sample size for outcomes to date of 28, the study did not have the power to be used in a predictive fashion. Indeed, we did not find a significant difference in maximum FMVD among the women who developed preeclampsia vs. those who did not. However, this is most likely a reflection of the low power due to small sample size (powered at only 33%). Using current means for difference in FMVD and S.D., we estimate that to achieve a power of 80% we would need 15 women to develop preeclampsia to detect a difference in maximum FMVD. Based on 20% average prevalence in our population, this would require a sample size of 75 high risk patients for our cohort.

Two studies have examined women at high risk for developing preeclampsia and have demonstrated a significant reduction in FMVD in women who developed

preeclampsia. The first, by Savvidou et al., enrolled 86 women in London, UK, at 23-25 weeks' gestation and looked at development of both IUGR and preeclampsia (23). Both women who developed IUGR (n=14) and preeclampsia (n=10) had a significant reduction in FMVD (3.58% ± 2.76% for preeclamptics vs. 6.17% ± 2.82 for IUGR vs. 8.59% ± 2.76% for normal outcome, $p<0.0001$ and <0.004 respectively). In the second study, by Takase et al., 43 high risk women in Saitama, Japan were enrolled during the second half of pregnancy (24). These women were high risk for having previous Preeclampsia based on either having renal dysfunction, family history of preeclampsia or hypertension, primigravity or advanced maternal age. Nine of 43 women (21%) developed preeclampsia and had a significant reduction in FMVD, measured at 60 seconds post cuff deflation (1.6 ± 1.0% vs. 11 ± 4.5%; $p<0.05$). The authors used at 3% cut-off value for FMVD to calculate a 90% positive predictive value (PPV) and 100% negative predictive value (NPV) for predicting the development of preeclampsia using brachial artery ultrasound technique.

Our high risk cohort differed from that of Takase et al., for several reasons. Half of our cohort had either pre-gestational or gestational diabetes as one risk factor. Our mean gestational age was lower than in their study. We had half as many women with previous preeclampsia. All of these reasons likely explain why Takase et al., were able to show a significant reduction in FMVD in women who subsequently developed preeclampsia in a small cohort of 43 women. Additionally, they were working with a homogeneous population of Japanese women while we had a diverse ethnic population. Differences in brachial artery hyperemic response based on ethnicity have not been examined in the literature but may account for a larger variation in FMVD in our cohort.

We did not achieve the statistical power to determine a significant difference for women who developed preeclampsia in our preliminary analysis. However, we found a striking difference in FMVD in a comparison based on adverse pregnancy outcomes. Eleven patients in our cohort had adverse pregnancy outcomes likely related to vascular dysfunction with three suffering intrauterine fetal demise (IUFD). The reduction in FMVD in the group with adverse outcomes (n=11) vs. favorable outcomes (n=17) is highly significant at a power of 94% (4.52% ± 4.23% vs. 11.39% ± 4.89%; $p < 0.001$). Using our previously established cut-off for maximum FMVD of 5%, the PPV of having and adverse pregnancy outcome is 90% and the NPV is 84%. No previous studies have examined the utility of brachial artery ultrasound in predicting adverse pregnancy outcomes related to vascular dysfunction.

When we examined the two outliers in FMVD values for preeclamptic group (FMVD=10.9%, see Fig. 5) and women who developed Preeclampsia in our high risk cohort (FMVD= 14.5%, see Fig 9), interestingly, both values were attributed to women who also had the HELLP syndrome with preeclampsia. These were the only cases of co-existing HELLP syndrome among our participants. We did have one preeclamptic women develop the HELLP syndrome post-partum, one week after the study and had reduced FMVD (2.4%). Fischer et al. examined forearm vascular reactivity in patients with Preeclampsia and HELLP syndrome and noted a reduction in vasodilation in the Preeclamptic group but no change in the HELLP syndrome and Preeclampsia group as compared to normotensive controls (28). Elevated FMVD values in our two preeclamptic women with co-existing HELLP syndrome appear to be consistent with Fisher's study results. Additional studies comparing FMVD response in preeclamptic

patients with and without the HELLP syndrome are needed to confirm these findings. We speculate that different pathogenic mechanisms may underlie the development of the HELLP syndrome.

