THE ROLE OF PLACENTAL GROWTH FACTOR IN EARLY ONSET PRE-ECLAMPSIA

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Pectora roborant cultus recti

A dissertation in partial fulfilment of the degree of Masters of Medicine (Obstetrics & Gynaecology) Stellenbosch University

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Date: December 2017

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ABSTRACT

Background

Placental growth factor (PlGF) is an angiogenic protein produced by the placenta of all pregnant women. It has been proven that levels in women with early onset pre-eclampsia are markedly reduced and its role in the prediction and diagnosis of pre-eclampsia is well established. The role of PlGF and other biochemical markers to predict poor outcome in pre-eclampsia and thus assist in decision for expectant management has been at the forefront of much research in recent years and shows promising results, especially when used as ratios (soluble-fms-like tyrosine kinase 1(sFlt-1)/PlGF) or in combination with other clinical parameters. There are currently no published studies on this topic pertaining to a South African context.

Study Objective

To evaluate the role of PlGF in predicting outcomes in women with suspected or already proven early onset pre-eclampsia.

Methods

A prospective study of 122 women between 24 and 34 weeks gestation referred to Tygerberg Hospital for management of suspected pre-eclampsia was undertaken. Eligibility was established by identifying women with hypertension and proteinuria or other diagnostic criteria admitted in the labour ward or special care unit. Consent was obtained prior to obtaining a blood sample for PlGF analysis and these results were only disclosed to the investigators once all patients were postpartum, thus not influencing inpatient management or timing of delivery.

Interventions

Immediate delivery for maternal and/or fetal complications versus expectant inpatient management involving close observation of maternal and fetal wellbeing with delivery only at the time of complication versus routinely at 34 weeks gestation. This was done without knowledge of PlGF values.

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Results

PlGF as a single biomarker was not useful in predicting adverse outcome in our 122 study participants, as majority of our patients (79.5%) had highly abnormal PlGF levels (<12pg/ml) regardless of whether they delivered routinely at 34 weeks gestation without any complication or if they developed major maternal, fetal and/or neonatal morbidity. PlGF was however useful in predicting time interval to delivery; as median times to delivery between the 3 groups, PlGF <12pg/ml, 12-100pg/ml and >100pg/ml, was 7, 19.5 and 53 days respectively. Furthermore, in cases where diagnosis of pre-eclampsia was not certain, a normal PlGF value correlated with patients found ultimately not to have the disease.

Conclusion

To our knowledge this is the first study in South Africa assessing the role of PlGF as an outcome predictor in pre-eclampsia. Our results are in keeping with those published in international literature, showing that patients with highly abnormal PlGF levels will not gain more than 2 weeks of expectant management. In other words, interval to delivery is directly proportional to PlGF value. In patients where diagnosis of pre-eclampsia was ambiguous due to underlying maternal disease causing proteinuria and hypertension, PlGF was a useful additional test to ensure accurate diagnosis. Moreover, the placental growth factor, although stated as the most useful single biomarker in diagnosis and prediction of pre-eclampsia, appears to have a limited role as a stand-alone test to prognosticate the disease.

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ABSTRAK

Agtergrond

Plasentale groeifaktor (PlGF) is 'n angiogene proteïen wat deur die plasenta van alle swanger vroue geproduseer word. Dit is bewys dat vlakke in vroue met vroeë aankoms pre-eklampsie betekenisvol verlaag is en dat PlGF se rol rol in die voorspelling en diagnose van van pre-eklampsie goed gevestig is. Die rol van PlGF en ander biochemiese merkers om swak uitkoms in pre-eklampsie te voorspel en dus behulpsaam te wees in die besluit vir afwagtende hantering was in die afgelope jare baie op die voorgrond in navorsing en toon belowende resultate, veral wanneer as ratio's gebruik (sFlt/PlGF) of in kombinasie met ander kliniese parameters. Daar is tans geen gepubliseerde studies oor hierdie onderwerp in die suid-Afrikaanse konteks nie.

Studie-doelwit

Om die rol van PlGF in die voorspelling van uitkomste in vroue met vermoedelik of reeds bewese vroeë aankoms pre-eklampsie te evalueer.

Metodes

'n Prospektiewe studie van 122 vroue tussen 24 en 34 weke swangerskapsduurte wat na Tygerberg Hospitaal verwys is vir behandeling van vermoedelike pre-eklampsie is onderneem. Geskiktheid is vasgestel deur vroue met hipertensie en proteïenurie of ander diagnostiese kriteria in die kraamsaal of die spesiale sorgeenheid te identifiseer. Toestemming is verkry voordat 'n bloedmonster vir PlGF-analise verkry is. Hierdie resultate is eers aan die navorsers bekendgemaak na geboorte en het dus nie die binne-pasiënt behandeling of tyd van verlossing beïnvloed nie.

Intervensies

Onmiddellike verlossing vir moederlike of fetale komplikasies teenoor afwagtende binne-pasiënt hantering wat noukeurige observasie van moederlike en fetale welsyn met verlossing ten tye van komplikasie versus roetine verlossing op 34 weke. Dit is gedeon sonder kennis van PlGF-waardes.

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Resultate

PlGF was nie bruikbaar as voorspeller van swak uitkoms as 'n enkele biomerker in ons 122 deelnemers nie omdat die meerderheid van pasiënte hoog abnormale PlGF vlakke (<12pg/ml) gehad het ongeag of hulle roetineweg op 34 weke verlos is sonder komplikasies of hulle moederliek, fetale of neonatale morbiditeit ontwikkel het. PlGF was egter waardevol in die voorspelling van tyd tot verlossing; mediane tyd tot verlossong tussen die drie groepe PlGF <12pg/ml, 12-100pg/ml en > 100pg/ml was 7, 19.5 en 53 dae respektiewelik. In sekere gevalle waar daar onsekerheid was oor die diagnose van pre eklampsie, is gevind dat n normale PIGF waarde korreleer met pasiente wat uiteindelik nie pre eklampsie het nie.

Gevolgtrekking

Volgens ons kennis is hierdie die eerste studie in Suid-Afrika wat die rol van PlGF as uitkoms voorspeller in pre-eklampsie ondersoek. Ons resultate is in pas met die in die literatuur wat aantoon dat pasiënte met hoogs abnormale PlGF nie meer as twee weke sal wen met afwagtende hantering nie. Met ander woorde, tyd tot verlossing is direk proporsioneel aan PlGF-waardes. In pasiënte waar die diagnose van pre-eklampsie onseker was as gevolg van onderliggende moederlike siekte wat hipertensie en proteïenurie veroorsaak, kan PlGF dien as bykomstige toets om akkurate diagnose te verseker. PlGF het skynbaar 'n beperkte rol as enkele toets om siekte te prognostiseer, ten spiyte van die stelling dat dit die enkel mees waardevolle biomarker in die diagnose en voorspelling van pre-eklampsie.

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INTRODUCTION

Pre-eclampsia is a grave complication of pregnancy that remains one of the leading causes of maternal morbidity and mortality worldwide. Maternal deaths due to hypertension in pregnancy in South Africa accounted for 14.8% of maternal mortality according to the Saving Mothers 2011-2013 report, preceeded only by non-pregnancy related infections and maternal haemorrhage (1). The illness is defined as new onset hypertension and either proteinuria or end organ damage after 20 weeks gestation (2).

Using biomarkers of placental disease to predict which women will develop pre-eclampsia has been at the forefront of research during the past decade and the role of PlGF in the pathophysiology of pre-eclampsia is now well established. Moreover, just to what extent PlGF and other biomarkers, for example soluble-fms-like tyrosine kinase 1 (sFlt-1) can aid us in not only predicting which women may go on to develop pre-eclampsia, but also which women with already established disease are at higher risk of severe maternal or fetal complications if not delivered timeously; is a new and exciting domain still open to ongoing research.

The placental growth factor was discovered in 1991 by an Italian scientist Maria Grazella Persico (3). It is an angiogenic protein belonging to the vascular endothelial growth factor (VEGF) family and is a reflector of placental function. It seems to be the most sensitive and precise single biomarker in prediction of pre-eclampsia (4). Studies so far have supported the fact that women with adverse outcomes related to pre-eclampsia have decreased levels of PlGF and that low levels correlate with shorter intervals between diagnosis and delivery(5–8). Delivery is the only known cure for pre-eclampsia and ensures best maternal outcome the earlier it is done. Conversely, the benefits of expectant management for a premature fetus to improve perinatal morbidity and survival are well known (9). Thus any tool that can aid our decision for expectant management and estimate how many weeks gestation we might gain without compromising maternal health may certainly prove invaluable.

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Literature Review

Pre-eclampsia and its complications

Preeclampsia is a disorder of widespread vascular endothelial dysfunction and vasospasm that occurs after 20 weeks gestation and can present as late as 6 weeks postpartum. Revised diagnostic criteria by the International Society for the Study of Hypertension in Pregnancy (ISSHP) states that pre-eclampsia is defined as de novo hypertension after 20 weeks gestation plus proteinuria or maternal organ dysfunction or uteroplacental dysfunction (10). Hence proteinuria is no longer a diagnostic requirement and proteinuric versus non-proteinuric hypertension are now 2 separate categories. Hypertension per se is a systolic blood pressure (BP) of at least 140mmHg or a diastolic BP of at least 90mm Hg on at least 2 occasions four hours apart. Incidence of pre-eclampsia varies in the literature, known to affect 2-8% of pregnancies but at least 1 in 10 women will have signs and symptoms of pre-eclampsia requiring investigation (11,12). There is a trend toward an increased incidence in recent years (7).

Of all hypertensive complications of pregnancy; pre-eclampsia is most likely to result in serious adverse events. In early onset disease major adverse maternal outcome occurs in 15% of women (13) whilst others deliver free of any complication. Mostly however, it results in deterioration of maternal and fetal condition. It is frequently asymptomatic, detected only through routine antenatal screening but can result in life-threatening events such as cerebral haemorrhage, seizures, pulmonary oedema, renal failure, placental abruption, haemolysis, liver failure and even death. Fetal complications include preterm birth, stillbirth, intrauterine growth restriction (IUGR) and neonatal (intensive care unit (ICU) admission (14). Historically a diagnosis of severe pre-eclampsia meant immediate delivery to prevent further maternal morbidity. Delivery is always in the best maternal interests whilst expectant management seeks only to better neonatal outcome. Gestational ages at delivery along with availability of neonatal ICU facilities are the biggest predictors of neonatal survival and there are benefits to every day gained in utero. Conversely however, an intrauterine fetal death (IUFD) occurring whilst patients are being managed expectantly is devastating, and compromises maternal health too if it results from a placental abruption. In a study performed at our hospital the placental abruption rate was 20% amongst severe preeclampsia (15).

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Pathophysiology of Pre-eclampsia

The exact pathophysiology of this complex, multisystemic disorder is poorly understood. Diffuse endothelial injury, vasospasm and increased capillary permeability are hallmarks of the disease. Traditional belief is that the primary pathology seems to be defective trophoblast invasion of maternal spiral arteries by the cytotrophoblast. A failure in remodeling means that uteroplacental perfusion is converted from a low to high pressure state. Nowadays however the pathophysiology is becoming clearer as we discover the central role of placental angiogenic proteins negatively affecting maternal endothelial function (16).

An imbalance between circulating angiogenic and antiangiogenic factors has emerged as a potential key pathway in the pathophysiology not only of pre-eclampsia but other great obstetrical syndromes such as also IUGR, IUFD, preterm labour and mirror syndrome to name a few (17). "Syncitioblast oxidative stress" is thought to be the reason for the trophoblast-derived markers being out of balance; the major proangiogenic PlGF and (VEGF) are underproduced and antiangiogenic molecules such as sFlt-1 and soluble endoglin (sEng) are overproduced. The consequence of this is widespread vascular inflammation and end organ damage hence affording us better understanding of the disease process. Because these biomarkers can now be measured in maternal serum, they can now be used as diagnostic and prognostic factors (18).

Evidence supports the concept of two different entities in pre-eclampsia. One end of the spectrum is early pre-eclampsia developing before 34 weeks gestation characterized by placental dysfunction, IUGR, abnormal Dopplers, multiorgan dysfunction and a 20 fold increased risk of maternal mortality (19,20). On the other end of the spectrum is late onset pre-eclampsia with minimal placental involvement and rather a maternal systemic inflammation. Patients with late onset disease, otherwise known as maternal or "term pre-eclampsia", more often have normal or large placentae with normal fetal growth, Dopplers and birthweight. Etiology is thought to be from chronic gestational vascular inflammation associated with diabetes, hypertension or obesity. Postulations of the mechanism include that the size of the term placenta may restrict intervillous perfusion (21).

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The Placental Growth Factor

PlGF is a potent angiogenic factor mainly expressed in trophoblastic tissue. It is a member of the platelet derived growth factor family along with VEGF. Both these molecules are thought to contribute to early placental development through trophoblast proliferation and implantation. PlGF expression appears to predominate in the syncytiotrophoblastic layer of the placenta and VEGF from the cytotrophoblast (22). They result in enhanced permeability of vascular endothelium. PlGF plays a central role in placentation process. PlGF receptors are found predominantly on trophoblast cells but also on endothelial cells. It is thought to stimulate angiogenesis during conditions of ischaemia, inflammation and wound healing (23). It has been found to directly affect trophoblast proliferation, differentiation and cell protection from apoptosis (24).

