
RCSI

# Aspirin for Optimising Pregnancy Outcome in Pregestational Diabetes: The IRELAnD Study (Investigating the Role of Early Low-dose Aspirin in pre-existing Diabetes) – Pilot Study

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## Declaration

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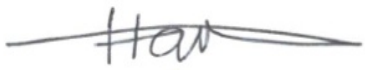

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# List of Abbreviations

| | |
|---|---|
| ACOG | American College of Obstetricians & Gynecologists |
| ADP | Adenosine Diphosphate |
| ASA | Acetyl Salicylic Acid |
| $Ca^{+2}$ | Calcium |
| C-AMP | Cyclic- Adenosine Mono Phosphate |
| CEMACH | Confidential Enquiries into Maternal and Child Health |
| CI | Confidence Interval |
| CLASP | Collaborative Low-dose Aspirin Study in Pregnancy |
| COX | Cyclooxygenase |
| CSR | Clinical Study Report |
| DIC | Disseminated Intravascular Coagulopathy |
| DVM | Delayed Villous Maturation |
| EBL | Estimated Blood Loss |
| ECCPA | Estudo Colaborativo para Prevenção da Pré-eclampsia com Aspirina |
| Epi | Epinephrine |
| GA | Gestational Age |
| GAD | Glutamic Acid Decarboxylase |
| GP | Glycoprotein |
| GDM | Gestational Diabetes Mellitus |
| Hb | Haemoglobin |
| Hb A1c. | Glycosylated Haemoglobin A1c |
| HLA | Human Leukocyte Antigen |
| HPL | Human Placental Lactogen |
| HR | Hazard Ratio |
| HSE | Health Service Executive |
| ICH | International Conference on Harmonisation |
| ICU | Intensive Care Unit |
| ISSHP | International Society for the Study of Hypertension in Pregnancy |
| IOL | Induction of Labour |

| | |
|---|---|
| IUGR | Intra-Uterine Growth Restriction |
| LDA | Low Dose Aspirin |
| LSCS | Lower Segment Caesarean Section |
| LTA | Light Transmission Aggregometry |
| MFMU | Maternal Fetal Medicine Units Network |
| MPV | Mean Platelet Volume |
| mRNA | Messenger-RNA |
| NICE | National Institute for Health and Care Excellence |
| NNT | Number Needed to Treat |
| NSAIDs | Non-Steroidal Anti Inflammatory Drugs |
| OR | Odds Ratio |
| PCR | Protein Creatinine Ratio |
| PECAM-1 | Platelet-Endothelial Cell Adhesion Molecule 1 |
| PE | Preeclampsia |
| PFA-100 | Platelet Function Analyser-100 |
| PGDM | Pre-Gestational Diabetes Mellitus |
| $PGE_2$ | Prostaglandin $E_2$ |
| PGI | Prostaglandin I |
| PIGF | Placental Growth Factor |
| PPH | Post-Partum Haemorrhage |
| PPP | Platelet Poor Plasma |
| PRP | Platelet Rich Plasma |
| RCOG | Royal College of Obstetricians & Gynaecologists |
| RCPI | Royal College of Physicians in Ireland |
| RCT | Randomised Controlled Trial |
| RD | Risk Difference |
| RR | Relative Risk |
| sEng | Soluble Endoglin |
| sFlt1 | Soluble Fms-like tyrosine kinase1 |
| SOL | Spontaneous Onset of Labour |
| $ThA_2$ | Thromboxane $A_2$ |
| 11-$DThB_2$ | 11-dehydrothromboxane $B_2$ |
| USPSTF | United States Preventive Service Task Force |

| | |
|---|---|
| VEGF | Vascular endothelial growth factor |
| vWF | Von Willbrand Factor |
| WHO | World Health Organisation |

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# Abstract

**Background**: The role of aspirin in reducing the risk of preeclampsia and improving perinatal outcome for pregnancies complicated by a diagnosis of pregestational diabetes mellitus has been investigated in few studies, which have revealed conflicting results. The arteriopathic characteristic of type I and type II diabetes, coupled with the pro-thrombotic nature of pregnancy, confer on aspirin a potential to ameliorate placental insufficiency-mediated morbidity frequently observed in these high risk pregnancies.

**Objectives:** To investigate the proportion of eligible women with pregestational diabetes who participated in the pilot phase of a randomised trial of low-dose aspirin therapy in pregnancy, compliance with the study protocol, as judged subjectively ( using self-reporting and pill count) and objectively ( using platelet aggregation testing) and analysis of all adverse events occurring after randomisation.

**Study Design**: A phase IV, multi-centre, randomised, open label trial investigating the use of aspirin for optimising pregnancy outcome in pregestational diabetes.

**Materials and Methods**: Participants were randomised to aspirin 75mg or no aspirin (controls). Aspirin (Nu-Seals®PA 943/6/1) was administered once daily by oral ingestion from the first trimester (initiated between $8^{+0}$ and $11^{+6}$ weeks) to 36 weeks' gestational age for women with pregestational diabetes mellitus (type I or II). The patient population included women with a singleton pregnancy < 12 weeks of gestation, with type I or type II diabetes of at least six months duration prior to conception.

**Results**: A total of 47 patients with pregestational diabetes were screened and 31 were deemed eligible ( 66%). Twenty-four patients agreed to participate in the study and were recruited, 13 in the aspirin group and 11 in the non-aspirin group. The proportion that presented prior to the gestational age cut-off of 12 weeks was 45/47 (96%). The participation rate among eligible women was 77% (24/31). The most common criterion for ineligibility was miscarriage (17%) and only 2/47 (4%) of women screened were already taking aspirin. Compliance was measured subjectively using diary card and pill count and that was supported by subjective test for platelet aggregation using light transmission aggregometry. Therefore 11 out of 13 patients were deemed compliant as per compliance criteria (85%).

.**Conclusions**: Platelet function testing was deemed to be informative for reflecting patient compliance with aspirin therapy, and for demonstrating non-response to aspirin among women judged to be compliant through other compliance-assessment methods. This pilot study is a precursor for a definitive large randomised controlled trial aiming to assess if low-dose aspirin initiated in the first trimester reduces obstetric complications related to placental dysfunction in the setting of pre-existing diabetes.

# Acknowledgements

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## Presentations & Publications

- Objective testing of compliance to aspirin, is it necessary?
  Hala Abu, Ann McHugh, Siobhan Corcoran, Sami Backly, Elizabeth Tully, Dermot Kenny, Fergal Malone, Fionnuala Breathnach
  The 10th International Symposium on Diabetes, Hypertension, Metabolic Syndrome and Pregnancy. 29th May- 1st June- Florence-Italy.

- Aspirin for Optimising Pregnancy Outcome in Pregestational Diabetes: The value of objective testing of study participant compliance. Pilot for The IRELAND Study (Investigating the Role of Early Low-dose Aspirin iN pre-existing Diabetes)
  Hala Abu, Ann McHugh, Siobhan Corcoran, Sami Backly, Elizabeth Tully, Dermot Kenny, Fergal Malone, Fionnuala Breathnach
  BMFMS Meeting 28th-29th March 2019- Edinburgh.

- The role of platelet aggregometry testing in evaluation of patient non-compliance versus non-response to aspirin therapy.
  Hala Abu, Ann McHugh, Siobhan Corcoran, Sami Backly, Elizabeth Tully, Dermot Kenny, Fergal Malone, Fionnuala Breathnach
  JOGS Meeting 23rd November 2018- Dublin.

- Platelet function testing in assessing patient compliance to aspirin administration in pregestational diabetes.
  Hala Abu, Ann McHugh, Siobhan Corcoran, Sami Backly, Elizabeth Tully, Dermot Kenny, Fergal Malone, Fionnuala Breathnach
  BMFMS Meeting 30-31st March 2017- Amsterdam.

- Aspirin for optimising pregnancy outcome in pregestational diabetes. Pilot study to assess feasibility and compliance.
  Hala Abu, Ann McHugh, Siobhan Corcoran, Sami Backly, Elizabeth Tully, Dermot Kenny, Fergal Malone, Fionnuala Breathnach
  RCSI Study Day 5th March 2017.

# Chapter 1: Introduction

## 1.1 Global impact of pre-eclampsia

Pre-eclampsia (PE) is a leading cause of maternal and fetal morbidity and mortality. It complicates 2% to 8% of pregnancies(1) and it is the most common hypertensive disease in pregnancy(2).

An increase in the observed incidence of this disease in the United States between 1993-1997 and 2001-2005 from 3.0% to 3.4%.respectively(1, 3), raised concern for an increase in prevalence, although this heightened incidence has not been sustained.

Over half a million women die each year from PE- related complications, mainly eclampsia and hypertension-induced cardiovascular accidents; 99% of these deaths occur in low to middle- income countries(1). Although the Confidential Enquiries into Maternal and Child Health (CEMACH) 2009-2012 in the United Kingdom(4), reported that maternal deaths from PE are at their lowest, maternal morbidity from the disorder remains substantial. Women with PE and eclampsia had a 3- to 25-fold increased risk of severe complications, including multiple organ failure, disseminated intravascular coagulopathy (DIC), and haemorrhagic stroke(4). Severe maternal morbidity (near-miss) cases were eight times more frequent in women with PE, increasing up to 60 times more frequent in women with eclampsia, when compared with women without these conditions(5).

As the placenta is believed to be the source of the disease, the consequences on the fetus have detrimental effects, such as intrauterine growth restriction (IUGR), prematurity, and even stillbirth.

The economic impact of PE stems from prolonged hospitalisation, need for caesarean section, and intensive care admission, in addition to prematurity, and the need for neonatal intensive care unit admission. Over a quarter of Intensive Care Unit (ICU) obstetric admissions in France are primarily for PE(6).

A population based study in Ontario, Canada, concluded that there was a substantial increase in the cost of caring for women with PE, attributed to an increased use of

spinal anaesthesia, maternal transfer to ICU, caesarean section delivery, neonatal transfer, newborn resuscitation, longer hospital stay for childbirth, and higher rates of preterm birth and low birth weight(6, 7).

A well-established hypothesis is that placental dysfunction, due to disordered early placental development and inadequate cytotrophoplast invasion, resulting in poor placental perfusion, is central to the disease process(8). This theory proposes an imbalance between decreased production of endothelial vasodilator mediators such as Prostaglandin $E_2$ and $I_2$ (Prostacyclin) and increased production of platelet Thromboxane A2 ($ThA_2$) which is a potent vasoconstrictor. This theory has led to the use of different antiplatelet agents, mainly aspirin to try to prevent the condition.

Early placental disease is believed to be followed months later by the clinical manifestations of PE, which reflect widespread endothelial dysfunction, resulting in vasoconstriction, ischaemia and increased vascular permeability(9).

The increased incidence of perinatal morbidity and mortality seen in pregnancies complicated by PE, although complex and multifactorial, is primarily due to the need for premature delivery and to the fetal effects of uteroplacental insufficiency(10). The risks posed by the PE to the fetus include severe IUGR, hypoxaemia, premature birth, intrauterine death (IUD) and birth asphyxia. Sibai & Barton also reported that severe PE is associated with high perinatal mortality and morbidity(11). The same authors indicated that perinatal mortality increases as the severity of PE increases. It is believed that PE is responsible for 12% of small for gestational age fetuses and one fifth of those born premature.(12) In addition to the increase in maternal and perinatal morbidity and mortality, PE has been found to confer long-term adverse effects on the mother and fetus. A Norwegian study that included over 600,000 women showed that women with PE have an 8-fold increase in deaths from cardiovascular diseases compared to women who did not have PE(13).

Another study that included over 30,000 women in the state of Washington from 1967 to 1998 showed that women with mild and severe PE had a 2-fold and 3-fold greater risk of cardiovascular events later in life, respectively(14).

While not all adverse perinatal outcomes in PE are attributed to placental dysfunction, any therapy that offers the potential to optimise placentation in this group deserves close attention.

### 1.2 Definition of preeclampsia

Preeclampsia is a hypertensive disorder with multisystem involvement that is exclusive to pregnancy. It was primarily defined by the occurrence of new-onset high blood pressure and a new-onset proteinuria in the second or third trimester of pregnancy or after 20 weeks' gestation. However, although this was the classic definition of PE, some women presented with high blood pressure with systemic manifestation of the disease without the presence of proteinuria. Many national and international guidelines have been published on the diagnosis and management of PE.

The International Society for the Study of Hypertension in Pregnancy (ISSHP)(15), defined PE as gestational hypertension of at least 140/90 mmHg on two separate occasions ≥4 hours apart accompanied by one of the following:

1) Significant proteinuria of at least 300 mg in a 24-hour collection of urine or 30 mg/mmol on protein/creatinine ratio (PCR) arising de novo.
2) Other maternal organ dysfunction: renal insufficiency (creatinine >90 umol/L; 1.02 mg/ dL), liver involvement (elevated transaminases – at least twice upper limit of normal ± right upper quadrant or epigastric abdominal pain), neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata), haematological complications (thrombocytopenia – platelet count below 150,000/microlitre, DIC, haemolysis).
3) Uteroplacental dysfunction in the form of fetal growth restriction.(15)

The American College of Obstetrics and Gynecology (ACOG) has modified the classical definition of PE in 2013(16), importantly specifying that significant proteinuria that meets the diagnostic threshold is not necessarily required for diagnosis of PE, as long as other criteria are met, such as:

1) Thrombocytopenia (platelet count less than 100,000/microlitre).

2) Renal insufficiency (serum creatinine concentration greater than 1.1mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease).
3) Impaired liver function (elevated blood concentrations of liver transaminases to twice normal concentration).
4) Pulmonary oedema.
5) New-onset cerebral or visual symptoms.

The national guideline for the Diagnosis and Management of Severe PE and Eclampsia published in 2011 by the Royal College of Physicians in Ireland (RCPI) updated its definition in line with other international guidelines in which proteinuria was not required for establishment of the diagnosis in the presence of maternal organ dysfunction(17),

On the other hand, the National Institute of Health and Care Excellence (NICE) (18) and World Health Organization (WHO) (19) guidelines specify that the presence of significant proteinuria is mandatory for the diagnosis of PE. However, NICE are currently revising this definition and a guideline is expected to be published in June 2019.

For the IRELAnD study, we followed the national guidelines in Ireland in defining PE, which reflect the ISSHP definition.

## 1.3 Pathogenesis of preeclampsia

The pathophysiology of PE has not been fully elucidated and it is certainly multifactorial, with vascular, immune, genetic and placental factors hypothesised to play an important role. It is suggested that the clinical features of this syndrome are caused by systemic endothelial dysfunction that result from a combination of diverse maternal factors and poor placental development. Maternal risk factors include obesity, advanced maternal age, black race and chronic hypertensive disease. It is believed that all these factors may contribute to inflammation, oxidative stress and vascular dysfunction all of which have been implicated in the aetiology of PE.

### *1.3.1 Trophoblast invasion*

The placenta is central to the pathogenesis of PE. There are multiple theories, with little agreement on the cause of PE. The most accepted mechanism is the one that

describes disruption of vascular remodelling and systemic anti-angiogenic response(8, 20). In normal pregnancy, cytotrophoblast cells, originating in the tips of anchoring villi for the fetal portion of the placenta, penetrate to invade the maternal endometrium(10). This process results in the transformation of spiral arteries into dilated, low resistance inelastic arteries, with very little maternal vasomotor control, which allows a dramatic increase in blood supply to the growing fetus(21). This endovascular trophoblast invasion happens in two stages during pregnancy. The first occurs in the first 8-10 weeks of gestation and represents an invasion into the decidual segment of spiral arterioles, while the second stage occurs between 16 to 18 weeks of gestation and represents an invasion into the myometrial segment of spiral arterioles(21). Therefore, even though PE is not diagnosed until clinical symptoms develop after 20 weeks' gestation, this vascular remodelling and intravascular change happen in the first and early second trimesters. Thus, poor trophoblast invasion is the early stage of the disease progression and, while it is hypothesised that improper remodelling of maternal spiral arterioles is a key factor in the development of PE, the same theory is suggested in cases of uteroplacental insufficiency, such as in IUGR, with or without hypertensive disease(20, 21).

### *1.3.2 Immunology*

Preeclampsia involves a host of immunologic and genetic factors. Among the other theories that have been proposed in the aetiology of PE is the alteration in the immune response to the allogenic fetus(20, 22, 23). This immune maladaptation may cause a shallow invasion of trophoblasts into the spiral arteries, leading to endothelial cell dysfunction, and the release of immune-mediated cytokines and proteolytic enzymes, leading to cell death and placental ischaemia(10). As PE is primarily a disease of first pregnancy, this suggests that exposure to the paternal antigen is protective, even if it was a pregnancy that resulted in a miscarriage(24). This protective effect is lost with a change in paternity(25). Additional evidence also implicates changing paternity as a risk factor for PE. In a study of Caribbean women(26), 61.7% of those with PE had new partner pregnancies, compared to only 10% among women with chronic hypertension, and 16.6% in controls. Furthermore, when three or four consecutive pregnancies were examined, rates of PE appeared to summate with each additional change in paternity. A previous pregnancy with the same father or a long period of sexual cohabitation with the father seems to offer protection from PE(26).

### *1.3.3 Endothelial cell dysfunction*

In normal pregnancy there is an 8-10 fold increase in endothelial production of prostaglandin $I_2$ ($PGI_2$)(27). This coincides with increases in $ThA_2$, which is primarily secreted from activated platelets but at a much lower level. This leads to a dynamic of biochemical events driven by $PGI_2$, a potent vasodilatory marker, a platelet inhibitor and renin angiotensin stimulator. In PE, the theoretical reduction in response to renin angiotensin system and increased sensitivity to angiotensin II and norepinephrine is evident(28). This lead to increased production of $ThA_2$ at the expense of $PGI_2$, which is increased 2-fold, compared to 8-fold in normal pregnancy, leading to a vasoconstrictive environment and reduced uteroplacental blood flow. Many markers of endothelial dysfunction, in addition to $ThA_2$ and prostacyclin, have been reported in PE. Increased levels of factor VIII–related antigen, total and cellular fibronectin, thrombomodulin, endothelin, growth factor activity, and a disturbance of the tissue plasminogen activator/plasminogen activator inhibitor balance supports the hypothesis that a more global endothelial cell dysfunction is intimately involved in the pathogenesis of PE(29, 30).

Vascular endothelial growth factor (VEGF), which is known to increase vascular permeability, has been reported to be reduced in the plasma of pregnant women with PE(22, 31). VEGF is a known potent angiogenic factor that has a vasodilatory effect by stimulating endothelial secretion of $PGI_2$ and nitric oxide. Another angiogenic modulator is placental growth factor (PIGF).

Maynard et al, found that certain potent angiogenic growth factors, VEGF, and PIGF play an important role in the aetiology of PE(32). Upregulation of soluble Fms-like tyrosine kinase1 (sFlt1), which is a variant of the VEGF receptor, reduces free or active forms of VEGF and PIGF, causing endothelial cell dysfunction.

### *1.3.4 Oxidative stress*

Oxidative stress reflects an imbalance between the formation of oxidative substances and the innate antioxidants that make up the endogenous defence system(33). Oxidative substances are often free oxygen radicals and peroxides. In abundance, they cause nuclear cell DNA and cell membrane damage. This model of oxygen dysregulation causes hypoxia /reperfusion injury, which results in poor invasion of

trophoblasts into the spiral arteries in the early stages of gestational life. Markers of oxidative stress have been found in the serum and placental tissue of pregnancies complicated by PE(34).

### *1.3.5 Inflammatory process*

It has been hypothesised that endothelial dysfunction is one aspect of a generalised inflammatory process(35). Fragments of syncytiotrophoblast have been seen in abundance in the serum of women with PE(35). It has been proposed that increased debris in the blood stream will provoke a systemic inflammatory process, which is present in all pregnant women in the third trimester(36). If that process exceeds the decompensating response, apoptosis and necrosis begins, leading to mass destruction of syncytiotrophoblast and major vasoconstriction.

## 1.4 Major risk factors for preeclampsia

Internationally published guidelines suggest the use of aspirin for the prevention of PE in pregnancies deemed to be at increased risk for the development of PE(37). NICE Guidelines include women with hypertensive disease in a previous pregnancy, or chronic kidney disease or autoimmune disease such as systemic lupus erythematosus or antiphospholipid syndrome and type 1 or type 2 diabetes in the high-risk group(18). Women perceived to be at high risk of PE are advised to take 75 mg of aspirin daily from 12 weeks until the birth of the baby or until development of PE. On the other hand, women who are primiparous or are over the age of 40 years or had a pregnancy interval of more than 10 years or have a body mass index (BMI) of 35 kg/m$^2$ or more at first visit or have a family history of PE or have multiple pregnancy are listed in the moderate group. Women with more than one moderate risk factor for PE are advised to take 75 mg of aspirin daily from 12 weeks until the birth of the baby.

Risk factors for development of PE can be categorised according to past medical or obstetric history, pregnancy-associated factors and familial factors.

### *1.4.1 Past medical or obstetric history*

Preeclampsia is a syndrome, primarily of first pregnancy(38). A large population-based study reported that nulliparous women were at increased risk of PE compared with

parous women(39). Parous women with a history of PE in their previous pregnancies are at heightened risk for developing the disease in their subsequent pregnancies. This association is particularly strong for early-onset, moderate and severe PE(39). The risk of recurrent PE was 12% for those who previously delivered at term and increased to 40% for those who delivered before 28 weeks of gestation(39).

A prolonged pregnancy interval is a risk factor for the development of PE(40, 41). In a large cohort study, a birth interval of more than 4 years increased the risk of PE in women who had no prior history of the disease (Odd Ratio (OR) 1.4, 95% Confidence Interval (CI) 1.2–1.6)(41).

Increased BMI is an important and potentially modifiable risk factor for PE with an attributable risk of 64%(42).

Compared with women with a BMI of 21, the adjusted risk of PE doubled at a BMI of 26 (OR 2.1, 95% CI, 1.4, 3.4]), and nearly tripled at a BMI of 30 (OR 2.9 95% CI 1.6-5.3).(43) Increases in pre-pregnancy BMI from normal weight to overweight or obese between pregnancies are associated with increased risk of PE in the subsequent pregnancy(44).

Extremes of age are linked to PE(36). In a large study that included over 76,000 women, advanced maternal age of over 40 years was associated with PE (OR, 1.49, 95% CI, 1.22-1.82); $P < 0.001$)(45). That was also evident among teenage pregnant women(46). A large population based study indicated an increased risk of PE/ eclampsia among the youngest group of teenagers ( 13-year-olds to 15-year olds) and that was observed even after adjustment for confounding factors such as socioeconomic status, misuse of drugs and inadequate prenatal care (adjusted OR, 3.7 (1.5 to 9.0), 95% CI, 0.6-1.7).(47)

### *1.4.2 Familial factors*

Multiple epidemiological studies suggest a familial tendency for PE(48). It has been reported that for women who experience PE, the rate of disease is higher in sisters (37%), daughters (26%) and granddaughters (16%) when compared with daughters-in-law (6%)(49). A recent review suggested that those with a family history of PE are at an increased risk for this disease, Relative Risk (RR) 2.90, 95% CI 1.70–4.93)(49).

