MAURITIUS RESEARCH COUNCIL

DIAGNOSTIC TEST FOR PREECLAMPSIA AT SSRNH IN MAURITIUS INOSITOL PHOSPHOGLYCAN-P TYPE

Final Report

December 2013

MAURITIUS RESEARCH COUNCIL

Address:

Level 6, Ebène Heights,
34, Cybercity,
Ebène 72201,
Mauritius.

Telephone: (230) 465 1235
Fax: (230) 465 1239
Email: mrc@intnet.mu
Website: www.mrc.org.mu

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PI Name and Address

Dr Lalita Dawonauth,

Department of Medicine,

Faculty of Science,

University of Mauritius,

MAURITIUS RESEARCH COUNCIL

FINAL REPORT

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LIST OF ABBREVIATIONS

AU	Arbitrary Units
AUC	Area under the Curve
BMI	Body Mass Index
BP	Blood Pressure
CHT	Chronic hypertension
DM	Diabetes Mellitus
ELISA	Enzyme Linked Immunosorbant Assay
GDM	Gestational Diabetes Mellitus
GH	Gestational Hypertension
HELLP	Hemolysis, Elevated Liver enzymes, low platelets
IADPSG	International Association of Diabetes and Pregnancy Study Groups
IPG-P	Inositol phosphoglygan-P type
MOH&QOL	Ministry of Health& Quality of Life

OR	Odds Ratio
PCR	Spot protein to creatinine ratio
PE	Preeclampsia
PIH	Pregnancy Induced Hypertension
ROC	Receiver Operating Characteristic curve

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SUMMARY

Objectives of this study:

- To determine the level of the molecule IPG- P type at clinical diagnosis of preeclampsia.
- To predict the occurrence of preeclampsia 1 week, 2 weeks and 4 weeks before clinical diagnosis of PE.
- To determine sensitivity, specificity, positive and negative likelihood ratios of the IPG-P test in preeclampsia

Introduction

There has been a study on the predictive value of the molecule inositol phosphoglycan-P (IPG-P) type in preeclampsia (PE). The study in Mauritius at the Sir Seewoosagur National Hospital has been done on 1050 pregnant women and the predictive value of the test has been established at diagnosis, 1 week and 2 weeks prior to the development of the disease at a significance value of $p<0.0001$ and at 4 weeks prior to PE at $p=0.01$.

Results

Number of cases confirming the predictive ability of the test: 82

Number of false positives: 1

Number of false negatives: 2

Preliminary results on 416 women have already been published in Journal of Reproductive Immunology: (Dawonauth et al 2014)

TableI : Diagnostic and predictive characteristics of the IPG-P test

	4 weeks before diagnosis	2 weeks before diagnosis	1 week before diagnosis	At diagnosis
Cut-off value of IPG/creatinine (U/nmol)	4.42	13.11	13.42	31.02
Area under curve	0.6316 (0.5116 – 0.7516)	0.8301 (0.7403 - 0.9199)	0.9119 (0.8739 – 0.9499)	0.9519 (0.9316 – 0.9721)
Odds ratio	3.048 (1.310 – 7.093)	20.23 (6.244 – 52.59)	44.00 (5.587 – 346.5)	

Relative risk	2.946 (1.295 – 6.701)	18.12 (6.785 – 60.34)	41.31 (5.324 – 320.5)	
Sensitivity	69.23% (48.21% - 85.67%)	82.61% (61.22% - 95.05%)	90.91% (58.72% - 99.77%)	95.65% (87.82% - 99.09%)
Specificity	57.53% (54.04% - 60.96%)	80.99% (78.11% - 83.63%)	81.48% (78.63% - 84.10%)	90.99% (88.80% - 92.87%)
Positive predictive value	0.0497 (0.0297 – 0.0775)	0.1098 (0.0674 – 0.1662)	0.0625 (0.0304 – 0.1119)	
Negative predictive value	0.9831 (0.9670 – 0.9927)	0.9939 (0.9846 – 0.9983)	0.9985 (0.9916 – 1.000)	
Likelihood ratio	1.630	4.345	4.909	10.61

Further Study

We would like to develop a dipstick test on urinary samples to predict preeclampsia before clinical diagnosis of the condition.

Further aims and objectives:

Extend the test to approximately 11,000 pregnant women yearly.

This would cost the Ministry of Health & Quality of Life Rs 8 million for an ideal number of tests of 12/pregnant woman done at each antenatal clinic appointment as from 20 weeks of pregnancy.

Importance of preeclampsia

Consequences of PE:

a) Maternal: morbidity / mortality
    i. Short term:
        1. Eclampsia

2. Cerebral hemorrhage
3. Disseminated Intravascular coagulation
4. HELLP
5. Acute Renal failure
6. Pulmonary edema
7. Pulmonary embolus
8. Adult respiratory distress syndrome
9. Cortical blindness
10. Abruptio placentae

ii. Long term:

1. Chronic hypertension
2. Ischemic heart disease
3. Stroke

b) Fetal: morbidity/ mortality

i. Short term:

1. Stillbirth
2. Spontaneous /iatrogenic abortion
3. Intrauterine growth restriction

iii. Long-term:

1. Neurodevelopmental delay

Implementation

Since we find that preeclampsia affects a significant proportion of pregnant women and there is a discrepancy between the prevalence as quoted in the literature and Mauritius, we believe that it is high time that we should screen pregnant women with this new test for PE.

I. Reasons for this discrepancy in Mauritius:

1. Chronic hypertension is at a prevalence in women at around 35%

2. Diabetes Mellitus is around 20%
3. Obesity around 20% and overweight women around 56%
4. Women carrying out leisure physical activity: only 16.5%

II. The average age of first pregnancy in women has increased due to women postponing reproduction in favor of their career.

III. Women have to cope with running a household as well as performing at work and this increases their level of stress.

IV. Now more and more women with diabetes and hypertension are interested in getting pregnant.

We shall envisage 3 scenarios in the implementation of the IPG test in the prediction of preeclampsia:

Scenario	**No. Of tests/ patient**	**Coverage: % patients tested X no.patients**	**No. Of patient tests**	**Costs in Rs**
A	3	8%x11,000	2640	159,000 (2% ideal no. of tests)
B	6	40%x11,000	26,400	1,590,000 (20% ideal no. of tests)
C	12	80%x11,000	105,600	6,340,000 (80% ideal no. of tests)

Scenario A:

We would be screening only women at very high risk of preeclampsia, those with established hypertension and proteinuria.

Advantages: low cost

Disadvantages: High risk of missing women with no clinical factors indicating impending preeclampsia.

Scenario B:

We would be screening only primiparas, those who are obese, diabetic, chronic hypertensive, previous history of hypertensive disorder of pregnancy, present multiple pregnancy.

Advantages: medium cost, less risk of missing PE

Disadvantages: Some risk of missing PE women

Scenario C:

We would screen almost 100% women at all visits

Advantages: Minimal risk of missing PE

Disadvantages: High cost, need to establish PE protocol to which medical/nursing staff need to adhere.

All scenarios: A,B and C will imply that hospital staff i.e Obstetricians , Intensive Care Unit doctors and medical staff of Accident& Emergency, midwives and other nurses looking after pregnant women will have to adhere to established protocol for preeclamptic women (or those suffering from complications of preeclampsia).

Laboratory technical staff will have to be formed on how to carry out laboratory tests for IPG-P.

I certify to the best of my knowledge (1) the statement herein (excluding scientific hypotheses and scientific opinion) are true and complete, and (2) the text and graphics in this report as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or of individuals working under their supervision. I understand that willfully making a false statement or concealing a material fact in this report or any other communication submitted to MRC is a criminal offense.

Principal Investigator Signature:	Date: 23/6/2014
DR LALITA DAWONAUTH L. Dawonauth	

Presentation of preeclampsia at SSRNH in Mauritius: a 2 ½ year survey starting December 2010.

Dawonauth Lalita[1], Rademacher Laurens[2], L'Omelette Arnaud Dominique[1], Jankee Sarojini[1], Lee Kwai Yan Man Yoon[1], Jeeawoody Razgia B[1]., Rademacher Thomas W.[3]

Abstract

This study was carried out to get an insight of the pregnancy characteristics of a sample of pregnant women presenting at one regional hospital, the Seewoosagur Ramgoolam National Hospital with its catchment area being the Northern part of the island of Mauritius.

The authors evaluated the benefit of using quantitative spot protein to creatinine ratios and IPG/creatinine ratios in the diagnosis and prediction of preeclampsia. The IPG/creatinine ratio was a highly sensitive and specific test for preeclampsia at diagnosis (AUC 0.9519 (95% CI 0.9316-0.9721) p value <0.0001, cut-off value 31.02, sensitivity 95.65%, specificity 90.99%, positive likelihood ratio 10.61), 1 week before clinical diagnosis (AUC 0.9119 (95% CI (0.8739-0.9499),p value <0.0001, cut-off value 13.42, sensitivity 90.91% (95% CI, 58.72% to 99.77%), specificity 81.48% (78.63% to 84.10%), positive likelihood ratio 4.909, 2 weeks before clinical diagnosis (AUC 0.8301 (95%CI 0.7403-0.9199), p<0.0001) cut off value, 13.11, sensitivity 82.61% (61.22% to 95.05%), specificity 80.99% (78.11% to 83.63%) positive likelihood ratio 4.345, 4 weeks before diagnosis (AUC 0.6316(95%CI 0.5116-0.7516),p value =0.01, cut-off value 4.42,

sensitivity 61.54 (40.57% to 79.77%), specificity 62.81% (60.94- 64.65%), positive likelihood ratio= 1.66

The IPG/ creatinine ratio test is a reliable diagnostic and predictive test.