In the future, we plan to complete our outcome analysis for all 40 subjects currently enrolled in our cohort study. We also hope to recruit more patients to the high risk cohort to achieve adequate power for the prediction of preeclampsia (a minimum of 35 additional participants). We would also like to examine Doppler Waveform parameters at an earlier time point, prior to maximum FMVD response. The small number of patients in our current cohort did not allow us to look at FMVD in specific high risk groups, such as diabetics, chronic hypertensives, obese women and women with previous preeclampsia. A larger cohort will allow us to examine the degree of endothelial dysfunction in specific high risk groups.

In conclusion, our results support the hypothesis that pregnant women who develop preeclampsia have impaired endothelial dysfunction as measured by brachial artery ultrasound. Preliminary analysis suggests that the impairment is present prior to development of clinical symptoms and brachial artery ultrasound represents a non-invasive tool to predict the development of preeclampsia and other adverse outcomes due to vascular impairment in high risk populations. Further research is needed to examine the degree of underlying endothelial dysfunction during pregnancy in separate high risk groups. Based on the literature and our preliminary data, there is strong potential for using brachial artery ultrasound to predict the development of preeclampsia and other vascular dysfunction related adverse outcomes. Current research using brachial artery

ultrasound has illuminated our understanding of the role of endothelial dysfunction in pregnancy and more studies applying this technique in pregnancy are necessary.

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PUBLICATIONS BASED ON THESIS WORK

1) Landres, I., Small, M., Ramsawak, S., Sirjusingh, A., Williams, K. 2004. Vascular dysfunction in preeclampsia assessed by brachial artery ultrasound. *AJOG*. 191-6:S40 (Abstract # 108). Poster presentation at the Society for Maternal-Fetal Medicine 25th annual meeting in Reno, Nevada on February 10, 2005.

Paper manuscript submitted to *AJOG* on March 1, 2005.

2) Landres, I., Small, M., Ramsawak, S., Sirjusingh, A., Williams, K. 2004. A comparison of doppler waveform parameters versus flow-mediated vascular dilation of the brachial artery in preeclamptic and normotensive women. *AJOG*. 191-6:S41 (Abstract #109). Poster presentation at the Society for Maternal-Fetal Medicine 25th annual meeting, Reno, Nevada on February 10, 2005.

3) Landres, I., Williams, K. 2004. Assessment of endothelial dysfunction in preeclampsia by brachial artery ultrasound.

Poster presentation at the Doris Duke Clinical Research Fellows Meeting, Asilomar, CA in June, 2004.

APPENDIX A:

Advertisements of research study

1. **Flyer content (used at YNHH perinatal unit).**

2. **Flyer content (used at Mount Hope Maternity prenatal clinics).**
   Note that flyer was modified to include maternal Trans-Cranial Doppler Study by Dr. Williams as well as small gift compensation.

3. **Brochure, English version** (modified contact info for Trinidad)

4. **Brochure, Spanish version** (translation verified by YNHH certified Spanish translator).

Are you here for a prenatal visit?

You Are Invited:

To participate in a research study on high blood pressure during pregnancy

- We will do an ultrasound of your arm to look at blood flow changes in pregnancy
- Ultrasound is safe and presents no risk to you or your baby
- The session takes about 15 minutes
- Please let your health provider know if you are interested in participating.

You can contact us for further questions:

Inna Landres
(page) 766-0557

Keith P. Williams, MD
(203)785-3091

Are you here for a prenatal visit?