In a study conducted in Utah, women who were the product of a pregnancy complicated by PE were three times as likely to have PE(50).

The paternal contribution to the disease was discussed in the same study. Both men and women who were the product of a pregnancy complicated by PE were significantly more likely than control men and women to have a child who was the product of a pregnancy complicated by PE. In the male study group, 2.7% were born of pregnancies complicated by PE, as compared with 1.3% offspring of the male control group.

### *1.4.3 Pre-existing medical conditions*

Many of the maternal risk factors associated with PE are similar to those associated with cardiovascular disease (diabetes, pre-existing hypertension, obesity, renal diseases and autoimmune conditions). While obesity prevalence is increasing worldwide, an increased global incidence of PE has also been observed(51).

Women with underlying chronic hypertension have a 10-25% risk of developing PE compared to the general population(52).

Duckitt et al carried out a systematic review in 2005 to predict the relative risk of PE, in relation to some pre-existing conditions, such as chronic hypertension, pregestational diabetes mellitus (PGDM), anti-phospholipid syndrome, and renal diseases(53). The risk of PE was increased in those with anti-phospholipid antibodies (RR 9.72, 95%CI 4.34 - 21.75) and PGDM (RR 3.56, 95%CI 2.54 - 4.99). In a population- based case control study(54), the prevalence of chronic hypertension was higher in women who developed PE than women who did not (12.1% *vs* 0.3%). In the same study, the prevalence of renal disease was found to be higher in women who developed PE, compared with those that did not (5.3% *vs* 1.8%)(54).

## 1.5 Pregestational Diabetes mellitus (PGDM)

The World Health Organization report published in 2016 showed that the number of people with diabetes has substantially increased over the last three decades(55). Between 1980 and 2014 the number of people affected with diabetes has risen from 108 to 420 million worldwide. The global prevalence if diabetes has grown from 4.7% in 1980 to 8.5% in 2004.

### *1.5.1 Pregestational diabetes and the risk of preeclampsia*

Pregestational diabetes represents a high-risk for the evolution of PE, with rates of PE within this group ranging between 9%(56) and 20%(57). Preeclampsia incidence is seen to rise significantly with increasing severity of diabetes and is also more common among women who have proteinuria at baseline(57).

Hanson et al estimated the overall risk of developing PE in women with type 1 diabetes to be approximately 21%, however, the risk was increased to between 36% and 54% among women with diabetes associated with microvascular disease(58). Preeclapmsia prevalence rose significantly with increasing severity of diabetes according to White classification (class B, 11%; class C, 22%; class D, 21%; class RF, 36%)(57). Preeclampsia was also more common among women who had proteinuria at baseline (28% vs 18%; OR, 1.75; 95% CI 1.02-3.01)(57).

Preeclampsia has a much higher incidence in women with type 1 diabetes mellitus (T1DM) than for type II disease (T2DM). It is well documented that there is a four-fold increase in the rate of PE among type 1 diabetics than in the non-diabetic population; 20% vs. 5%, respectively(58). Preeclampsia is diagnosed in 15-20% of pregnancies with T1DM and 10-14% in pregnancies of women with T2DM(59). Obesity has been long recognised as a shared risk factor for both PE and type II PGDM and has been consistently linked to the risk of developing PE. However, Knight et al. showed an increased risk of PE in women with PGDM, even after women were matched for BMI(59). PGDM was linked to early, as well as late PE. A large population-based study (Washington Trial) that included over 45,000 women delivered between 2003-2008 in Washington State, showed that PGDM was a risk factor for both early-and late-onset PE (before and after 34 weeks' gestation, respectively); however, there was a stronger correlation between the latter and PGDM.(40)

The risk of PE has been shown to depend on multiple key risk factors in patients with PGDM such as the level of glycosylated haemoglobin A1c (HbA1c) and the presence of proteinuria. The Diabetes And Preeclampsia Intervention Trial (DAPIT), showed a strong correlation between glycemic control in patients with T1DM and the risk of PE.(60) In early pregnancy, Hb A1c level ≥ 8.0% was associated with a significantly increased risk of PE (OR 3.68, 95% CI 1.17-11.6) compared with optimal control. At both 26- and 34-weeks' gestation, there was a progressive and significant increase in

the odds of PE for suboptimal control (Hb A1C ≥6.1%) compared with the referent value (HbA1C <6.1%), with ORs ranging from 2.09 to 3.81 and 1.78 to 8.01, respectively. In another Finnish study, an improvement in glycaemic control judged by measuring HbA1C in the first trimester resulted in a reduction in the incidence of PE. The adjusted odds ratios for PE were 1.6 (95% CI 1.3-2.0) for each 1% increment in the HbA1c value at 4-14 (median 7) weeks of gestation(61).

In addition to glycaemic control, microvascular complications such as retinopathy and nephropathy are independent risk predictors for PE among women with diabetes. In the same study, PE was five times more prevalent in women with T1DM without nephropathy compared to a non-diabetic control population(62). On the other hand, diabetic women with known nephropathy had a 6-fold increase in the likelihood of developing PE compared to diabetic women with no nephropathy, which means diabetic women with nephropathy had a 30-fold increased chance of developing PE compared to non-diabetic women(56). The highest incidence of PE is observed among women with diabetes who are nephropathic and hypertensive at baseline(56). It is of importance to mention that distinguishing diabetic nephropathy from PE can be clinically challenging so; it is wise to assume that PE exists when the diagnostic dilemma cannot be solved.

### *1.5.2 Gestational insulin resistance*

Insulin resistance plays a major role in the aetiology of type II diabetes, and in the pathogenesis of dyslipidaemia, hypertension and coronary heart disease. In addition, it is characteristic of a normal pregnancy due to elevation of placental lactogen, placental growth hormone, estradiol and cortisol that have antagonistic action to insulin(63). Women with higher levels of fasting insulin, which is an indirect way of measuring insulin resistance, are at increased risk of developing a hypertensive disorder in pregnancy(56). Mid-trimester insulin resistance increases significantly with increasing BMI and is associated with a significant increase in risk of developing PE even after adjustment for maternal BMI, advanced maternal age and non-Caucasian race (64). Interestingly, preeclamptic women who are non-diabetic have been found to demonstrate residual insulin resistance after delivery, in addition to a 3-fold elevated risk for developing T2DM(64).

### *1.5.3 Angiogenic imbalance*

Studies that have investigated the role of angiogenic/anti-angiogenic factors in PE among women with PGDM have yielded conflicting results. The underlying cause for the increased risk of PE in women with PGDM is not fully understood. However, Yu et al suggested that hyperglycaemia could cause histological changes similar to those found in PE(65). The author concluded that PGDM can lead to abnormal placentation by inducing oxidative stress, leading to placental hypoxia and increasing anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt1), a vascular endothelial growth factor receptor and soluble endoglin (sEng). He also concluded that elevated levels of sFlt1 and elevated sFlt1/PlGF ratio are predictive of PE in pregnant women with T1DM. The largest trial examined plasma angiogenic factors among 540 women with T1DM. In comparison to women who did not develop PE, those who develop the disease (n=94) had increased levels of plasma sFlt-1 and sEng, and lower PlGF(66). Power et al. looked at these factors among low-risk and high-risk patients. They concluded that the pattern of elevated concentrations of sFlt1 and sEng, and low PlGF in high-risk pregnant subjects with pre-existing diabetes or chronic hypertension or multiple pregnancy or history of PE in previous pregnancy who developed PE is similar to that reported in low-risk pregnant women who developed the condition(67). However, there was a modest difference in these factors among women with PGDM who did not develop PE. A more recent study supported the previous study and showed that the median rise of sFlt1/PlGF ratio at 35-40 weeks was three-fold higher in women with PGDM than in non-diabetic controls(68). Table (1.1) summarises the findings in the above mentioned studies.

Understanding of the influence of angiogenic factors on the placental haemodynamic environment has led to an assessment of the effect of aspirin on the secretion of these factors in the human placenta. In hypoxic conditions, aspirin inhibits the production of sFlt1 in the cytotrophoblasts, and thus results in pro-angiogenic activity(69).

### *1.5.4 Haptoglobin (Hp).*

Haptoglobin is primarily produced in the liver and is functionally important for binding free haemoglobin from lysed cells in vivo, thereby preventing its toxic effects and oxidative damage generated by free iron in the vascular system of the kidneys. In addition to that it has pro-angiogenic and antioxidant properties. Multiple studies have

suggested its involvement in the pathophysiology of PE.(32, 70, 71) Different phenotypes (Hp1-1, 2-1, 2-2) have different properties, with Hp 1-1 recognised as potently antioxidant and Hp2-2 as a potent angiogenic. Small studies have produced heterogeneous results, in relation to the influence of haptoglobin phenotype on the risk of PE in diabetic women(70, 72). Weissgerber et al. conducted a secondary analysis of two large randomised controlled trials for the use of vitamin C supplementation to reduce the risk of PE(71, 73). They concluded that there was no relationship between the development of PE and the Hp phenotype.

## 1.6 Classification of Diabetes Mellitus

There are 2 different methods of classifying diabetes in pregnancy. The first is the American Diabetes Association (ADA) classification and the second is the White classification.

### *1.6.1 American Diabetes Association classification*

Diabetes mellitus is a systemic disorder characterised by the presence of hyperglycaemia due to impairment of insulin secretion, defective insulin action or both. The majority of cases of diabetes can be broadly classified into four major categories: Insulin-dependent diabetes mellitus (IDDM) or T1DM and non-insulin-dependent diabetes mellitus (NIDDM) or T2DM, gestational diabetes mellitus (GDM) and other specific types. All except GDM can be categorised under pregestational diabetes (PGDM).

### *1.6.2 Insulin dependent diabetes mellitus (IDDM) or (T1 DM)*

Type 1 diabetes accounts for 5-10% of those with diabetes and results from cellular-mediated autoimmune destruction of B-cells in the pancreas. The rate of cell destruction is variable, such that the presentation varies from childhood to adulthood, with rapid destruction in the former and slow destruction in the latter. Autoantibodies to islet cell or insulin or glutamic acid decarboxylase (GAD) or tyrosine phosphatases have been found in 85-90% of those with IDDM.(74) This type of diabetes has strong links also to human leukocyte antigen (HLA)(75), particularly to DQA and DQB genotype. In addition to the autoimmune nature of this category and the genetic predisposition, there

are environmental factors that are still poorly defined. Most patients with T1DM are below the age of 25 but it can occur at any age.

### *1.6.3 Non-Insulin dependent diabetes mellitus (NIDDM) or (T2 DM)*

This type of diabetes accounts for 90-95% of patients with diabetes. The aetiology ranges from predominant insulin resistance with relative insulin deficiency to a predominant secretory defect with insulin resistance(76). Unlike IDDM, NIDDM has a strong genetic predisposition; however, the genetics of this form of diabetes are complex and not clearly defined. Most patients with this type of diabetes are obese as obesity itself can cause some degree of insulin resistance. Women who have had a prior history of gestational diabetes are prone to develop type II diabetes(77). It can occur in isolation or it can be part of a much broader underlying disorder including the metabolic syndrome, which is a cluster of biochemical and physiological abnormalities, such as obesity, high blood pressure and dyslipidaemia.

### *1.6.4 Gestational diabetes mellitus*

Gestational diabetes refers to glucose intolerance with onset or first recognition during pregnancy. The prevalence ranges from 1 to 14% of pregnancies, depending on the population studied, and it accounts for nearly 90% of all cases of diabetes in pregnancy(55).

### *1.6.5 Other specific types of diabetes*

This includes a wide variety of relatively uncommon conditions, such as diabetes that resulted from a monogenetic defect of B-cell function(75). This form of diabetes is characterised by onset of hyperglycaemia at an early age, usually before 25 years. It is referred to as maturity-onset diabetes of the young (MODY) and is characterised by impaired insulin secretion with minimal or no deficiency in insulin action. It is inherited as autosomal dominant pattern with six different genetic loci have been identified at different chromosomes; the commonest is the mutation at chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor -1a (HNF-1a)(78).

Included in this category of miscellaneous rare forms of diabetes, is diabetes that results from pathology in the exocrine pancreas, such as pancreatitis, infection, trauma

and pancreatectomy. Endocrinopathies that result in over- production of hormones that can antagonise the action of insulin, such as cortisol, growth hormone and glucagon can cause diabetes.

Certain drugs can impair insulin secretion either permanently, such as intravenous Pentamidine, or temporarily, such as glucocorticoids.

### *1.6.6 White classification of gestational diabetes*

The White classification, named after Priscilla White, who pioneered research on the effect of diabetes types on perinatal outcome, is widely used to assess maternal and fetal risk and it is based on the onset and duration of the diabetes and the presence of vascular disease(79, 80). It distinguishes between gestational diabetes (type A) and diabetes that existed before pregnancy (PGDM). These two groups are further subdivided based on age at onset, duration of disease and the presence or absence of vascular complications.

There are two classes of gestational diabetes:

- Class $A_1$: gestational diabetes; diet controlled
- Class $A_2$: gestational diabetes; medication controlled

The second group of diabetes, which existed before pregnancy, can be divided into these classes:

- Class B: onset at age 20 or older or with duration of less than 10 years
- Class C: onset at age 10-19 or duration of 10–19 years
- Class D: onset before age 10 or duration greater than 20 years
- Class E: overt diabetes mellitus with calcified pelvic vessels
- Class F: diabetic nephropathy
- Class R: proliferative retinopathy
- Class RF: retinopathy and nephropathy
- Class H: ischaemic heart disease
- Class T: prior kidney transplant

The White classification is based largely on women with T1DM(81). The increasing rates of childhood obesity and the overall increase in obesity in the population has resulted in more women of reproductive age with type II diabetes(82).

## 1.7 Placenta and pregestational diabetes

Maternal diabetes is associated with concentration of various cytokines, hormones and metabolites in the maternal circulation. These changes are likely to affect the placenta; hence receptors, transporters and enzymes the primary target of these molecules, are expressed in asymmetrical pattern(83). It has been postulated that the diabetic environment may have profound effects on placental development and function.

The placenta of diabetic women has attracted much attention largely due to the presence of changes in placental structure that may contribute to the associated pathology that is linked to diabetes such as PE, pregnancy loss, IUGR(84).

### *1.7.1 The placenta in early diabetic pregnancy*

In normal pregnancy, the development of the placenta is characterised by three distinct periods(85). It starts with implantation of blastocyst into the endometrial surface. The placental structure then continuously develops by series of differentiation and proliferation process of trophoblast cells that leads to placental villi(86). The majority of villi floats freely in the intervillous space but some invades into the decidua and those are the ones that give the integrity and anchor the placenta(87). A proportion of cytotrophoblast accumulates and invades the spiral arterioles and remodels them into low resistance arterioles(88). Shallow invasion has been implicated in PE and IUGR(87, 88) and since these adverse outcomes are present in higher proportion in pregnancies complicated with PGDM, this suggests that a maternal diabetic environment has an adverse impact on trophoblast invasion with demonstrable alterations in first trimester placental development, with significantly reduced placental vascularisation(89).

The invasion process is complex and requires multiple chemical factors such as Leptin and the oxidative stress-associated isoprostanes that aids invasion and metalloproteinases that inhibits it. It was found that the placental amount of matrix metalloproteinase that is associated with tissue remodelling during invasion is increased in placentae of mother, with PGDM while it is reduced in the normal population(90). In

patients with PGDM the imbalance between those two powerful factors and the tendency towards invasion inhibition is a recognised finding(91).

Another factor that plays a role in placental development is cytotrophoblast proliferation that is believed to be altered in patients with PGDM secondary to lack of Human Placental Lactogen (HPL) in the first trimester(90).

The placental vessels are developed during the first trimester and is regulated by various growth factors and cytokines. These include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), placental growth factor (PlGF), tumor necrosis factor (TNF), Interleukin 8 (IL-8) and insulin like growth factors 1 and 2 (IGF1, IGF2); most are altered in PGDM(91, 92). The full pathophysiology is not well understood and certainly it is an area that needs more studies initiated.

### *1.7.2 Placenta in the third trimester*

As in the first trimester, the circulating maternal and fetal concentration of cytokines, hormones and growth factors in the third trimester are changed in diabetes compared to non-diabetic patients(84, 87). Multiple studies found that maternal diabetes enhances the surface area of the capillary wall by elongation and enlargement of diameter and higher branching of villous capillaries(83, 90).

Despite the fact the surface area is increased, it is dysfunctional for the transport of nutrient and electrolytes. Various studies described structural changes of placenta in maternal diabetes at term of gestation. These changes do not occur in each diabetic pregnancy and it seems independent of type of diabetes (pregestational or gestational); however, it is more profound in the pregestational group(93). These changes were observed as well in a well-controlled diabetic pregnancy. Huynh et al performed a systematic review to delineate the histopathological findings and their association with maternal diabetes(94). It showed that delayed villous maturation (DVM) is the most common finding in PGDM placentae. This is defined as absent or inadequate terminal villi of the placenta and is associated with chromosomal abnormalities, GDM, and an adverse outcome(95). Higgins el al showed that DVM was significantly associated with PGDM compared to non-diabetic pregnancies (8% vs 2.8%, $P < .05$; RR 2.8 [95% CI 1.03-7.6]), and prenatal or intrapartum intrauterine death (8.6% vs 0%, $P < 0.05$)(96). Along with increased volume and surface area of parenchymal tissue, DVM was the

placental abnormalities most consistently reported with maternal diabetes(94, 97). Teasdale at el found increased non-parenchymal and parenchymal tissue in the placentas of women with well-controlled White class B and C diabetes(98). On the other hand, Boyd et al. found increased parenchymal volumes, on average 12% larger than placentas from normoglycemic controls, but a decrease in non-parenchymal tissue in women with well-controlled T1DM, compared to normoglycemic controls(99).

Placental weight tends to be heavier in diabetes, similar to fetal weight but the weight gain is more pronounced in the placenta than in the fetus, as is reflected in a higher placental-to-fetal weight ratio than in normal gestation(100).

The aetiology of miscarriage in diabetic women has been linked to placental pathology, as the placental structure is altered and the surface exchange area is widened as a result of hyper proliferation and hyper-vascularization(101).It is believed that placental modification can occur in both women with pregestational and gestational diabetes, but as most of the placental structure is formed in the first trimester, it seems the extent of damage is more pronounced in women with PGDM. A state of hyperglycaemia can cause excessive trophoblast proliferation and that subsequently results in delayed villous maturation and delayed trophoblast invasion. This mechanism could explain the higher incidence of spontaneous miscarriage, PE and IUGR with diabetes. In addition, a well-studied hypothesis of poor placental development and trophoblast invasion in type 1 diabetic women is dysregulation of matrix metalloproteinases (MMPs) in the first trimester caused by the diabetic environment(102).

In addition to structural modification in placentae of pregnant women with diabetes, placental metabolic and endocrine functions are altered too. There exists oedema and over-presentation of Hofbauer cells (placental macrophages) and this contributes to a greater release of placental cytokines such as leptin, tumour necrosis factor-α (TNF-α) and interleukins, and subsequently modifies placental metabolic and endocrine functions.(103)

### 1.8 Platelets and haemostasis

The platelet surface is a dynamic structure, through which a host of membrane receptors reacts to a number of activating agents such as agonist and adhesive proteins.(104) Some of the most important receptors involved in physiological and

pathological haemostasis include the glycoprotein (GP) I-IX, V complex, the integrin, (GP IIb-IIIa complex), GP VI receptors, thrombin receptors, adenosine diphosphate (ADP) receptor, Thromboxane receptors, P-Selectin, platelet-endothelial cell adhesion molecule 1 (PECAM-1), and receptors for coagulation factors(105).

Platelet activation occurs in response to endothelial injury. When platelets encounter the sub-endothelial matrix, endothelial collagen and von Willebrand factors (VWF) are exposed to circulating blood, platelets tether and adhere to both collagen and VWF via glycoprotein Ia/IIa receptor, leading to release of Calcium ($Ca^{+2}$). Subsequently, $Ca^{+2}$ leads to conformational changes to glycoprotein IIb/IIIa receptors, so they are able to bind to fibrinogen, leading to dense granule formation which leads to Intracellular signalling to induce the release of ADP which stimulates phospholipase $A_2$ enzyme to produce Arachidonic Acid (AA)(106). and Thromboxane, which are soluble agonists that further activate platelets and trigger the binding of plasma fibrinogen to the platelet GP IIa-IIb receptor, thereby forming a platelet plug.(107) This pathway toward platelet activation is illustrated in figure (1.1).

Another route for platelet activation is through the release of ADP from dense granules, which further perpetuates platelet activation by binding to ADP-specific receptors (P2Y1) and promotes the action of phospholipase $A_2$ on membrane phospholipid compounds to produce AA.

Arachidonic acid is the first precursor that is a by-product of membrane bilayer phospholipids formed by the action of phospholipase $A_2$. It then follows one of two pathways, either the cyclooxygenase (COX) pathway that produces $ThA_2$ or prostaglandins (PG). Alternatively, via the lipoxygenase enzyme pathway that synthesises leukotrienes. PG is differentiated into PGE ($PGE_1$ and $PGE_2$), PGI and PGD. $PGE_1$ and $PGE_2$ both have a vasodilatory effect and while $PGI_2$ is a weak inhibitor of platelet aggregation, $PGE_2$ is a weak platelet aggregator (108)(see figure 1.2). $PGI_2$ and $ThA_2$ are substantially more potent than $PGE_2$, which is manufactured in the vascular endothelium and which is a potent vasodilator and inhibitor of platelet aggregation. Thromboxane $A_2$, is the most important platelet activator and functions by inducing expression of fibrinogen receptors (GP IIb/IIIa) on the platelet membrane and by binding to $ThA_2$ receptors on the surface of other platelets, thereby triggering their activation(108).

### *1.8.1 Platelets in normal pregnancy*

It is well known that haematological changes in pregnancy include a small reduction in the platelet count, which leads to activation of megakaryocytes to stimulate production of platelets from the bone marrow. As those platelets are young, they are bigger in size, leading to an increased Mean Platelet Volume (MPV)(109).

Various studies of small numbers of patients have suggested that the activation of platelet aggregation is increased in the third trimester of pregnancy(110-112). However, these studies used different agonists such as ADP, AA, Thrombin, and Adrenaline. Different media were used as well that ranged from whole blood, platelet-rich plasma or using washed platelets.

Other studies have suggested that this platelet hyperaggregability may be due to elevated intracellular $Ca^{2+}$ mobilisation, increased $ThA_2$ formation, and reduced production of cyclic Adenosine Monophosphate (cAMP), an inhibitory intracellular second messenger in platelets(113).

Different studies suggest an overexpression of CD40 Ligand (CD40L), which creates a proinflammatory medium leading to platelet activation(114, 115).

In conclusion, platelet reactivity in a healthy pregnancy is incompletely understood and remains a controversial issue. Indeed, it is plausible that heightened platelet aggregation in pregnancy may not result from the same pathway in all patients. Therefore, further studies in this field are needed to better define what precise mechanism underlies platelet hyperaggregation in different clinical scenarios such as diabetes, and crucially, which patients may benefit from antiplatelet therapy.