In normal pregnancy – PlGF gradually rises from 15 weeks of gestation and peaks at around 28-32 weeks gestation, falling thereafter until delivery (31). Differences in PlGF levels between normal and pre-eclamptic pregnancies is most marked before 35 weeks gestation (6). Lower levels of PlGF in pre-eclampsia have been proven and is postulated to be due to two possible mechanisms – one being syncytiotrophoblastic underproduction as already discussed and the other being that PlGF binds to circulating sFlt-1 leaving less free PlGF when sFlt-1is in excess from decidual hypoxia and oxidative stress in pre-eclampsia (18). This theory is yet to be confirmed.

Decreased concentrations of angiogenic growth factors may cause a placental microvasculopathy seen in pre-eclampsia. But it may be just as likely though that reduced concentrations of these growth factors are a result of abnormal placental function (25). It is a classic chicken/egg debate. A review article by Widmer et al. show significant differences in PlGF and sFlt-1 levels after 25 weeks gestation as compared to samples obtained during the first and early second trimesters in women who went on to develop pre-eclampsia (26). This implies that the biomarkers have a relationship with the pathophysiology of the disease rather than the cause.

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Predicting pre-eclampsia and adverse outcome using PlGF – The evidence to date

It has been proven that predicting outcome of pre-eclampsia by looking at variables such as severity of hypertension or urinary protein lacks accuracy and is subject to interobserver bias (28). As already discussed, unnecessary iatrogenic premature delivery resulting from suspected pre-eclampsia has major implications on the patient, the neonate and the health system in general. Expert opinion suggests that around 20% of pregnant women with new gestational hypertension and 30-50% of pregnant women with quantitatively measured proteinuria will be confirmed to have pre-eclampsia (29). Detection of high risk patients in the first trimester allows for pharmacological intervention in the form of low dose aspirin. Furthermore, detection of at risk patients in second trimester has the benefit of closer surveillance of the pregnancy and escalation of care to a tertiary setting. Timing and content of antenatal care could be tailored to the patient-specific risk. Accurate risk stratification into 3 categories of no pre-eclampsia, mild pre-eclampsia and severe pre-eclampsia would cause significant reductions in morbidity associated with iatrogenic preterm delivery (30). Furthermore, a biochemical test that could identify women with minimal risk, would prevent clinician and patient anxiety and prolonged inpatient hospital stays, especially in those with borderline blood pressures and proteinuria.

Biomarkers like PlGF and sFlt-1 can now be isolated from maternal circulation and abnormal concentrations in early second trimester (signifying abnormal placental development with impaired production) are being used to predict pregnancies destined to develop pre-eclampsia (27). There are 27 different studies to support the fact that PlGF concentration in blood was lower in women who went on to develop the disease (34). Reference ranges are shown in the table below.

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Table 1: Recommended cut off values for the Triage PlGF test (29)

Result	Classification	Interpretation
PlGF < 12pg/ml	Test positive - highly abnormal	Highly abnormal and suggestive of patients with severe placental dysfunction and at increased risk for preterm delivery
PlGF ≥ 12 pg/mL and <100pg/ml	Test positive - abnormal	Abnormal and suggestive of patients with placental dysfunction and at increased risk for preterm delivery
PlGF ≥100 pg/ml	Test negative - normal	Normal and suggestive of patients without placental dysfunction and unlikely to progress to delivery within 14 days of the test

If taken one step further it now stands to reason that these biomarkers could be used to diagnose and predict outcome in pre-eclampsia, especially seeing as alterations of concentrations of these substances in maternal circulation precede the clinical onset of pre-eclampsia by several weeks to months, more specifically from 11-9 weeks before clinical onset with substantial reductions occurring at 5 weeks (2,31). Most studies on PlGF have been conducted in first and second trimesters looking at its role as a predictor of pre-eclampsia later on (32). Lack of adequate biomarkers to predict disease progression once diagnosed with pre-eclampsia makes it difficult for the treating clinician. Consequences of not knowing with certainty include prolonged often unnecessary hospitalization, overuse of other resources such a blood tests, ultrasound, IOL and neonatal intensive care services for premature babies – all of which have huge psychological and physical implications for the mother as well as financial and resource burden on the health care system.

The clinician is left pondering the following questions. Would it be incorrect to postulate that of women already confirmed to have pre-eclampsia, then those with highly abnormal PlGF values (<12pg/ml) would have worse outcomes than those with higher values? Also could the PlGF value be used to predict adverse outcome hence allowing clinicians to deliver these patients prior to it occurring? In a study by Rana et al. adverse outcomes did appear to be meditated by dysregulation of angiogenic factors (30). Studies have proven PlGF as a single biomarker strongly correlates with duration of pregnancy and time until

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delivery (6,12,30). Evidence supports that the addition of sFlt-1/PlGF ratio significantly improves prognostic performance in prediction of pre-eclampsia complications and is superior to all other parameters used such as degree of hypertension, proteinuria, uric acid, ALT and creatinine (5,30). Schnettler et al. looked at cost benefits of including sFlt-1/PlGF and concluded that it could reduce costs significantly by decreasing false positives and increasing true negative results with a reduction in antenatal admissions and monitoring (35).

Meler et al. evaluated the role of PlGF in prediction of maternal complications in patients with already established (not just suspected) pre-eclampsia and came to some interesting conclusions (7). Firstly, that very low levels of PlGF (<12pg/ml) in patients before 32 weeks gestation is a standard finding regardless of severity and this may be what limits its ability to grade disease severity or prognosticate maternal outcome. Of note is that more than two thirds of their patients recruited had PlGF<12pg/ml; in comparison to Sibiude et al's study of 96 patients with suspected pre-eclampsia where only 35% had PlGF values less than 12pg/ml (8). Secondly, because hypertension and proteinuria still remain such poor markers of disease severity, and some patients with suspected pre-eclampsia may not fulfil criteria completely, PlGF can assist in defining pre-eclampsia and identifying patients at risk of adverse outcome. Moreover, it might help differentiating pre-eclampsia from benign gestational progression of chronic renal disease, lupus, thrombocytopaenia and chronic hypertension. Superimposed pre-eclampsia is reported to affect 26% of women with chronic hypertension and 22-75% of women with chronic kidney disease(37,38). In a study by Bramham et al. PlGF is the best performing biomarker for women with underlying chronic disease and superimposed pre-eclampsia requiring delivery within 14 days, comparable to levels in pre-eclamptic women without chronic hypertension or renal disease. Equally they found PlGF levels was also significantly higher in the chronic hypertensive/renal patients who did not have superimposed pre-eclampsia, thus implying a substantial placental contribution to the development of superimposed pre-eclampsia (39). If PlGF is used in defining superimposed pre-eclampsia in the future, then interpreting ambiguous clinical parameters may become easier.

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Another interesting question is the role of PlGF in eclampsia. A study compared PlGF levels of severe pre-eclamptic women versus eclamptic women. It was low in both categories with no significant difference (17).

The landmark PELICAN study of 625 women with suspected pre-eclampsia, Chappel et al. concluded that PlGF has sufficiently high sensitivity and negative predictive value to be integrated into algorithms allowing stratified management with appropriate surveillance (6). From the PELICAN study we also know that interval to delivery was markedly different between the normal, abnormal and highly abnormal groups of PlGF, and that one can also use PlGF to predict need for delivery above many other commonly used signs and tests in current practice. They also found it to be a marker of IUGR of placental origin. The study concluded that PlGF value predicted delivery within 2 weeks. Values less than 12pg/ml had an average delivery time of only 9 days and levels less than 100pg/ml had an average delivery time of 23 days and levels above 100pg/ml had an average time to delivery of 62 days (6,40). Sibiude's study mentioned earlier also concluded that lower values were related to adverse outcome and interval to delivery less than 15 days (8).

Disappointingly though, despite all the excitement of better prognostication of disease, there are no diagnostic or predictive tests for pre-eclampsia that have been proven to improve pregnancy outcome (14). Furthermore majority of biomarkers predict disease in the advanced stages when there is already end organ damage (6). Aspirin started before complete trophoblast invasion at 18-20 weeks gestation is the only prophylactic treatment proven to result in a reduction in pre-eclampsia (36), so the main problem of a successful screening tool is that prophylaxis is limited but it would allow for closer prenatal monitoring of patients who did screen positive.

It is important to understand that PlGF's predictive power exists only for early-onset but not late-onset pre-eclampsia due to the formers' antecedent pathology of poor placentation. Term pregnancies show syncytiotrophoblast stress similar to those of pre-eclampsia, thus making biomarkers redundant in distinguishing normal pregnancies from those with late onset disease (21).

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Role of other biomarkers and combination tests

Other cytokines have been implicated in the etiology of pre-eclampsia. These include Granulocyte colony stimulating factor (GCSF), Endothelin-1 and Human chorionic gonadotrophin (HCG), Activin A but current data on the role of these molecules in pre-eclampsia is inconsistent (27). PlGF is more sensitive and precise than any other single biomarker as a predictor of pre-eclampsia because it reflects placental function (4). sEng and sFlt-1 are biomarkers that increase markedly in pre-eclampsia. It is speculated that sEng acts in concert with sFlt-1 to amplify endothelial dysfunction and induce clinical signs of pre-eclampsia (33). Furthermore, it has been shown that trophoblastic tissue – in response to hypoxia stimulates sFlt-1 synthesis and inhibits PlGF production (21).

Another point of interest is the role of uterine artery Doppler in combination with PlGF as a predictive marker of pre-eclampsia after 20 weeks gestation. There is some encouraging evidence (7,41). Speculation is that abnormal uterine artery Dopplers is a marker of maternal endothelial dysfunction. Combination of sFlt-1, PlGF and Doppler ultrasound of uterine arteries at 20 weeks gestation can allow prediction of pre-eclampsia with 83% sensitivity and 95% specificity (41). Newer studies are thus looking at the application of biomarkers in routine screening as a new approach to antenatal care.

Conclusion

Redman's concept of a race is so apt – "sometimes delivery wins, sometimes an abnormal outcome comes first" (18). Advantages for the fetus of expectant management must be weighed against potential dangers to the mother (44). Delayed delivery for fetal benefit in dedicated tertiary units specialized to manage pre-eclampsia and its complications is proven to be safe (45). The benefits of an accurate pathophysiological biomarker that could enable individualized assessment of each suspected or confirmed pre-eclamptic woman with the potential to reduce morbidity but also minimize health care expenditure and improve pregnancy experience for the patient would be revolutionary. PlGF shines a spotlight on pregnancies at highest risk, and alerts clinicians to which patients need closer surveillance or lower thresholds for delivery.

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OBJECTIVE

The purpose of our study was to measure PlGF levels in women diagnosed with pre-eclampsia before 34 weeks gestation then evaluate results against the certain maternal, sonographic, fetal and neonatal characteristics that will be discussed in detail below. Primary outcome was to see if lower levels of PlGF could predict adverse events and aid clinicians in deciding whether pre-eclamptic patients qualify for expectant management based on their risk for complications if they remained undelivered. Ability to differentiate early onset pre-eclampsia that would or would not be associated with poor outcome could certainly be useful to guide clinical management. Secondary outcomes included using PlGF to predict interval from diagnosis of disease to delivery. Moreover, we wanted to see if normal values might be helpful in excluding diagnosis of pre-eclampsia when ambiguous clinical parameters were present.

MATERIALS AND METHODS

A total of 122 women, admitted to Tygerberg hospital with suspected pre-eclampsia between July and September 2015 were prospectively included in the study. This study aimed to be a pilot study; hence no formal power analysis to determine sample size was performed. Inclusion criteria were women of any age with singleton or multiple pregnancy between 24 and 34 weeks of gestation with either confirmed or suspected pre-eclampsia admitted for stabilization and work-up/confirmation thereof. One additional EDTA tube of blood (3-5mls) was collected from the patients at the time of routine venipuncture either on the day of hospitalization or when biochemistry was repeated prior to delivery. Patients were sourced from the labour ward or special care unit of Tygerberg hospital. No patients had sequential PlGF levels done, it was a once off pre-delivery test done within 1 week of admission to hospital. The blood was centrifuged within 4 hours of collection, labeled with the hospital number, aliquoted and stored at -40°C for later analysis by a trained anatomical pathologist familiar with the Triage PlGF instrument. This test is a fluorescence immunoassay with a measurable range of 12-3000pg/ml. The PlGF results were not divulged to myself, the patient or anyone involved in the research study or ongoing care and clinical management of the patient, whilst in hospital or upon discharge. Only once data was captured for all 122 patients post-delivery did the laboratory analyst disclose the results to the researcher. Clinical data were

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ascertained and included age, gravidity, parity, body mass index (BMI), results of routine blood tests obtained at booking visit, smoking status, comorbid medical conditions, gestational age at booking, diagnosis and mode of delivery as well as clinical information including degree of hypertension, haematological and biochemical results and that of a quantified protein in a 24 hour urine collection. Uterine and umbilical artery Doppler indices and sonographic evidence of IUGR were also captured. These measurements were done by a trained sonographer. All pregnancy outcomes were recorded including maternal complications related to pre-eclampsia, as well as birth weight, Apgar scores and early neonatal outcome. Of importance was looking at the time interval from initial suspected diagnosis (in many cases this was earlier than admission date to Tygerberg) to delivery and interval between sample retrieval date until delivery.