You Are Invited:

To participate in a research study on high blood pressure during pregnancy

- We will do an ultrasound of your arm and your head to look at blood flow changes in pregnancy

- Ultrasound is safe and presents no risk to you or your baby

- The session takes about 30 minutes

- Please let your health provider know if you are interested in participating

To thank you for your time and help you will receive a baby gift of your choice.

Questions and Answers:

What is Preeclampsia?

• Preeclampsia is a disorder characterized by development of high blood pressure during pregnancy. Preeclampsia can harm both the mother and her baby and it is the leading cause of maternal and infant illness around the world.

What does the study involve?

• We will be using an ultrasound machine to examine blood flow in your arm before and after inflation with a blood pressure cuff

What is the time commitment?

• It should take about 15 minutes each visit
• We would like to see you twice during your pregnancy

What are the risks and benefits?

• Ultrasound is safe and presents no risk to you or your baby
• Blood pressure cuff inflation might cause mild discomfort
• By participating in this study you will help to better our knowledge of how to treat future patients with preeclampsia

Is my personal information kept secret?

• All information we get about you will be kept confidential and your name will be known only to the researchers involved in this study.

For more information:

YOU CAN CONTACT US

- **Inna Landres, YMS**
  (page) 766-0557
  inna.landres@yale.edu

- **Keith P. Williams, MD**
  (203) 785-3091

- If you have questions concerning your rights as a research subject you may contact the Human Investigation Committee at 203 785-4688

You are invited:

TO PARTICIPATE IN A RESEARCH STUDY

- **To find women who have or may develop blood pressure problems during pregnancy (preeclampsia)**

- **We will use an ultrasound test of your arm to measure blood flow changes during your pregnancy**

- **Each session takes about 15 minutes and we can coordinate it with your prenatal visits**

Preguntas y Repuestas

Qué es Preeclampsia?

• Preeclampsia es una enfermedad caracterizado por empiezamiento del alta presión sanguínea durante el embarazo. Preeclampsia puede dañarse la madre y su bebé y es una mayor causa mundial del enfermedad maternal y infantil.

Qué tipo del estudio es?

• Vamos a utilizar el ultrasonido para examinar el flujo sanguíneo del brazo antes y despues del inflación con un aparato para medir la preción

Qué es la obligación del tiempo?

• Cada sesión dura unos 15 minutos
• Queríamos verle dos veces durante su embarazo

Qué son los riesgos y beneficios?

• El ultrasonido no presenta ningun riesgo a Ud. o su bebé
• La inflación con un aparato para medir la presión puede causar molestia menor
• Su participacion en este estudio vaya a ayudarnos entender como mejor atender pacientes con preeclampsia

Va a estar segura mi información personal?

• Sí, toda su información estará confidencial y su nombre va a estar conocido solamente a los investigadores del este estudio

Para mas informacion:

- **Inna Landres, YMS**
  page: (203) 766-0557
  inna.landres@yale.edu

- **Keith P. Williams, MD**
  (203) 785-3091

- Si tiene preguntas sobre sus derechos como participantes de investigación, puede llamar al Comité del Investigación Humano (Human Investigation Committee) al (203) 785-4688

Le invitamos:

A PARTICIPAR EN UN ESTUDIO DE IVESTIGACIÓN

- **Para encontrar mujeres quien tiene o van a tener problemas con presión sanguínea durante su embarazo (preeclampsia)**

- **Vamos a utilizar el ultrasonido del brazo para medir los cambios del flujo sanguíneo durante su embarazo**

- **Cada sesión dura unos 15 minutos y podemos coordinarla junta con sus visitas prenatal**

APPENDIX B

DATA COLLECTION

1. **Participant data collection sheet**

2. **Maternal/Perinatal Outcomes data collection sheet**

YALE UNIVERSITY SCHOOL OF MEDICINE
YALE NEW HAVEN HOSPITAL MEDICAL CENTER
DATA COLLECTION SHEET
PREDICTION OF PREECLAMPSIA –STUDY