### *1.8.2 Platelets in patients with preeclampsia*

As discussed in section 1.8.1, Dundar demonstrated an increase in MPV in normal pregnancy.(109) He also suggested that this increase is more substantial in patients with PE and that the increase is evident from 24 weeks' gestation up to birth(109).

Various studies investigating platelet reactivity in patients with PE have reached conflicting results. The hypothesis of overexpression of CD 62 and 63 was supported by a study published by Françoise in 2002(116). This was a small study that included 30

patients in the PE group and they were compared to women who were either normotensive or had gestational or pregnancy-induced hypertension. It concluded that platelet activation is increased in PE but not in other hypertensive disorders or in normal pregnancies. That theory was supported by two other studies(117, 118). P-selectin expression, which is a platelet and leucocyte recruiter, has been linked to platelet activation and found to be increased in patients with PE(119).

β-thromboglobulin, also called Pro-Platelet basic protein, is a chemokine that is released in large amounts after platelet activation. It has been found to be present in substantial amounts in women with PE(120).

Another study on a small number of patients with PE found that the Platelet endothelial cell adhesion molecule-1 (PECAM-1) appeared to be markedly elevated in preeclamptic patients, compared to normotensive pregnant women(118).

A cross sectional study by Babic (2011) on preeclamptic patients showed increased platelet activation in the form of a reduction of concentration in cAMP (OR:0.81; 95%CI 0.57- 1.14; $p<0.05$) and cyclic guanosine mono-phosphate, cGMP (OR:.78; 95% CI 0.55–1.09; $p<0.059$); both are inhibitory for platelet activation and aggregation(121).

Thus, the precise determination of the extent of platelet activation, both in normotensive pregnancy and in each of the hypertensive disorders of pregnancy (preeclampsia and gestational hypertension), remains ill defined.

### 1.9 Aspirin

Aspirin has been investigated for the prevention of PE, owing to its negative effect on thromboxane production. An imbalance between prostacyclin and thromboxane plays a key role in the development of PE, and it is believed to result from shallow placental invasion and ischaemia that occur shortly after implantation, very early in the first trimester of pregnancy.

Aspirin is one of the oldest medications that is still in use in modern world. It was first used by the Sumerians and Egyptians, who referred to it in their ‘Ebers papyrus’, an ancient medical text by the Salix Alba (mid 1500 BC). It was not until the mid-17$^{th}$ century that it was heavily researched and thesis submitted to the University of Oxford,

detailing its effectiveness in clinical practice as an anti-inflammatory agent and anti-pyretic(122). In 1971, Vale, Samuelson, and Bergstrom were awarded the Nobel Prize for describing the mechanism of action of aspirin, and clinical research investigating the antiplatelet effects of aspirin began(123). By far, aspirin is still one of the most researched drugs in the world, with an estimated 700 to 1,000 clinical trials conducted each year(122).

### *1.9.1 Aspirin pharmacodynamics*

Aspirin is a weak acid that acts principally on COX enzymes that have 3 isoforms. At a low dose (0.45mg/kg, 60-150 mg), it targets COX-1 and to a lesser extent COX-2. This leads to the irreversible inhibition of the enzyme and, subsequently, the reduction of $ThA_2$ activity levels by 95% within 5 days of administration(124). This leads to permanent inhibition of platelet aggregation for the entire life-span of the platelet, which is usually 8-9 days in healthy individuals.

It is important to mention that there are other sources for production of $ThA_2$ from non-dependent COX pathways in monocytes(125). That means, that theoretically, aspirin will fail to inhibit the production of $ThA_2$ completely in some cases(126). This is one of the principal theories for suboptimal response to aspirin, or aspirin resistance, in some clinical scenarios.

### *1.9.2 Aspirin as an antiplatelet therapeutic agent*

Aspirin inhibits platelet prostaglandin synthesis, i.e. $ThA_2$, prostacyclin, and other prostaglandins. These prostanoids are generated by the enzymatically-catalysed oxidation of AA, which is itself derived from membrane phospholipids(127). Aspirin acts as an acetylating agent, where an acetyl group is covalently attached to a serine residue in the active site of the COX enzyme. This differentiates aspirin from other non-steroidal anti-inflammatory drugs (NSAIDs) (such as diclofenac and ibuprofen), which are reversible inhibitors of COX enzymes(128).

Aspirin is rapidly absorbed in the upper gastrointestinal tract and results in measurable inhibition of platelet function within 60 minutes. Enteric coating of aspirin significantly delays its absorption(129). The plasma half-life of aspirin is only 20 minutes; however, because platelets cannot generate new COX, the effects of aspirin last for the duration

of the life of the platelet(127).

The dose of aspirin required to obtain adequate platelet inhibition has been studied extensively. A single dose of 100 mg of aspirin effectively abolishes the production of $ThA_2$ in normal individuals.(130) Single doses below 100 mg result in a dose-dependent effect on $ThA_2$ production(131). This inhibitory effect is cumulative on repeat dosing, with complete suppression of platelet thromboxane synthesis estimated to occur within a few days after daily dosing of 20 to 50mg, with more rapid suppression occurring with larger doses of 150-300mg.(132) In the literature, low dose aspirin has been described for aspirin intake between 60-150 mg/day(133, 134).

### *1.9.3 Platelet response to aspirin*

Despite its high efficacy, safety, and low cost, aspirin may not benefit all patients equally(135).

Different terminologies have been used for patients who show suboptimal response to aspirin such as ‘aspirin non-responders’, ‘aspirin non-responsiveness’, and ‘aspirin resistance ‘and ’aspirin treatment failure’. Hankey et al defined aspirin resistance as the inability of aspirin to reduce platelet production of $ThA_2$ and thereby platelet activation and aggregation(136). However, many studies defined aspirin resistance based on clinical manifestation such as recurrence of cardiovascular and cerebrovascular ischaemia(137). Snoep et al conducted a meta-analysis of 15 studies to find the correlation between biochemically proven aspirin resistance and clinically evident aspirin resistance in the form of new onset thrombotic events in cardiac patients. It showed that patients who were biochemically identified as having aspirin resistance were more likely to also have "clinical resistance" to aspirin because they exhibited significantly higher risks of recurrent cardiovascular events, compared to patients who were identified as aspirin sensitive by laboratory-based criteria(138).

Because of a lack of consensus in defining aspirin resistance and because the majority of studies that have investigated this matter recruited diverse patient populations, had different aspirin regimens and utilised different assays for assessment of the effect, a wide range of prevalence of aspirin resistance was reported between 0.4%(139) to 70%.(140) varying with the assay used and the populations studied.

The mechanism of platelet suboptimal response to aspirin therapy has been thoroughly investigated over the last 20 years. Various reasons have been postulated to explain why that phenomenon happens; including inadequate dosing, genetic polymorphisms of COX-1, increased platelet turnover.and other genes involved in thromboxane biosynthesis, the latter has received much attention in the last few years with advances in the field of pharmacogenetics. Recently, gene expression analysis identified >60 genes described as the 'aspirin response signature'. This will have great potential to enhance patient care by reducing on-treatment clinical events, adverse drug reactions, and health care-related costs(141).

It has been well recognised that platelets can be activated independently of $ThA_2$ synthesis and therefore may not be fully inhibited by aspirin intake. This can occur via platelet membrane receptors for ADP (P2Y1) as well as thrombin, epinephrine, and glycoprotein receptors for collagen to a lesser extent(142, 143). A case control study showed the response of platelets to ADP was much more pronounced in aspirin-resistant individual(144).

In addition to that, $ThA_2$ can be synthesised by different cells such as monocytes and neutrophils via the COX-2 pathway and this cannot be inhibited by aspirin intake. The COX-2 isoenzyme is more readily available in megakaryocytes and immature platelets, which are present in abundance during pregnancy because of increase platelet turnover(145). ,Rocca et al demonstrated that COX-2 was observed in <10% of circulating platelets from healthy controls compared to 60% in patients with thrombocytopenia, which is a high platelet regenerating condition(145).

In addition, some studies have suggested that COX-1 gene mutations that decrease its affinity to aspirin have also been identified(146, 147).

Aspirin irreversibly blocks COX-1 activity and, because platelets lack a nucleus, and therefore a synthetic ability to produce prostaglandins, this inhibition lasts for the whole life span of platelets. Various randomised controlled trials have demonstrated that daily low dose aspirin therapy can suppress COX-1(123, 129, 132, 145) .

Perneby showed that a dose of aspirin as low as 37.5 mgs daily is sufficient to achieve full platelet COX inhibition, as evidenced by >98 % suppression of serum thromboxane B2 and almost abolished AA- induced aggregation in platelet- rich plasma 2–6 h after

dosage administration(148). Patrono further demonstrated that a single oral dose of 50–100 mg of aspirin, dose-dependently inhibits the activity of platelet COX(149).

Specific to pregnancy, it is noteworthy that a prospective study by Sibai showed that 60–80 mg of aspirin per day reduced $ThA_2$ production in over 90% of pregnant women(133). Roberts et al. noted that 50 mg of aspirin per day reduced $ThA_2$ production in over 95% of cases without altering prostacyclin production, whereas 100–300 mg of aspirin per day completely inhibited $ThA_2$ production, and also inhibited prostacyclin(150).

On the other hand, Caron et al conducted a prospective cohort study on pregnant women on aspirin to determine the rate of platelet non-responsiveness to aspirin. This study concluded that just over 28% showed a lack of platelet function response to aspirin 81 mg and that was in most cases overcome with higher dosing(151).

In obstetric patients, a number of studies have been carried out in pregnant women at high risk of PE, in order to determine responsiveness to aspirin therapy. This includes two prospective cohort studies, one case-control study and a dose escalation study from groups in the UK, Canada and Poland (152-155). In these high-risk obstetric populations, suboptimal platelet response to aspirin was identified in 29–39% of participants, and was associated with increased PE risk, preterm birth and delivery of small-for-gestational-age infants. Assessing resistance was measured objectively using thromboxane metabolites detection in the urine(155), or platelet function analyser (PFA-100) (153, 154)or light transmission aggregometry (LTA)(152).

### *1.9.4 Tests of platelet response to aspirin*

Lack of aspirin response has been measured at a biochemical level, such as testing for platelet aggregation or measuring the active form or metabolites of some end-point products such as $ThA_2$. On the other hand, the lack of clinical response in cardiac patients, manifested by further ischaemic events despite taking optimal doses of aspirin, is another way of measuring response to aspirin. In the field of obstetrics, clinical manifestations of aspirin resistance could be considered in the context of the development of adverse perinatal effects such as PE and intrauterine growth restriction (IUGR).

Because aspirin does not inhibit platelet function regulated by pathways other than $ThA_2$, an appropriate assay evaluating aspirin efficacy should be specific to the platelet COX pathway. For this reason, LTA using AA as an agonist is an excellent assay to detect platelet inhibition by aspirin.

A number of assays have been developed to measure platelet reactivity and, hence, to indirectly quantify aspirin's antiplatelet effect. These studies, however, are notable for limited reproducibility(106). Some assays are laboratory- based and require extensive expertise to operate, whereas others have been specifically developed for point-of-care testing(156). Although some assays study global haemostasis, most platelet function assays target a specific phase of platelet function, from platelet adhesion to platelet activation and aggregation(156). Most of these assays measure platelet aggregation that may not necessarily be specific for $ThA_2$-induced platelet reactivity.
Therefore,laboratory studies may falsely indicate an individual as aspirin resistant if platelets are aggregating from a trigger other than $ThA_2$, even though aspirin might be effectively inhibiting COX-1, its sole target (106).

Of the COX-specific assays available to quantify the antiplatelet effect of aspirin, LTA using AA as an agonist. It is considered the historical gold standard because of its relatively high specificity for platelet COX; AA is used as the agonist to exploit the specific pathway affected by aspirin (COX-dependent $ThA_2$ synthesis).

COX-specific assays include LTA when AA is used as an agonist, the current gold standard. This test assesses in vitro the platelet-to-platelet clump formation in a glycoprotein (GP) IIb/IIIa-dependent manner.

LTA is based on the measurement of the increase in light transmission through the optically dense sample of platelet rich plasma (PRP) after the addition of the exogenous platelet agonist(157) This assay will be discussed in details in chapter 2.

Verify Now®*applies the same principle of LTA using whole blood, which can be used at the bedside and can be effective in clinical settings(158). However, there exists a lack of consistency between LTA and tests that use whole blood in addition to the increased possibility of an unexpected interaction between blood elements and platelets leading to false results(159).

Measurement of the stable serum and urinary metabolites of $ThA_2$, $ThB_2$ and 11-dehydrothromboxane $B_2$ (11-DThB2) has been used in various studies(160-162). These tests do not directly measure platelet reactivity and because it is not formed in the kidney, detection of this metabolite in the urine reflects systemic $TxA_2$ formation, which largely, albeit not exclusively occurs in the platelets.

*Verify Now® (Accumetrics, Inc., San Diego, CA; formerly known as Ultegra Rapid Platelet Function Assay) was developed as a point-of-care, whole-blood, semiautomated, cartridge-based platelet function test to determine the response to antiplatelet agents.

It has been calculated that about 30% of the urinary metabolite derives from extra-platelet sources.(163). And as mentioned in section 1.9.2, $ThA_2$ can be produced by platelets through the action of COX-2 and can be synthesised by other cells such as monocytes, macrophages, endothelial cells(164).

In contrast, if platelet function assays rely on ADP, collagen or epinephrine for platelet activation, the assays are considered to assess non-COX-dependent pathways. Non-COX-specific assays include the PFA-100® system, a point-of-care system using citrated whole blood(165). It assesses platelet aggregation under high shear, mimicking platelet-rich thrombus formation after injury to a small vessel wall under flow conditions(166). This test does not depend on the COX-1 pathway and hence it is not specific for aspirin intake. Despite the fact it is the most widely used test in assessing platelet function, it is ability to adequately quantify platelet response to aspirin remains debatable. In addition to that, most studies that have compared PFA-100 to LTA have reported poor correlations between the two assays, independently of the agonist used, with higher proportions of aspirin resistance with the former((108, 167).

Lordkipanidzé et al concluded in her study that "*since PFA-100 methodology is not specific to the aspirin-sensitive COX pathway, it seems less suitable for the detection of aspirin resistance*".(167)

A broad range of cut-offs for all these tests has been presented.(131). These cut offs were proposed based on studies that targeted male volunteers. Consequently, there is limited applicability of these cut-offs to obstetric populations(108, 168).

Direct comparison of different laboratory methods to detect aspirin resistance showed very weak or no correlation. Lordkipanidzé *et al*. compared the performance of six major tests (AA-induced aggregation with LTA; ADP-induced aggregation with LTA; whole blood aggregometry; PFA-100, VerifyNow, urinary 11-dehydrothromboxane $B_2$) in 201 patients with stable coronary artery disease receiving ≥ 80 mg aspirin daily. Overall, the correlation among the various platelet function tests was very poor. The prevalence of aspirin resistance was relatively low with the more specific tests, AA-induced platelet aggregation (4%) and VerifyNow (6.7%), while it was > 50% when unspecific tests, such as platelet aggregation induced by ADP (51.7%) and PFA-100 (59.5%), were used(167).

There is no official guideline recommending one assay over another, and platelet function testing is not recommended for routine clinical testing in patients requiring aspirin therapy. There are few data on the cost/benefit ratio of these tests and there is still no consensus on a platelet function test which could be used effectively in daily clinical practice.

In conclusion, A suboptimal response to aspirin does not currently have a uniform definition nor has an adequately sensitive and specific diagnostic test emerged because of the limitations previously mentioned. Platelet testing is proved to be of benefit in identifying women with suboptimal response to aspirin, but the results should be interpreted in light of clinically significant outcomes. There is a need to develop a clinically meaningful definition of aspirin resistance, based on data linking aspirin-dependent laboratory tests to clinical outcomes in patients.

In my opinion; finding a reproducible and valid measure/ test that detects platelet function changes in relation to aspirin therapy along with efforts to elucidate the mechanism of aspirin resistance should be the focus of the future studies in order to enable clinicians to optimise anti-platelet therapy in aspirin resistant patients This ambition will require large-scale, prospective and randomised clinical investigations.

### *1.9.5 Compliance with aspirin*

To assess the clinical impact of suboptimal platelet response to aspirin, it is vital that true non-responsiveness can be distinguished from non-compliance. Multiple studies suggested that the predominant cause of suboptimal aspirin effect is non-compliance

(143, 164, 169). In cardiovascular research, non-compliance to aspirin intake is estimated to be between 30-50%(136, 164). It has been estimated, for example, that approximately 9% of patients who suffered myocardial infarction who were presumed to be aspirin resistant were aspirin resistant because of non-compliance(170).

The existing literature demonstrates that there is no consensus on robust assessment of compliance(171).

A meta-analysis by Navaratnam focused on aspirin resistance, showed that 45/87 studies involved relied upon patients' reports of compliance, in the form of a qualitative assessment using a questionnaire or diary card which might not be accurate(131). In a more recent metanalysis by the same author on assessment of adherence to aspirin intake among obstetric and cardiology cohorts, aspirin compliance was poorly described and a threshold for acceptable adherence was noted to be included in the methods section in only 24% (6/25) of obstetric trials.(172) Assessment of adherence to aspirin can be subjective in the form of an adherence questionnaire, interviews and self-reporting. However; these methods lack accuracy and are vulnerable to recall bias if not completed contemporaneously. In the same metanalysis, subjective methods were reported as the most widely used method in assessing compliance in cardiology trials (93%) and second most common method in obstetrics trials (48%) after semi-subjective methods. Semi- subjective assessment methods of medication compliance include pill counting and diary card review, and examination of prescription records in addition to observed dosing. However, such methods all remain an indirect way of assessing adherence and they all are vulnerable to tampering and pill dumping. These methods were the most commonly used methods in obstetrics trial (67%), but they were used infrequently in cardiology trials. Continuous intake observation is not feasible on an outpatient basis; pill counts can be misleading when medications are being discarded instead of ingested.

Objective measurement of the aspirin metabolite (salicylic acid) can be deceptive due to aspirin's short half-life (about 30 minutes) and would fail to identify individuals who only take their medications in anticipation of a doctor's appointment(173). In addition, aspirin metabolite monitoring has not been tested during pregnancy. Measuring the metabolite of thromboxane in the serum and urine does not directly measure platelet reactivity and is not platelet specific.

### *1.9.6 Use of aspirin in the prevention of preeclampsia in high-risk populations*

The era of use of aspirin to prevent cardiovascular disease started in the early 1950s. It was not until the mid-1980s that aspirin was investigated for prevention of PE and IUGR in obstetric patients when 102 women at high risk of developing PE were recruited by Beaufils(174). This study demonstrated that the prevalence of PE was significantly reduced in the aspirin group compared with the untreated group (0/48 vs 6/45 $p < 0.05$). Since then, numerous randomised trials were conducted to assess the efficacy of aspirin in the prevention of PE in high-risk women and they have yielded conflicting results. Initial studies suggested a protective effect (174-178). Subsequently, some larger studies failed to identify a benefit to aspirin therapy (179-181). Because of the discrepancy in the results of the trials that could be attributed to differences in inclusion criteria, timing of initiation of administration of aspirin and different dosages of aspirin, the CLASP trial ( Collaborative Low Dose Aspirin in Pregnancy) tried to interrogate these heterogeneous studies, and in doing so concluded that the overall reduction of PE attributed to low-dose aspirin therapy was only 12% (180). Caritis et al found no benefit to prescribing low dose aspirin (60 mgs) to prevent PE; however, 50% of women who were randomised to the aspirin group were above 20 weeks' gestation at the time of recruitment.(52)

Because of the major debate between studies, the first meta-analysis in the efficacy of aspirin in the prevention of PE in high-risk patients was published by Duley in 2001. It included 39 trials and over 30,000 patients.(182) Patients were sub-classified into four high-risk groups: patients with previous severe PE, PGDM, chronic hypertension, and renal or autoimmune diseases. The remainder were considered to be in the moderate risk group. The observed reduction in PE incidence was modest (15%) with the use of aspirin (32 trials, 29,331 women); (RR= 0.85, 95% CI 0.78- 0.92). There was no heterogeneity with the results, even with variation of risk status, dose of aspirin, gestation at trial entry, or the use of a placebo. Four individual studies (with a combined weight of 27%) did not support the use of aspirin, the largest trial of which included 6275 patients and which had a RR of 1.14 (95% CI, 0.94-1.38). Duley correctly reports that most large studies of the efficacy of aspirin for preventing and treating PE are disappointing, in that aspirin has little beneficial effect. It is plausible that recruitment late in gestation may be responsible for the failure to observe a benefit to aspirin therapy in reducing the risk of preeclampsia. Of note, most patients in these trials were

recruited in the second and third trimesters of pregnancy. Trophoblast invasion occurs mainly in the first and second trimesters and is most active in the first; the defects in trophoblast invasion associated with PE are certainly present from about 16 weeks' gestation. It therefore seems that most patients were recruited after the primary pathology developed, which may explain the limited impact of aspirin.

Since then, more than 11 meta-analyses and systematic reviews have been published on aspirin and many of them have been based on relatively small studies.

Another Cochrane review by the same author in 2007 included 59 trials of over 37,000 women, which showed a 17% overall reduction in the risk of PE associated with use of aspirin when combining studies of different design. This author stratified patients according to risk-status for PE and concluded that aspirin may confer a 25% reduction in PE for women deemed to be at high risk for developing the disease. PGDM constituted a high risk factor in this analysis.(182)

Another systematic review by Coomarasamy in 2003 included 14 RCTs of over 12,000 patients. This review targeted high-risk populations and stratified them into women with a history and/or family history of PE, chronic hypertension, PGDM, or renal disease. The overall reduction of PE in all four groups was modest, at 14%.(183)

In 2005 a systematic review by Ruano(184) included 22 RCTs and evaluated the effectiveness of low dose aspirin in low- and high-risk populations in preventing PE. High risk populations were defined as those who had essential hypertension, PGDM, chronic renal disease, previous severe PE, renal failure or BP>160/110. This meta-analysis specifically compared low-risk vs. high-risk patients and showed how little effect low dose aspirin has in preventing PE in high-risk populations. It also highlighted that a different dosage of aspirin or timing of administration did not correlate with relative risk of PE. Despite this meta-analysis focusing on randomised trials and agreeing on strict inclusion criteria, there was a clinical heterogeneity that was overcome by evaluating the gestational age at which low dose was initiated, the duration of administration and the dosage of aspirin. A lower dose of aspirin was used in earlier studies (50-75mg/day). In fact, there was little difference demonstrated with different aspirin dosage. Some studies included within this meta-analysis suggested greater efficacy when aspirin treatment began before 17 or 20 weeks' gestation.

However, the author concluded that the causes for the conflicting results may be explained by the difference in inclusion criteria.