As part of the pre-eclampsia work up, a 24 hour urine collection was commenced to quantify total protein levels. The results of which assisted in making a definitive diagnosis. In the patients who did not have a DUP it was generally because some maternal or fetal factor disqualified them from expectant management and they were delivered prior to commencement or completion of the test.

Once all data was captured, the laboratory assistant then divulged PlGF results to allow analysis of PLGF against outcome and patient demographics. Patients were sorted into 3 groups depending on their PlGF values - Normal (≥ 100pg/ml) = 3, Abnormal (12-99pg/ml) = 2, and Highly Abnormal (<12pg/ml) = 1. The great majority of participants - 97 patients (79.5%) had PlGF values <12pg/ml. 16 patients (13.1%) had abnormal values in category 2 and 9 patients (7.4%) had normal PlGF values above 100pg/ml. In the results, I will refer to these categories mentioned above as Groups 1, 2 and 3 when comparing captured patient characteristics and pregnancy outcomes to PlGF value.

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ETHICAL ASPECTS

Informed consent for the investigations described herein was obtained for all women. They understood that they would not be paid to participate in the study and that the results of the blood test would be unknown to all but the laboratory analyst until after delivery and hence would not be used at all in their further management. All that was needed from them was an extra 3ml of blood, taken at the time of routine venipuncture. Women were given the option to decline participation. The Ethics Committee of Stellenbosch University gave approval for the study (S14/10/223). Informed Consent in Afrikaans or English (Addendum A) was obtained from each participant prior to her blood sample being collected.

- The test results were not divulged to anybody including the researcher, supervisor, patient or treating clinicians until all the patients in the study had delivered and the data captured. At this point the results were emailed by the laboratory assistant to the principle researcher and her supervisor for analysis.
- The blood was collected at the time of routine venipuncture thus not causing further patient discomfort
- There was no deviation from standard patient management.
- Patient confidentiality was not compromised in any way.
- The data was collected personally by the researcher

DIAGNOSTIC CRITERIA

Pre-eclampsia was diagnosed based on ISSHP revised diagnostic criteria - Normotensive women who developed a BP ≥ 140mmhg systolic or ≥90mmHg diastolic on 2 occasions at least 4 hours apart after 20 weeks gestational age and proteinuria ≥ 300mg/24hr urine collection or protein creatinine ratio ≥ 30mg/mmol or persistent ≥ 1+ proteinuria on dipsticks in the absence of a DUP. If proteinuria was absent, pre-eclampsia was still diagnosed in hypertensive patients with maternal organ damage or IUGR signifying uteroplacental dysfunction. Maternal organ dysfunction included renal insufficiency, liver involvement, neurological or haematological complications.

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All patients were managed according to our departmental policy which requires a certain standard of care involving initial stabilization with hourly observations in the labour ward, blood pressure control, magnesium sulphate administration for seizure prophylaxis (if severe pre-eclampsia or imminent signs) as well as for fetal neuroprotection before 32 weeks gestation, corticosteroid administration for fetal lung maturity and cardiotocograph (CTG) monitoring if pregnancy was 27 weeks gestation or more. Haematological and biochemical investigations was done on all patients at admission and repeated the next day and in patients who were deemed candidates for possible expectant management there was commencement of a DUP. Failure to control blood pressure on maximal antihypertensive medication or the development of major fetal and/or maternal complications were reasons for delivery. In the absence of the above, the patient was deemed stable and referred to the Special Care unit, where a team of consultants evaluated the patient for expectant in-patient management. Elective delivery was performed routinely at 34+0 weeks gestation in confirmed pre-eclamptic patients who did not develop any complication necessitating delivery before this point.

STATISTICAL ANALYSIS

Continuous data were compared by analysis of variance if data were normally distributed, or otherwise with the rank sum test. Ratio's were compared using the chi2 test. Where applicable, a p-value of less than 0.05 was considered to be statistically significant.

BUDGET

Funding was obtained via the Harry Crossley Foundation to the amount of R17 466. This afforded us the finances necessary to purchase 5 boxes of PlGF test devices (25 per box) as well as the Triage MeterPro analyser. The Alere company provided us, free of charge, with a centrifuge machine as well as a laboratory assistant at NHLS who assisted in centrifuging, storing and analyzing the serum. No budget provision for this study was necessary from the Western Cape Department of Health.

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RESULTS

Of the 122 subjects recruited for suspected pre-eclampsia, 115 (94.3%) were singleton pregnancies and 7 (5.7%) carried twin pregnancies. Forty-four were nulliparous (36.1%). No women were excluded from analysis after entry into the trial, even if they were subsequently found not to have pre-eclampsia after all. Missing maternity records accounted for 4 incomplete entries in the data capture. As much information was obtained telephonically from the patients themselves, from the birth register and the hospital information system.

We compared baseline characteristics to PlGF value. Mean age of the participants was 27.7 years with an average BMI of 29.1kg/m^2. 21 women (17.2%) of the study population was human immunodeficiency virus (HIV) infected and 19 patients (15.7%) were smokers.

Table 2: Demographic data of women

Characteristic	Population mean (SD)
Age (years)	27.7 ± 6.10
BMI (kg/m^2)	29.1 ± 6.84

Chart 1: Smoking status

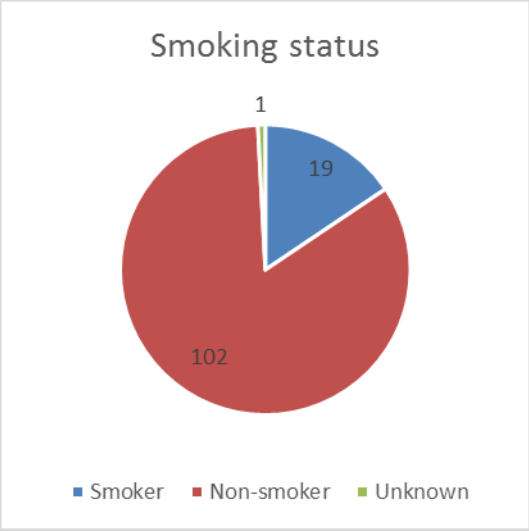
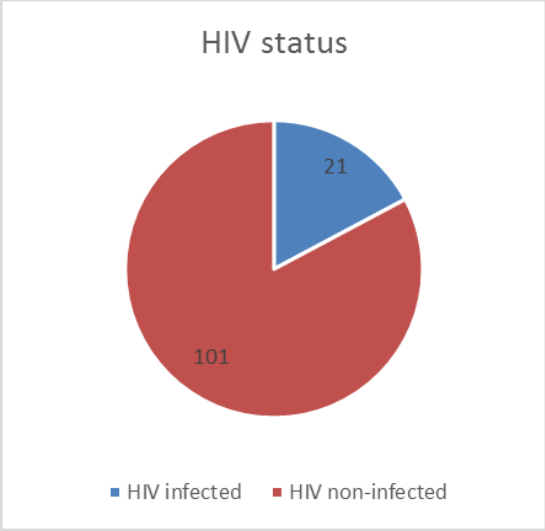


Chart 2: HIV status



Average gestation at booking and diagnosis was 16 weeks 1 day and 28 weeks 5 days respectively. 80 patients had an early ultrasound performed before 24 weeks gestation. Mean gestational age at delivery was 31 weeks 0 days ± 3.36 and median interval gained through expectant management was 8 days. To compare we also calculated time from

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when the PlGF blood was taken up until delivery, giving a median of 4 days. Four of the patients included in the study unfortunately already had intrauterine fetal deaths at the time of enrollment. Moreover, we looked at booking blood pressures as well as highest recorded blood pressures. Average of the latter amongst participants was 170/105 mmHg.

Table 3: Gestation at delivery and time gained expectantly in all 122 subjects

	Gestational age at delivery (weeks)	Time Gained Expectantly (days)
Mean (SD)	31w 0days ±3.36	15.14 ±19.71
Median (range)	31w 5days (21wd2-40w6d)	8 (0-122)

Amongst the study sample, 84% of the patients had a quantified DUP done. The mean urinary protein excretion was 2.4g/24hr. The highest result was 17.63 g/24hr.

When evaluating haematological and biochemical markers of the study participants we noted platelet counts and creatinine levels. If platelet count was <100 $x10^9$/L we then recorded liver transaminases and lactate dehydrogenase. Seventeen (13.9%) patients had platelet counts lower than 100 $x10^9$/L and of these 7 had haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, 2 had "evolving" HELLP syndrome where liver enzymes were the upper limit of normal in the presence of thrombocytopaenia. Eight patients had isolated thrombocytopaenia.

Thirty patients (24.5%) had creatinine values above the normal threshold for pregnancy of 75µmol/L. Highest creatinine was 159 µmol/L, but this particular patient was diagnosed at 16 weeks gestation with an underlying chronic renal lesion and a DUP of 7.17g/24h. She never went on to develop pre-eclampsia.

Comorbid conditions analysed were chronic hypertension and diabetes mellitis. Twenty-four patients (19.6%) were known with chronic hypertension and 5 patients (4%) were diabetics.

In terms of ultrasound and Doppler indices, we looked at umbilical artery and uterine artery Doppler as well as evidence of IUGR defined by estimated fetal weight below the 10th percentile. One hundred patients (81.9%) which equates to 107 fetuses, had umbilical artery Dopplers (UAD) with 71.9% of these being normal (peak-systolic to end-

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diastolic blood flow velocities of less than 95th percentile). Uterine artery Doppler (UtAD) was done for 88 patients (72.1%) and more than half these (51 patients) had bilateral notching (57.9%). 20 patients of the 88 had unilateral notching.

Chart 3: Umbilical Artery Doppler indices

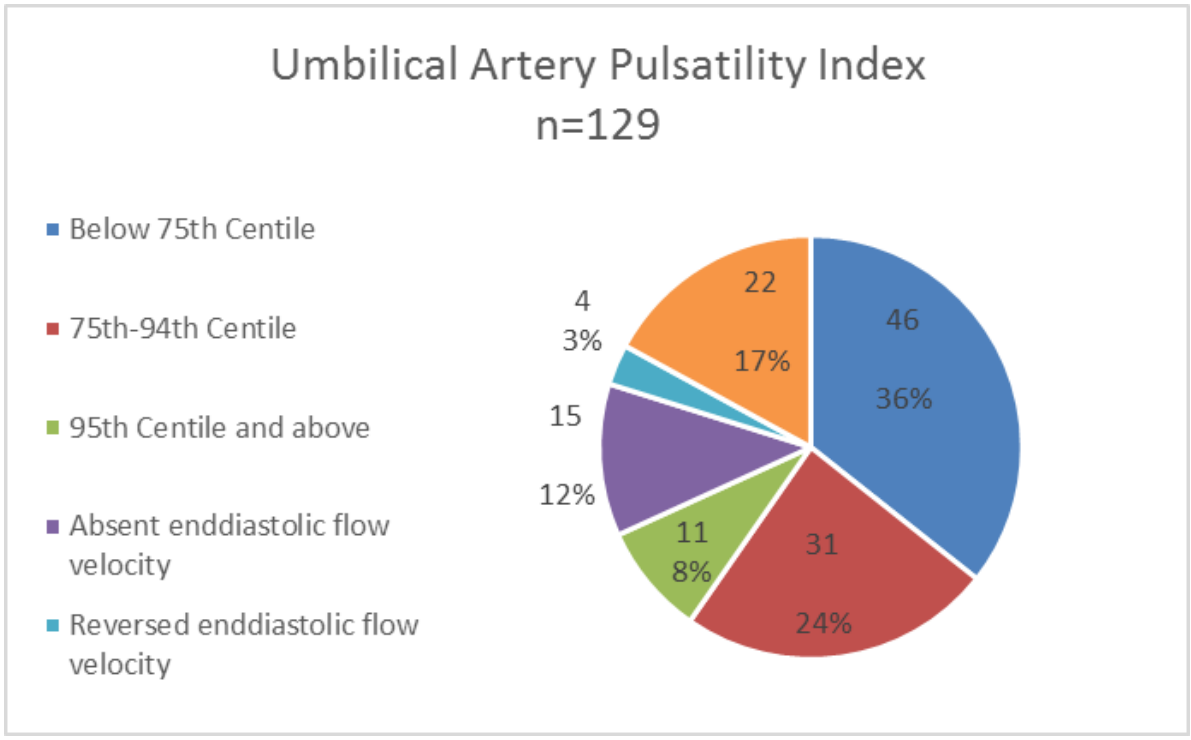
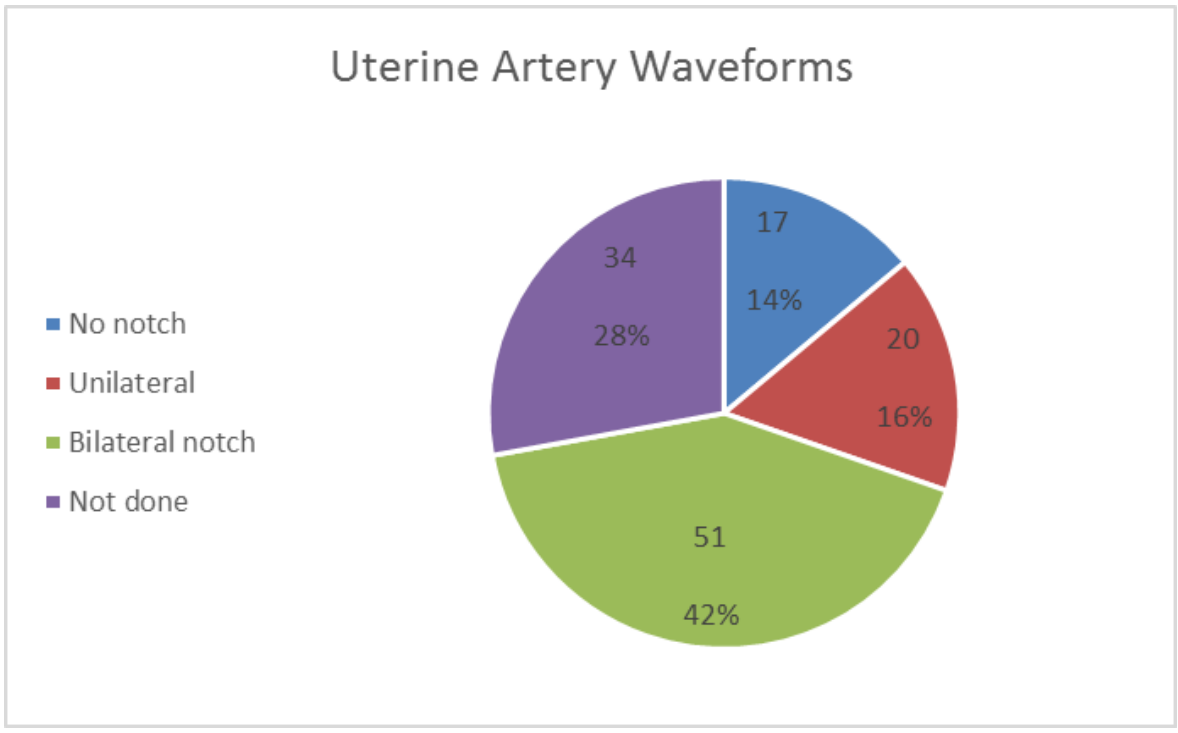