Introduction

Mauritius is a small island in the Indian Ocean. The estimated resident population of the island of Mauritius by the end of 2011 was 1,250,349. In the same year 14,002 live births were registered, giving a crude birth rate of 11.2/1000 mid-year resident population. There are 5 main regional hospitals in the island, with a total of 2,328 beds, where the health service is free. In the private sector, there are 17 private health institutions with a total of 706 beds.

Hypertensive disorders of pregnancy account for considerable maternal morbidity and mortality after hemorrhage and abortion in Mauritius. Using quantitative proteinuria, preeclampsia is a pregnancy complication that afflicts approximately 8.2% of all pregnancies in Mauritius.(Dawonauth et al., 2014).

The number of maternal deaths registered in 2011 was 5, and the maternal mortality rate per 100,000 livebirths was 36 in 2011.("stats-reports-2011.pdf,") (MOH&QOL, Health Statistics Report, 2011).

Current tests employed to detect preeclampsia are qualitative measurements of proteinuria by dipstick. In some cases when BP levels and dipstick proteinuria levels of these women come under control, these women get sent home. A few come back in

emergency to the hospital with diagnoses such as eclampsia and abruptio placentae . In some cases these emergencies are accompanied by fetal or neonatal deaths.

Blood pressures are easily measured in antenatal clinics. Cnossen et al published a metaanalysis in 2008,(Cnossen et al., 2008) that included 34 studies to determine the accuracy of using systolic and diastolic pressures to predict preeclampsia. In the second trimester, at a specificity of 90%, the sensitivities of diastolic and systolic blood pressures were only 35% and 24% respectively. The conclusion was that blood pressure measurements in the early trimesters had very modest predictive capacities for detecting preeclampsia (Lindheimer et al., 2009).

In Mauritian hospitals, proteinuria is assessed by dipstick assay and although 24H proteinuria is accepted by international consensus among obstetricians, this is rarely assessed in our local context. Dipstick analysis is largely done in an unsupervised fashion by nurses. This is due to the low cost and ease with which it can be performed. Waugh et al's data on urines from hypertensive women found a high false negative rate where up to 65% of women with <1+ proteinuria on dipstick analysis had significant proteinuria (Maybury and Waugh, 2005). This suggests that the correlation between dipstick urinanalysis and 24-hour protein estimation is, at best, imprecise.

In order to sidetrack the above problems, we have found that urinary IPG level is an attractive candidate to study in preeclampsia. It shows a high sensitivity and specificity at clinical diagnosis of preeclampsia and as a biomarker can predict preeclampsia 4 weeks before clinical diagnosis (Dawonauth et al., 2014).

Materials and Methods:

Patients were recruited at SSRN Hospital in the northern part of the island as described in (Dawonauth et al., 2014).

This prospective, longitudinal study took place at the SSRNH with the approval of the National Ethics Committee of the Ministry of Health & Quality of Life. Written, informed consent was obtained from recruited patients.

1050 pregnant women attending the antenatal clinic at SSRNH were initially recruited, but complete records were available on only 1008 pregnant women. All patients were followed up longitudinally in this study, with urine samples collected every 1-4 weeks. On the day of a routine morning visit, the baby's gestational age, mother's level of BP, history of DM±CHT, and any previous history of HBP, PIH or PE were determined. The weight, height and BMI were noted. Proteinuria was determined with dipstick. Urinary specimens were collected and stored at +4°C for up to 4 days, then transported and transferred to -20°C until required for assay. Urine samples were collected in the morning as a midstream specimen, at regular visits at the clinic, on the days of admission, and, in the case of preeclamptic patients, post-partum.

The IPG-P content was assayed blind in triplicate using a previously described ELISA-based assay (Williams et al, 2007) modified to assess the levels in a quantitative fashion (Scioscia et al. 2012). The IPG-P levels were divided by creatinine levels to measure the IPG/creatinine ratio in U/nmol. Morning MSU were also assayed for protein and creatinine levels.

Urine samples were collected at regular visits of the patient at the clinic and the IPG/creatinine levels calculated. IPG levels were assessed quantitatively using an ELISA method and adjusted to creatinine in order to calculate the IPG/creatinine ratio in U/nmol.

Gestational hypertension (GH) was diagnosed if patients had a systolic blood pressure of ≥140 mmHg or higher and/or a diastolic blood pressure ≥90 mmHg or higher, on 2 occasions (at least 6 hours apart) after 20 weeks of gestation, but did not have a protein/creatinine ratio greater than 30.0 mg/mmol or albumin dipstick ≥+1 . Patients with chronic hypertension were not included in this group. Preeclampsia was diagnosed if the woman had gestational hypertension with significant proteinuria. Gestational diabetic women (GDM) did not have chronic DM or gestational hypertension or preeclampsia. GDM women were classified by obstetricians according to the latest International Association of Diabetes and Pregnancy Study Group criteria.

Results

We recruited 1050 pregnant women at SSRN hospital, after getting their written informed consent, but complete information was available for only 1008 pregnant women.

1008 pregnant women were included in this study: of these, 878 were non-preeclamptics (87.1%) of whom 596 (59.2%) had normal pregnancies, 85 (8.4%)

developed preeclampsia of whom 13 (1.29%) had both GDM and PE; 128 (12.7%) with only gestational hypertension and 80 (7.9%) developed only gestational diabetes.

Table 1 shows the patient characteristics in terms of age group, ethnicity, preexisting chronic and gestational diseases and diagnoses during the current pregnancy. The pregnant women were also assessed with respect to tobacco and alcohol consumption. Table 1 shows that the majority of pregnant women did not smoke or consume alcohol.

There was a weak history of family related hypertension during pregnancy (1.88%) but a strong family history of chronic hypertension (35.1%) and type 2 Diabetes Mellitus (37.7%).

We are having an increasing number of pregnancies in older nulliparas, hence following the present trend in the Western World.

The ethnic origin of our pregnant sample is similar to the ethnic distribution in Mauritius, where Hindus, Tamil, Telegus, Marathis and Muslims are grouped together as being of Indo-Mauritian ethnic group. The Creole group is very diverse group where people have originated from Madagascar, East and West Africa and have intermarried with other groups, thus there is a reasonable admixture in this group.

Spot urine protein/ creatinine ratios (PCR) were significantly higher in PE than in controls at booking and at delivery ($p<0.0001$). IPG/ creatinine levels were significantly higher in preeclamptics than in controls, at booking and at delivery ($p<0.0001$).

The clinical characteristics expressed non-Gaussian distribution. Spearman's correlation was carried out for the search of relationship between the clinical characteristics and IPG/creatinine ratio. There was a weak correlation of IPG/creatinine with maternal age, maternal weight and body mass index as well as with the gestational age of the baby.

There was an association with a previous history of pregnancy induced hypertension ($p=0.01$). No association was found between smoking and IPG/creatinine levels. No association was found between the baby's sex and median IPG/creatinine levels in mothers.

ROC curves were generated to identify cut-off values for IPG/creatinine ratio at diagnosis and for a period at least two weeks but not more than one month prior to diagnosis (predictive period). At diagnosis, the sensitivity and specificity of the IPG/creatinine test is high with a high positive likelihood ratio and negligible negative likelihood ratio, at a cut-off value of 31.02 AU/nmol. 1 week prior to diagnosis the predictive value of the test, that is, sensitivity and specificity were also high at a cut-off value of 13.42 AU/nmol. 2 weeks and 4 weeks prior to diagnosis, cut-off values were respectively 13.11 and 4.42 AU/nmol The cut-off values of IPG/creatinine 2 weeks prior to diagnosis and at diagnosis were significantly different ($p<0.001$).

Discussion

We describe in this study the presentation of preeclampsia at one hospital in the northern part of the Island of Mauritius. For a more thorough study we should have

included patients from other regional hospitals where delivery of pregnant women is also carried out. However, we were limited in terms of finance and manpower available to conduct such a study.

The prevalence of preeclampsia in our sample is 8.4%, when we used quantitative proteinuria with a cut-off value of 30 μg/μmol. This prevalence is higher than the prevalence found from 1980-2010 in the USA in a recent study (Ananth et al., 2013). Our sample was derived from a low to medium risk group for preeclampsia, as women who were completely healthy were less likely to voluntarily give ≥1 samples of urine.