A meta-analysis by Askie(185) in 2007 based on 27 trials on 31,678 women, concluded that aspirin is effective in preventing PE, although the effect, a 10% reduction in PE incidence, was too modest to warrant routine use in all women. The authors concluded that, despite a very large dataset, 'the evidence base for particular groups of high-risk women remains limited'. Pre-existing diabetes was identified in 905 randomised women in this meta-analysis and the authors calculated a RR for PE of 0.76 (95% CI 0.56 to 1.04), thus failing to demonstrate a statistically significant effect. No information is provided on gestational age at recruitment for this sub-analysis, but very few studies were included that recruited women <16 weeks. Two thirds of the overall dataset were recruited after 20 weeks' gestational age. However, if started early in pregnancy, the treatment may be effective.(186, 187) Askie et al noted a more marked benefit when aspirin treatment started before 20 weeks of gestation (RR 0.87; 95% CI 0.79–0.96) and when the dosage was ≥ 75 mg/day (RR 0.77; 95% CI 0.61–0.97).

Another meta-analysis by Bujold included 34 RCTs, including 11,348 women with follow-up for the outcome of PE.[117] Low-dose aspirin, which started at 16 weeks or earlier, was associated with a significant reduction in PE (RR 0.47, 95% CI 0.34–0.65 and the prevalence of PE was 9.3% in the aspirin group compared with 21.3% in the control group.

A relatively recent meta-analysis in 2007 by Roberge had strict inclusion criteria, which led to only five studies on a combined total of 556 women to be included in the final analysis(188). The criteria were (1) treatment with aspirin or placebo initiated at or before 16 weeks of gestation, (2) information provided on preterm and term PE, and (3) judged to be low-risk or unclear risk of bias. When compared to controls, aspirin initiated ≤16 weeks of gestation was associated with a major reduction in the risk of preterm PE (RR 0.11, 95% CI 0.04–0.33); however, it had no significant effect on term PE (RR 0.98, 95% CI 0.42–2.33).

A systematic review by Henderson et al. concluded that low dose aspirin administered after the first trimester of pregnancy could reduce the risk of PE by at least 10%.(189)

The most recent meta-analysis (Xu ting-Tang 2015) included 29 randomised studies and concluded that aspirin is effective in reducing PE (OR, 0.71; 95% CI, 0.57–0.87) and severe PE (OR, 0.37; 95% CI, 0.23–0.61) if started before 16 weeks' gestation in high risk populations(190). This included all women with either antiphospholipid syndrome, chronic renal disease, essential hypertension, PGDM, history of PE in previous pregnancies, a family history of PE or multiple pregnancies.

Few studies have investigated the role of aspirin in preventing PE specifically in women with PGDM. Only two randomised trials have recruited women with PGDM without chronic hypertension or established renal disease (ECPPA 1996; Caritis 1998)(52, 181). The latter trial was a large multicentre Maternal Fetal Medicine (MFMU) Network study that investigated the role of aspirin in prevention of PE for high-risk women, which included a subgroup of 471 women with PGDM. Although this study did not demonstrate a difference in the incidence of PE between aspirin and placebo groups, women were recruited in the second trimester (mean gestational age at recruitment 18 weeks +/- 4 weeks).

The dose of aspirin used in randomised trials ranges from 50mg to 150mg. For some women, particularly those with type II diabetes-related obesity, the commonly-used 60mg dose may be too low to exert a full effect on $ThA_2$ production. Recent work by one of the co-Principal Investigators for this submission (Kenny 2013)(191) indicates that 20% of patients in Ireland with established coronary artery disease are inadequately 'protected' by aspirin, as evidenced by a thromboxane $B_2$ level of >2.2 (indicating platelet aggregation of greater than 20%). Age, hypertension, and weight were identified as risk factors for an inadequate aspirin response. Furthermore, randomised trials of this nature are potentially compromised by an inability to confidently ensure patient compliance. The use of platelet function assays offers the potential, both to determine whether body mass index and gestational age influence the ability to achieve an optimal biologic drug effect and to confirm patient compliance.

A meta-analysis by Roberge in February 2017 looked into timing of administration and dosage of aspirin(192). A total of 45 RCTs were included in the analysis and the results showed that aspirin had a significant impact on the incidence of PE if administered before 16 weeks' gestation and that was in agreement with most of the previous meta-analyses. However, the highlight of this meta-analysis was that the effectiveness of

aspirin was not only dependent on the gestational age at initiation of treatment but also on the dose of the drug as it has a dose-response effect.

The Aspirin for Evidence-Based Preeclampsia Prevention Trial (ASPRE) is the most recent, multicenter, randomised, double blinded placebo study in this field that studied the impact of using aspirin at 150 mg daily started between 11-14 weeks' gestation in pregnancies deemed to be at high risk for the development of PE based on a first trimester serum screening test. The primary objective of this study was to evaluate the effect of a prophylactic low-dose aspirin administered in the first trimester of pregnancy on the incidence of delivery with PE before 37 weeks of gestation in women identified at high risk of preterm PE using combined screening for detection of PE. The secondary objectives were to study the effects of aspirin on the incidence of early (delivery before 34 weeks of gestation) PE, the incidence of IUGR, fetal death, perinatal death, admission to neonatal intensive care, a composite measure of neonatal morbidity and mortality and placental abruption. Testing was offered to 26,941 pregnant women, of those 2641 were at high risk of PE and eligible for inclusion. Among the patients included, 1776 were randomised to aspirin or a placebo(193). This study demonstrated that the occurrence of preterm PE was significantly reduced by aspirin (0.38; 95% CI 0.20–0.74; $p$ = 0.004). Preterm PE occurred in 13 of 798 participants (1.6%) in the aspirin group, as compared with 35 of 822 (4.3%) in the placebo group. Of note, just 7% of patients in the ASPRE study had PGDM.

Specifically pertinent to the IRELAnD study, is the issue of aspirin effect on preeclampsia in pregnancies complicated by pregestational diabetes.

## 1.10 Safety of aspirin in pregnancy

### *1.10.1 Animal data*

Results of animal studies suggest that COX-1 inhibition may lead to cardiac, midline, and diaphragm defects.(194) Another study has shown some evidence of developmental toxicity in rats, although studies in rabbits did not demonstrate an increase in fetal anomalies(195). Single large doses of 500–625 mg/kg during organogenesis have led to a range of anomalies, including craniorachischisis, gastroschisis and umbilical hernia, cleft lip, diaphragmatic hernia, heart malrotation, and

supernumerary ribs and kidneys; however, lower doses have not been associated with the same range of adverse effects(196).

### *1.10.2 Human data*

The use of low dose aspirin in pregnancy is common, with between 4 and 8% of women reporting gestational use in a US report(197). The use of aspirin is less prevalent in European countries. There are few data that give an estimate of the split between low dose use and the use of aspirin at much higher doses as an anti-inflammatory, analgesic or antipyretic. The approximately 50-fold dose difference needs to be considered when interpreting studies of gestational aspirin exposure.

Two cohort studies using data from registries - one of which used reports from the first prenatal care visit and the other lacked data regarding patient's compliance to NSAIDs intake and over the counter administration - generally found no increased risks of birth defects after NSAIDs exposure(198, 199).

A 2003 meta-analysis that included 22 studies assessed the association between early pregnancy aspirin exposure and congenital anomalies(200). When the overall malformation rates were compared for studies that reported this outcome, there was no association between aspirin exposure and congenital anomalies (OR 1.33; 95% CI 0.94-1.89). When analysed by type of study, an association was identified in case-control studies (OR 1.64; 95% CI 1.30-2.04) but not in cohort studies or the included RCTs (OR 1.03; 95% CI 0.94-1.13). This difference in outcome by study type could be explained by maternal recall bias in the case control studies. There is also potential for confounding by maternal indication for aspirin use, e.g. hyperthermia or influenza infection. The meta-analysis could not assess the effect of aspirin dose, as this was not reported in most included studies.

First trimester exposure to aspirin from the 8$^{th}$ week of pregnancy for the prevention of PE was reported in a 2011 RCT(201). Eighty-two women received 100mg of aspirin daily. No adverse fetal consequences were reported. Another RCT evaluating preconceptional and first trimester exposure to aspirin involved randomising 1228 women to aspirin or placebo, six months prior to conception, and until 36 weeks of gestation and that showed no adverse effect of early aspirin administration on the fetuses.(202) Another trial, assessing the efficacy of aspirin for the prevention of PE in

high-risk women who were started on aspirin at 9-14 weeks' gestation, was prematurely terminated for its slow recruitment rate (203).

A US case control study found an association between aspirin use at conception and miscarriage (Hazard Ratio (HR) 4.3; 95% CI 1.3 - 14.2).(204) None of the aspirin use appeared to be low dose aspirin in the reported results. Aspirin did not appear to adversely affect miscarriage rates in the Cochrane review of aspirin for in-vitro fertilisation(205).

Another US case control study assessed the relationship between first trimester aspirin use and congenital cardiac defects(206). Risk of maternal recall bias was reduced by the use of control infants with non-cardiac malformations. The cases comprised 1381 infants with structural cardiac defects. There was no association between exposure and a range of tested congenital heart defects. A similar study published in 2012 found moderate associations between aspirin use in early pregnancy and a range of defects including anencephaly/craniorachischisis, anophthalmia/microphthalmia, cleft palate, and amniotic bands(207). This study was limited by potential differential recall of medication exposure between cases and controls, multiple testing, and a lack of information on what proportion of aspirin exposures were low dose.

The most commonly reported adverse effect associated with aspirin or saliclyate exposure from case control studies is gastroschisis because of the hypothesis of vascular disruption and vasoactive effect of NSAIDs.(208-211) This finding has not been consistently reported, with US and Hungarian case control studies finding no association(212, 213). To avoid recall bias in Nogard's study(213). the control group comprised children with congenital abnormalities, apart from those included in the study (neural tube defect, gastroschisis, and cleft lip+/- palate). Two reviews concluded that first trimester aspirin exposure might be associated with gastroschisis(214, 215).

A large prospective cohort study was conducted by the Norwegian Institute of Public Health, which did not find associations between exposure to any NSAIDs in the first 12 weeks of gestation and the occurrence of birth defects such as congenital heart defects and orofacial clefts(216). However, they did observe a non-statistically significant increased risk of ventricular septal defects after exposure to multiple NSAIDs in the first 12 weeks of gestation, mainly Ibuprofen and Kaptoprofen. The same study reported an

increase in spontaneous miscarriage. It recommended that aspirin not to be used in the third trimester due to an increased risk of premature closure of the ductus arteriosus.

A case control trial that investigated the association between ventricular septal defect and exposure to recreational drugs, NSAIDs, and cough remedies showed a possible association between NSAID and ventricular septal defect(217).

A large US cohort study included over 50,000 pregnant women, of whom approximately 15,000 had some degree of aspirin exposure in the first four months of pregnancy(218). After adjusting for differences between exposed and non-exposed pregnancies, there was no association between exposure and a range of subgroups of congenital anomalies.

A 2012 study found an association between aspirin exposure and quadriparetic cerebral palsy among a population of extremely low gestational age newborns(207). Aspirin use was reported by 5.6% of mothers, though indications for use or dosage were not described. Women who reported aspirin use had important differences from the rest of the population that may have affected outcomes, e.g. less likely to be single or receiving Medicaid and a higher prevalence of cervical insufficiency. Others have criticised the conclusions of the study, noting that aspirin use may identify a particularly high-risk group for adverse outcomes (219).

The United States Preventive Service Task Force (USPSTF) found adequate evidence that low-dose aspirin, as a preventive medication, does not increase the risk of placental abruption, postpartum haemorrhage, or fetal intracranial bleeding. It also found adequate evidence that low dose aspirin, as a preventive medication in women at increased risk for PE, does not increase the risk of perinatal mortality (220). It concluded as well that evidence on long-term outcomes in offspring exposed in-utero to low-dose aspirin is limited, but no developmental harms were identified by 18 months of age in the one study reviewed.

Henderson et al (2014),(189) was unable to determine if this observation was significant because of the level of heterogeneity within these small studies. There was no evidence that prophylactic low dose aspirin significantly affects the risk of other complications affecting the mother or fetus, including postpartum haemorrhage (PPH), spontaneous miscarriage, caesarean birth, neonatal haemorrhage, low Apgar score, or neonatal ICU

admission(189).

In conclusion, the body of evidence of the safety profile of low dose aspirin in pregnancy on the mother and fetus is reassuring.

### *1.10.3 Aspirin therapy and placental pathology*

A histological finding in the placenta of preeclamptic patients is usually consistent with villous ischaemia (increased syncytial knots, thickening of trophoblastic basement membrane, villous hypovascularity, villous agglutination and infarction), fibrinoid necrosis of uterine vessels and acute atherosis; more tortuous or densely distributed spiral and basal arteries than normal(221).

Despite an improvement in outcomes in the aspirin treated pregnancies with PE, histological evidence of uterine vascular pathology persisted in the majority of women with a prior complicated pregnancy demonstrating similar placental lesions(222, 223).

On the other hand, a recent study showed that aspirin use during pregnancy was associated with a reduced risk of hypoxia-related placental pathology in the third trimester in high-risk population [OR and 95% CI in the 1st, 2nd, and 3rd trimesters: 0.55 (0.31, 1.00), 0.76 (0.49, 1.17), and 0.53 (0.29, 0.94), respectively](223). It concluded as well that the longer duration of aspirin use in pregnancy the lower the risk of hypoxia-related placental pathologies in the high-risk population.

## 1.11 Study rationale

Importantly, although NICE has issued guidance on the prevention of hypertension in pregnancy - which includes a recommendation that women deemed to be at high risk for hypertension in pregnancy be considered for low dose aspirin after 12 weeks' gestational age, and PGDM is listed as a criterion of 'high risk' - this recommendation is not supported by randomised-trial evidence demonstrating that aspirin therapy benefitted women with diabetes. Furthermore, this guidance is at variance with international guidelines on the management of diabetes in pregnancy, none of which includes a recommendation that aspirin is prescribed to this group.(16, 18, 19, 224) This reflects the dearth of studies that have been done on this high-risk subgroup and the

paucity of evidence gleaned from meta-analyses, in favour of aspirin therapy in this group.

We observed the liberal prescription of aspirin in pregnancy without a solid background in the form of randomised trials to support its use. Our research group decided to investigate the role of this antiplatelet therapy, specifically in optimising pregnancy outcome among the high-risk cohort with PGDM.

### 1.12 Clinical practice at international units

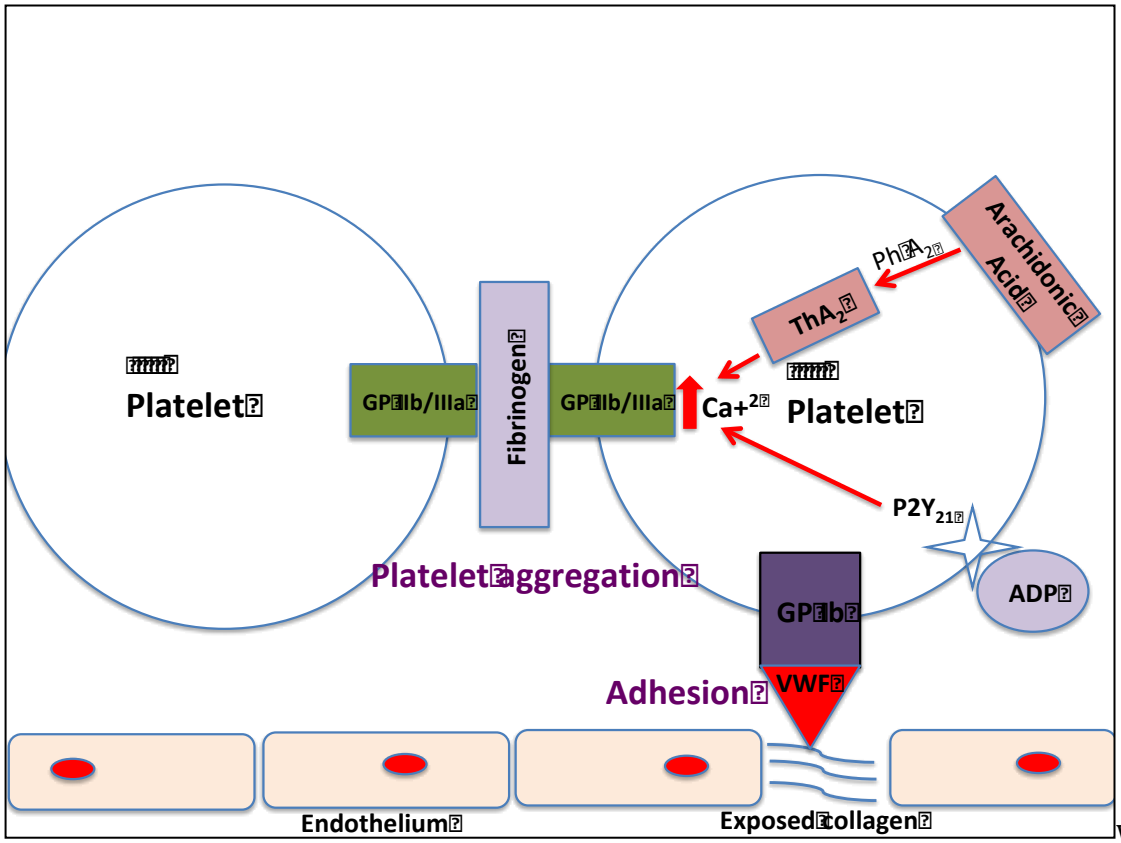
The World Health Organization recommends low dose aspirin (75 mg) before 20 weeks of pregnancy for women at high risk for PE(19). The US Preventive Services Task Force recommends low dose aspirin (81 mg/d) after 12 weeks' gestation in women at high risk for PE(16). The national guidelines for the management of hypertension in pregnant women in Canada and the United Kingdom also recommend prophylactic low dose aspirin for women perceived to be at increased risk (16, 224). However, these guidelines are all issued in the context of an acceptance for the fact that the evidence for a beneficial effect of aspirin on reducing perinatal morbidity or improving maternal outcomes, for particular subgroups of patients such as women with PGDM, is very weak or inconclusive. Nevertheless, the global use of low dose aspirin remains patchy, perhaps in large part because of controversy surrounding its efficacy.

| | No. of participants | PlGF | sFlt1 | sEng | sFlt1/PlGF ratio |
|---|---|---|---|---|---|
| **Yu *et al* 2009(65)** | 151 | No change | Increased | Increased | Increased |
| **Powers *et al* 2010(67)** | 194 | Reduced | Increased | Increased | Increased |
| **Holmes *et al* 2013(66)** | 540 | Reduced | Increased | Increased | ncreased |
| **Cohen *et al* 2014(68)** | 159 | Reduced | Increased | --- | Increased |

PlGF: Placental Growth Factor
sFlt1: Soluble Fms-like tyrosine kinase 1
sENG: Soluble Endoglin

**Table 1.1 The variation in balance of angiogenic/ antiangiogenic factors in development of preeclampsia in women with PGDM.**

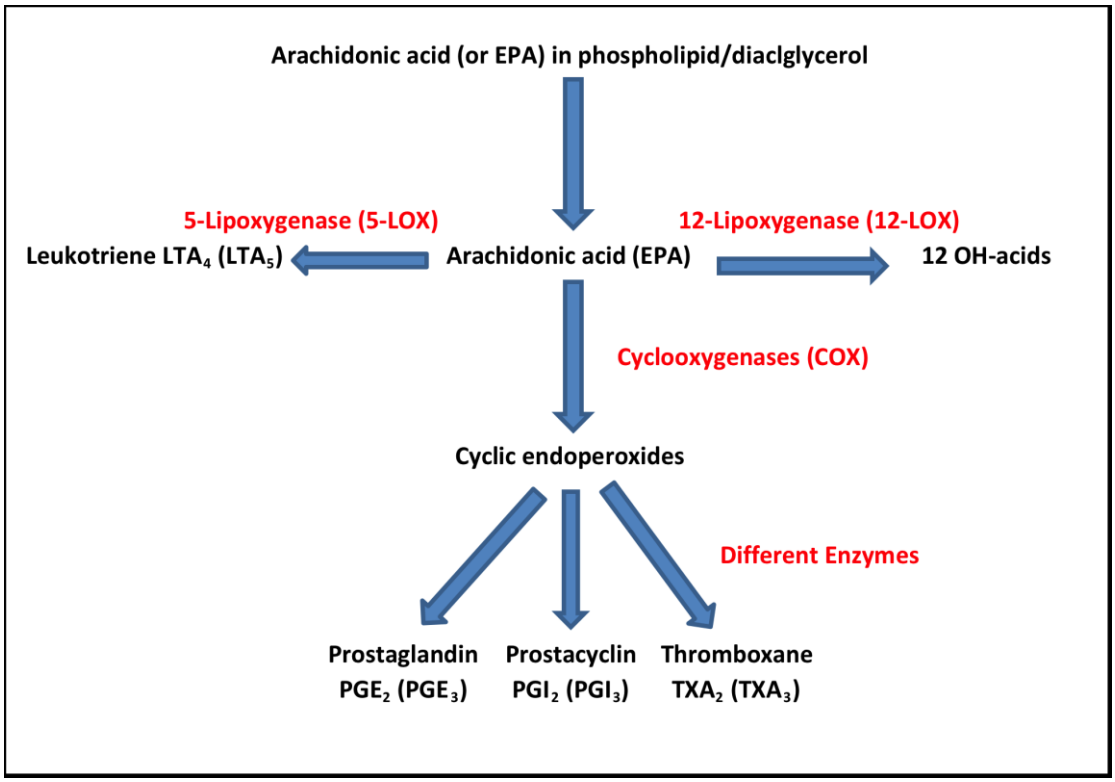
This table demonstrates the variation in different biomarkers in 4 main studies that investigated the role in the balance of angiogenic/ antiangiogenic factors in development of preeclampsia in women with PGDM.




VWF: Von Willebrand Factor
GP: Glycoprotein
ADP: Adenosine diphosphate
$ThA_2$: Thromboxane $A_2$
$PhA_2$: Phospholipase $A_2$
$Ca^{+2}$: Calcium

**Figure 1.1. Platelet activation pathway**

Platelets are activated via several different membrane receptors. When endothelium is injured, the subendothelium exposes von Willebrand factor (VWF) that binds to GP Ib, causing platelet adhesion. Thrombin, $ThA_2$, and ADP bind to the thrombin receptor, $ThA_2$ receptor, and P2Y12, respectively. This causes an increase in intracellular calcium ($Ca^{2+}$) and a decrease in cAMP, leading to platelet contraction and GP IIb/IIIa activation. Activated GP IIb/IIIa on adjacent platelets bind to fibrinogen (final common pathway) leading to platelet aggregation and thrombus formation.




EPA: Eicosapentaenoic acid

**Figure 1.2. Synthesis of Eicosanoids from Arachidonic Acid**

Arachidonic acid is released from a bilayer cell membrane phospholipid. It is cleaved by phospholipase $A_2$ and takes one of two major pathways. 5-Lipooxygenase converts it to Leukotrienes. The more important pathway is through cleavage via cyclooxygenase which results in the production of different endoperoxides or prostaglandins.

# Chapter 2: Materials and Methods

## 2.1 Study title

Aspirin for Optimising Pregnancy Outcome in Pregestational Diabetes: Pilot for The IRELAND Study (Investigating the Role of Early Low-dose aspirin in pre-existing Diabetes).