Chart 4: Uterine Artery Doppler Waveform



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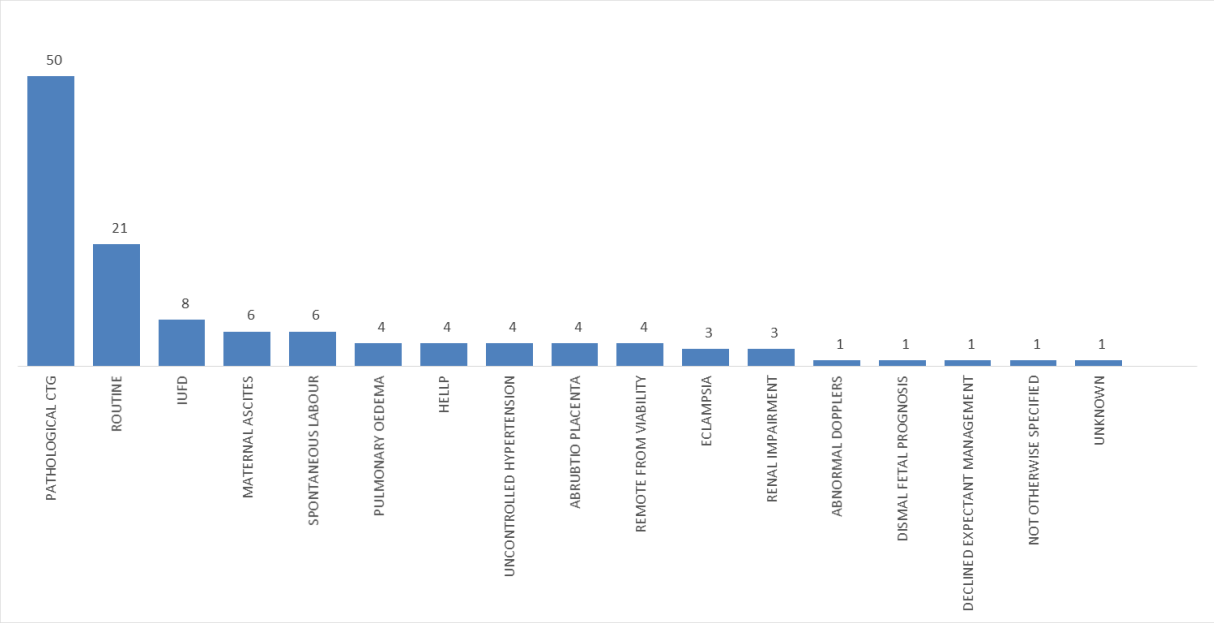
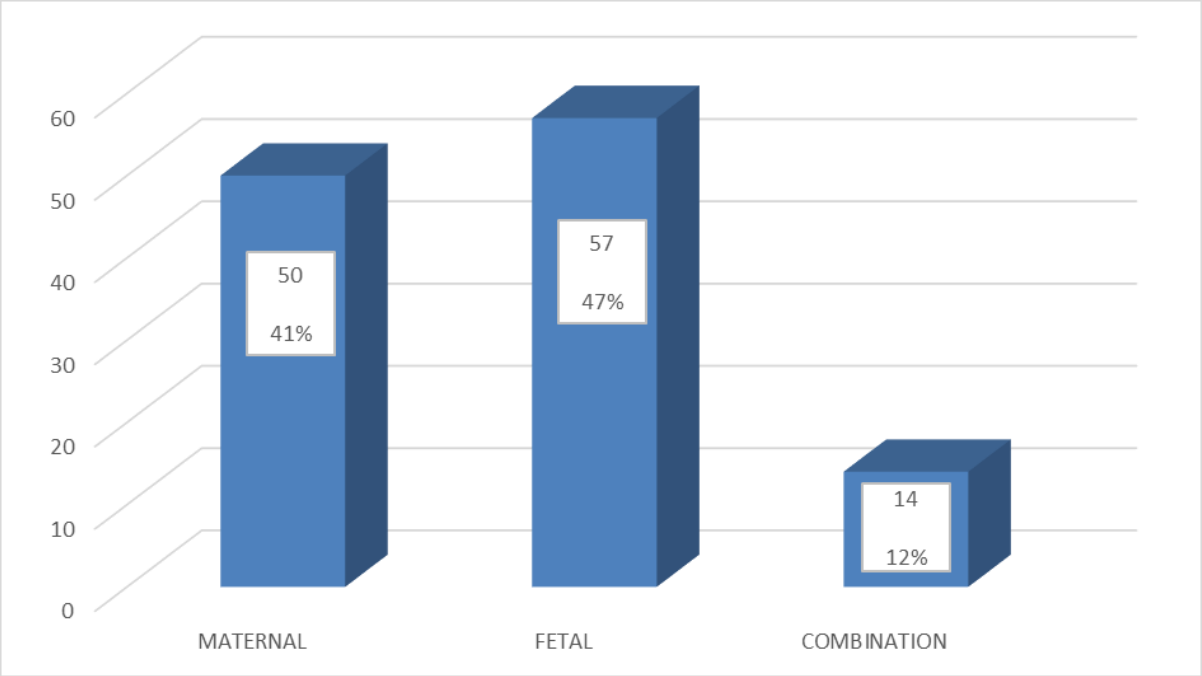
Forty-two (34.7%) of our patients underwent IOL - either due to a maternal or fetal complication or routinely at 34 weeks gestation. The indications for delivery are shown in graph below.

Graph 1: Indications for Delivery



Graph 2: Maternal versus Fetal indications for delivery



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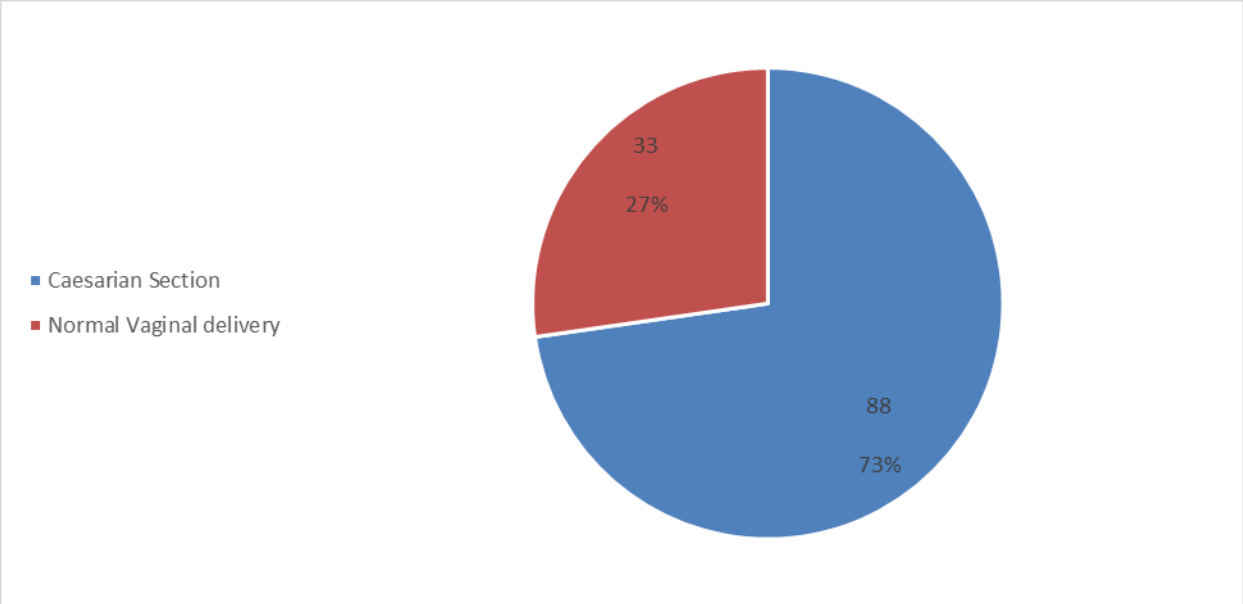
The commonest reason for delivery was pathological CTG in 50 patients (41.3%), the next being routine delivery at 34 weeks gestation as per Tygerberg Hospital pre-eclampsia protocol (17.3%). We then grouped all the indications for delivery into maternal reasons, fetal reasons and a combination of the two (for example a patient who was delivered for renal impairment and persistently suspicious CTG). Maternal reasons for delivery comprised 50 patients (41.3%) and 57 patients (47.1%) delivered for purely fetal reasons. 11.6% of the patients delivered for a combination. Overall 33 women (27.3%) delivered vaginally and 88 by caesarian section (72.7%).

Chart 5: Mode of delivery (n=121)



Neonatal analysis included capturing Apgar scores at 1, 5 and 10 minutes; birthweights and neonatal outcome until day 7 of life or discharge from hospital, whichever came first. ICU admission, neonatal morbidity or mortality after day 7 as well as neurodevelopmental outcome was not captured, hence it is not a true reflection of intact survival. Mean birthweight was 1406grams. There were 112 livebirths (86.8%) and 3 of these were early neonatal deaths. Seventeen stillbirths equated to a perinatal mortality rate of 155/1000 live births.