Also given that diabetes, obesity and hypertension are three of the strongest risk factors for PE, high rates of PE in populations with higher proportions of diabetic, obese and hypertensive women would be expected. This island is well known for its very high prevalence of diabetes mellitus, obesity, chronic hypertension, dyslipidemia, metabolic syndrome and ischemic heart disease. Indeed, the prevalence of diabetes presented in the last Non-Communicable Diseases (2009)(" ncd-2009.") report would give Mauritius the second highest figure of any country in the world. Maternal factors predisposing to preeclampsia include, gestational diabetes mellitus : OR: 3 (Suhonen and Teramo, 1993) chronic hypertension: OR 9 (Rey and Couturier, 1994) and obesity OR 5.2 (95%CI 2.4-11.5)(Ros et al., 1998).

According to data derived from the United States natality statistics for 1993, the total number of pregnancies among women 35 years or older is increasing especially among

nulliparas. This shift in child -bearing patterns, which is also occurring to a certain extent in Mauritius, may be due to marriage or remarriage later in life or because of delaying pregnancy until completion of educational or career goals (Sibai, 2001).

Smoking rate during pregnancy is very low in Mauritius considering the fact that women get very strong messages from the culture, the media and health workers to avoid smoking. On the other hand smoking among men is very prevalent. A small proportion of women (6.55%) continued taking alcohol during the index pregnancy but 1.09% quit alcohol when they became pregnant.

Blood pressures are already elevated at systolic 130.6± 22.05mm Hg/ diastolic 81.61± 15.94 mm Hg at booking at the antenatal clinic in hospitals. These values may be because some women are booking late during their pregnancies. In control women, systolic BP is 111.6± 9.39, diastolic 69.5± 7.14 mm Hg. Even in non-pregnant women during their reproductive years, the author regularly observes young women who are living healthily with a systolic BP of 90 mm Hg .

10.71% of our sample had a previous history of pregnancy induced hypertension. Recurrence of a hypertensive disorder in pregnancy is a well-known phenomenon. (Sibai)

The IPG urinary test has several criteria to make it a useful diagnostic test for preeclampsia including the fact that it is a simple, non-invasive test. It is an easy to perform test at any time in pregnancy and in cases where there is any doubt as to the diagnosis, this assay can be used as a confirmatory test. It is a highly sensitive and

highly specific test with a very high positive likelihood ratio and negligible negative likelihood ratio. In its current ELISA format, it provides a potentially low- cost test, the price of which could be further reduced if made into a dipstick format. When tested two weeks prior to clinical diagnosis, this assay maintained a high sensitivity and specificity, 84.2% and 83.6% respectively, with AUC at 0.862. This is a minimum predictive estimate since in a few cases, the levels of IPG-P rose very rapidly, within days, to attain very high levels. The time of initial rise in IPG levels prior to clinical diagnosis of preeclampsia can be very variable, but it does not rise as early as the first trimester as shown by a paper from Scioscia (Scioscia et al., 2011), who found that in the first trimester, IPG levels were not elevated in women who subsequently developed preeclampsia. In contrast amniotic fluid levels may be high during this period and placental leakage defects not sufficient enough to result in maternal pathology (Scioscia et al., 2007)(Burton et al., 2011). We observed that some PE women had cyclical elevations in IPG/creatinine levels and when levels of IPG were measured on consecutive days, the levels of IPG remained high and values were not spuriously elevated.

REFERENCES

Ananth, C.V., Keyes, K.M., Wapner, R.J., 2013. Pre-eclampsia rates in the United States, 1980-2010: age-period-cohort analysis. BMJ 347. doi:10.1136/bmj.f6564

Burton, G.J., Scioscia, M., Rademacher, T.W., 2011. Endometrial secretions: creating a stimulatory microenvironment within the human early placenta and implications for the aetiopathogenesis of preeclampsia. J. Reprod. Immunol. 89, 118–125. doi:10.1016/j.jri.2011.02.005

Cnossen, J.S., Vollebregt, K.C., de Vrieze, N., ter Riet, G., Mol, B.W.J., Franx, A., Khan, K.S., van der Post, J.A.M., 2008. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. BMJ 336, 1117–1120. doi:10.1136/bmj.39540.522049.BE

Dawonauth, L., Rademacher, L., L'omelette, A.D., Jankee, S., Lee Kwai Yan, M.Y., Jeeawoody, R.B., Rademacher, T.W., 2014. Urinary inositol phosphoglycan-P type: Near patient test to detect preeclampsia prior to clinical onset of the disease. A study on 416 pregnant Mauritian women. J. Reprod. Immunol.101-102:148-52. doi:10.1016/j.jri.2013.06.001

Heading 1 - ncd-2009.pdf, n.d.

Lindheimer, M.D., Roberts, J.M., Cunningham, F.G., Chesley, L.C., 2009. Chesley's hypertensive disorders in pregnancy. Academic Press/Elsevier, Amsterdam; Boston.

Maybury, H., Waugh, J., 2005. PROTEINURIA IN PREGNANCY – JUST WHAT IS SIGNIFICANT? Fetal Matern. Med. Rev. 16, 71–95. doi:10.1017/S0965539504001445

Rey, E., Couturier, A., 1994. The prognosis of pregnancy in women with chronic hypertension. Am. J. Obstet. Gynecol. 171, 410–416.

Ros, H.S., Cnattingius, S., Lipworth, L., 1998. Comparison of Risk Factors for Preeclampsia and Gestational Hypertension in a Population-based Cohort Study. Am. J. Epidemiol. 147, 1062–1070.

Scioscia, M., Gumaa, K., Whitten, M., Selvaggi, L.E., Rodeck, C.H., Rademacher, T.W., 2007. Inositol phosphoglycan P-type in healthy and preeclamptic pregnancies. J. Reprod. Immunol. 76, 85–90. doi:10.1016/j.jri.2007.03.016

Scioscia, M., Williams, P.J., Gumaa, K., Fratelli, N., Zorzi, C., Rademacher, T.W., 2011. Inositol phosphoglycans and preeclampsia: from bench to bedside. J. Reprod. Immunol. 89, 173–177. doi:10.1016/j.jri.2011.03.001

Sibai, B.M., 2001. Hypertensive disorders in women. Saunders, Philadelphia, PA.

stats-reports-2011.pdf, n.d.

Suhonen, L., Teramo, K., 1993. Hypertension and pre-eclampsia in women with gestational glucose intolerance. Acta Obstet. Gynecol. Scand. 72, 269–272.

Williams, P.J., Gumaa, K., Scioscia, M., Redman, C.W., Rademacher, T.W., 2007. Inositol phosphoglycan P-type in preeclampsia: a novel marker? Hypertension 49, 84–89. doi:10.1161/01.HYP.0000251301.12357.ba

Table1: Summary characteristics of pregnant women

	Number	Percenta
Ethnic Origin		
Indo-Mauritian	658	65.28%
Creoles	337	33.43%
Mixed	13	1.29%
Pre-existing disease		
Chronic HBP	15	1.49%
T2DM	30	2.98%
Previous gestational disease		
PIH	108	10.71%
GDM	20	1.98%
Current pregnancy		
Chronic HBP	11	1.09%
GDM	79	7.85%
PIH	128	12.72%
HBP+ superimposed PE	5	0.5%
PE+GDM	13	1.29%
Smoking		
Yes	18	1.79%
Previous to diagnosis of pregnancy	22	2.18%
No	968	96.03%
Alcohol		
Yes	66	6.55%
Previous to diagnosis of pregnancy	11	1.09%
No	931	92.36%

Table2: Family History of pregnant patients

Family History	Number	Percentage %
PIH	19	1.88%
Chronic Hypertension	354	35.1%
T2DM	380	37.7%

Table3: Obstetric characteristics of pregnant women

	PE: Mean ± SD	**Controls: Mean ± SD**	**P value**
Age in years	28.18 ± 6.34	26.23 ± 5.99	0.005
Booking BMI (Kg/m2)	28.40 ± 8.38	25.31 ± 6.92	0.0002
Booking BP (mm Hg)			
Systolic	130.6 ± 22.05	111.6 ± 9.39	<0.0001
Diastolic	81.61 ± 15.94	69.5 ± 7.14	<0.0001
At delivery BP (mm Hg)			
Systolic	139.6 ± 17.1	113.3± 9.86	<0.0001
Diastolic	87.19 ± 14.27	71.75 ± 7.41	<0.0001
Booking proteinuria (µg/µmol)	9.72 (0.51-47.31)	4.65 (1.45-52.74)	<0.0001
Delivery proteinuria (µg/µmol)	111.2 (20.5-289.2)	11.07 (7.64-18.69)	<0.0001
Type of delivery			
Emergency CS	36	167	
Elective CS	30	153	
Normal Delivery	15	382	
Gravidity	2.18 ± 1.41	2.23 ± 1.37	0.71
Parity	0.71 ± 0.92	0.83 ± 0.99	0.27
Booking IPG/Creat	9.72 (0.51-	4.65 (1.45-52.74)	<0.0001

(U/nmol)	47.31)		
Delivery IPG/Creat (U/nmol)	84.12 (19.74-162.9)	3.75 (0-9.92)	<0.0001

Table4: Clinical characteristics and correlation with IPG/Creatinine levels.