## 2.2 Study objectives

The purpose of this pilot is to determine rates of patient participation and compliance with aspirin therapy, in addition to exploring the effect of low-dose aspirin on platelet function throughout pregnancy in this high-risk population.

### *2.2.1 Primary objectives (Feasibility)*

1. **Recruitment issues**: To determine the proportion of women eligible and willing to participate in a RCT of aspirin use in pregnancies complicated by PGDM.

2. **Compliance issues*:*** Subjectively ( using self-reporting and pill count) and objectively ( using platelet aggregation testing).

3. **Satisfaction of participants.**

4. Operational & logistical issues relating to recruitment, data collection, transport and storage of samples.

### *2.2.2 Secondary objectives (Exploratory)*

- Assessing platelet aggregation using Light Transmission Aggregometry (LTA) in light of usage of aspirin in women with PGDM.

## 2.3 Study design

This was a pilot, prospective, randomised study, for women with PGDM (type 1 and type 2) for at least six months prior to pregnancy presenting to the Combined Obstetrics Endocrine service in two tertiary maternity units (Rotunda Hospital and Coombe Women and Infant's University Hospital).Participants were randomised into two groups, one group was for the use of oral "aspirin", acetylsalicylic tablets (Nu-Seals®) 75mg daily from first trimester, and the other part was the control or non-aspirin group. This study was a precursor to a multicentre double-blinded placebo-controlled randomised trial, involving all 7 tertiary referral obstetric centres participating in the Perinatal Ireland Research Consortium (Rotunda Hospital, National Maternity Hospital, Coombe Women's and Infant's University Hospital, University College Hospital Galway, Cork University Maternity Hospital, Mid-Western Regional Maternity Hospital, Limerick, and Royal Jubilee Maternity Hospitals, Belfast).

## 2.4 Patient cohort

### *2.4.1 Screening and pre-recruitment*

All pregnant women with PGDM attending the endocrine service in the Rotunda and Coombe Hospital were identified by a specialised Diabetes midwife who would usually review them either the clinic or when they are admitted early in gestation for certain reasons, mainly blood glucose stabilisation or insulin introduction for patients who were not on insulin, pre-pregnancy. The researcher was then alerted that a potentially eligible participant was registering for prenatal care. Patient Information Leaflets were provided for patients who were deemed eligible for study entry. It was estimated that around 40 patients in the Rotunda Hospital would attend over a 12-month period who would be potential candidates for inclusion in the study.

### *2.4.2 Inclusion criteria*

People who satisfied all of the following were considered for inclusion in the study:

1. All women with type I or type II diabetes, at least 6 months' duration prior to conception.
2. Were able to speak and read English.
3. Had a singleton pregnancy at <12 weeks' gestational age, with documented fetal cardiac activity prior to recruitment.

4. Were willing to sign voluntarily a statement of informed consent to participate in the study.

### *2.4.3 Exclusion criteria*

The following patients were excluded from participation in the study:

1. Aspirin hypersensitivity (prior bronchospasm/ urticarial/ angioedema with aspirin).
2. Peptic ulcer disease.
3. Known bleeding diathesis.
4. Multifetal gestation.
5. Severe early-onset preeclampsia in a previous pregnancy.
6. Patient already on aspirin.
7. Under 18 years of age.
8. Miscarriage prior to randomisation.

The rationale for excluding multiple gestations and patients with early onset severe preeclampsia from a previous pregnancy was because of the potential for developing preeclampsia in those high risk groups, who will likely be on Aspirin at an early gestation, in accordance with NICE guidelines.

The pre-recruitment visit was performed either in the diabetic clinic or in the prenatal ward if patients were admitted to hospital. Patients were screened as early as 5 weeks and up to 11 weeks' gestation. During the visit, the researcher explained to the patient the purpose of the study, provided a Patient Information Leaflet, and then offered an appointment for recruitment. In some cases, the pre recruitment and recruitment visit were on the same day if the patient was willing to do so and pregnancy was between $8^{+0}$ and $11^{+6}$ weeks' gestation.

### *2.4.4 Number of participants*

Participants who met eligibility criteria were randomised to enteric-coated aspirin 75mg (Nu-Seals® aspirin) or no aspirin. Aspirin was prepared and packaged at a central location. A computer-generated randomisation programme using an excel sheet sequence was used to assign patients to aspirin or control (no aspirin) groups.

### *2.4.5 Period of study*

Recruitment began in June 2015 and the last patient recruited in February 2016, over an 8 months period. All patients recruited were seen in the Obstetrics Diabetic Service on a monthly basis until 36 weeks' gestational age:

- $8^{+0}$ - $11^{+6}$ weeks; (pre-recruitment/recruitment visit)
- $12^{+0}$ - $16^{+6}$ weeks; (second study visit)
- $17^{+0}$ – $21^{+6}$ weeks; (third study visit and anatomy scan)
- $22^{+0}$ - $26^{+6}$ weeks; (fourth study visit and fetal Echocardiogram scan)
- $27^{+0}$ - $31^{+6}$ weeks; (fifth study visit and departmental growth scan)
- $32^{+0}$ – $35^{+6}$ weeks (sixth study visit and departmental growth scan)
- $36^{+0}$ (seventh study visit, departmental growth scan, stop aspirin intake, plan for delivery)

Platelet function testing was carried out at each visit of the study at 4-weekly intervals

### *2.4.6 Recruitment*

Once a patient met the inclusion criteria and agreed to participate in the study, a consent form was provided and the following maternal details was collected at enrolment (pre-recruitment or recruitment visit)

- **History**

- Maternal age
- Weight and height
- Ethnicity
- Smoking, alcohol and Drug use
- Medical history
- Medication use within 4 weeks of recruitment
- Obstetric history
- Medical/surgical history
- Family history of PE or diabetes.
- Pre-existing complications of diabetes (retinopathy, nephropathy or neuropathy)

- Drug allergies including Aspirin

- **Physical examination**

- Height (in meters) was recorded, to enable the body mass index (BMI) to be calculated.
- Weight was measured at every visit using the same scales, with the participant wearing a similar amount of clothes (shoes/runners off).

- Systolic, diastolic blood pressure (mmHg) and Mean Arterial Pressure (MAP) were measured using an automated device. Automatic, calibrated, blood pressure monitors with adjustable cuffs, small (<22cm), normal (22-32cm) or large (>32cm) cuff was used depending on mid-arm circumference, to measure the blood pressure and the average of two to three consecutive readings, at 1-minute intervals, was recorded. The Royal College of Physicians Ireland's (RCPI) criteria for PE is blood pressure of 140 mm Hg systolic or higher or 90 mm Hg diastolic or higher, which occurs after 20 weeks of gestation in a woman with previously normal blood pressure and proteinuria, with a urinary excretion of 0.3 g protein or higher in a 24-hour urine specimen.
- Early first trimester ultrasound scan was performed (transabdominal or transvaginal) to confirm fetal cardiac activity and gestational age.

- ***Clinical laboratory tests***

- Standard prenatal testing for blood type, red-cell antibody status, HIV, Rubella IgG, Hepatitis B, Syphilis, and Varicella IgG was included, as per standard obstetric care. Baseline evaluation of the following laboratory indices of glycaemic control was recorded 4-weekly, until 36 weeks' gestation:

  a) Fructosamine
  b) Glycosylated haemoglobin (HbA1c)

- Baseline evaluation of the following indices of renal function:

  a) Creatinine clearance (in the first trimester of pregnancy)

  b) 24-hour urinary microalbumin excretion (in the first trimester of pregnancy and repeated in the third trimester)

c) 4-weekly measurements of serum Creatinine and Urea.

- Platelet Function Assay: Serum specimens were collected from study participants at randomisation and 4-weekly thereafter, to determine platelet function indices, thus ensuring treatment compliance and checking for aspirin resistance.

- ***Concomitant medication***

Any medications that were considered necessary for the participant's welfare and would not interfere with the study medication were given in accordance with clinical indications and at the discretion of the investigator. History of duration and dose of folic acid supplementation was specifically sought and recorded. A record of all medication taken by research participants in the 6 weeks before visit 1, along with the concomitant medication the research participant took throughout the study, was recorded on the appropriate page of the Case Report Form.

- ***Fetal anatomy and fetal echocardiogram***

Ultrasound examination of fetal anatomy was offered at 18 – 24 weeks, for excluding major structural abnormality in the fetus, and a targeted fetal echocardiogram was performed at 24 weeks' gestation in all participants, in accordance with departmental policy.

- ***Fetal growth scan***

Ultrasound examination of fetal growth was offered from 28 weeks onward on a 4-weekly basis, in keeping with local hospital guidelines for patients with PGDM**.**

## 2.5 Randomisation

Following recruitment, patient randomisation was performed within the centre. Patients were assigned a study number and an intervention (aspirin or no aspirin), in an open-label fashion. Randomisation was performed via the Perinatal Ireland restricted-access web-based portal for study personnel only. Women underwent computer-generated randomisation to one of two antenatal management protocols:

- **Aspirin group arm 1:** Aspirin therapy 75mg daily commenced after satisfactory routine first trimester assessments, to include sonographic confirmation of fetal cardiac activity. Monthly blood was drawn during pregnancy up to week 36 for platelet function, serum fructosamine, and renal profile, with quantification of microalbuminuria in the first and third trimesters. Prenatal care was delivered as standard through the Combined Obstetric Endocrine service. Aspirin was provided free of charge to the participants through the local pharmacy in the hospital
- **Control group arm 2:** No aspirin therapy. Monthly blood was drawn during pregnancy up to week 36 for platelet function, serum fructosamine, and renal profile, with quantification of the microalbuminuria in first and third trimesters. Prenatal care was delivered as standard through the Combined Obstetric Endocrine service.

## 2.6 Blinding

This pilot phase was an open-label study. No blinding of either the research participant or of the investigator occurred. Both the research participant and the investigator were aware of the investigational medicinal product that the research participant had been allocated to receive. A pilot study is not a hypothesis testing study. In general, the fundamental purpose of conducting a pilot study is to examine the feasibility of an approach that is intended to ultimately be used in a larger scale study. On the other hand, a hypothesis testing clinical trial is designed to compare randomised treatment groups in order to draw an inference about efficacy/effectiveness and safety in the patient population, based on sample results. The primary goal in designing such a study is to minimise the bias in the estimate of the treatment effect.

## 2.7 Assessment of platelet aggregation

Platelet aggregometry has been historically used to test for platelet function. Light transmission aggregometry (LTA) has been described since the early 1960s. The general principles on which LTA is based, have not changed since O'Brien and Born first described it(157).

Platelet-rich plasma (PRP) is stirred in a cuvette that is placed between a light source and a photocell at 37 degrees Celsius. The ability of platelets to aggregate in-vitro in response to exogenous aggregating agents such as AA, ADP or EPI is measured. The plasma is cloudy as the platelets are circulating around in the sample; this will allow little light to pass through. Upon addition of the agonist, platelet aggregates and the sample becomes clearer, allowing more light to pass through. Transmission of light is detected by the photocell and recorded as a function of time. Therefore, as a general rule, Photo-optical measurement of light transmission increases in relation to agonist-induced platelet aggregation. Various agonists have been used in-vitro to induce aggregation such as AA, ADP, Thrombin, Epinephrine, Collagen, and Ristocetin. In our laboratory, we used AA. PPP is used to calibrate the system. The PPP represents 100 percent aggregation. The device records the rate and maximal percentage of this increase from 0% (maximal optical density of PRP) to 100% (no optical density of platelet-poor plasma) by a photometer. A cut off, of 20% value, was determined to distinguish poor from good responders, in keeping with international guidelines. This signal is converted automatically in a graphic curve that parallels the increase in light transmission during the platelet aggregation. The benefits of using LTA are various; not only is it historically the gold standard method in detecting platelet aggregation, but it is sensitive to antiplatelet therapy. It is a manual sample processing and it can be affected by some pre-analytical conditions such as PRP preparation, haemolysis, and low sample volume. It requires highly skilled laboratory technicians and experience in performing and interpreting platelet function. It is reproducible and subjected constantly to an ongoing standardisation process.

Equipment for experimentation

- Eppendorf Centrifuge 5810 R
- Light transmission aggregometer PAP-4
- LEC Medical LSC119UK Fridge freezer
- HOTpoint RZFM151 Freezer

All equipment was serviced as per manufacturer’s recommendations.

### *2.7.1 Blood draw and sample labelling*

1. The patient was given the option of sitting or lying down during blood draw.

2. A tourniquet was applied to upper arm of the patient, who was then asked to make a fist.
3. Non-sterile gloves as per routine blood drawing protocol were used. The area where potential puncture site (usually cubital fossa of non-dominant arm) was cleaned using alcohol wipes.
4. 19 gauge needle was used as a larger bore needle would ensure the least disruption to platelets, and prevent aggregation.
5. The tourniquet was removed immediately once the needle was in the vein and blood was drawn slowly, as platelets are very sensitive to shear. Blood was allowed to flow naturally into the syringe. Blood flowed directly into the anticoagulant, with gentle mixing.
6. 20 mL of venous blood was drawn using a 19-gauge butterfly needle connected to a green 10 mL Starstedt anticoagulant bottle containing 3.2% (w/v) trisodium citrate (1:9 volume of citrate to blood, final citrate concentration of 0.32%).
7. Samples were labelled with patient initials, study number, gestational age, and time of blood draw.

### *2.7.2 Sample transportation*

Rotunda Hospital to RCSI St Stephen's Green:

1. Blood samples were stored at room temperature, as cooling can lead to activation of platelets.
2. Samples were transported from phlebotomy in the Rotunda Hospital to RCSI St Stephens Green. The blood was transported in a blood transport box via Med Lab pathology for analysis in Molecular and Cellular Therapeutics Laboratory by the research assistant.
3. Samples were transported to RCSI within 150 minutes of blood draw. In the event that there was a delay in transportation, the sample was processed in the research laboratory in the school of midwifery building in the Rotunda Hospital. The processed samples were stored at room temperature (1mL of PPP should be stored at -20 degrees Celsius) and transported to RCSI laboratory for analysis.
4. Samples were analysed within 240 minutes of blood draw (platelets become unstable over time).

5. In the event that there was a problem with the blood sample, such as blood becoming clotted or the sample being insufficient, the researcher was informed. If another sample was not possible to obtain, a blood sample was taken at the next patient visit and a protocol deviation form filled.

### *2.7.3 Coombe Hospital to RCSI St Stephen's Green*

1. Blood samples were stored at room temperature.
2. Samples were transported from phlebotomy in the Coombe Hospital to RCSI St Stephens Green. The blood was transported in a blood transport box via MedLab pathology for analysis in Molecular and Cellular Therapeutics Laboratory by the research assistant.
3. Samples were transported to RCSI within 150 minutes of blood draw.
4. Samples were analysed within 240 minutes of blood draw (platelets become unstable over time).

### *2.7.4 Sample preparation*

*Preparation of Platelet Rich Plasma (PRP):*

1. Blood was centrifuged at 170 G (RCF) for 10 minutes at room temperature, to prepare PRP. No break should be applied to the centrifuge to prevent platelet activation.
2. The supernatant was removed (approximately 3-5 mL of PRP per 10 mL of blood) carefully, so as not to disturb the red blood cells (RBC), using a Pasteur transfer pipette and added to a fresh 15 mL falcon tube.
3. The PRP were stored at room temperature until needed.

*Preparation of Platelet Poor Plasma (PPP)*

1. The remaining RBC layer from step 2 was centrifuged at 1500 G (RCF) for 3 minutes at room temperature (no break should be applied to the centrifuge).
2. The supernatant (approximately 1-3 mL of PPP) was remove carefully, so as not to disturb the RBC layer), using a Pasteur transfer pipette and added to a fresh 15 mL falcon tube.
3. The RBC layer was discarded into a yellow sharps bin.

4. 1 mL of PPP at -20 degrees Celsius was stored in an Eppendorf and the remaining PPP was used for calibration of the light transmission aggregometer (PAP-4).

Experimentation (platelet function testing)

*PAP-4 setup (light transmission aggregometer) calibration*

1. 250 µL of PPP was added to a siliconised, flat-bottomed test tube 7.25 x 55 mm and used to calibrate the aggregometer prior to experimentation. Each well containing a sample of interest was calibrated.
2. The PPP tube was added to each measured aggregation well of interest; The channel button (i.e. channel 1, 2, etc.) on each well was activated to calibrate until “PPP set” was displayed on the machine for each well. The PPP represented 100% aggregation.

### *2.7.5 Aggregation*

Stir bars and the PRP samples were added to test tubes prior to the addition of agonists that induce platelet aggregation. The final reaction volume in each test-tube was 250 µL (225 PRP + 25 of AA).

1. The stir bars were added to the test tubes followed by 225 µL of PRP. This was performed in the loading wells prior to the addition of test tubes to measured aggregation wells.
2. Once samples were ready, measured aggregation wells were then added.
3. The channel button on each well was pressed to begin.
4. 15 seconds were allowed to pass prior to the addition of 25 µL of AA.
5. Post addition of the AA, aggregation was allowed to run for 4 minutes before the channel button was pressed on each well to stop the reaction.
6. A light beam passing through the PRP sample was measured. As platelet aggregates, more light passed. This determined the percentage of aggregation.
7. A print out was given from the machine, whereupon the results were written down and stored in an appropriate folder.
8. The stir bars were removed using a magnet and washed with hot water to clean. The stir bars were reused for future experiments.

9. Used glass test tubes were discarded into yellow sharps bin.

Controls:

- NEGATIVE Control: No addition of AA to PRP, just stirred for 4 minutes = no aggregation.
- POSITIVE Control: Healthy control (free from any medication known to affect platelet function i.e. no aspirin) = to confirm AA was working.

### *2.7.6 Analysis*

- At the end of the study, graph pad prism software version 7.0a was used to graph aggregation results. Those patients demonstrating ≤ 20 % aggregation were deemed aspirin responders and those with > 20 % aggregation were considered non-responders.
- All analysis and experimentation was performed in the temporary lab in the School of Midwifery building in Rotunda Hospital or in Lab 5, third floor RCSI St Stephen's green by the research assistant.

## 2.8 Assessment of compliance

Compliance was assessed subjectively by counting the tablets returned by the participant at each visit at 4-weekly intervals. The total number of tablets taken was calculated by subtracting the number of tablets returned from the number of tablets prescribed. In addition, compliance was measured by diary card, which was given to the patient after randomisation to the aspirin group. Each participant was required to tick on the card daily when they have taken the aspirin tablet. A record of all investigational medicinal products dispensed and returned was documented on the investigational medicinal product dispensing and accountability log.

Compliance was calculated as a percentage of the reported intake of tablets to the total number participants were expected to have taken between the date of randomisation and the date of the visit at 36 weeks' gestation or the date of delivery if delivery occurred <36 weeks. Non-compliance for more than 15% of the trial period was considered a significant deviation from the study protocol.

Objectively, platelet aggregation was tested along with the diary card and pills count at 4-weekly intervals. A patient was only considered non-compliant if all tests indicated non-compliance.

### 2.9 Safety reporting

The safety of the investigational medicinal product (aspirin) was assessed through the recording, reporting and analysis of baseline medical conditions and adverse events in line with good clinical practice (GCP).

- ***Serious adverse events (SAE).***

Any untoward medical occurrence or effect that at any dose resulted in death or was life-threatening or required hospitalisation or prolongation of existing hospitalisation, or resulted in persistent or significant disability, a congenital anomaly or birth defect or important medical events that might have jeopardised the subject or might have required an intervention to prevent one of the above characteristics/consequences

- ***Suspected unexpected serious adverse reactions (SUSARs)***

An adverse reaction, the nature or severity of which was not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational medicinal product or summary of product characteristics for an authorised medicinal product).

- ***Assessment of causality***

All adverse events were assessed for relatedness to aspirin. A causal relationship to aspirin was reported as adverse reactions. The assessment of whether the SAE was likely to be related to treatment according to the following definitions:

- Unrelated

  Where an event was not considered to be related to the study medication.

- Possibly related

Although a relationship to the study medication cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

- Probably related

The temporal relationship and absence of a more likely explanation suggest the event could be related to the study medication.

- **Reporting procedures for serious adverse events**

All SAEs were reported within 24 hours of occurrence to the Safety Team for review by the Chief Investigator except for those that the protocol identified as not requiring immediate reporting. As a minimum the initial report contained the unique patient study identifier, provided the symptom or diagnosis, identified the seriousness criteria, and provided the name and contact details of the reporter. Additional information where applicable were provided in a follow-up report.

A paper SAE report form was completed by the investigator, scanned and emailed to a dedicated email address hosted by Perinatal Ireland 'safetyteamPNI@rcsi.ie'. The following personnel (Safety Team) had access to the safety mailbox:

- Dr Fionnuala Breathnach, Chief Investigator
- Ailbhe Cullen, RCSI CRC Director of Nursing
- Dr Elizabeth Tully, Perinatal Ireland Programme Manager
- Carole Schilling, RCSI Quality and Regulatory Affairs Manager

The sponsor would need to report all SUSARs to the competent authority (the HPRA in Ireland) and the ethics committees concerned.

The HPRA would report any SUSARs which occured during the trial to Eudravigilance (EV) on behalf of the investigator and sponsor to facilitate the Pharmacovigilance Department at the HPRA in fulfilling their obligation to submit SUSAR reports to EV within legislative timelines, the Sponsor would require to report SUSARS to the HPRA well in advance of the legislation timeline:

- SUSARs that were not fatal or life-threatening required reporting to the HPRA within 9 calendar days in an unblinded manner
- SUSARs that were fatal or life threatening require reporting to the HPRA within 3 calendar days in an unblinded manner. The Sponsor would submit reports to the HPRA using the online reporting form found at the following link: http://www.hpra.ie/homepage/medicines/regulatory-information/clinical-trials/reporting-serious-adverse-events.

- ***Data and Safety Monitoring Board (DSMB) roles and responsibilities***

The DSMB is an independent group of experts that advises the RCSI and the study investigators. The members of the DSMB serve in an individual capacity and provide their expertise and recommendations. The primary responsibilities of the DSMB are to: 1) periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy, and 2) make recommendations concerning the continuation, modification, or termination of the trial. The DSMB considers study-specific data as well as relevant background knowledge about the disease, test agent, or patient population under study.
The DSMB is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines and voting procedures prior to initiating any data review. The DSMB is also responsible for maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it.
The DSMB reviewed each protocol for any major concern prior to implementation. During the trial, the DSMB reviewed cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, DSMB members were satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review were sufficient for evaluation of the safety and welfare of study participants. The DSMB assessed the performance of overall study operations and any other relevant issues, as necessary.

- ***Statistics***

For the pilot phase of the IRELAND study, only simple descriptive statistics were used to present the data. These data were then used to inform the planning (feasibility and

sample size) of a further definitive RCT to be conducted across several Perinatal Ireland centres.

No formal hypothesis was generated and no interim analysis was planned.