DATA ENTERED BY: **DATE:**

Study Number _____ **Initials** _____
EGA/EDD _____
Date of Birth ____ / ____ /_____
Pre-pregnancy weight _____lbs or _____ kg
Height _____ ft _____inches or _____ cm
Ethnicity Hispanic/White/Black/Asian/Other _____
Cigarette Use No_____ Yes _____ No. Years ____
Amt/day pre-preg____ Amt/day preg ____
Alcohol Use No_____ Yes _____
Amt/day pre-preg____ Amt/day preg ____
Drug Use No_____ Yes _____ Specify:_____

MEDICAL HISTORY

Check here if NONE ☐

1. Diabetes	**Check if yes**				
Class:	**previous pregnancy**	**current pregnancy**	**Mother**	**Sisters**	**Other**
A1 = gestational					
A2 = gestational-insulin					
B = < 10 yrs; after 20 y/o					
C = 10-19 yrs; 10-20 y/o					
D = > 20 yrs; before 10 y/o					
F = renal disease					
H = coronary artery disease					
R = retinopathy					
T = renal transplant recipients					
2. Hypertension					
3. Thromboembolic disease					
4. Coagulopathy					
5. Preterm delivery					
6. Preeclampsia					

Medical History	Check if yes In general	Check if yes During this pregnancy
7. Renal disease		
8. Heart disease		
9. Chron. Hypertension		
10. Thyroid disfunction		
11. Tromboembolic disease		
12. Anemia		
13. sickle cell anemia		
14. Other hemoglobinopathy		
15. Coagulopathy		
16. Pneumonia		
17. SLE/CTD/joint diseases		
18. Drug Sensitivity		
19. Allergies_____		
20. Blood transfusions		
21. Asthma		
22. Liver, GI disease		
23. Rh incompatibility		
24. Other (specify)		

PAST OBSTETRIC HISTORY

Grav.	Term	Preterm ≥ 20wks	SAB < 20 wks	TAB	Ectopic	Multiples	Live Children

PREVIOUS PREGNANCIES

Preg. #	GA @ delv	Date / Yr	Birth Wt.	Sex	Outcome 1-7	Complication 8-18	Placental Pathology 19-21
1							
2							
3							
4							
5							
6							

1: Vaginal
2: forceps
3: vacuum
4: c-section
5: miscarriages
6: elective termination
7: med. Indicated termination
8: spont PTL
placenta
9: PPROM
10: PTD for maternal indications
11: PTD for fetal indications
12: Preeclampsia
13: IUFD
14: IUGR
15: abruptio
16: hypertension
17: diabetes
18: Other
19: chorio
20: abruption
21: infarcts

Polyhydramnios/Oligohydramnios	_____	GA at Dx	_____
IUGR	_____	GA at Dx	_____
Preeclampsia	_____	GA at Dx	_____
PIH	_____	GA at Dx	_____
PTL	_____	GA at Dx	_____
PPROM	_____	GA at Dx	_____
		GA at Tx	_____
Gest. DM	_____	GA at Dx	_____
diet control	_____		
insulin control	_____		

Comment: _____
_____
_____

MEDICATIONS
Check (3) if yes

____ anti-HTN	____ analgesics	____ sedatives/anti- depressants
____ insulin	____ antibiotics	____ hormones
____ anti-asthma	____ steroids	____ antifungal medication
____ thyroid-medication	____ heparin	
____ diet-supplements	____ LMWH (*low molecular weight heparin)* started @ _____wks	
____ prenatal vitamins/ minerals/iron	____ low dose aspirin	dose _____
	____ tocolytics	

____ INH/Vit B6

other _____ _____ _____
_____ _____
_____

Maternal/Perinatal outcome variables:

Study Number_____ **Initials**___ **Date**_____

1. EGA admission to study_____ wks

2. **Diagnosis**
(may cite more than one)