Clinical characteristics	Spearman r	95% confidence interval	p-value
Age	0.116	0.006587 to 0.2224	<0.033
Weight	0.166	0.05586 to 0.2724	<0.003
Height	0.005	-0.1049 to 0.1157	0.9205
BMI	0.177	0.06540 to 0.2832	<0.002
Weight of baby	-0.058	-0.1678 to 0.05277	0.2894
Gestational Age	0.251	0.1458 to 0.3512	< 0.001

Table 5: Characteristics of IPG/Creatinine test at diagnosis and before clinical diagnosis

	4 weeks before diagnosis	2 weeks before diagnosis	1 week before diagnosis	At diagnosis
Cut-off value of IPG/creatinine (U/nmol)	4.42	13.11	13.42	31.02
Area under curve	0.6316 (0.5116 – 0.7516)	0.8301 (0.7403 -0.9199)	0.9119 (0.8739 – 0.9499)	0.9519 (0.9316 – 0.9721)
Odds ratio	3.048 (1.310 – 7.093)	20.23 (6.244 – 52.59)	44.00 (5.587 – 346.5)	
Relative risk	2.946 (1.295 – 6.701)	18.12 (6.785 – 60.34)	41.31 (5.324 – 320.5)	
Sensitivity	69.23% (48.21% - 85.67%)	82.61% (61.22% - 95.05%)	90.91% (58.72% - 99.77%)	95.65% (87.82% - 99.09%)
Specificity	57.53%	80.99%	81.48%	90.99% (

	(54.04% - 60.96%)	(78.11% - 83.63%)	(78.63% - 84.10%)	88.80% - 92.87%)
Positive predictive value	0.0497 (0.0297 – 0.0775)	0.1098 (0.0674 – 0.1662)	0.0625 (0.0304 – 0.1119)	
Negative predictive value	0.9831 (0.9670 – 0.9927)	0.9939 (0.9846 – 0.9983)	0.9985 (0.9916 – 1.000)	
Likelihood ratio	1.630	4.345	4.909	10.61

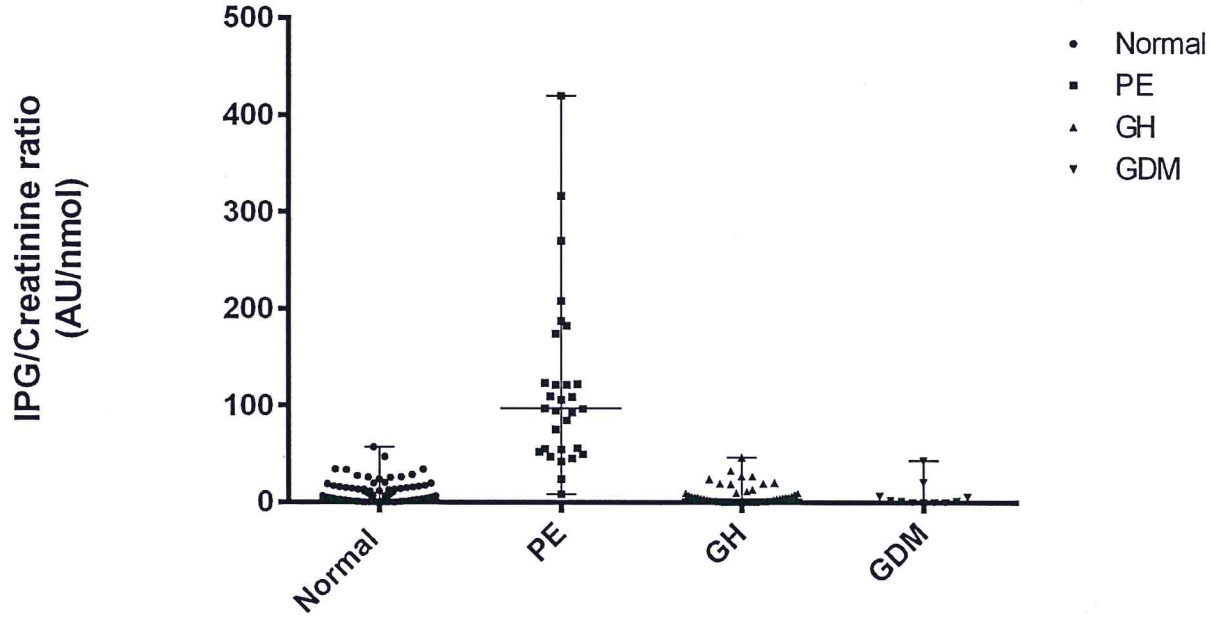

Figure1. Scatter plot of IPG/Creatinine in different patient groups

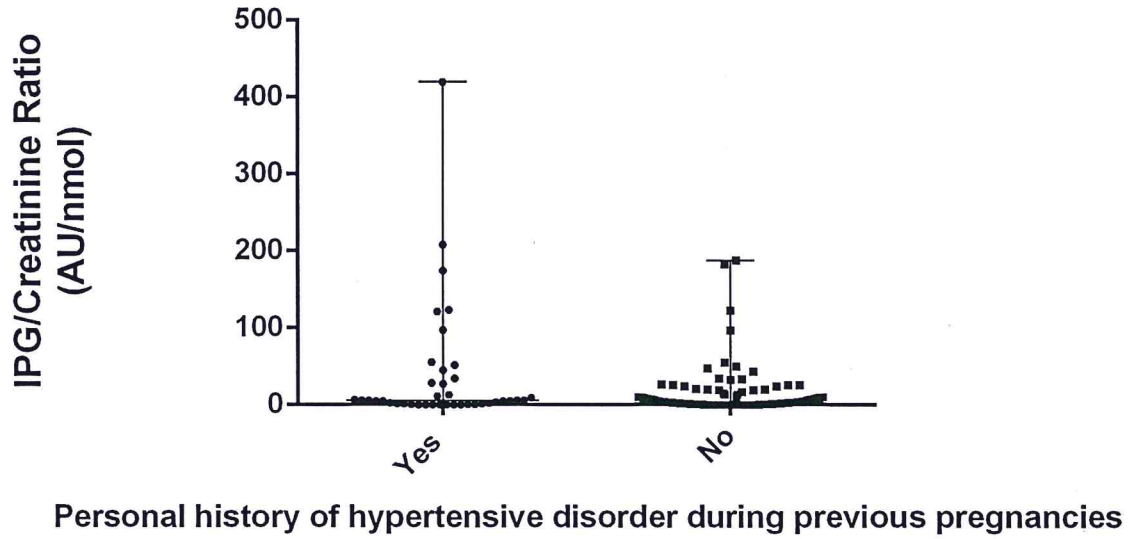
IPG/Creatinine Ratio (AU/nmol)
500
400
300
200
100
0
Yes
No
Personal history of hypertensive disorder during previous pregnancies

Figure 2

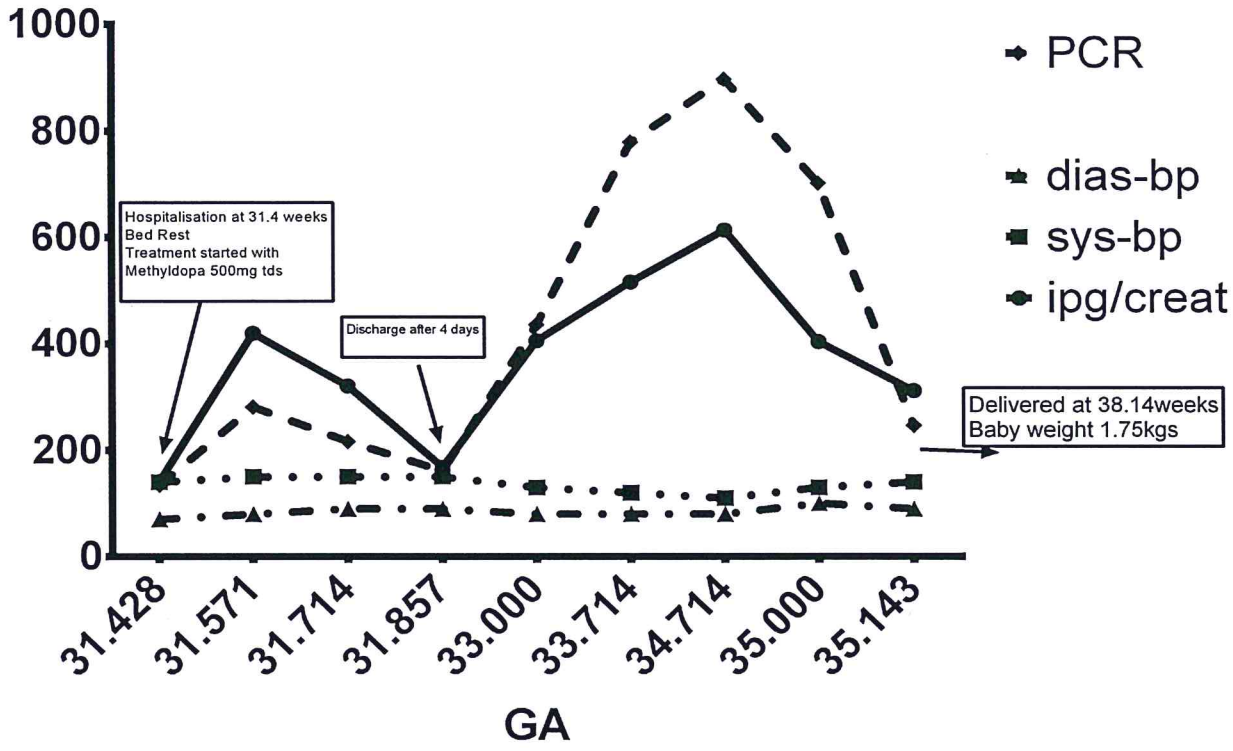

Figure3: Repetitive measurements of IPG/Creat and PCR in 1 patient at different gestational ages.