Table 4: Birthweights

	Birthweight (grams)
Mean	1406 ±587
Median	1350 (360-3200)

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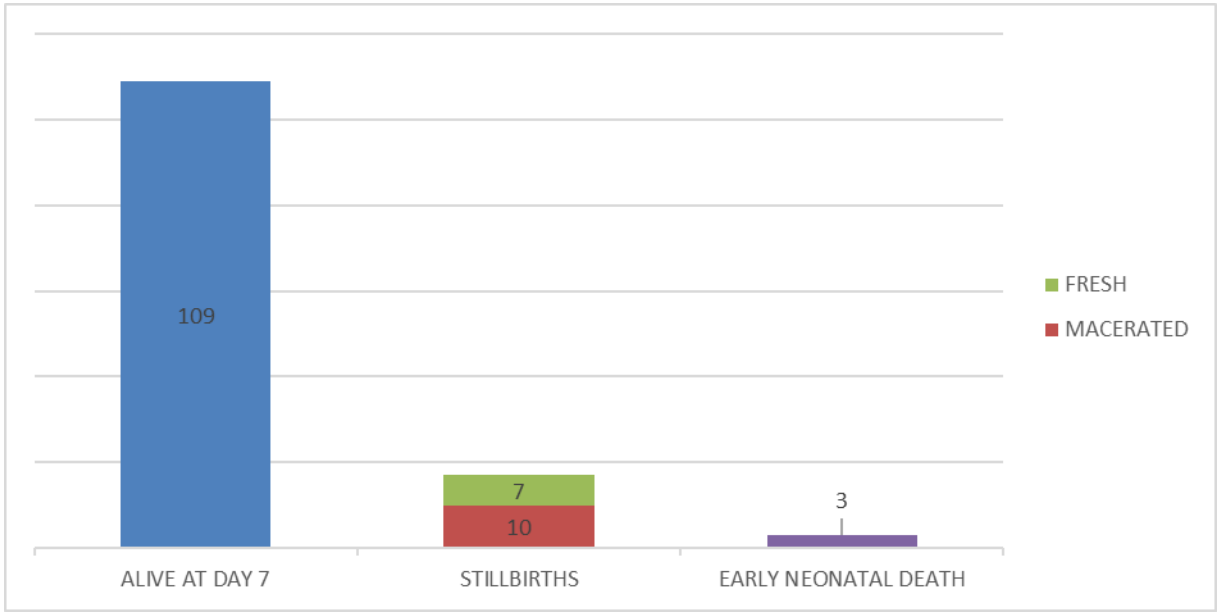
Graph 4: Perinatal Outcome



Adverse maternal outcomes occurred in 39 of the 122 subjects enrolled in the study (Graph 5). The most common adverse fetal outcome was IUGR defined by growth <10th centile on ultrasound where small for gestational age was excluded by previous ultrasound measurements and/or normal Dopplers. 21 patients (of which 12 had live births) never had the privilege of a formal ultrasound once diagnosed with pre-eclampsia, either because they were delivered prior to the ultrasound being performed or the fetus had demised in utero. Hence IUGR was likely under-reported as criteria for diagnosis required the presence of a scan. As Dopplers have been discussed and tabulated already they were not included in the fetal outcome graph that follows.

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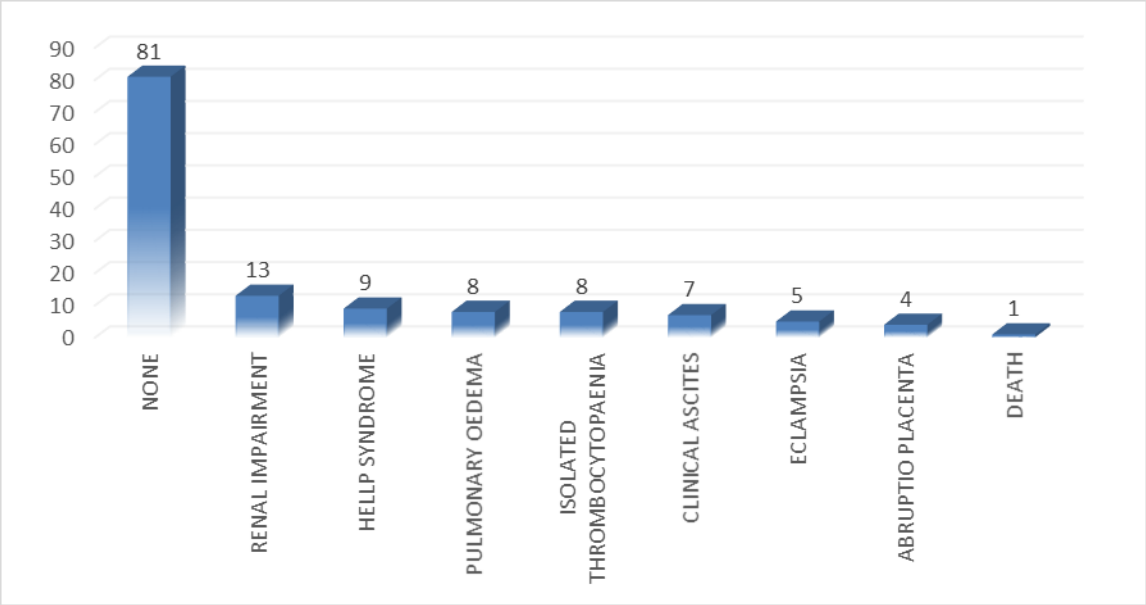
Graph 5: Maternal Adverse Outcome



Graph 6: Adverse fetal outcome

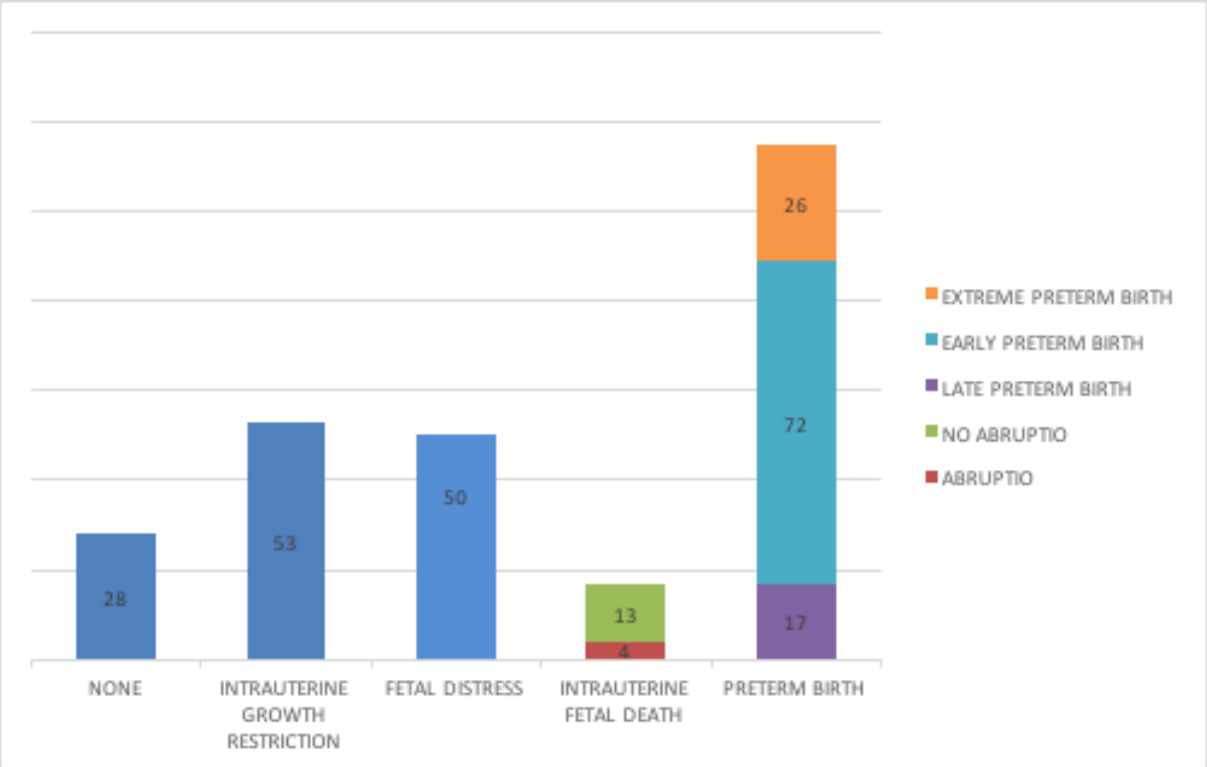


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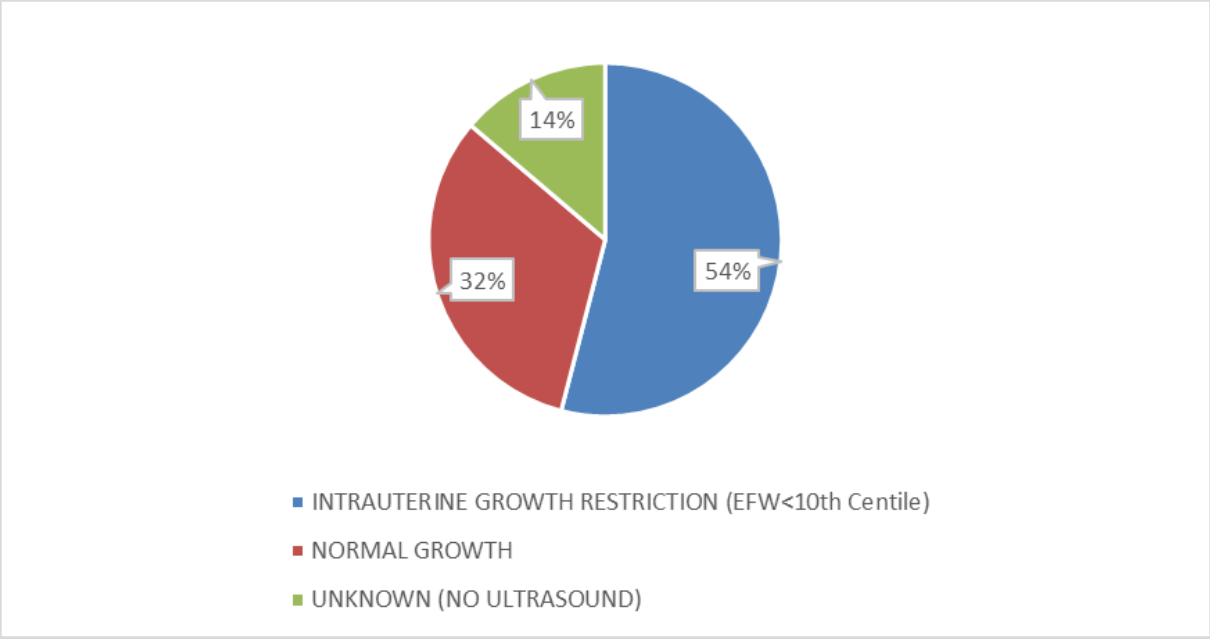
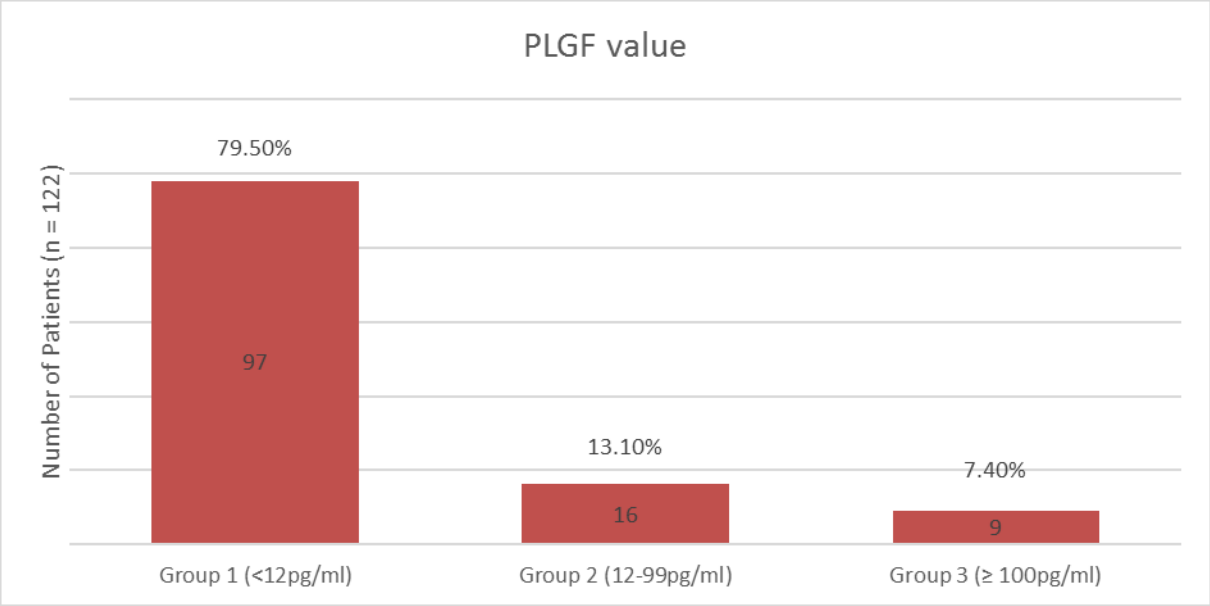
Chart 6: Incidence of IUGR amongst live births (n=105)



Stratification of Data according to PlGF Result

PlGF values of the 122 participants were grouped into the 3 categories represented in the graph below.

Graph 7: Stratification of patients according to PlGF value



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Patient characteristics that were statistically significant when compared against PlGF value were the following, each of which I will discuss in detail.