- ***Determination of sample size subjects***

Sample sizes of 24 have been recommended for pilot studies. (93, 225) The pilot phase was exploratory, targeted at closely interrogating the biologic effect of antiplatelet therapy in diabetic women during pregnancy. It was expected to inform a definitive randomised placebo-controlled study with respect to patient participation and dropout rates.

- ***Analysis sets***

Two populations were used to describe the data:

1) Intention-to-Treat: all subjects randomised to the treatment arm in the study and completed all assessments

2) Per-Protocol: all subjects randomised who underwent all assessments up and who did not violate protocol criteria (entry criteria)

## 2.10 Data handling and record keeping

All data were stored securely. Details of outcome measures and adverse events were documented in hospital healthcare records, individual research participant CRFs and in an encrypted electronic database. We adhered to hospital protocols pertaining to healthcare record use and storage. To protect the research participant's identity, a unique identification code (Study Number) was assigned by the Investigator to each study participant and used in lieu of the participant's name when adverse events and/or other study-related data were reported. This coded form of identification, instead of the participant’s name, appeared on all documents/databases and was cross-referenced by the participant's date of birth. All paper records were kept in a locked filing cabinet within respective units. Research participant data were stored also on a dedicated, protected research electronic database at the RCSI facility where a server stored the data. This server had managed access and password protection. The information that this server contained was backed up every 24 hours. Desktop and laptop computers

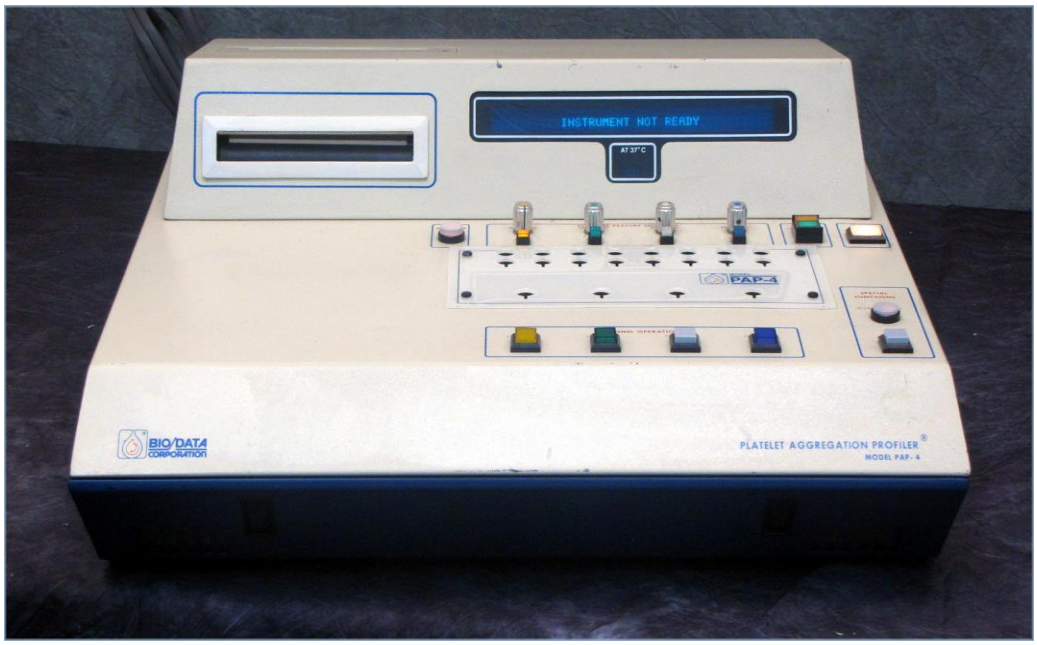
were used for data collection and entry onto this server. All reads and writes to the database were recorded with date, time and user. Users had a unique internal identification and data entry required an electronic signature, which consists of system password and username.

### 2.11 Quality control and quality assurance procedures

To ensure good clinical practice, the sponsor monitored the progress of all clinical investigations being conducted. A clinical monitor, who was a representative of the sponsor, had the obligation to follow this study closely. In addition to conducting a site visit prior to initiation of enrolment, the clinical monitor visited the study facilities regularly and utilised telephone and written communication on an on-going basis. During periodic visits to the designated study site the monitor reviewed the source documents used in the preparation of the CRFs to verify the accuracy and completeness of the information contained in those reports in preparation for retrieval. Source documents were all the information in original records and certified copies of original records of clinical findings, observations, or other activities in the study, which were necessary for the reconstruction and evaluation of the study. All data generated during this study, and the source documents from which they originated, were subject to inspection by the sponsor or its agent, health authorities, and regulatory agencies. On completion of the study the sponsor's representative made a final visit to the site to collect remaining CRFs, study medication, and other supplies.

### 2.12 Ethics

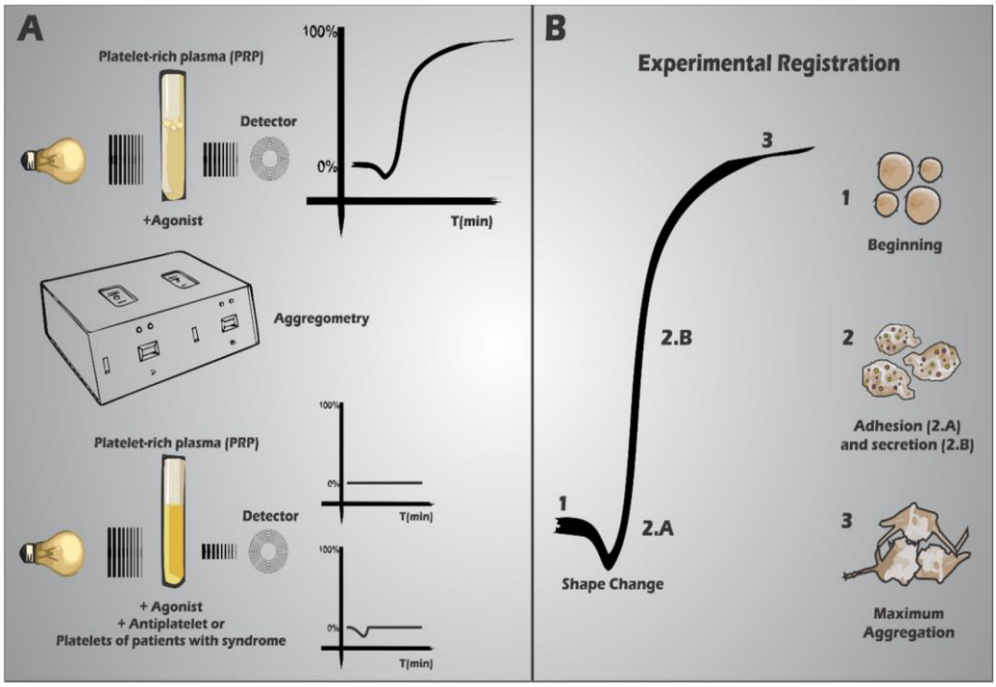
Before initiating this study, the Study Protocol, Patient Information Leaflet and Informed Consent Form, applicable advertising, and any other written information given to participants were reviewed and approved by a constituted Institutional Review Board/ Independence Ethics Committee IEC/IRB. A signed and dated statement that all documents submitted for review have been approved by the Committee were given to the Sponsor or designee before the study was commenced at the site. Ethical approval for the IRELAnD study was sought from the National Maternity Hospital Ethics Committee.




***References:*** Royal Brisbane and Women's Hospital - Brisbane/AU

**Figure 2.1. Light transmission aggregometry**.

It includes channels for performing aggregation tests in PRP when desired. The system we used is a PAP-4 light transmission aggregometer.




Reference: International Journal of Molecular Sciences 15(10):17901-17919 · October 2014

**Figure 2.2. Tests of platelet function through light transmission aggregometry (**A**) and its experimental record (**B**).**

(A) Normal platelets (up), disabled or treated with the antagonists (down) are activated by agonists such as ADP, thrombin and arachidonic acid, resulting in different records; (B) experimental graphical profile of aggregometry assay using normal platelets, appearing at (1) the beginning of the test after adding the agonist, followed by (2.A) shape change. Then, platelets' adhesion and aggregation occur (2.A—primary aggregation) and, if the stimulus is adequate, there is the granule secretion (2.B—secondary aggregation) and maximum platelet aggregation (3).

# Chapter 3: Results

The results of our study are presented as follows

- Section 1 – Description of study cohort and feasibility data
- Section 2 – Compliance and patients' narratives
- Section 3 – Safety data and monitoring
- Section 4 – Delivery and neonatal outcome
- Section 5 – Participants' satisfaction questionnaire

## 3.1 Description of study cohort and feasibility data

The total number of women screened in both hospitals was 47. Of these 31 were eligible to entry criteria. Seven out of those 31 women did not consent to study. Twenty-four patients gave initial consent for the study, two withdrew at different stages of the study, one had late fetal demise and 21 continued to delivery. See Figure 3.1. Sixteen (34%) patients were not eligible for study participation. The most common reason cited for ineligibility was first trimester pregnancy loss. Eight out of 16 had a miscarriage before 12 weeks' gestation (this represented 17% of total number of patients screened. Two had difficulty communicating in English and two were taking aspirin in the first trimester before the recruitment phase. Other causes cited for ineligibility are outlined in Figure 3.2.

There was no statistical difference between groups when baseline characteristics were compared (ethnicity, gravidity, parity, smoking status, onset of conception or gestational age at recruitment, BMI, systolic and diastolic blood pressure readings. All patients in both groups had a normotensive blood pressure reading at the time of recruitment in the first trimester. See Table 3.1.

When comparing BMI between both groups, there was no statistically significant difference. None of the participants were in the underweight category and 14/23 were classified as overweight or obese. See Figure 3.3.

Sixteen patients almost 70% of total cohort) were Caucasian. Five were Asian and only two patients (9%) were black African. See Figure 3.4.

Age was equally distributed between both groups. The mean age was 32 years with range between 18 and 42 years. See Figure 3.5.

Attendance at the preconception clinic was higher among women with type I diabetes than type 2. See Figure 3.6.

## 3.2 Compliance and patient narratives

### *3.2.1 Compliance*

Non-compliance as prespecified in the study protocol, was considered if 15% of the overall pill intake was missed judged objectively by pills count and diary card and that was confirmed objectively with platelet aggregation testing > 20% at the period of the missed pills.
Based on that assumption, we have identified 2 out of 13 patients in the aspirin group who were considered non-compliant based on the above criteria (15.4%) which is in keeping with rate of non-compliance in the literature from international sources.
Figure (3.7) demonstrates the disparate degrees of platelet aggregation observed according to whether participants were taking aspirin or not. A platelet aggregation level of 20% or less was observed in participants on aspirin throughout the active phase of treatment. No suppression of platelet aggregation was observed in the control group.
In addition to that, we identified a patient in the aspirin group (patient 15) whose platelet aggregation levels were above the 20% mark all through pregnancy despite the fact that subjective measures indicated that she was taking aspirin as prescribed. That was evident, however intermittently in 2 more patients (patient 4 and 12) on two different occasions.

### *3.2.2 Patients' narratives*

Patient (1):

This patient withdrew consent to participate in the study before randomisation. Demographics and other clinical data were not collected. The patient did not volunteer a reason for withdrawal from the study.

Patient (2): Non-aspirin group

This patient had T2 DM with no known microvascular complications. She had a planned pregnancy; however, she did not attend a preconception clinic. She had a BMI of 29.2 $kg/m^2$ at the time of recruitment and was on metformin 500 mg twice a day that was stopped at 7 weeks' gestation. She was commenced on insulin and folic Acid 5 mg once daily at a gestational age of 7 weeks. She was recruited at $9^{+4}$ weeks' gestation and randomised to the non-aspirin group. She regularly attended her study visits, which were 8 visits in total. Platelet aggregometry results throughout the study period are demonstrated in the line chart, see Figure 3.8. All platelet activity was recorded as above the 20% threshold line, which indicates absence of any suppressive agents to platelet aggregation. She had no serious adverse events; hospital admissions or PE features during the study period. Her last study visit was at $37^{+2}$ weeks' gestation and she was induced at $39^{+1}$ weeks and achieved a vaginal delivery with an uneventful postnatal course. She delivered a male infant of 4.1 kgs with Apgar Scores of 9 and 10 at 1 and 5 minute of life, respectively. No admission to the neonatal unit was required in the first month of life.

Patient (3): Aspirin group

This patient was a 39-year-old multiparous woman with T2 DM without known microvascular complications. She did not attend the preconception clinic and was on metformin 1000 mgs twice daily and folic Acid 5 mgs once a day until 7 weeks' gestation when she was commenced on insulin. There was no significant medical history, but she reported a family history of deep vein thrombosis. After establishing viability with a first trimester scan, she was consented, recruited at $11^{+4}$ weeks' gestation and randomised to the aspirin group. The baseline platelet aggregometry on the day of her visit was 91% and she was given 82 tablets of aspirin 75 mgs and a diary card. She was scheduled for an appointment for 4 weeks. At her second, third and fourth visits, aspirin pills were counted, the diary card was checked, and she was deemed compliant with study medication intake. This coincided with platelet aggregation of 11%, 16% and 6%, respectively. There were no recorded serious adverse events during that period of the study. At the fifth visit at 25 weeks' gestation she reported loss of the medication diary. Platelet aggregation was 76% that indicated poor compliance with the study medication. A new diary card was provided, a protocol deviation form was filled, and she was encouraged to comply with study regulations. At 32 weeks' gestation she attended for a sixth study visit, pill count was correct as well as

the diary card. Platelet aggregation was 13%. At 36 weeks' gestation she had a last study visit when she was asked to stop taking the study medication. Pills were not available for counting, but her diary card showed discrepancy in pill intake by 12 pills. Platelet aggregation supported the suspicion of non-compliance with aggregation of 69%, see Figure 3.9. The patient was deemed generally non-compliant based on the study criteria for non-compliance. At $38^{+2}$ weeks' gestation, the patient went into labour and was still taking aspirin despite being told to stop taking it at 36 weeks. She had an uncomplicated vaginal delivery of a live born male infant weighing 3.4 kgs. A protocol deviation form was filled for the incident and categorised under intentional product misuse.

Patient 4: Aspirin group

A 34-year-old primiparous woman, who had T1 DM. She had a preconception counselling session and had an optimal glycaemic control. Along with diabetes she had hypothyroidism and she was taking levothyroxine 50 microgram daily. She was recruited to the study at $10^{+3}$ weeks and was randomised to the aspirin group. Baseline bloods were taken at that visit including platelet aggregation, which was recorded at 75%. During her enrollment in the study she had 7 further visits at 14, 18, 22, 25, 29, 33 and 36 weeks' gestation, during which the platelet aggregation levels were 15, 6, 3, 7 and 10%, respectively. This is demonstrated in Figure 3.10. On her seventh visit her platelet aggregation was 69% despite the fact that she reported taking aspirin on a daily basis. Pill counts showed 2 pills missed but the diary card did not. On her last visit at $36^{+3}$ weeks, aspirin was stopped 3 days prior to her visit. A blood sample was taken but was not sufficient for analysis. On the basis of objective testing by way of platelet aggregation evaluation, diary card and only 2 pills missing on pill count, the patient was deemed compliant throughout pregnancy. At $37^{+3}$ weeks, while attending antenatal clinic for a routine visit, blood pressure was noted to be 150/95 and she had proteinuria and facial swelling. She was admitted for investigation of suspected PE and 24 hours urinary protein collection was 500 mg. Labour was induced at $37^{+5}$ weeks and normal vaginal delivery was achieved of a 3.4 kgs male infant and Apgar Scores of 9 and 9 at 1 and 5 minutes of life, respectively. She continued to have an uncomplicated postnatal course and made an uneventful recovery. There were no neonatal complications in the first 4 weeks of life.

Patient 5: Aspirin group

A 30-year-old, African, multiparous patient who had T2 DM for 3 years prior to conception. She did not attend the preconception counselling clinic and was commenced on insulin and folic Acid on week 6 of gestation, when metformin was stopped. She had no prior microvascular complications and did not record PE in previous pregnancies. She was recruited at $10^{+5}$ weeks' gestation and was randomised to the aspirin group. Baseline platelet aggregation was 86%. Her second study visit was at 15 weeks. A diary card and pills were checked, and the patient was deemed compliant with study medication. Platelet aggregation was 5%. On visit 3; at 19 weeks she expressed a desire to withdraw from the study due to difficulty in obtaining blood samples. Her decision was respected, and a withdrawal form was completed.

Patient (6): Non-aspirin group

A 37-year-old Caucasian, who had T1 DM for 21 years. She was $P2^{+1}$ with two previous lower segment cesarean sections and a first trimester miscarriage. She had no significant medical or family history apart from an episode of postnatal hyperthyroidism after the second pregnancy in 2012 that resolved 6 months later. She attended the preconception clinic 3 years prior to conception and had a spontaneous conception. She was recruited at $10^{+2}$ weeks and was randomised to the no-aspirin group. Baseline platelet aggregation was 76%. Her second visit was at 14 weeks; a scan was performed and showed a regular fetal heart activity. Platelet aggregation was 76%. See Figure 3.12. A third study visit was scheduled for 4 weeks later. At 18 weeks she attended for third study visits and she recorded uncomplicated pregnancy course with no evidence of bleeding or abdominal pain. A bedside scan showed regular fetal heart activity, normal liquor volume and a low-lying posterior placenta. Platelet aggregation was 25%. A fourth study visit was scheduled for 4 weeks later and a routine anomaly scan was scheduled for 2 weeks. At $20^{+1}$ weeks, she presented to the emergency room with vaginal bleeding and lower abdominal pain that started a few hours prior to her presentation. A scan showed fetal demise and no amniotic fluid, suggesting the possibility of preterm premature rupture of membranes. Her biochemical and haematological profile were all normal and there was no evidence of early PE. She proceeded to have a vaginal delivery of a 340-gram fetus. She declined postmortem examination. The placenta was sent for histopathological examination and that showed

no evidence of abruption or retroplacental clots. The cause for mid-trimester intrauterine fetal demise was not determined by detailed pathology examination. She made a good recovery in the postnatal period. This event was considered as a SAE and was reported to the DSMB as per study protocol.

Patient 7: Non-aspirin

A 32-year-old Asian woman had been diagnosed with T2 DM two years prior to conception. She was primiparous and attended the preconception clinic before embarking on this pregnancy. She was on metformin 1000 mg twice a day and folic Acid 5 mg once daily. She had a liver adenoma resected at the age of 22 years and she was a smoker of 10 cigarettes a day for the last 10 years. At $11^{+4}$ weeks she was recruited and randomised to the non-aspirin group. Baseline platelet aggregation was 12% despite the fact that there was no suggestion in her drug history of intake of antiplatelet therapy. In her other six visits over the study period, the platelet aggregation results were all above the 20% cut off, reflecting non-suppression of platelet aggregation, see Figure 3.13. At 23 weeks' gestation, she was admitted for blood glucose stabilisation and introduction of Insulin. She had 2 SAEs related to insulin (Novarapid and Humalin S) allergic reaction in the form of intense skin itch and facial swelling. This reaction required hospitalisation for 4 days. Neither event was related to the investigational medicinal product. Labour was induced at $38^{+6}$ weeks for diabetes and a vacuum delivery of a male infant ensued, weighing 3.5 kgs with Apgar Scores of 9 and 10 and at 1 and 5 minutes of life. The patient had no postnatal complications and made a good recovery. There was no neonatal hospital admission in the first month of life recorded.

Patient 8: Non-aspirin group

This participant was a white Irish primiparous woman with T2 DM. She was morbidly obese in the pre-pregnancy period before she had a gastric band 18 months prior to conception, which resulted in dramatic loss of weight of approximately 11 stone. Her BMI at booking was 30kg/$m^2$ and she did not attend the preconception clinic. She had hypercholesterolemia treated with low fat diet and essential hypertension on Amlodipine 2.5 mgs. She attended the diabetes service in the Rotunda Hospital at 6 weeks' gestation where amlodipine was stopped, and folic Acid 5 mgs was commenced. A

recruitment appointment was scheduled for $10^{+5}$ weeks' gestation at which a viable singleton pregnancy was confirmed, and she was randomised to the aspirin group. A platelet assay testing in the first visit of the study that was before the commencement of aspirin was 0% despite the fact the patient declined being on antiplatelet therapy. Second study visit was at 15 weeks' gestation and at that stage patient had uneventful pregnancy. A diary card was checked along with aspirin tablet count that deemed the patient compliant. Venous blood was drawn from patient for platelet assay testing along with routine study bloods, however, the sample was not sufficient to be processed and a plan was to wait for the third study visit to obtain bloods. A protocol deviation form was filled, and patient was scheduled for a third visit in 4 weeks' time. A third and a fourth visits were scheduled at 19, and $22^{+6}$ weeks respectively and patient was compliant with aspirin intake by diary card, pill count and platelet aggregation testing were 0, 11% respectively, see Figure 3.14. She had no admissions to hospital and blood pressure was within normal range (112-122/60-72). Since then, the patient had multiple presentations and admissions for various reasons. At 17 weeks' gestation she reported left calf pain radiating to the groin. She was admitted and commenced on therapeutic low molecular weight Heparin (LMWH), Tinzaparin. A left lower limb Doppler scan was negative for deep vein thrombosis (DVT) and she was discharged home the following day. At $27^{+2}$ weeks gestation the patient presented to the emergency department with reduced fetal movement for 2 days. She was admitted for observation and a departmental scan showed normal fetal growth, liquor volume and Umbilical artery Doppler. She was discharged home the following day. Soon after, she had another presentation with abdominal pain radiating to the groin area and she was admitted to the antenatal ward for 24 hours for observation and was discharged home. At $31^{+1}$ weeks she presented to emergency department with vaginal spotting and reduced fetal movement. Bleeding was very minimal and fetal movements were appreciated as soon as the patient was admitted to the hospital. Fetal scan and cardiotocograph (CTG) monitoring were all normal and patient was discharged home. Two weeks later she presented again to the emergency department with reduced fetal movement that improved after admission to hospital. She had another episode of vaginal bleeding that settled spontaneously at $33^{+5}$ weeks' gestation. She was admitted for observation for 24 hours and was sent home thereafter. At $34^{+5}$ she was admitted through diabetic clinic with one episode of mildly raised blood pressure (145/98 mmHg) without proteinuria. She was admitted for blood pressure monitoring, 24 hours urinary protein collection and

other biochemical work up. Blood pressure settled without antihypertensive treatment and 24 hours protein collection was within normal range, with no laboratory features consistent with PE. The patient was sent home after being in the hospital for 2 days and seen in the day care unit for blood pressure monitoring. The last SAE was completed for this participant when the patient presented to emergency department with vomiting and diarrhea. A diagnosis of viral gastroenteritis was established, and the patient was admitted to an isolated room for intravenous fluid hydration and was sent home the following day.