Pregnancy	Delivery (weeks)	IPG/Creat (baby)	PCR (baby)
Single	35.86	52.71	88.09
Twin	31.57	47.73	165.91

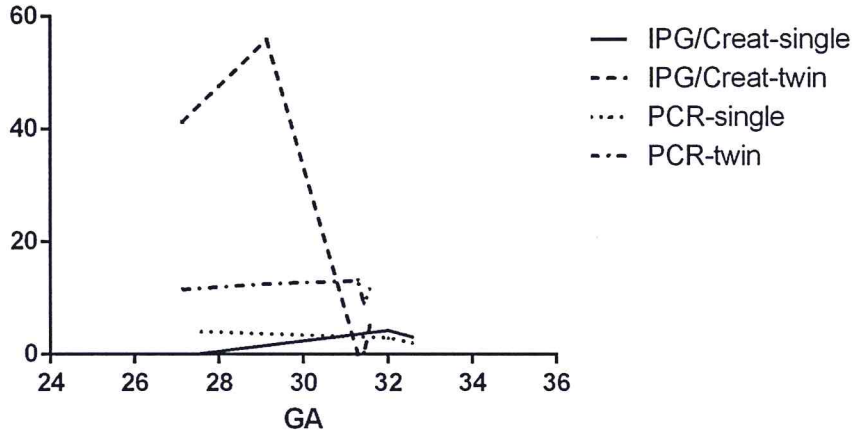

Figure 4: Measurement of IPG/creat and PCR in a single and a twin pregnancy.

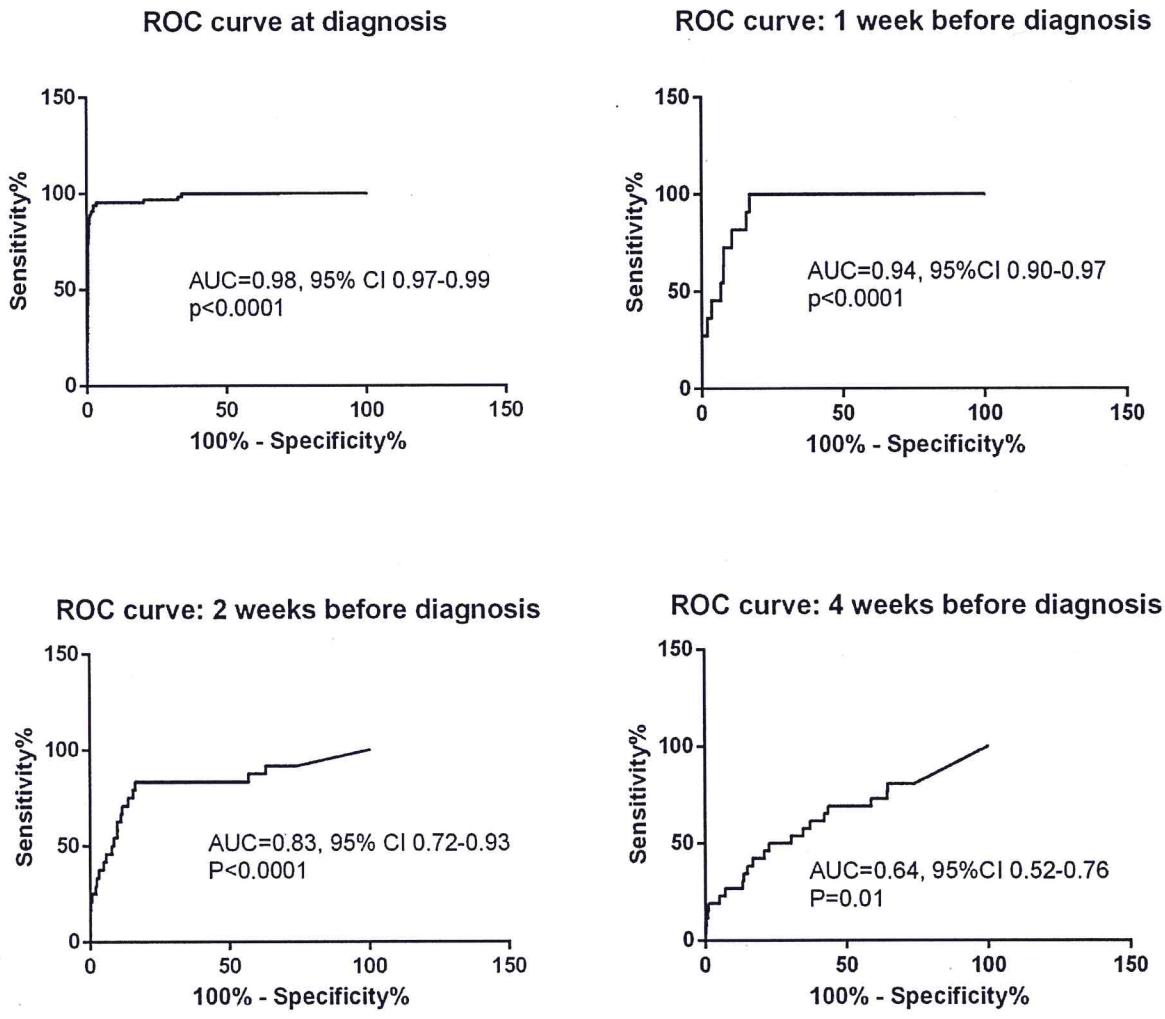

Figure 1

Figure 5a,b,c,d: ROC curves

RESEARCH PROTOCOL

1. *Project Summary*

Preeclampsia, also known as toxemia, is a serious obstetrical complication. The condition is characterized by high blood pressure (140/90 mmHg or more) and proteinuria. Maternal syndrome develops during pregnancy later than 20 weeks of gestation. If left untreated, it usually leads to eclampsia. The causative factors are young or elderly primigravidae, family history of preeclampsia, pregnancy complications like multiple pregnancy , medical disorders such as hypertension, kidney disease and diabetes. Heredity also has a role to play. The pathogenesis of preeclampsia has been known to be inadequate perfusion of the placenta which causes it to release specific hormones or chemical substances that results in endothelial damage, alteration in metabolism and inflammation Urinary measurements have been performed of the content of inositol phosphoglycans, IPG-P type and IPG-A type, which are putative insulin second messengers in preeclampsia. Recently, it was found that there is an association between IPG-P type and preeclampsia. It was discovered that IPG- P type was increased in the placenta, amniotic fluid and urine of women prone to preeclampsia.

Aims:- To determine the correlation between the molecule IPG- P type and preeclampsia.

- To analyse urinary content of albumin and creatinine.

-To propose a pathophysiological mechanism where the relationship between IPG-P and Preeclampsia could be explored.

Methodology:

Recruitment women who consent as candidates for the study at SSR National Hospital in the Obstetrics Department. A midstream urine specimen will be collected from all the women who have given their consent to participate in the study. And these patients will be followed up and urine sampling will be done every2- 4weeks. At least 2 specimens must be obtained. The

specimens will be stored at -20°C till they will be used for the assay. A polyclonal antibody- based ELISA will be used to detect levels of inositol phosphoglycan P- type (P-IPG).

Expected Output: This project can be useful in screening pregnant women and identifying those who are at risk of developing preeclampsia in Mauritius, thus providing a screening test whereby women can be identified before the clinical appearance of the disease, hence giving doctors and nurses a tool for better management of the condition.

2. *Project Justification*

It has been difficult to identify a single screening test for preeclampsia up till now. If a test that could correctly predict cases of preeclampsia in the second and maybe in the first trimester of pregnancy could be identified, this would successfully identify those pregnancies which could lead to significant morbidity (short-term and long-term) and mortality in affected women and their babies. P-IPG assessment can be performed directly on mid-stream urine specimens by using an antibody-based test. This may lead to the development of a dipstick that measures levels of P-IPG, thus representing a non-invasive test suitable for routine use in clinical practice.