- Age
- BMI
- Gestational age at delivery
- Interval from diagnosis to delivery
- Interval from PlGF until delivery
- Highest Diastolic BP
- Birthweight
- Uterine Artery Dopplers
- Comorbidities (specifically diabetes mellitus and renal lesions)
- Induction of labour
- Reason for delivery

Factors which did NOT seem to have any relevance to PlGF value (P value >0.05) included

- Gravidity *(p = 0.332)*
- Parity *(p = 0.164)*
- Rhesus/Syphilis/HIV status *(p = 0.78/0.101/0.075)*
- Smoking *(p = 0.498)*
- Gestation at booking *(p = 0.123)*
- Gestation at diagnosis *(p = 0.182)*
- Booking systolic and diastolic blood pressures *(p = 0.898/0.479)*
- Highest Systolic blood pressures *(p = 0.169)*
- DUP *(p = 0.364)*
- Platelet count and serum creatinine value *(p = 0.388/0.780)*
- Umbilical Artery Dopplers *(p = 0.401)*
- Chronic Hypertension *(p = 0.268)*
- Mode of delivery *(p = 0.079)*
- Adverse Maternal Outcomes *(p = 0.212)*
- Apgar score at 1, 5 and 10 minutes *(p=0.062/0.203/0.227)*

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Age

Average age of patients was significantly higher in group 2 (p value 0.03), with the youngest women (14 years old) being in Group 1. The significance of this is uncertain.

BMI

BMI did appear highest in Group 3 who had normal PlGF values (Average BMI 28,8 kg/m^2 in Group 1 versus 35,7 kg/m^2 in Group 3).

Gestation at Delivery

Amongst group one – the average gestation at delivery was 30 weeks and 2 days, group 2 was 32 weeks 3 days and group 3 was 35 weeks 3 days. This was statistically significant (P value 0.00) implying patients with a normal PlGF values have a higher chance of delivering closer to term.

Highest diastolic blood pressures

These were significantly greater amongst group 1 (P value 0.014) with a mean of 106 mmHg, with group 3 at 92.8 mmHg. Groups 1 and 2 had mean systolics of 170 mmHg and in group 3 the mean systolic was borderline severe also – 157. These systolic values were not significantly different (P value 0.169) between groups as was found with the diastolic pressures between groups.

Birthweight

97 babies born to 96 mothers in Group 1 with an average birthweight of 1279.22g was significantly lower (P value 0.00) than the average birthweight of group 2 (1598.94g) which in turn was lower than group 3 (2195.45g).

Table 3: Statistically Significant Demographic Data of women stratified according to PlGF Value

	Group 1 (<12pg/ml)	**Group 2 (12-99pg/ml)**	**Group 3 (≥ 100pg/ml)**	**All**	**p-value**
Age (years)	27.06 ± 5.98	31.31 ± 5.35	28.22 ± 6.88	27.7 ± 6.1	0.033
BMI (kg/m^2)	28.82 ± 6.55	27.4 ± 3.99	35.71 ± 11.45	29.06 ± 6.83	0.021
Gestation at Delivery (days)	212.31 ± 21.57	227.46 ± 14.09	247.77 ± 28.74	216.9 ± 23.54	0.000
Highest Diastolic Blood Pressure (mmHg)	105.96 ± 13.04	104.92 ± 12.66	92.77 ± 11.14	104.84 ± 13.23	0.016
Birthweight (g)	1279.22 ± 507.63	1598.94 ± 461.8	2195.45 ± 761.5	1406.41 ± 587.55	0.000

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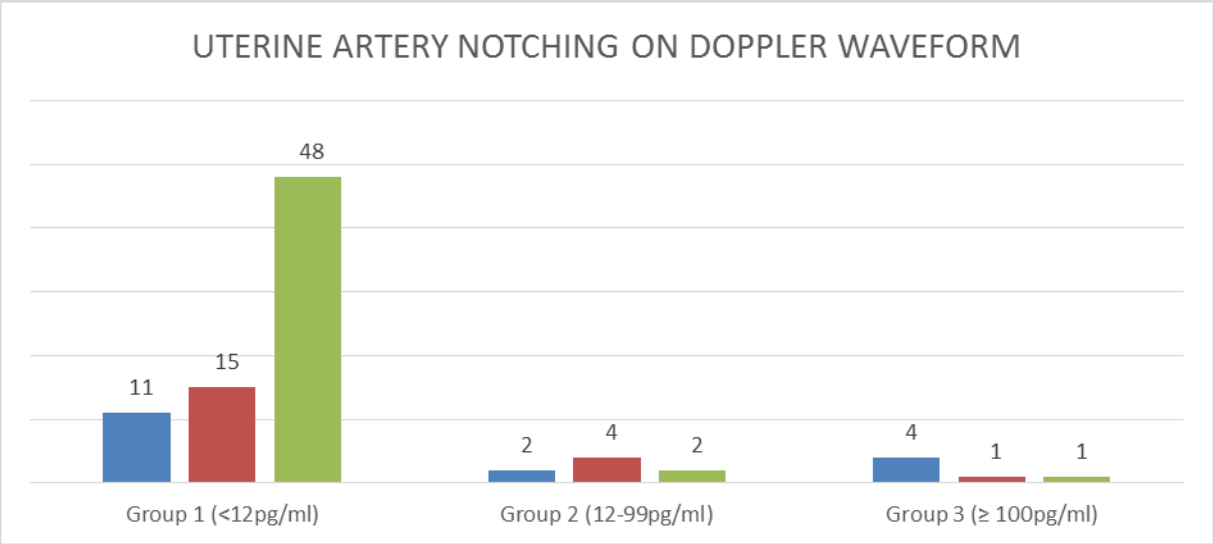
Uterine Artery Dopplers

These were measured in 88 women. Amongst Group 1, 63 had bilateral or unilateral notching, and only 11 had no notching whilst in the 6 patients who had uterine artery Dopplers done in Group 3, 4 had no notching, 1 had unilateral notching and 1 had bilateral notching. Abnormal uterine artery Dopplers amongst patients with a highly abnormal PlGF value was statistically significant.

Graph 8: Uterine Artery Dopplers amongst PlGF groups (P Value = 0.006)



Diabetes Mellitus and Renal lesions

These were the two comorbidities that appeared proportionally more common amongst group 3 compared to group 1 although due to very small numbers it is difficult to comment on this. Only 3 patients in the study were confirmed to have underlying renal disease – 2 of these were in group 3 were found not to have pre-eclampsia after initial work up. 5 patients had diabetes – 3 fell into group 1 and 2 were in group 3. Chronic hypertension with superimposed pre-eclampsia was not significantly different between the groups.

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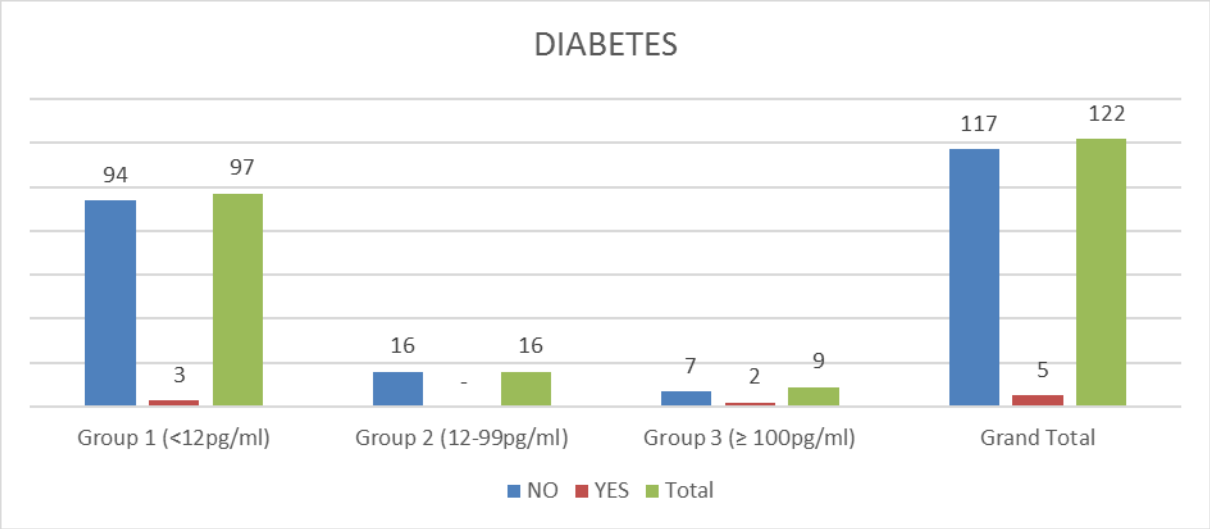
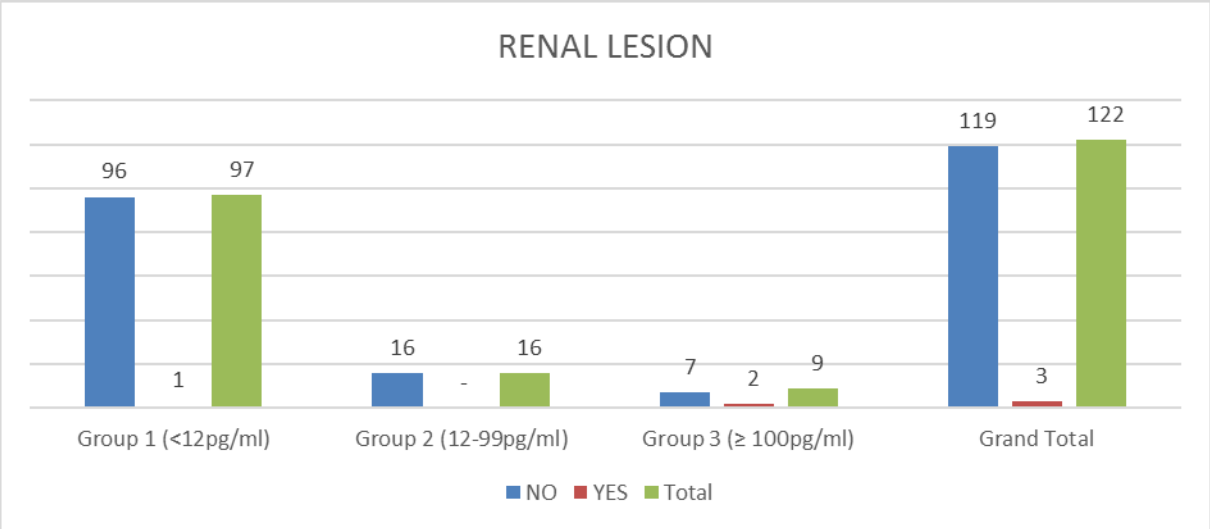
Graph 9: Prevalence of diabetes amongst PlGF Groups (P Value =0.000)



Graph 10: Prevalence of Renal Lesions amongst PlGF Groups (P Value = 0.000)



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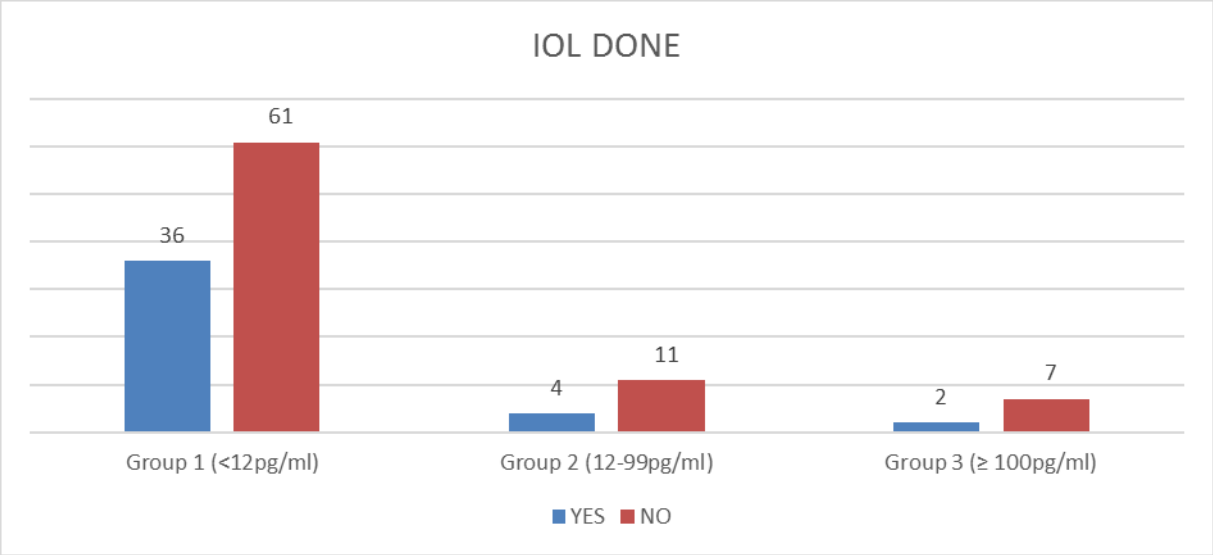
Induction of labour (IOL)

IOL was done in 37% of group 1 women, 25% of group 2 and 22% of group 3.

Graph 11: IOL in women stratified according to PlGF value (P Value = 0.004)



Indication for Delivery

Almost half of patients with abnormal PlGF values in groups 1 and 2 were delivered for fetal distress (Group 1 42.2%; group 2 50% and combined 43.36%) compared to group 3 where 11.11% delivered due to a pathological CTG. In group 1 only 13.4% of patients reached 34 weeks gestation and were induced routinely without any complication.

In group 3, with a normal PlGF value 44% of patients delivered routinely at 34 weeks gestational age without any adverse outcome occurring during their period of expectant management.