A 5$^{th}$, 6$^{th}$ and 7$^{th}$ visits were scheduled at 27$^{+5}$, 32$^{+5}$ and 36$^{+5}$, during which patient was still compliant with aspirin intake judged by the diary card and the pill count as well as platelet aggregation (4,6 and 8%, respectively). She was diagnosed with mild pregnancy induced hypertension at 35 weeks' gestation. Of note, aspirin was stopped 5 days prior to bloods withdrawal in visit 7, which explains the low aggregation rate. A further blood was taken randomly at 38 weeks that showed platelet aggregation of 64%. The patient was induced at 39$^{+2}$ for reduced fetal movements and had an operative vaginal delivery (vacuum) of a live female infant weighing 3.3 kgs with Apgar Scores of 9 and 10 in the first and 5$^{th}$ minutes of life, respectively. She did not have confirmed PE and had an uneventful postnatal course. There was no admission to the neonatal unit.

Patient 9: Non-aspirin

This is a 38-year-old, white Irish multiparous woman who had T1 DM for over 21 years and was on an Insulin pump. She had hypothyroidism and was on levothyroxine 150 mcg once daily in addition to regular vitamin B12 injections for treating pernicious anaemia. She attended the preconception-counselling clinic 3 years prior to conception. She had no microvascular complications of diabetes. A recruitment visit was initiated at 9$^{+4}$ weeks' gestation and she was randomised to the no-aspirin group. She had regular study visits on a four- weekly basis. Figure 3.15 demonstrates the results of platelet aggregation per visit. Platelet aggregation levels were above the threshold of 20%, suggesting non-usage of anti-platelet therapy, with exception of visit 3, as there was an unexplained decline in platelet aggregation to below 20%. She had no serious adverse events, hospital admissions or PE features during the study period. At 38$^{+1}$ weeks, she had an elective caesarean section, as she was deemed unsuitable for induction of

labour. A live male infant weighing 3.5 kgs was delivered. There were no postnatal complications and the baby did not need admission to the neonatal unit.

Patient (10): Aspirin group

This patient had Maturity Onset Diabetes of Youth (MODY), diagnosed one year prior to conception and was poorly controlled on insulin with a BMI of 30.3 kg/m$^2$. She had an unplanned pregnancy and attended the diabetes service in the Rotunda Hospital at 6 weeks' gestation. At that stage she was diagnosed with hypothyroidism and commenced on thyroxine 25 mcg and folic Acid 5 mgs once daily. She was recruited at $9^{+6}$ weeks' gestation and was randomised to the aspirin group. On her second visit she reported missing one pill of the study medication, which was consistent with her diary card. This incident was repeated on the third visit, when the diary card and pill count confirmed two pills missed. Platelet aggregation profiles for the first, second and third visits were below the 20% threshold. Figure 3.16 highlights platelet aggregation results over the study period. The patient was deemed compliant with study protocol throughout the study as no report of missed pill in the next three study visits. Aspirin was stopped at $36^{+0}$ weeks as per study protocol and her last and eighth visit was at $37^{+4}$ weeks' gestation. A blood sample was obtained but was not sufficient for analysis. There were no reports of antenatal admissions to hospital, PE or SAEs prior to delivery. Labour was induced in accordance with the hospital protocol for managing women with PGDM at $39^{+5}$ weeks' gestation and had a vaginal delivery of a live fetus weighing 3.6 kgs and Apgar Scores of 8 and 9 at first and fifth minute of life. She had a primary postpartum haemorrhage secondary to uterine atony and the resultant haemorrhage was quantified at 700mls., This was managed medically by giving an infusion of 40 IU oxytocin and misoprostol 1 gram rectally. The patient made a good recovery and did not require additional intervention. Haemoglobin concentration was 9.7 g/dl on the second day of the postnatal period.

Patient 11: Non-aspirin group

This patient was 24 years old, primiparous, white Irish woman who had T1 DM for 8 years and had a BMI of 25.2 kg/m$^2$. She had a planned pregnancy and attended our Diabetes service at 6 weeks' gestation. She was recruited and randomised at $9^{+4}$ weeks' gestation to the no-aspirin group. She attended for her subsequent study visits

at 14, 18, 22, 28, 32 and 36 weeks. Platelet aggregation results are shown in Figure 3.17 and they were all above the 20% cut-off. The pregnancy was uneventful apart from the fact that it was a macrosomic fetus with an abdominal circumference above the 95$^{th}$ centile from 32 weeks' gestation with normal liquor volume. It was challenging to stabilise her blood glucose levels especially towards the end of the pregnancy. For that reason, she was induced at 37$^{+6}$ weeks' gestation and she had a category 3 caesarean section, which indicates that there was no immediate threat to life of mother and fetus, but early delivery was required for failure to progress in the first stage of labour. A live female infant was delivered weighing 4.6 kgs who did not require admission to the neonatal unit. She had a primary PPH due to uterine atony with EBL 1100 ml. She did not require blood transfusion despite the fact that her haemoglobin dropped from 11.6 g/dL to 7.2 g/dL.

Patient 12: Aspirin group

This patient was a 35 years old Asian, multiparous woman with T2 DM who was diagnosed two years prior to conception and was on metformin 1000 mg twice a day. She was diagnosed with Empty Sella Syndrome and hypopituitarism in 2010 and was started on cabergoline for hyperprolactinaemia which was stopped in August 2015 a year before conception. She was also on thyroxine replacement therapy for hypothyroidism and was closely monitored in the diabetic service in the Mater hospital. The index pregnancy was unplanned, and she was commenced on folic acid and insulin at 6 weeks' gestation and metformin was stopped. She was recruited and randomised at 8$^{+4}$ weeks' gestation to the aspirin group. During the course of the pregnancy she reported missing 3 pills over the second, third, fourth and fifth visits in total and that was confirmed with pill diary and pill counting. Platelet aggregation is shown in Figure 3.18. On her sixth visit at 26$^{+3}$ weeks' gestation, platelet aggregation was 69% despite the fact that the patient was reported by pill count and diary to be compliant with taking aspirin during that period. Platelet aggregation dropped to below 20% cut-off on the seventh visit. Aspirin was stopped at 36 weeks' gestation and she was induced at 38$^{+2}$ weeks and had a vaginal delivery of a live male infant, weighing 3.5 kgs and Apgar Scores of 8 and 9 at first and 5$^{th}$ minutes of life, respectively. Mother and baby made an uneventful postnatal recovery. She had one SAE when she was admitted at 36$^{+4}$ weeks' gestation with possible upper respiratory tract infection. She was started on intravenous

co-amoxiclav and was kept under observation for 24 hours and all throat swabs came back negative so, she was discharged home the following day.

Patient 13: Non-aspirin group

This was a primiparous white Irish woman who was 35 years of age and who had T1 DM for nearly two decades. She was on an insulin pump and she had no known microvascular complications secondary to diabetes. She attended a preconception clinic in 2012 and was started on folic acid 5 mgs once daily since. She was recruited at $8^{+2}$ weeks' gestation and was randomised to the non-aspirin group. During the study period, she attended 4-weekly visits and the pregnancy followed an uneventful course. Platelet aggregation was tested at each visit and results are shown in Figure 3.19. At $37^{+5}$ weeks' gestation, she presented to the emergency department with per vaginal watery leak and prelabour rupture of membranes was confirmed. She had category (3) caesarean section, as she was not favorable for induction of labour. A live female fetus weighing 3.2 kgs was born with Apgar Scores of 9 and 10 at 1 and 5 minutes of life, respectively. She had a primary PPH due to uterine atony and the EBL was 1000 ml. She was managed medically and did not require blood transfusion.

Patient 14: Non-aspirin group

This was a 32-year-old white Irish, multiparous woman with T2 DM. She had no other medical illnesses and was recruited at $11^{+1}$ weeks' gestation and was randomised to the non-aspirin group. During the study period, she had 4 weekly visits and her pregnancy followed an uneventful course. However, a growth scan at 32 weeks' gestation showed a fetus measuring above the 95$^{th}$ centile for abdominal and head circumferences. Amniotic fluid volume was within the normal range. Platelet aggregation analysis was taken at each study visit and the results are shown in Figure 3.20. She was scheduled for an elective caesarean section at 39 weeks' gestation as she had a previous one. A live born male baby was delivered weighing 4.5 kgs and Apgar Scores of 8 and 9 at first and fifth minutes of life, respectively, who did not need admission to the neonatal unit. This participant's postnatal period was uneventful.

Patient (15): Aspirin group

This patient was an 18-year-old European primiparous woman, with T1 DM for 6 years. She had no other medical illnesses and she was not on any medications except for insulin injections. She had an unplanned pregnancy and was commenced on folic acid 5 mgs once daily at 7 weeks' gestation when she attended the diabetic service in the Rotunda Hospital. She was recruited to the study and randomised to the aspirin group. Platelet aggregation at recruitment/ first visit before commencement of aspirin was 78%. In the next 6 visits and despite the patient self-reporting full compliance with medication intake and the confirmation of that by counting the pills and diary card, platelet aggregation failed to drop below the target level of < 20%. Figure 3.21 shows the platelet aggregation pattern throughout the study period. The patient was considered to be a non-responder rather than non-compliant in this situation. Unfortunately, a sample of blood was not obtained on visits 6 and 7 to compare platelet aggregation when aspirin was stopped at 36 weeks' gestation.

She presented to the emergency department at $36^{+5}$ weeks' gestation feeling generally unwell with a high temperature and right flank pain. Biochemical markers showed c-reactive protein (CRP) of 86 mg/L and white blood cells (WBC) of 16,200/µl, but normal electrolytes and creatinine. She was commenced on co-amoxiclav and gentamicin intravenously as per hospital guideline for pyrexia and sepsis in pregnancy. The patient's clinical condition improved over the next 48 hours and urine culture was negative for bacteria. Although the source of infection was not established, the patient went home on oral cephelexin, a first-generation cephalosporin. At $37^{+5}$ weeks she attended the diabetes clinic for a routine antenatal appointment and her blood pressure was 150/99 mmHg with evidence of proteinuria. She was admitted and a PE diagnosis was established on the basis of high blood pressure that required labetalol 200 mgs twice a day and significant proteinuria (> 300mg/d). Induction of labour was commenced and she was delivered by an emergency LSCS for evidence of fetal distress on CTG. A live born female fetus was delivered weighing 3.8 kgs, Apgar Scores of 8 and 10 at 1 and 5 minutes of life, respectively and cord pH of 7.3 (arterial) and 7.28 (venous). There was no admission to the neonatal unit and mother had an uneventful antenatal course.

Patient (16): Aspirin group

This study participant was a 25-year-old, Asian, multiparous woman who was diagnosed with T2 DM after the delivery of her last child 2 years earlier. She had an

unplanned pregnancy and attended our diabetic services at $6^{+4}$ weeks' gestation when she was commenced on insulin and folic acid 5 mgs once daily and metformin was stopped. She was recruited at $11^{+6}$ weeks' gestation and was randomised to the aspirin group. She had 6 further study visits and she was deemed compliant in taking aspirin as prescribed, judged by diary card and platelet aggregation. Aspirin was stopped at 36 weeks' gestation and a caesarean section was scheduled for 39 weeks as the patient had 2 previous caesarean sections. A live male infant was born weighing 3.6 kgs, with an uneventful neonatal course. She had a primary PPH in the operating theatre and recovery room due to uterine atony that was managed medically. EBL was1400 ml. SAE form was filled and submitted.

Patient (17): Non-aspirin group

This patient was a 30-year-old, primiparous Irish woman who had T1 DM for over 12 years and stage one diabetic retinopathy in both eyes. She attended the preconception clinic after she had laser surgery for both eyes and was commenced on folic acid 5 mgs once daily. She conceived spontaneously and was recruited at $11^{+3}$ weeks' gestation and randomised to the no-aspirin group. Her platelet aggregation results throughout the study period are shown in Figure 3.23. She had an uneventful antenatal course until 34 weeks' gestation when a routine fundoscopy showed evidence of deteriorating diabetic retinopathy and vitreous haemorrhage. After discussion with the consultant ophthalmologist, the decision was made to expedite delivery by elective caesarean section at $36^{+2}$ weeks' gestation. She delivered a live infant weighing 3.3 kgs with uneventful postnatal course for the mother and baby.

Patient (18): Non-aspirin group

This was a 41-year-old white Irish multiparous woman, who had T2 DM for 6 years. She conceived through in vitro fertilisation and was previously on folic acid and had satisfactory glycemic control. She attended the diabetic service at 6 weeks and was recruited at $8^{+3}$ weeks and randomised to the no-aspirin group. Baseline platelet aggregation was 10% despite the fact that she denied using antiplatelet therapy. The only declared medications in the first trimester were oral folic Acid and insulin. Figure 3.24 shows platelet aggregation over the next six visits and through the study period as they were all above the 20% threshold. The patient had an uneventful pregnancy and

was induced at $38^{+6}$ weeks' gestation as per protocol for managing patients with PGDM. However, she had an emergency caesarean section secondary to fetal distress evident on CTG. A live infant weighing 2.8 kgs was delivered with Apgar Scores of 9 and 10 at 1 and 5 minutes of life, respectively. No neonatal admission over the first month of the neonatal period was required. She developed a primary PPH due to uterine atony that was managed medically. The EBL was 1000 ml. Haemoglobin post event was 10.8 g/L and she did not require blood transfusion.

Patient (19): Aspirin

This participant was a 36 years old multiparous, Asian woman with T2 DM for 3 years prior to conception. She was on metformin 850 mgs three times a day. She also had essential hypertension for 18 months and was on labetalol prior to conception, continued through pregnancy at a dose of 200 mg twice a day. She had a background diabetic retinopathy of the left eye that was surgically corrected prior to conception. She attended the preconception clinic and was on folic acid 5 mgs once daily for 6 months before pregnancy. At $10^{+6}$ weeks' gestation, she was recruited and randomised to the aspirin group. Baseline platelet aggregation was 62% at the first visit. She had 6 more study visits over the course of the pregnancy accompanied by platelet aggregation assessment except for the last and seventh visit at $36^{+6}$ weeks' gestation. Figure 3.25 shows the results of platelet aggregation per visit. She was compliant in taking aspirin all through pregnancy and she only missed 2 pills in total that was evident on pill count, diary and platelet aggregation results. She had two fundoscopic assessments at 24 and 32 weeks. The latter showed background retinopathy in the right eye. A planned caesarean section was performed at $39^{+2}$ weeks' gestation as she had undergone caesarean delivery previously. A live born male infant was delivered weighing 3.3 kgs, who did not need admission to the neonatal unit. The patient had a primary PPH due to uterine atony that was managed medically. An EBL of 1400 ml was reported. She did not require blood transfusion. Aspirin was stopped 23 days prior to delivery.

Patient 20: Aspirin group

This participant was a white American patient who was 28 years of age, primiparous and who had T1 DM for 16 years. She was on an insulin pump and she achieved good glycemic control prior to conception. She had no microvascular complications of

diabetes. She was recruited and randomised at $8^{+3}$ weeks' gestation to the aspirin group. She had regular study visits on a 4- weekly basis and it was noticed that she missed a small number of pills on visits 4, 5, 6 and 7. She missed a total of 16 pills and that was confirmed on pill count and diary card. Platelet aggregation on visits 4 and 6 were 70% and 69%, respectively, which was above the cut-off for patients on aspirin and that confirmed non-compliance, see Figure 3.26, The blood sample obtained on visit 5 was not sufficient to be analysed. She was deemed non-compliant by study criteria. At $35^{+3}$ weeks' gestation, she attended the emergency department complaining of vomiting and diarrhea and was diagnosed with viral gastroenteritis. She was admitted to an isolation unit and had supportive therapy and fluid replacement. Initially she had ketosis secondary to dehydration, but all blood work up including full blood count, serum electrolytes and arterial blood gases were normal, and that excluded diabetic ketoacidosis. She was kept under observation for 24 hours and was discharged home; The patient was admitted with gastroenteritis and diabetic ketoacidosis. Aspirin was stopped at 36 weeks and she was induced at 39 weeks and had vacuum delivery of a live born female baby weighing 3.5 kgs. She had a primary PPH from uterine atony in the delivery suite and was managed with 10 IU oxytocin, intramuscular injection followed by 40IU oxytocin infusion. Bleeding settled and EBL was 900 ml in total. Haemoglobin dropped from 11.2 before delivery to 7.4 g/L, but she did not require blood transfusion.

Patient 21: Aspirin group

This patient was a 41-year-old, black African multiparous woman, who had T2 DM for 4 years. She had an unplanned pregnancy and attended the diabetic service in the Rotunda Hospital at 7 weeks' gestation and was recruited and randomised at $8^{+1}$ to the aspirin group.

Platelet aggregation for the period of the study and through 8 visits is shown in Figure 3.27. She was compliant with study medication and she only 3 missed tablets in total; that was on visit 5 of the study. She had an uneventful antenatal course until $29^{+5}$ weeks' gestation when she was admitted via the diabetes clinic after a routine visit as she was complaining of cough and shortness of breath for one week that was managed with oral co-amoxicilav (500 mgs/125 mgs) by her local general practitioner. A chest examination was negative, and she was admitted for observation and some preliminary

work up including throat swab, chest x-ray, full blood count and arterial blood gases which came back all within normal range. The patient was discharged home. Aspirin was stopped at $36^{+0}$ weeks. She went on to a spontaneous onset of labour at $37^{+6}$ weeks and delivered a live male infant weighing 4.1 kgs who did not need admission to the neonatal unit. The patient developed a mild PPH of 600 ml due to uterine atony that was managed with 40 IU oxytocin and 1 gm misoprostol rectally. Hb post-delivery was 10.0 g/dL and she did not require blood transfusion.

Patient 22: Aspirin group

This was a 36-year-old white Irish woman who had T1 DM for 20 years. She attended the preconception clinic 2 years prior to conception. She had a background history of retinopathy in both eyes secondary to long-standing diabetes. She was recruited and randomised to the aspirin group at $8^{+0}$ weeks' gestation. Platelet aggregation is demonstrated in Figure 3.28. She was fully compliant with study medication and that was judged by diary card, pill count and platelet aggregation results. She had an uneventful antenatal course until 30 weeks' gestation when the fetal abdominal circumference was measuring above the 95$^{th}$ centile for gestational age.

At $33^{+6}$ weeks gestation she presented to the emergency department with generalised itch. Bile Acid level was 35 µmol/L and liver enzymes were deranged. A diagnosis of Obstetric Cholestasis was made and patient was started on ursodeoxycholic acid (ursofalk) and vitamin K. Aspirin was stopped in anticipation of preterm delivery. She had a spontaneous vaginal delivery at $35^{+0}$ weeks of a live male baby weighing 3.7 kgs. He was admitted to the neonatal unit with transient tachypnoea of the newborn and was discharged to the postnatal ward on day 3 of life. Mother and baby made a good recovery. The patient had a blood test 6 weeks later to monitor liver enzymes, which had normalised at that stage.

Patient 23: Non-aspirin

This patient was 29 years old Asian, primiparous woman with T2 DM for 16 months prior to conception. She was recruited and randomised at $10^{+2}$ weeks to the no-aspirin group. She had an uneventful course of pregnancy until $29^{+3}$ weeks' gestation when a routine scan showed asymmetric growth restriction with normal liquor volume and

Umbilical artery Doppler. A plan was made to perform serial ultrasound-based fetal surveillance every 2 weeks. Platelet aggregation over the study period is illustrated in Figure 3.29.

Delivery was planned by induction of labour at $37^{+5}$ weeks. Vacuum delivery of a live-born male infant weighing 2.3 kgs with Apgar Scores of 8 and 9 at 1 and 5 minutes of life, respectively. The baby was admitted to the neonatal unit for observation over 48 hours and was then discharged to the postnatal ward. Both mother and baby had an uneventful postnatal course.

Patient 24: Aspirin group

This was the only patient who was recruited from the Coombe Women's and Infants' University Hospital. She was a 36-year-old white Irish multiparous woman who had T1 DM. She had no known microvascular complications from diabetes. She was recruited and randomised at $7^{+4}$ weeks to the aspirin group. Baseline platelet aggregation was taken in the first visit before the commencement of aspirin, but the sample was late arriving to the laboratory for processing. At $9^{+5}$ weeks' gestation she was admitted to hospital with the upper respiratory tract infection, and throat swabs confirmed Influenza B infection. Serial platelet aggregation testing over the study period showed an aggregation pattern that remained below 20%.

The patient was compliant with the study medication throughout the study period and that was confirmed with the diary card, pill count and platelet aggregation testing. See Figure 3.30. A growth scan performed at 32 weeks confirmed a large for gestational age fetus with all measurements above the $95^{th}$ centile, but normal liquor volume. Aspirin was stopped at 36 weeks' gestation and elective caesarean section was planned at $39^{+1}$ weeks for previous caesarean section. A live born male infant was delivered weighing 4.7 kgs with Apgar Scores of 9 and 10 at 1 and 5 minutes of life, respectively. Both mother and fetus made a satisfactory postnatal recovery.

### 3.3 Serious adverse events

There was a total of 29 SAEs between both groups (14 in the aspirin and 15 in the non-aspirin group). There were 2 cases of PE in the aspirin group; those cases developed after cessation of aspirin treatment at 36 weeks' gestation. There was one case of

pregnancy induced hypertension in the non-aspirin group that developed at late gestation. There were 3 cases of PPH in the non-aspirin group compared to 5 cases in the aspirin group. All the cases in the aspirin group delivered at least 3 weeks after cessation of aspirin. Platelet aggregation was not tested after aspirin was stopped, as this was not part of the study protocol. Pregnancy related SAEs included one case of obstetric cholestasis in the aspirin group and a case of late fetal demise at 20 weeks' gestation, a case of IUGR, a case of suspected DVT, two cases of reduced fetal movements and two cases of vaginal bleeding, all in the non-aspirin group. Only one case of diabetes related SAEs (vitreous haemorrhage) was identified in the non-aspirin group and no cases were seen in the aspirin group in relation to this particular subcategory. The SAEs in the others subcategory included 5 cases of infection-related conditions such upper respiratory tract infection (3 cases), viral gastroenteritis (one case) and possible pyelonephritis (one case). All these cases were in the aspirin group. The cases in the others subcategory in the non-aspirin group were two cases of allergy to insulin and a case of gastroenteritis. Figure 3.31 demonstrates the frequency of SAEs between both study groups.

## 3.4 Delivery and neonatal outcomes

The median gestational age of delivery was 38 weeks +/- 1 day in both groups. There were fewer spontaneous vaginal births in the non-aspirin group compared to the aspirin group (11% vs 50%).There were two admissions to neonatal units, one in each group, and they were for a 24 hour period for possible transient tachypnoea of newborn in one case and low birthweight in the other. Table 3.4 demonstrates the delivery outcomes between both study groups which will be discussed in detail in the Discussion chapter.

### *3.4.1 Results of placental pathology*

Ten placentae had a histopathological examination by a pathologist specialised in perinatal pathology; 5 were in the aspirin group including the patient who had PE at term, and 5 in the non-aspirin group including the patient who had a late fetal demise at 20 weeks' gestation.

On microscopic examination using haematoxylin and eosin stain; the placental tissue of 4 out of 9 patients had DVM. They were equally distributed between aspirin and non-

aspirin group. The remaining 5 placentae showed normal histological features in the form of normal placental villi with central connective tissues core covered by trophoblastic cell layers. The placenta of the patient who had a late fetal demise was excluded, as DVM is a feature of the third trimester.