Urinary excretion of P-IPG starts before the clinical evidence of the disease and in some cases several weeks earlier and by doing a specific ELISA test we could measure P-IPG in the urine of a group of women in the General population. Sensitivity for the test has been found to be 100% (In studies an overt PE patient has never been missed) and specificity of the test for PE were found to be between 69% to 100%, depending on the study, with the majority being on the upper end of specificity. Predictive sensitivity within 2 weeks before the onset of clinical symptoms is around 95%, within 3 weeks about 90% and within 4 weeks, around 80%.If >1 test result are taken over time for a single patient, there can be improved specificity. The only other test available commercially to predict preeclampsia is the sFlt-1/PlGF assay, which is a blood test, with a sensitivity of 59 to 100%, specificity of 43 to 100%, positive likelihood ratios of 1.4 to infinity, negative likelihood ratios of 0.0 to 0.7(Chesley's Hypertensive disorders of pregnancy, 2009).

This will be the first biomarker being used in Mauritius to detect PE.

Ethical Clearance for the project has already been obtained from the Ministry of Health and Quality of life National Ethics Committee held on 17th November 2010.(attached)

Carrying out the project in Mauritius will allow us to bring this test to Mauritius and provide us with the expertise to calibrate our tests so that they may be internationally quality controlled.

Also in cases where results of the test are not clear, we shall be able to carry out confirmatory tests at Sylus Pharmaceuticals Limited.

In the first instance if our tests prove to be reliable, the University will be able to carry out the tests for our hospitals and in the second phase technicians in hospitals themselves will be trained by the University of Mauritius to carry the tests in Hospital laboratories.

The test will allow us to estimate false negative and false positive women, who are detected by current tests and give them a more reliable diagnosis.

This test will increase the awareness of this disease for Obstetricians and midwives and encourage them to update their predictive and management guidelines for preeclampsia.

Women who test repeatedly negative on the test, when they test positive with conventional markers, can be reassured and sent home after a non-invasive test, instead of being admitted to hospital, in a very anxious state as to their and their babies' health.

Diabetic and hypertensive women can be encouraged to take the risk of pregnancy, as the fact that they may turn preeclamptic can be successfully monitored.

If the sFlt-1 commercially available ELISA assay is used as a comparable assay, the test will cost about £31/ sample for clinicians and estimating an average of ten tests/patient, it will cost £310/woman. A recent paper assessing the cost-benefit of the sFlt/PlGF diagnostic test for PE revealed a consensus that the diagnostic test in question will make a saving to the NHS,UK of £945./pregnant women(Hadker et al, 2010) .

The P-IPG test can be offered cheaper, will accurately distinguish PE from gestational hypertension and is non-invasive.

As follow-up, with the help of Sylus Pharmaceuticals, we shall be able to manufacture our own antibodies for the tests.

3. *Project Description*
   Objectives

- To determine the correlation between the molecule IPG- P type and preeclampsia, prospectively.
- To determine the urinary content of albumin and creatinine.
- Comparison of a small sample of the urine specimens of babies whose mothers have suffered from PE .
- Propose pathophysiological mechanism implicating IPG-P in preeclampsia

State of knowledge in the field:

Preeclampsia is a pregnancy specific syndrome that complicates approximately 2% of pregnancies, and is one of the major causes of maternal, fetal and neonatal morbidity and mortality. Also preeclampsia can have long-term consequences as women who experienced it represent a new high risk group for cardiovascular diseases later in life.

In 2000, the National High Blood Pressure Education Program Working Group revised the criteria for diagnosis that include the presence of hypertension and proteinuria after 20 weeks of gestation. Preeclampsia is defined as a systolic blood pressure of 140 mm Hg or higher, or a diastolic blood pressure of 90 mm Hg or higher that occurs after 20 weeks of gestation on two occasions, at least 6 h apart, within 7 days in a woman who previously had normal blood pressure, associated with proteinuria of 0.3 g/dL or more of protein in a 24-h urine specimen, or 0.1 g/l (≥1 + on the dipstick), in at least two random samples collected 6 or more hours apart (Cetin et al. 2011). In severe preeclampsia the systolic blood pressure level is 160 mm Hg or higher or the diastolic blood pressure level at or above 110 mm Hg and urine protein is 5 g or more in a 24-h urine specimen or 3 + on the dipstick.

Preeclampsia may have an early onset (preeclampsia starting before 34 weeks of gestation) or late onset (preeclampsia starting after 34 weeks of gestation). Since there is no effective intervention to prevent or treat preeclampsia, there is a danger of progression with the development of eclampsia, which is associated with convulsions and death. The common treatment following conservative management is delivery of the baby to save the mother's life. In the cases of late onset, the baby is born at or around term, but in the cases of early onset, the delivery is pre-term and the newborns are premature babies with all the related complications. (Cetin et al. 2011)

Various studies indicate clinical differences in the etiology and manifestation of early and late onset preeclampsia related to perfusion of the placental vasculature, fetal blood flow to essential organs, the development of intrauterine growth restriction and the level of decidual inflammation.

There are important differences in the pathophysiology of early- versus late-onset preeclampsia. While impaired placentation and fetal growth restriction are typical of early onset preeclampsia,

late onset preeclampsia is associated with a well-grown offspring and thought to occur when physiological hemodynamic and metabolic changes overwhelm a woman's ability to maintain a normal blood pressure and insulin metabolism.

Currently, pre-existing risk factors serve as common practice to predict preeclampsia. Before a woman has ever been pregnant, those at risk of preeclampsia can be identified from their profile of classical cardiovascular, renal disease or metabolic risk factors. A woman's own low birth weight puts her at twice the risk of future preeclampsia. However, although the frequency of preeclampsia among patients with prior risk is higher, prior risk by itself is not an accurate measure of preeclampsia.

Measurement in early pregnancy of a variety of biological, biochemical, and biophysical markers implicated in the pathophysiology of preeclampsia has been proposed to predict the development of the syndrome. New technologies may also allow the detection of new molecules for the establishment of a metabolic sign of preeclampsia in early pregnancy. (Cetin et al 2011)

In the past decades, a novel family of putative insulin mediators, inositol phosphoglycans (IPGs), was discovered and described to exert many insulin-like effects. These molecules are derived from lipidic glycosylphosphatidylinositol precursors in the plasma membrane by the action of a specific phospholipase D and released outside of the cell. They are then transported back into the cytoplasm where they act as allosteric activators and/or inhibitors of a large number of enzymes and transduction proteins involved in the control of cell signalling and metabolic pathways. These molecules allowed a better understanding of insulin action mainly identifying the specificity of metabolic responses after the activation of the insulin receptor. One of the molecules belonging to this family of mediators, namely IPG P-type, was shown to activate, among other enzymes, pyruvate dehydrogenase phosphatase (PDH-Pase), glycogen synthase phosphatase, and glycerol-3-phosphate acyltransferase. The activation of key phosphoprotein phosphatases plays a major role in the regulation of the disposal of glucose by oxidative metabolism via PDH, and by the nonoxidative route of storage by glycogen synthesis, both pathways classically known to be regulated by insulin.

A considerable amount of literature now exists that supports the concept that the inositol second messenger system (IPG-P) is involved in this guidance of the metabolic pathway

for glucose to glycogen (i.e. activation of glycogen synthase, etc.) (Kunjara et. al. 2000) When the placenta becomes exposed to a normal oxygen environment, once the blood flow through the maternal lacunae is established, a disconnect between the metabolic phenotype (Warburg) and oxygen tension could occur. Also the glycogen source from the endometrial glands will cease and the placenta will need to start producing glycogen or transporting glucose directly from maternal blood sources (insulin action required). In preeclamptic placenta , accumulation of syncytiotrophoblast glycogen continues and is present during the disease process right up to delivery (Kunjara, et al. 2000). The levels of IPG-P are high in the preeclamptic syncytiotrophoblast, but synthesis does not occur in this location (Deborde et al. 2003). In contrast the IPG-P levels are low in normal gestational matched placenta (Scioscia et al. 2007). The amniotic fluid of both normal and pre-eclamptic women has very high levels of IPG-P, preeclamptic amniotic fluid having a higher level. (Paine et al. 2006). Amniotic fluid therefore is a potential source of toxin if IPG-P is released in the maternal blood and it has pathological consequences. In preeclampsia, there may be a thinner glycocalyx at the syncytiotrophoblast, which leads to the leakage of IPG-P into maternal circulation then into her urine. A strong correlation exist between urinary IPG-levels in preeclamptic women and many of the disease markers (Kunjara et al. 2000). The amniotic fluid reservoir is known to have consequences for both normal and preeclamptic women. In normal women leakage of the IPG-P occurs near or at the onset of labour and may be involved in a parturition signal or post-partum pre-eclampsia (Paine et al, 2003). In contrast leakage of IPG-P from 30 weeks onward into the maternal blood probably contributes to the disease syndrome of pre-eclampsia (Rademacher, et al., 2007)

Relation to longer-term National goals:

1. Savings at the level of Health Services (as described above)
2. There is a national vision to make Mauritius become a knowledge hub: Therefore understanding of the pathophysiology of some medical disorders as understood by the international community becomes important.
3. It will help Obstetricians update national guidelines as how to detect and manage preeclampsia.