All indications for delivery were divided into maternal, fetal or combination. In group 1, 42.3% delivered for maternal reasons, 50.5% delivered for fetal reasons and 7.2% for a combination. In contrast group 3, three of the nine patients delivered for maternal reasons, only one for fetal reasons and five for a combination. This was statistically significant.

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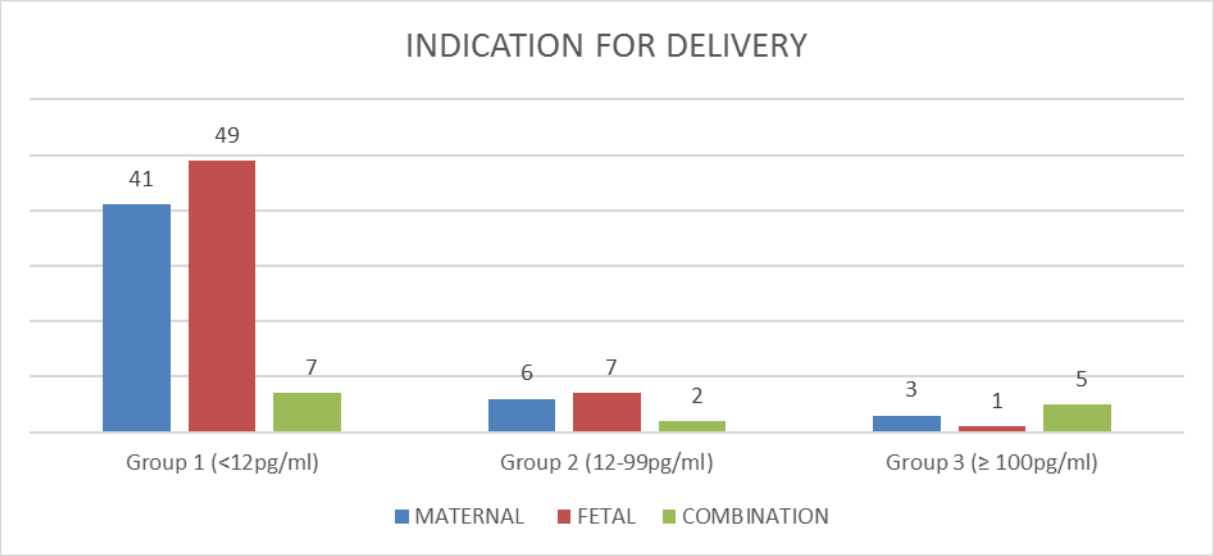
Graph 12: Indication for Delivery amongst women in each PlGF group (P Value = 0.000)



Time from diagnosis until delivery

Time in days from when PlGF was drawn until delivery also differed greatly between the groups – strengthening the notion that PlGF is an accurate predictor of delivery within 14 days. In group 1 the average time was 5.19 days whereas group 3's mean was 45 days. Many of our patients were diagnosed several days or weeks gestation prior to being incorporated in the study – especially if they had their workup for suspected pre-eclampsia done at referring hospital, thus if one looks at time from diagnosis until delivery it differs somewhat from the above figures but remains significant (p=0.00) supporting our statement. Time from diagnosis until delivery gives a mean of 11 days in group 1; 17 days in group 2 and 57 days in group 3. Thus those with severe pre-eclampsia and a PlGF value of <12pg/ml would not gain more than 2 weeks of expectant management; as confirmed in other studies.

Table 4: Days gained on expectant management between groups

Difference in days between diagnosis to delivery	Group 1 (<12pg/ml)	Group 2 (12-99pg/ml)	Group 3 (≥ 100pg/ml)	Grand Total	p-value
Average	10.91 ± 11.81	17.07 ± 12.81	56.77 ± 40.05	15.14 ± 19.7	0.000
Median	7 (0-56)	19.5 (2-43)	53 (3-122)	8 (0-122)	

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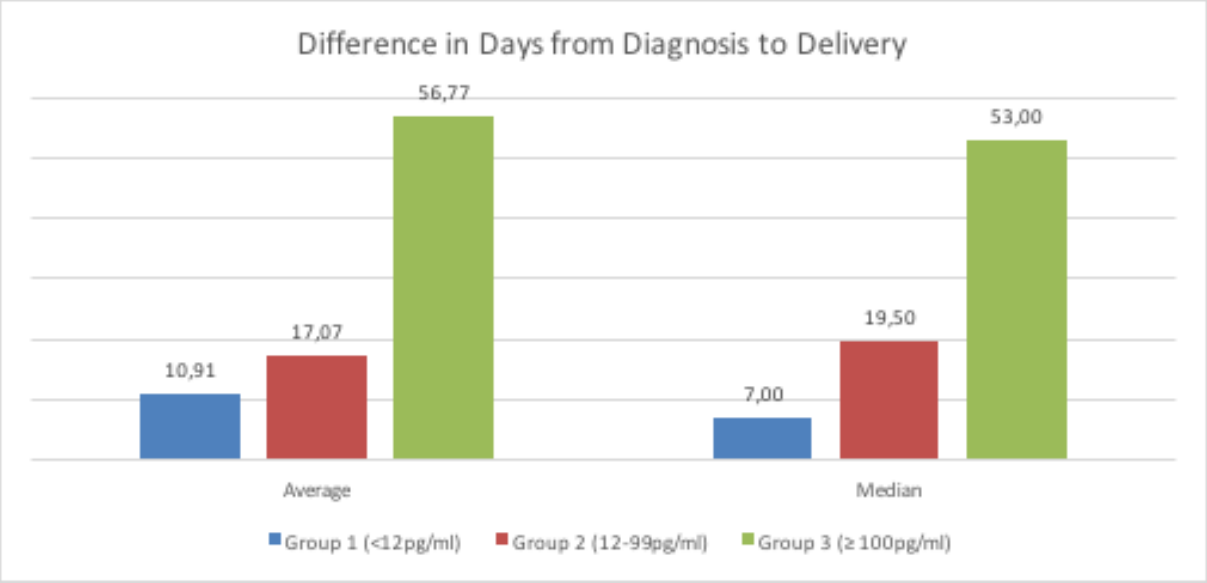
Graph 13: Days gained expectantly between groups



Adverse Outcome

All adverse outcomes were captured amongst participants and listed in the graph below. Looking at the data retrospectively it is evident that knowing the patient's PlGF values prior to delivery would have not been useful in predicting maternal complications (P value 0,212). However, when analyzing the data on perinatal complications, there were statistically significant differences between the 3 groups (P value 0.001), which did not change irrespective of whether we included or excluded patients with no other complication where IUGR was not known (no ultrasound). Adverse perinatal outcome was significantly more prevalent in the patients with highly abnormal PlGF values compared to those in group 3.

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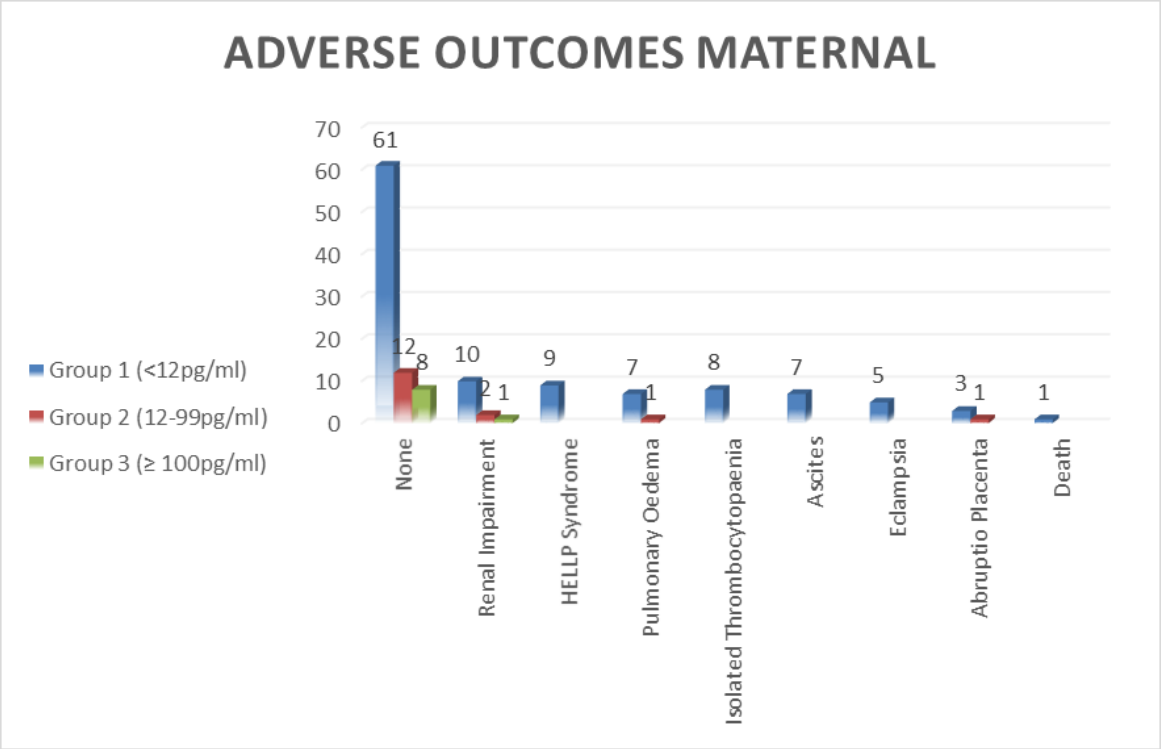
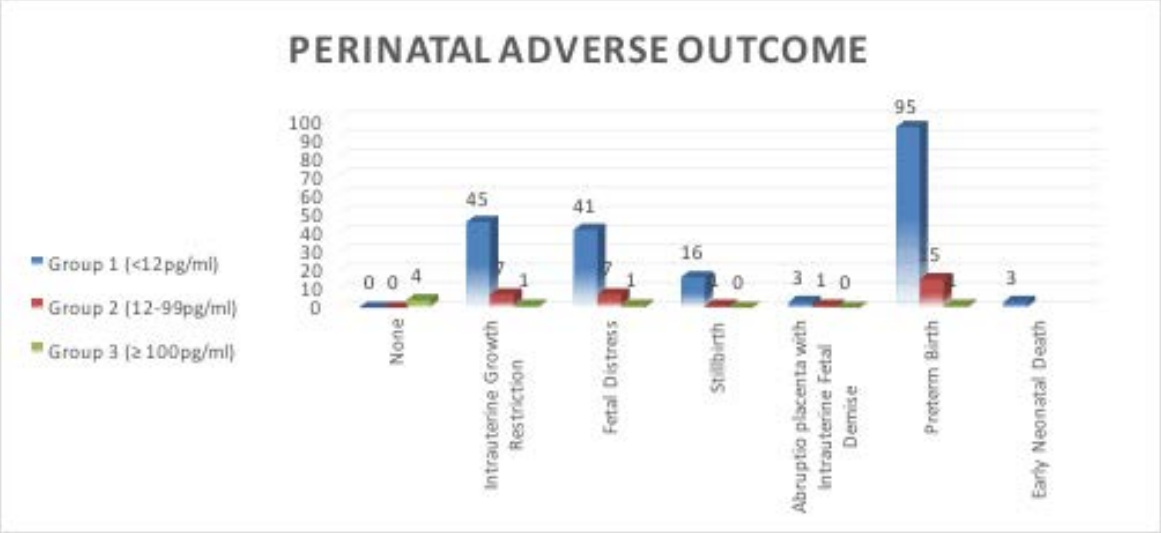
Graph 14: Adverse Maternal Outcomes (P Value = 0.212)



Graph 15: Adverse Perinatal Outcomes (P Value = 0.001)



One patient who presented with seizures and proteinuria was later diagnosed with seizures secondary to neurocysticercosis and not eclampsia and delivered at term, in one other the diagnosis of pre-eclampsia was revised to metabolic syndrome and in another chronic hypertension with a renal lesion then allowed outpatient management as diagnosis of pre-eclampsia was doubtful. Others were more thought to have chronic hypertension with a renal lesion and were offered outpatient management after review by maternal fetal medicine specialists. These patients delivered closer to term but were

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not then excluded from our study as important secondary outcomes were extrapolated from their higher PlGF values.

In four patients, whose DUP result came back less than 0.3 with no other clinical or biochemical factors convincing of pre-eclampsia, they were discharged and had uneventful pregnancies delivering at term. One obese subject with proteinuria of 0.8g/24hr and only mild hypertension present since booking was labeled metabolic syndrome and had successful outpatient management with close surveillance, delivering at term. Another patient carrying DCDA twins declined hospital management and discharged herself against doctors' advice after being diagnosed with severe pre-eclampsia at 25 weeks gestation with urine protein quantification of 0.39g/24hrs. She came back in spontaneous labour at 39 weeks gestation with no maternal or fetal complications at all. Conversely another patient who was discharged home when her urine result came back 0.23g/24hrs; only to present 6 weeks later with an abruptio placenta IUFD secondary to pre-eclampsia.

The one maternal mortality in our study was a woman who presented to Tygerberg Hospital having had multiple eclamptic seizures. She was in renal failure and had HELLP syndrome. Emergency caesarian was done for poor maternal condition but she unfortunately did not recover neurologically and died 2 days postpartum.