PIH _____
Preeclampsia _____
HELLP _____
DM _____
Oligohydramnios _____
IUGR _____
Other (specify) _____

3. **Complications in labor and delivery**

1=Eclampsia
2=DIC
3=Acute Renal Failure
4=Fetal death
5=HELLP
6=Concealed hemorrhage
7=Maternal pyrexia
8=Anesthetic accident
9=vertical incision
10=rupture of old scar
11=maternal fits
12=infected liquor
13=membranes ruptured >24 hr
14=cord round neck
15=cord prolapse
16=true knot in cord
17=retained placental
18=placenta previa diagnosed in labor
19=Other

Intrapartum care with antihypertensives
Treatment with MgSO4
Highest uric acid
Platelet count
Elevated liver enzymes
24 hour protein
Serum Creatinine elevated
Serum urea elevated
Hemoglobin
Hematocrit
Fibrinogen

PREGNANCY OUTCOME

Termination Spontaneous/Elective @ GA_____ wks

If yes:

Elective Genetic Medically indicated

reason_____

Date delivery _____ / _____ / _____

GA at delivery _____ weeks _____days

AROM SROM

Induction Indication

Mode of delivery:

Vaginal / Forceps / Vacuum / C-section /

If operative, what was indication: _____

Intrapartum Complications

Temp>100.4 Abruption Meconium Vag. Bleeding

Cord Prolapse FHR requiring delivery FHR.160bpm>10mins Antibiotics

Other_____

Placental Pathology (if available):

chorioamnionitis _____

abruption _____

infarcts, vascular pathology _____

Placental weight _____

Other _____

Postpartum Complications

Anemia PPH Retained Placenta manual removal /curretage

Systemic Infection UTI Wound Infection CS/Episiotomy

Other: _____

PERINATAL OUTCOME VARIABLES

Neonatal Outcome
Check (3) if yes

Baby A		**Baby B**	
Ballard	_____**Wks.**	**Ballard**	_____**Wks.**
Birthweight	_____gram	Birthweight	_____gram
Apgar score	_____1_____5	Apgar score	_____1_____5
Cord PH	_____	Cord PH	_____
Sex	Boy / Girl	Sex	Boy / Girl
Chartnumber child:	_____	Chartnumber child:	_____
IUFD (*intrauterine fetal death)*	_____	IUFD (*intrauterine fetal death)*	_____

Adverse neonatal outcome
Check (3) if yes

	Baby A	**Baby B**	**Baby C**	**Baby D**
RDS				
TTN				
IVH				
BPD				
NEC				
Hypoglycemia				
Hypocalcemia				
Hyperbilirubinemia				
Sepsis suspected				
Sepsis confirmed				
LBW (< 2500 gr)				
VLBW (< 1500 gr)				
SGA				
LGA				
Demise				
ROP stage/zone				
NICU duration				
NND				
Days of Hospitalization				
NICU + step down				

PRENATAL LABS

TEST	RESULTS	Date	comments
GC	**Pos / Neg**		
Chlamydia	**Pos / Neg**		
Urine C & S	**Pos / Neg**		
B Strep	**Pos / Neg**		
Creatinine clearance (mL / min)			
Urine 24 hrs protein (mg/24 hr)			
Hgb (< 20 wks)g/dL			
Hct (< 20 wks) %			
MCV (< 20 wks)fL			
RDW (< 20 wks)%			
AFP(in MOM)			
hCG(in MOM)			
E3(in MOM)			
Folate ng/ml			
B12 pg/mL			
RPR	(non) reactive		
HIV	NEG/POS		

ADMIT Tests/Labs

TEST	RESULTS	Date	comments
BP (range, highest)			
Urine Protein			
Hgb/Hct			
Platelets			
Uric Acid			
BUN/Creatinine			
LDH			
BiliD/BiliT			
AST/ALT			
ALK			
PT/PTT/INR			
Beta Strep			
HIV			
Cultures			

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