General Plan of work:

Methodology

This prospective, longitudinal study is taking place at the SSRNH in Mauritius with the approval of the Medical Ethics Committee of the Ministry of Health and Quality of Life and written informed consent has and will be obtained from recruited patients, who accept to participate in the study. 1500 patients attending the antenatal clinic at SSRNH will be recruited consecutively for this study. (We are putting down a recruitment period of 8 months as the prevalence of preeclampsia is around 2% in the Mauritian population)

All patients will be followed up longitudinally with urine sampling usually every 2–4 weeks. On the day of a routine visit to the antenatal clinic, a woman's gestational age, level of BP, history of DM or gestational DM, any previous history of HBP, PIH, preeclampsia will be determined. The weight, height and BMI at booking will be noted. Any proteinuria during the visits will be determined with a dipstick. Urine is collected as a mid-stream sample in the morning. Urinary specimens will be collected and stored at −20°C until required for assay. Urine samples will be collected from same patient after each 2-4 weeks and at least 2 specimens must be obtained. Then, their babies' sample of urine will be collected together with the babies' clinical data.

The P-IPG content will be assessed blind in triplicate using a previously described ELISA-based assay(Williams et al. , 2007). Briefly, the procedure will be carried out on urine samples directly without previous extraction of IPG-P. Samples will be diluted to 1:500 and heated at 90°C for 15 mins to ensure the exposition of P-IPGs, which are usually bound to proteins. The assay will be then performed using rabbit polyclonal antibodies anti-IPG-P (dilution 1:10,000; incubation 30 min). The results will be standardized against a polynomial standard curve generated by serial dilutions of high positive IPG PE urine. In terms of the research protocol a number of successful prospective studies have been carried out in London and some European countries, and although a further such study in Mauritius will be useful in strengthening the data associated with the IPG diagnostic it will be important to move onto a more progressive study design. The study will follow previous protocols with the exception that if a greater than an estimated baseline result is obtained with the IPG diagnostic, those women would then undergo increased biweekly monitoring, where further urine samples could be taken. It is felt that now sufficient evidence exists to suggest that a high IPG result correlates with the onset of preeclampsia, and therefore not only will this protocol be useful in a study sense, but will also lead to better clinical outcomes for the patients. A study such as this if successful will be pinnacle in establishing the IPG assay as a diagnostic test both nationally and internationally.

Statistical Analysis

Sample size calculation will not be carried out as the difference in urinary output of P-IPG between PE and controls is expected to be high as derived from an analysis of data published on urinary P-IPG and PE (Williams et al, 2007). The study will then be based on prospective recruitment of 1500 patients with a normal risk pregnancy to obtain about 30 cases of preeclampsia (2%) (Poonyth et al, 2003).

Distribution of raw data will be assessed by the method of Kolmogorov and Smirnov. Continuous variables will be compared by using the one-way analysis of variance between groups (ANOVA) or the Kruskal-Wallis test (non-parametric one-way ANOVA) if a normal distribution is not confirmed. Differences among groups will be assessed by Bonferroni multiple comparisons tests, according to results of the Kolmogorov-Smirnov test. Categorical variables will be evaluated using Chi-squared and Fisher exact tests with Yates' correction as appropriate.

Receiver-operating characteristic (ROC) curves will be calculated to determine the best threshold value of urinary P-IPG to predict PE. Trade-offs between sensitivity and specificity will have to be made to choose the cut-off value for a screening test and it depends on the implications of the test/disease. We have decided to place a premium on sensitivity rather than on specificity as the costs of missing a case are high (both economically and clinically) since close care can improve outcomes. After definition of a threshold value, the assessment of test effectiveness will be calculated by using a 2 × 2 table and expressed as sensitivity, specificity, and positive and negative predictive values. The likelihood ratio will be calculated for the selected cut-off value derived from ROC curves (Paine et al ,2010).

Linear correlation of Pearson will be used to verify significant linear relationship between P-IPG values and urinary protein content. Given the possible relationship between protein content and severity of PE (and, perhaps, higher P-IPG output), the correlation between proteins and P-IPG will be studied in all samples and in urinary specimens of women who developed PE before the diagnosis was made.

International Collaboration

We shall seek the collaboration of Professor Thomas W. Rademacher and Laurens Rademacher from Sylus Pharmaceuticals Ltd., UK, to help us start off this assay in our University laboratory. Professor Rademacher will supply us with sufficient antibody to start off the test on 8800 samples.(attached letter). Laurens Rademacher will visit Mauritius in early February to introduce the ELISA- based assay to the University of Mauritius.

(As he has already visited Mauritius where he was staying at Le Victoria Hotel we have included his per diem allowance at a cost of Rs 52,032. For his hotel stay. (Attached receipt)).

Expected Output

- Ability to differentiate between preeclampsia and gestational hypertension
- Ability to predict preeclampsia before clinical detection
- Offer test as a screening tool to Ministry of Health and Quality of Life.

4. Project Activities, Cost components and Milestones (attached)
5. Validation and Dissemination of Results

   There will be close collaboration between Professor Rademacher and the principal investigator to see to it that the P-IPG test is well set up in Mauritius and the former will make sure that the quality of the assay is not impaired. Also, in cases of disagreements, Professor Rademacher will be able to calibrate our tests.

   The results of the research will be disseminated by way of papers in international scientific journals. The papers will be authored jointly by the PI, Professor Rademacher and other close collaborators.

   The authorization of the MRC will be requested in writing before the results of the research are made public.

6. Prior Research work undertaken by PI

   I have done my PhD thesis on "The Molecular Genetics of Pregnancy Induced Hypertension in Mauritius" at the University of Mauritius, so I have extensive knowledge on the disease itself and on how Obstetrics Departments operate at the national level.

   Internationally, the main research groups on IPG are:

   - University College London/ CISC Spain
   - University of Virginia, Hunter College, USA

   The test has been recently registered with the MHRA (UK and EU) and are seeking a CLIA lab in the USA to begin testing in that domain. Sylus Pharmaceuticals Ltd is in discussion with

some of the major diagnostic companies for the commercialization and marketing of the kit. Commercial versions of the kit will require Beta Test Sites and Mauritius could be in line for this status. This could include licensing rights if Mauritius then decided to offer a testing service throughout the region including parts of Africa.

A patent of invention of the presented ELISA method (Williams, 2007) has been filed in 2003 (No. EP1295122). Sylus Pharmaceuticals has a financial interest in the patent.

7. Result of previous work financed by MRC.

   Nil of note

8. Referees

   Professor Xavier Jeunemaitre

   PARCC-Centre de Recherche HEGP,

   INSERM-U970

   56, Rue Leblanc,

   75015, Paris,

   France

   Professor Konrad Morgan,

   Vice-Chancellor,

   University of Mauritius,

   Reduit,

   Mauritius

9. This research work is being partly funded by Sylus Pharmaceuticals Ltd in terms of antibodies provided for the ELISA assay. (attached letter).

   Funding is being sought from the MRC in terms of some biochemicals to be used for the assay, the purchase of an ELISA reader as the one in our department is faulty and the salaries of a research assistant and a nursing officer as we are in great need of some staff to assist us in data and urine collection. It will also cover per diem allowance of Laurens Rademacher for his stay in Mauritius.

10. Facilities, equipment and other resources.

    (Attached excel sheet)

REFERENCES

Cetin I, Huppertz B, Burton G, Cuckle H, Gonen R, Lapaire O, Mandia L, Nicolaides K, Redman C, Soothill P, Spencer K, Thilaganathan B, Williams D, Meiri H.(2011) Pregenesys pre-eclampsia markers consensus meeting: What do we require from markers, risk assessment and model systems to tailor preventive strategies
Placenta.32S1:S4-S16.
Chesley's hypertensive disorders in pregnancy (2009). Tests to predict preeclampsia. Chp11. Edited by M.D.Lindheimer, J.M. Roberts, F.G.Cunningham,3rd edition
Deborde S, Schofield JN, Rademacher TW (2003). Placental GPI-PLD is of maternal origin and its GPI substrate is absent from placentae of pregnancies associated with pre-eclampsia. J Reprod Immunol. 59(2):277-94

Hadker N, Garg S, Costanzo C, Miller JD, Foster T, van der Helm W, Creeden J (2010).Financial impact of a novel pre-eclampsia diagnostic test versus standard practice: a decision-analytic modeling analysis from a UK healthcare payer perspective. J Med Econ 13(4):728-37.