DISCUSSION

The role of PlGF in pre-eclampsia lies in being able to predict interval to delivery. In patients with values <12 pg/ml, the average time gained from diagnosis to delivery with expectant management was 11 days, and 73.2% of the patients with this highly abnormal value delivered with 2 weeks of diagnosis. Those with slightly higher but still abnormal PlGF values (12-100pg/ml) delivered on average within 17 days. Out of the 9 patients in our study with normal PlGF values (above 100pg/ml) – only 1 delivered within 15 days of diagnosis and the mean interval to delivery in this group was much longer, on average 57 days or just over 8 weeks.

Our findings did not support the role of PlGF in predicting adverse maternal outcome. This is because the vast majority of our patients had severe early pre-eclampsia and not

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mild to moderate disease, this is also reflected by their highly abnormal PlGF levels in 79.5% of the study population. The link between adverse outcome and PlGF levels could not be proven. For example; the eclamptic patient who demised versus the severe early onset pre-eclamptic who delivered routinely at 34 weeks gestation both had PlGF values of <12pg/ml. In women with severe early onset pre-eclampsia, it can be expected that her PlGF value will be less than<12pg/ml; but knowing this cannot help us predict her chances for having an eclamptic seizure or on the contrary delivering at 34 weeks gestation without complication. A highly abnormal value can alert the clinician to the fact that there is profound placental disease and risks of complications exist, but this is already established by the mere fact that they have been diagnosed with the severe early onset syndrome. The management would not change based on PlGF value; as all these patients need inpatient care with very close maternal and fetal surveillance anyway.

Perinatal outcome however did correlate with PlGF level, with fetuses and neonates of mothers with abnormal values suffering more IUGR, fetal distress, stillbirth and early neonatal death. Whether one could say PlGF can be used as a predictor of poor perinatal outcome may be debated by the fact that patients in group 3 with normal values likely did not have pre-eclampsia. Hence those babies would, by the mere absence of placental disease, be less affected in utero and out, as iatrogenic preterm delivery would also be avoided. Thus it again highlights that PlGF is a useful diagnostic marker of pre-eclampsia where diagnosis is complicated due to maternal factors such a underlying chronic disease and obesity causing metabolic syndrome and that perinatal outcome is in fact directly related to the presence or absence of pre-eclampsia, as is already well known, rather than PlGF value per se.

Some other interesting observations were that majority of women in our study were multigravida. This contradicts the notion of pre-eclampsia being associated with nulliparity. This is in keeping with findings from other studies (44). Meta-analysis has shown that maternal cigarette smoking is associated with a significant reduction in risk of pre-eclampsia. Based on an in vitro experiment the mechanism might be that smoke decreases sFlt-1 and increases PlGF (46). Despite this postulation, we did not find more smokers in group 3 compared to group 1.

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Trend toward higher BMI's in Group 3 can be understood due to the fact that these patients presented initially with what looked to be pre-eclampsia but was possibly only mimicking it because of their underlying illnesses and comorbidities such as metabolic syndrome as a consequence of their obesity. The thinner phenotypes in general were the younger, healthy primigravidas who had no other reason to have hypertension and proteinuria other than pre-eclampsia; thus correlating with a lower average BMI.

The significantly higher diastolic BP in group 1 could imply that the severity of the degree of pre-eclampsia could be predicted by a combination of highly abnormal PlGF and other parameters such as diastolic BP. Interestingly, more of our participants with severe hypertension (SBP ≥160mmHg and/or DBP ≥110mmHg) were labelled as so because of the systolic element rather than the diastolic. Systolic blood pressure is regarded as the strongest predictor of maternal end organ damage (48), but SBP differences between the groups was not statistically significant.

Evidence for the significance of unilateral compared to bilateral notching is confounding; some studies found similar rates of adverse perinatal outcome amongst those with unilateral notching compared to general population and other studies showed that both unilateral and bilateral notching is associated with a high incidence of developing IUGR and pre-eclampsia (47). In our study bilateral notching was significantly more prevalent in the patients with highly abnormal PlGF values.

Clinical ascites as a reason for delivery is a protocol employed by our hospital because it signifies a significant capillary leak with a higher chance of pulmonary or cerebral oedema.

In all the examples mentioned in results section of the patients discharged with normal DUP results, the patient where diagnosis was revised to metabolic syndrome, along with the DCDA twin mother who declined in hospital treatment as well as the patient who was readmitted with an abruptio placenta – the PlGF value correlated with outcome.

Indeed, the predictive value of PlGF in diagnosing pre-eclampsia has already been well established in literature and the normal values in women in this study whose diagnoses were revised supports this notion, as in the neurocysticercosis case. In patients where a

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diagnosis of pre-eclampsia is difficult due to proteinuria being present possibly from another pre-existing condition, a normal PLGF value would allow the clinician to decide with a greater degree of confidence that the patient is very unlikely to have pre-eclampsia and can be offered outpatient management until term.

A last comment from our research is that PlGF was not useful in predicting maternal complications (Graph 4) and will also not be useful in distinguishing mild pre-eclampsia from severe pre-eclampsia of early onset.

STUDY STRENGTHS

This was a prospective study of a group of high-risk patients admitted for pre-eclampsia evaluation. Data was collected and recorded once all patients were postpartum and outcomes recorded in a blinded fashion prior to the release of the PlGF results. Hence the influence of the angiogenic factor level on clinical care and decision making by the clinicians treating the patients was not possible.

STUDY LIMITATIONS

Firstly, this was a study of a small number of subjects from a single centre. Furthermore, the neonatal outcomes were probably not a true reflection of intact survival as morbidities and neurodevelopmental outcome were not reported, neither was mortality past day 7 known. IUGR was only recorded if the patient had a formal ultrasound with Dopplers, hence likely an under-reflection of fetuses compromised by placental insufficiency, and at birth, neither Ballard scores were reviewed nor birthweights correlated to gestational age. A small number of our patients had been diagnosed with an IUFD around the time of PlGF collection which may have contributed to a lower value than what would have been if the fetus were alive. We acknowledge that a PlGF/sFlt-1 ratio would have been of a more prognostic benefit than PlGF alone. And finally, a bias exists by the fact that PlGF values were not done on all the women at the same gestation but with a variance of 10 weeks, thus making interpretation of comparisons less valid.

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CONCLUSION

In conclusion, this study strengthens and supports the findings from previous studies and provides further evidence that an abnormal PlGF value in women with suspected pre-eclampsia does correlate with shorter time until delivery. Not more than 2 weeks of expectant management is likely in patients in the highly abnormal group and may be stretched to 3 weeks if the PlGF value is abnormal but more than 12pg/ml. Time to delivery was markedly different between the 3 groups.

To our knowledge this is the first study in South Africa assessing PlGF as a predictor of adverse outcome in pre-eclampsia. Further studies on a larger cohort of patients would need to be done that possibly include sequential levels of PlGF and other angiogenic and antiangiogenic factors before drawing any strong conclusions on their role. Incorporating PlGF to assist in confirming or excluding diagnosis of pre-eclampsia in confounding or ambiguous cases will certainly prove helpful, in those with normal values thus preventing unnecessary intervention, iatrogenic preterm delivery and prolonged inpatient management with massive cost saving benefit and amelioration of maternal anxiety.

Lastly, our study found that PlGF was not a useful prognosticator in predicting adverse maternal outcome, although statistically significant correlation did occur when evaluating perinatal complications. This finding must be interpreted with caution however, as normal values likely implied absence of pre-eclampsia which in itself denotes better fetal and neonatal outcome. Although the placental growth factor has been stated as the most useful single biomarker in diagnosis and prediction of pre-eclampsia, it would appear its role in prediction of adverse maternal outcome would only be when used in combination with sFlt-1, as evidenced in other trials but is out of the scope of this study. We conclude from this small study that the role of PlGF as a stand-alone marker for this purpose is unlikely to convey any benefit.

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ADDENDUM A – CONSENT FORM

THE ROLE OF PLACENTAL GROWTH FACTOR IN PREDICTING OUTCOMES OF PRE-ECLAMPSIA

Dear .....

The doctors caring for you told us that you have been diagnosed with pre-eclampsia. This is a serious complication of pregnancy which we diagnose when the mother has high blood pressure and protein in her urine. Pre-eclampsia could affect you or your baby, or both of you. While delivery of the baby may be a good choice for you (the mother), it may be bad for your baby as it is still long before your baby should normally be born. If your baby is born too early, it has a bigger chance of dying or suffering complications of its lungs, brain or gut. If your baby is born too late, you have a higher risk of developing problems of the brain, heart, lungs, liver or blood. The specific risks are different for each patient and your own doctor haS already explained your specific problems, or will do so soon.

The doctors caring for you have to decide whether it is best for you and your baby to be born now or whether they can safely deliver the baby a little later when it has a smaller chance of complications. They will do several tests to help them make this decision, including blood tests which will probably be done at least daily over the first few days. They may also consider one or more tests to find out how well your baby is. We will not be involved in any decisions about your management. This is the task of your own doctors.

However, we are asking if you will be willing to take part in a research project which we are doing in Tygerberg Hospital. The study is called: The role of placental growth factor in predicting outcomes of pre-eclampsia. We will be measuring your blood level of PLACENTAL GROWTH FACTOR. This is a substance that comes from the placenta. There is evidence that the baby needs to be born earlier when this level is low. We want to investigate whether this test will help doctors in their decision about when to deliver the baby if the mother has pre-eclampsia.

For this we need two millilitre (half a teaspoon) of your blood. If you are willing to take part in our study, we will ask your doctor to draw this extra blood when your next blood is drawn. This is all that is required for the study - we will not be drawing any blood on our own. We will store your blood for a while and then do the test on our machine. We will not use the result to decide when your baby should be born. We plan to get blood from 100 patients with pre-eclampsia, all at Tygerberg Hospital. When all these babies have been born, we will study all the results to see if this test can be used in women who have pre-eclampsia in future. If the test is useful, it may make the decision about when to deliver a bit easier. Your result will also be used in the study, unless you tell us not to do that. If you decide that we should not use your result, even after your blood was taken, please contact dr Deall. Once you tell us you will take part, you will receive a number which will be used in our analysis. This means that no-one will be able to identify your result when we look at all our results.

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You do not have to agree to have the extra blood taken. Your doctors will treat you exactly the same whether you decide to take part in the study or not. There are also no extra risks for your health, because your doctors will have to draw your blood in any case to be able to treat you in the best way.

The person doing this study is Dr Tracey Deall. She is the principal investigator and you can contact her at [redacted]. She is doing the study with Professor DW Steyn. No-one else is involved. We will not require anything more from you once the blood was drawn. You will not need to pay for the test. The two researchers will pay all costs. You will also not be paid for taking part.

The Health Research Ethics Committee of Stellenbosch has approved the research. They will also be able to inspect our records at any time. The committee can be contacted at (021) [redacted]. We have also planned our study according to what is done worldwide. We will do the study according to the International Declaration of Helsinki.

If you agree to take part in the study, please sign below. If you have any uncertainties, please ask the person who explains this study to you.

Signature Date

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ADDENDUM B – ETHICS APPROVAL

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02-Dec-2014 Deall, Tracey TC

Approval Notice New Application

Ethics Reference #: S14/10/223 Title: Evaluation of placental growth factor (PlGF) and its role in the assessment of pre-eclampsia

Dear Dr Tracey Deall,

The **New Application** received on **15-Oct-2014**, was reviewed by Health Research Ethics Committee 2 via Committee Review procedures on **19-Nov-2014** and has been approved. Please note the following information about your approved research protocol:

Protocol Approval Period: **19-Nov-2014 -19-Nov-2015**

Present Committee Members:

[redacted]

Please remember to use your **protocol number** (**S14/10/223**) on any documents or correspondence with the HREC concerning your research protocol. Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or

monitor the conduct of your research and the consent process.

After Ethical Review:

Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit. Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372 Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

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Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms [redacted] at Western Cape Department of Health [redacted]@pgwc.gov.za Tel: +27 21 [redacted]) and Dr [redacted] at City Health [redacted]@capetown.gov.za Tel: +27 21 [redacted]). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research. For standard HREC forms and documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at 219389207.

Included Documents:

Declaration DW Steyn Research Protocol CV DW Steyn Consent Form Protocol Synopsis Declaration TC Deall Application Form Cover Letter

General Checklist CV TC Deall

Sincerely,

Mertrude Davids HREC Coordinator Health Research Ethics Committee 2

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ACKNOWLEDGEMENTS

I would like to thank my study leader, Professor DW Steyn, for his guidance and mentorship through this 2 year endeavour, for the many hours spent reviewing my data and doing the statistical analyses.

Secondly my sincere gratitude to my assistant at the NHLS, Dr. Megan Rensburg, for processing the samples, without whom I would have a study with no PlGF results.

Then to Sister Erika Van Papendorp, for her assistance in patient recruitment, and for superseding expectations by her ever willingness to help.

And lastly to my patient and understanding husband Warwick, for his unwavering support and confidence in me.