Kunjara S, Greenbaum AL, Wang DY, Caro HN, McLean P, Redman CW, Rademacher TW (2000). Inositol phosphoglycans and signal transduction systems in pregnancy in preeclampsia and diabetes: evidence for a significant regulatory role in preeclampsia at placental and systemic levels.Mol Genet Metab. 69(2):144-58.
Paine MA, Scioscia M, Gumaa KA, Rodeck CH, Rademacher TW (2006). P-type inositol phosphoglycans in serum and amniotic fluid in active pre-eclampsia.J Reprod Immunol. 69(2):165-79.
Paine MA, Scioscia M, Williams PJ, Gumaa K, Rodeck CH, Rademacher TW(2010). Urinary inositol phosphoglycan P-type as a marker for prediction of preeclampsia and novel implications for the pathophysiology of this disorder. Hypertens Pregnancy.29(4):375-84.
Poonyth L, Sobhee R, Soomaree R.(2003) Epidemiology of preeclampsia in Mauritius Island. J Reprod Immunol. 59(2) : 101-109.
Scioscia M, Gumaa K, Kunjara S, Paine MA, Selvaggi LE, Rodeck CH, Rademacher TW(2006). Insulin resistance in human preeclamptic placenta is mediated by serine phosphorylation of insulin receptor substrate-1 and -2.J Clin Endocrinol Metab. 91(2):709-17.
Scioscia M, Gumaa K, Whitten M, Selvaggi LE, Rodeck CH, Rademacher TW (2007). Inositol phosphoglycan P-type in healthy and preeclamptic pregnancies.J Reprod Immunol. 76(1-2):85-90. Review.
Scioscia M, Gumaa K, Rademacher TW (2009). The link between insulin resistance and preeclampsia: new perspectives.J Reprod Immunol.82(2):100-5. Review.
Rademacher TW, Gumaa K, Scioscia M.(2007) Preeclampsia, insulin signalling and immunological dysfunction: a fetal, maternal or placental disorder?J Reprod Immunol. 76(1-2):78-84. Review.
Williams PJ, Gumaa K, Scioscia M, Redman CW, Rademacher TW (2007). Inositol phosphoglycan P-type in preeclampsia: a novel marker?Hypertension. 49(1):84-9.

DATA COLLECTION SHEET& ETHICAL CLEARANCE MOH&QOL

UNIVERSITY OF MAURITIUS

FINANCE SECTION

DIAGNOSTIC TEST FOR PREECLAMPSIA AT SSRNH IN MAURITIUS: INOSITOL PHOSPHOGLYCAN P TYPE (IPG-P)

Statement of Receipts & Payments

for the period 01 January 2012 to 31 October 2013

Project Ref: N4 2011 0805

	Rs Budget	Rs Actual
Receipts	1,226,000.00	**1,008,000.00**
SP197874-MRC		608,000.00
SP200061-MRC		400,000.00
Payments		
Consumables	261,600.00	**268,333.89**
Unilab Ltd-PO 24490		1,400.01
Supplies Solution-PO 24307		38,128.00
MSM Ltd-PO 24356		37,518.75
Biswal Trading Co Ltd-PO 24306		18,000.00
Ducray Lenoir-PO 24554		1,581.25
Sera Laboratories International Ltd		11,946.00
Ducray Lenoir Ltd-PO 25058		2,673.75
Unilab Ltd-PO 25426		2,500.10
Robert Le Maire Ltd-PO 25459		1,245.00
Biswal Trading Co Ltd-PO25424		38,999.97
Ducray Lenoir Ltd-PO 25425		34,396.50
Ducray Lenoir Ltd-PO 25960		3,196.54
Ducray Lenoir Ltd-PO 25809		14,805.12
DucrayLenoir Ltd PO- 22789		18,630.00
Elsevier Ltd - Medical Bookshop		12,731.47
Unilab Ltd-PO 25460		26,220.00
Unilab Ltd-P027197		1,794.00
Unilab Ltd-PO 27290		2,049.93
Marcello Stationery		517.50
Equipment	200,000.00	**138,502.76**
Biswal Trading Co Ltd-PO 24265		84,510.00
Robert Le Maire Ltd-PO 24664		14,000.00
Le Warehouse Ltd-PO 25197		5,380.00
Biswal Trading Co Ltd-PO 24711		5,179.99
Harel Mallac Technologies-PO 24441		24,610.00
Graphpad Software Inc-Prism 6 Mac Single		4,822.77
Salaries (RA/Technicians)		
RA-L'Omelette Arnaud Dominique	360,000.00	**274,111.50**
Local Travel		
RA-L'Omelette Arnaud Dominique	30,000.00	**15,628.00**
Documentation/Publications	30,000.00	**9,564.00**
DHL Mauritius Ltd		1,974.00
AAPCA Mauritius		3,967.50
Le Defi Plus Ltee		3,622.50
Computing Services	30,000.00	-
Per Diem (for setting up test)		
Accommodation-Mr Larens Rademacher	61,400.00	**55,441.74**
Salaries (Nursing Officer)		
Nursing Officer-Razgia Bibi Jeeawoody	253,000.00	**230,000.00**
Total Expenses	1,226,000.00	**991,581.89**
Remaining Balance		**16,418.11**

CONSENT FORM

I have been explained the purpose and the implications of the study and I, Mr/Mrs/Miss, ...,

Responsible Party of Mrs/Miss ..... agree to participate in the study (Role of P-IPG in Pre-eclampsia).

I, hereby agree to give the relevant clinical information and to provide urine sample necessary for this study. Confidentiality is ensured.

.....

Signature of Responsibility Party/Patient

Data Collection Sheet

DATE:

ID - IPG:

HOSPITAL:

NAME:

HOSPITAL NO:

AGE:

ETHNIC ORIGIN:

ADDRESS:

TEL NO:

PAST MEDICAL HISTORY:

- PIH
- PE
- PREVIOUS GESTATIONAL DIABETES
- PREVIOUS CHRONIC HYPERTENSION
- OTHERS

PERSONAL HISTORY:

SMOKER
ALCOHOL CONSUMPTION
DIABETES MELLITUS
OTHERS

PAST SURGICAL HISTORY:

PRESENT DRUG HISTORY:

MENSTRUAL HISTORY:

- REGULAR/IRREGULAR MENSES
- MENARCHE

CONTRACEPTION USE:

FAMILY HISTORY:

- HBP
- PIH (GH+PE)
- OTHERS

INFERTILITY:

DATE FIRST SEXUAL COHABITATION:

MARRIED LIFE: DATE OF MARRIAGE:

OBSTETRIC HISTORY: DATE OF BOOKING:

NUMBER OF PREGNANCIES:

NUMBER OF DELIVERIES:

NUMBER OF ABORTIONS:

NUMBER OF LIVE BIRTHS:

LAST MENSTRUAL PERIOD:

EXPECTED DATE OF DELIVERY:

GESTATIONAL AGE:

HEIGHT:

WEIGHT AT BOOKING:

BMI BOOKING

BP AT BOOKING:

PREVIOUS PREGNANCIES:

DIPSTICK AT BOOKING:

.....

.....

BABY

MALE/FEMALE:

DOB: TIME:

WEEKS OF GESTATION:

INDICATION FOR CS:

WEIGHT OF PLACENTA:

WEIGHT OF BABY

HEAD CIRCUMFERENCE:

URINE OF BABY COLLECTED 1 DAY AFTER DELIVERY:

YES/NO:

TIME AND DATE OF COLLECTION:

DATE 1: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS1 DRUGS1	DATE 5: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS5 DRUGS5
DATE 2: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS2 DRUGS2	DATE 6: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS6 DRUGS6
DATE 3: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS3 DRUGS3	DATE 7: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS7 DRUGS7
DATE 4: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS4 DRUGS4	DATE 8: URINE COLLECTION (WEEKS) BP (after 15 mins) WEIGHT PROTEINURIA BY DIPSTICK DIAGNOSIS8 DRUGS8

Ministry of Health and Quality of Life

Mauritius

MHC/CT/NETH/DAW

8 November 2011

Dr Lalita Dawonauth
Year 1 Coordinator
Department of Medicine
Faculty of Science
University of Mauritius

Dear Madam

Request for Ethical Clearance

I am directed to inform you that the Ethical Committee of the Ministry of Health and Quality of Life has considered your application for Ethical Clearance in respect of your project

2. I am pleased to inform you that the Ethical Committee has approved, in principle, the award of Ethical Clearance to the above project subject to the conditions laid down in the Annex.

3. You may wish to note that this Ministry will collaborate for the project and will facilitate access to patients and their files at the SSRN Hospital

Yours faithfully,

(Dr N. Gopee)
Director-General Health Services
for Supervising Officer

MINISTRY OF HEALTH AND QUALITY OF LIFE

The National Ethics Committee

Decision

Project Protocol	:	MHC/CT/NETH/DAW
Applicant	:	Dr Lalita Dawonauth
Project Title	:	Role of IPG-P type as a diagnostic test for pre-eclampsia in Mauritius.

The National Ethics Committee Meeting held on 21 October 2011 has

Awarded Ethical Clearance

to the above project proposal.

You are also requested to:-

(a) submit a Progress Report every month;
(b) Notify the Ethical Committee of any amendment of recruitment of material or of consent form, or of information to be submitted to the research participants;
(c) Report to the Ethical Committee any serious or unexpected, unforeseen circumstances;
(d) Report to the Ethical Committee termination of the project;
(e) Provide relevant information to the Ethical Committee for ongoing review;
(f) Give a copy of the Final Summary on the Final Report to the Ethical Committee.
(g) Ensure that confidentiality is respected throughout the project.
(h) Ensure that the question on ethnic group is optional.

MINISTRY OF HEALTH * P-Louis, Mauritius *

MINISTRY OF HEALTH * P-Louis, Mauritius *

(Dr N. Gopee)
Director-General Health Services
for Supervising Officer