The placenta of the case of term PE was in the aspirin group and the histological findings were consistent with uteroplacental deficiency in the form of thickened blood vessel walls and narrow lumen, hydropic changes in chorionic villi and fibrinoid necrosis despite the use of aspirin.

The case of late fetal demise at 20 weeks was a normal sized placenta with evidence of a small retroplacental clot, but it was likely a post-mortem change.

### 3.5 Acceptability of the study

Figure 3.32 demonstrates the satisfaction of patients who participated in our study. Nineteen patients out of 24 participated in a questionnaire about their overall satisfaction with the study. Sixteen out of the 19 involved were overall very satisfied with the study and felt they gave informed consent to participate in the study after fully discussing it with the researcher and that was appropriately done in a suitable place and time. All participants felt that they had the option to withdraw from the study without having an impact on their clinical care.

With regard to convenience, figure 3.33 shows that 4 out of 19 felt they had to attend multiple appointments as study and regular antenatal appointments were not scheduled simultaneously. Two out of 19 had to take extra time off and 3 out of 19 found withdrawing blood every 4 weeks was inconvenient. In addition, one participant withdrew from the study because of overly onerous blood draws.

Figure 3.34 shows that no patients experienced any level of anxiety regarding the study; however, only one patient reported that she suffered a complication secondary to the usage of Aspirin as she had a postpartum haemorrhage. Fifteen were happy to participate in the definitive study, should one start and they were pregnant again.

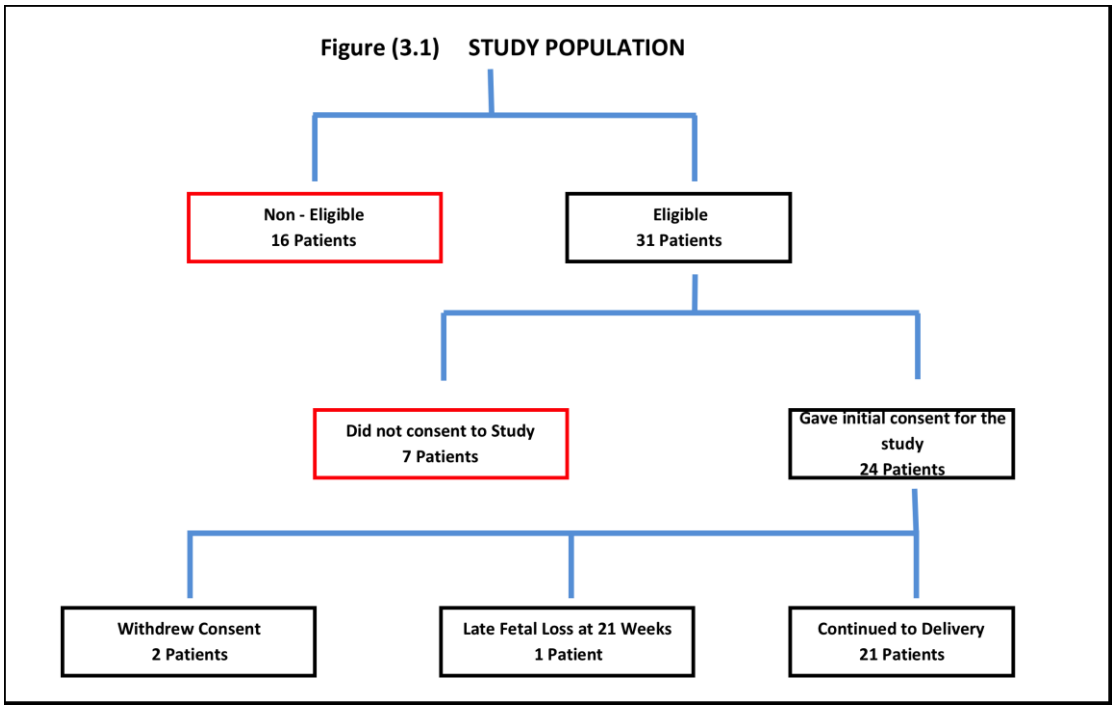



**Figure 3.1. Study population**

This demonstrates the number of subjects contributing to the population for this study.

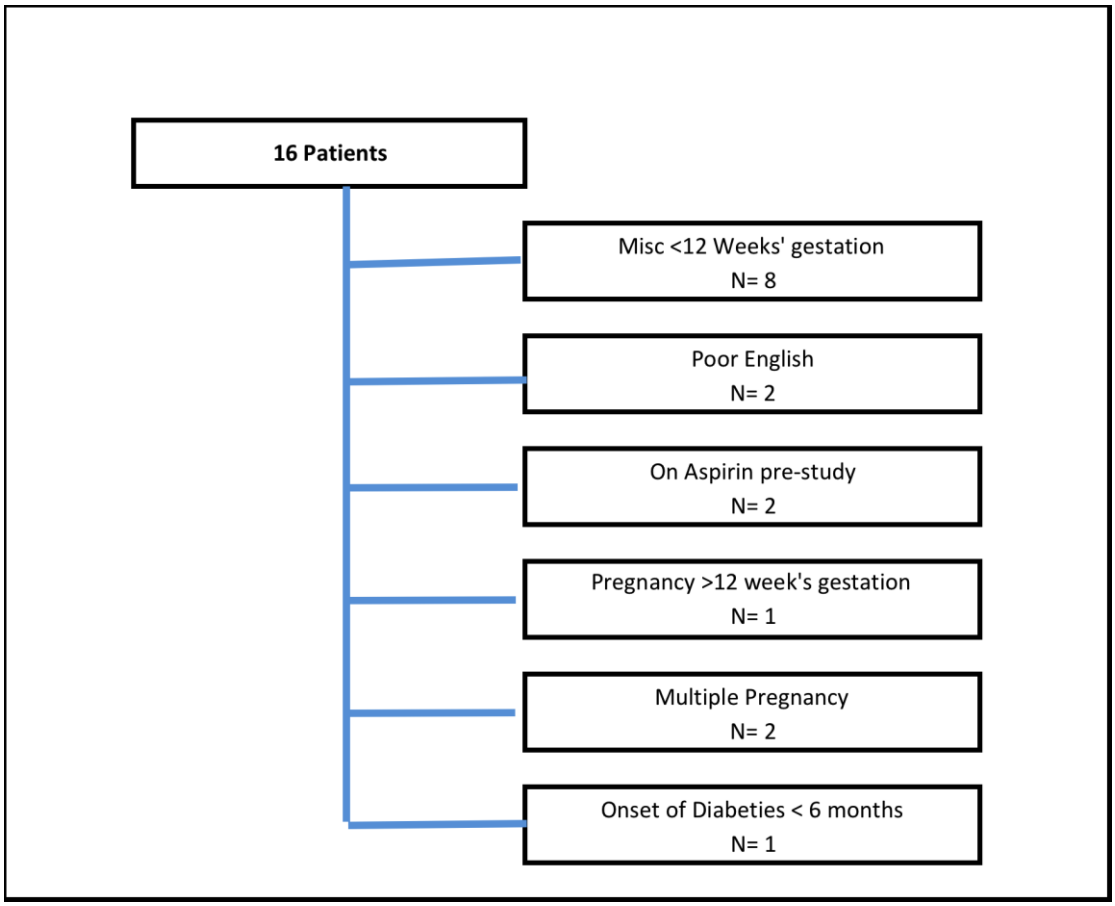



**Figure 3.2. Patients who were ineligible for the study entry**

This figure shows the number of women who were deemed ineligible for entry to the study and shows different subgroups.

| Characteristic | | Aspirin (N=13) | Non-aspirin (N=10) | P-value |
|---|---|---|---|---|
| Diabetes type | Type 1 | 6 (46%) | 5 (50%) | 0.855 |
| | Type 2 | 7 (54%) | 5 (50%) | |
| Ethnicity | African | 2 (15%) | 0 | 0.399 |
| | Asian | 3 (23%) | 2 (20%) | |
| | Caucasian | 8 (80%) | 8 (80%) | |
| Gravida | | 3 ± 2 | 2 ± 1 | 0.411 |
| Nulliparity | | 6 (46%) | 5 (50%) | 0.855 |
| Current smoker | | 1 (7.7%) | 1 (10%) | 0.846 |
| Spontaneous Conception | | 12 (92%) | 7 (70%) | 0.162 |
| GA at Recruitment (weeks) | | 10 +/- 2 | 10 ± 1 | 0.759 |
| BMI ($kg/m^2$) | | 30 ± 6 | 25 ± 3 | 0.088 |
| SBP (mmHg) | | 121 ± 15 | 122 ± 11 | 0.713 |
| DBP (mmHg) | | 69 ± 10 | 69 ± 8 | 0.853 |

GA=Gestational Age
BMI: Body Mass Index
SBP: Systolic Blood Pressure
DBP: Diastolic Blood Pressure

**Table 3.1. Maternal characteristics between the aspirin and non-aspirin groups**

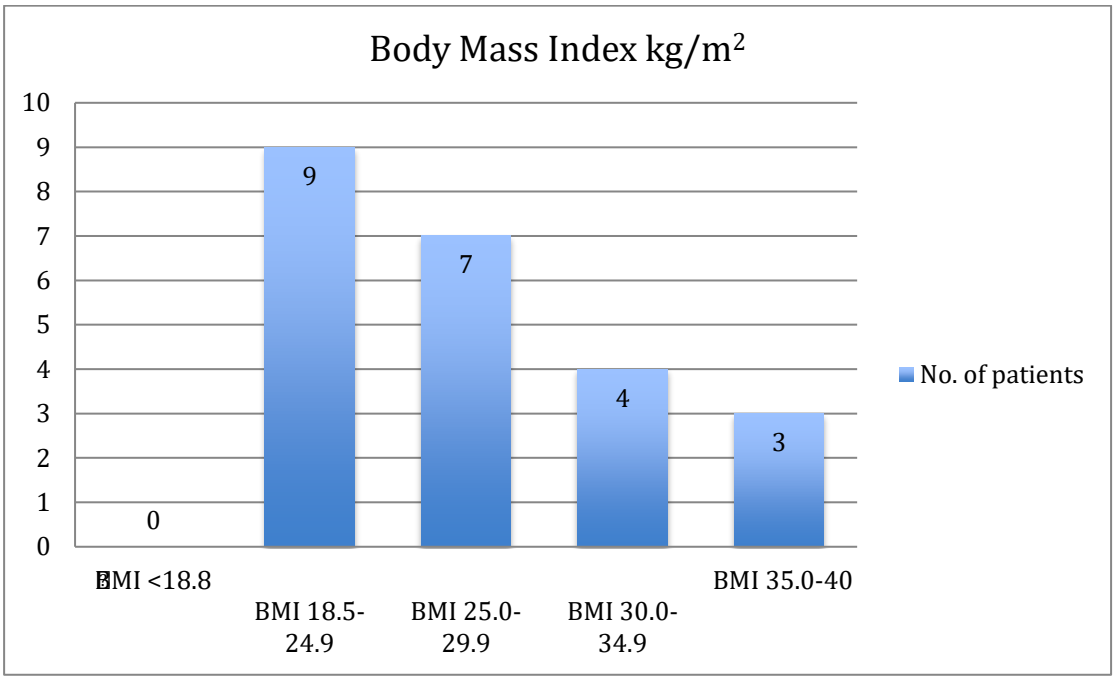



**Figure 3.3. Distribution of body mass index of study cohort**

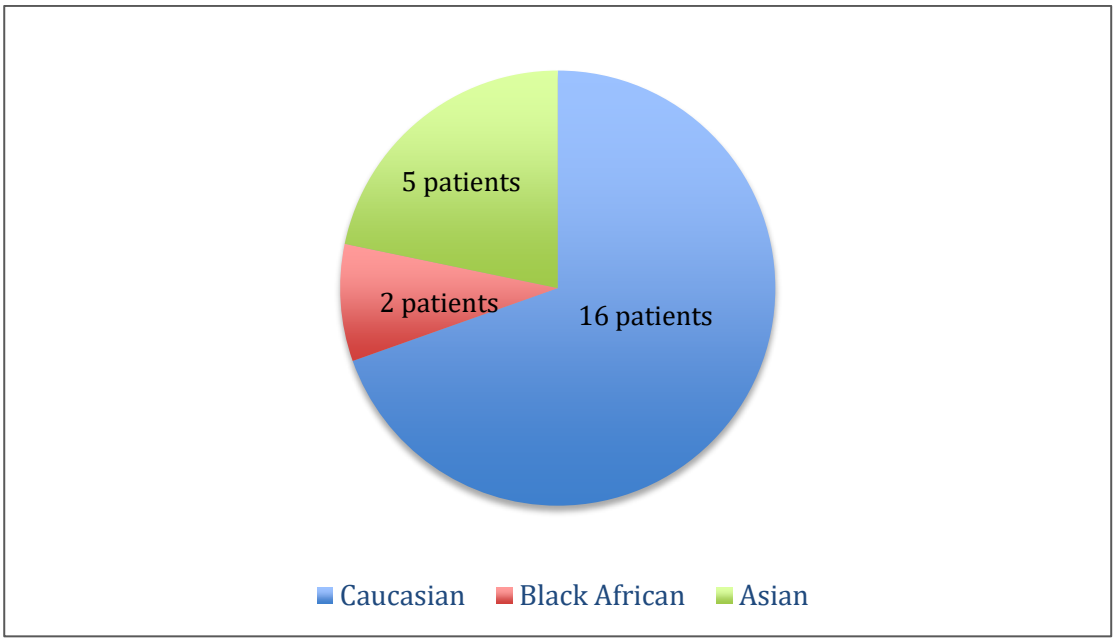



**Figure 3.4. Ethnicity of study cohort**

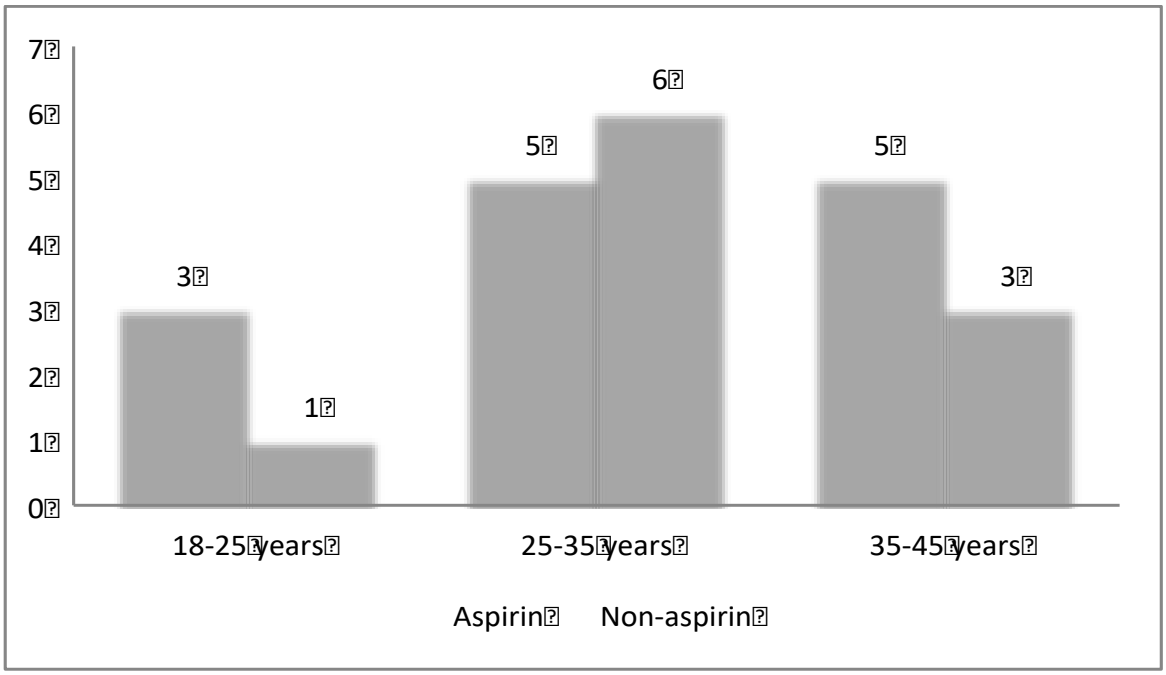
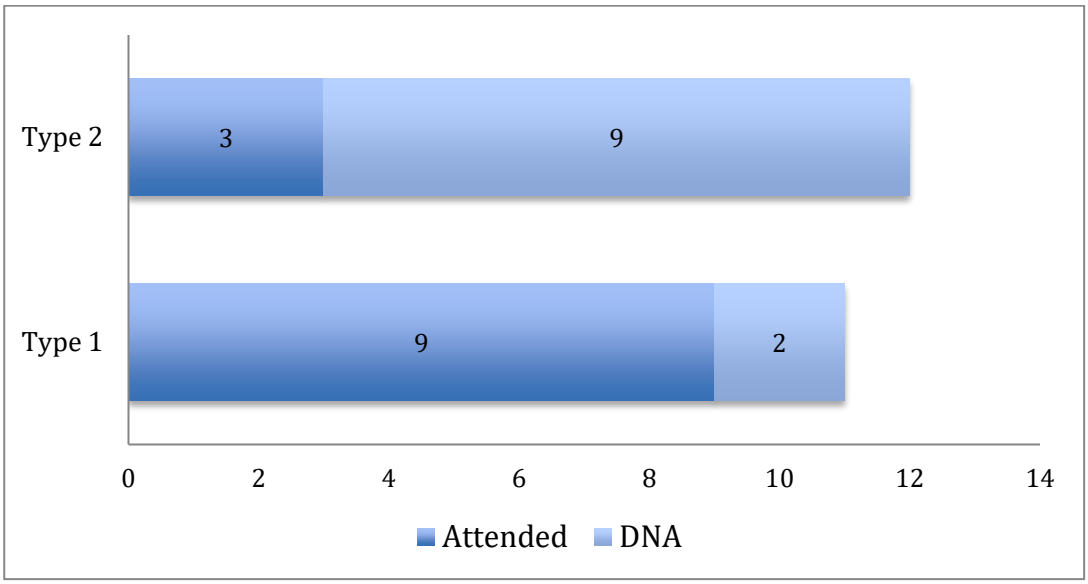



**Figure 3.5. Age of participants in years**




DNA= Did Not Attend

**Figure 3.6. Attendance to preconception clinic**

This figure depicts attendance at the preconception clinic and compares between type 1 DM and type 2 DM groups.

V=Visit

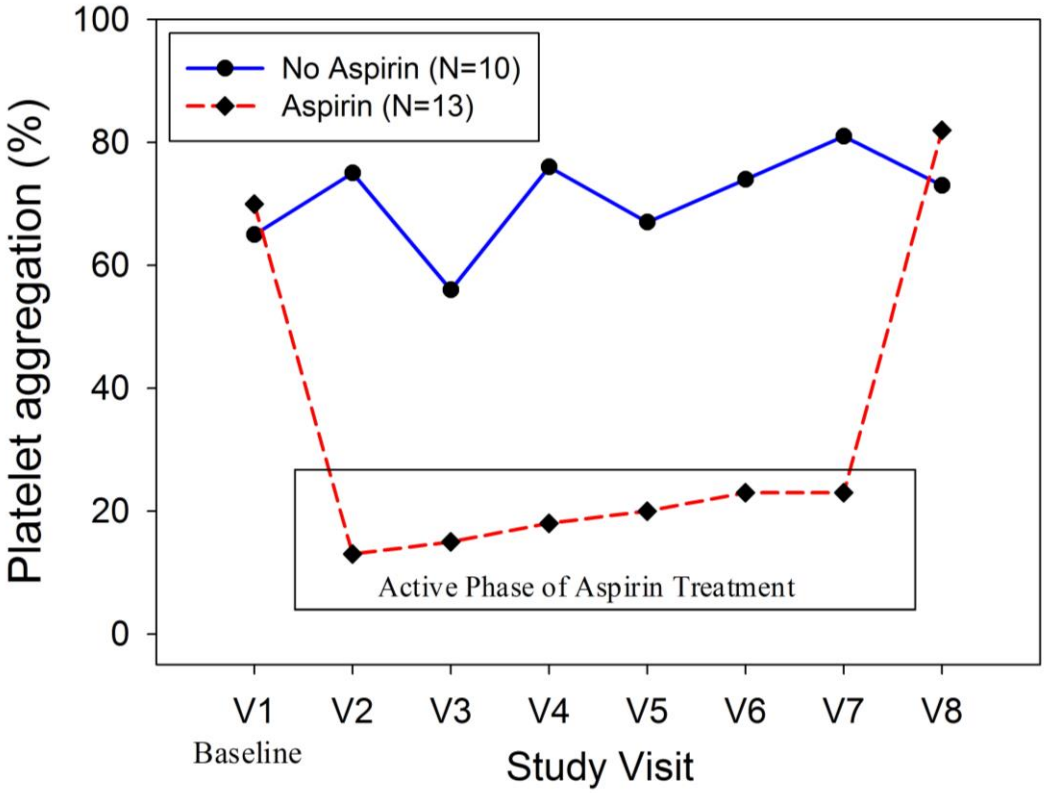
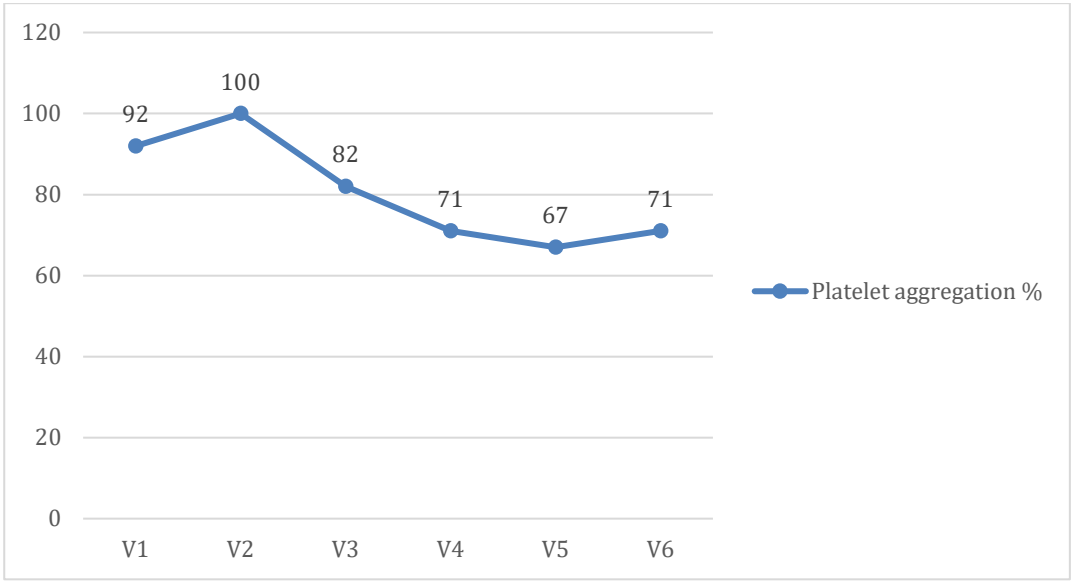


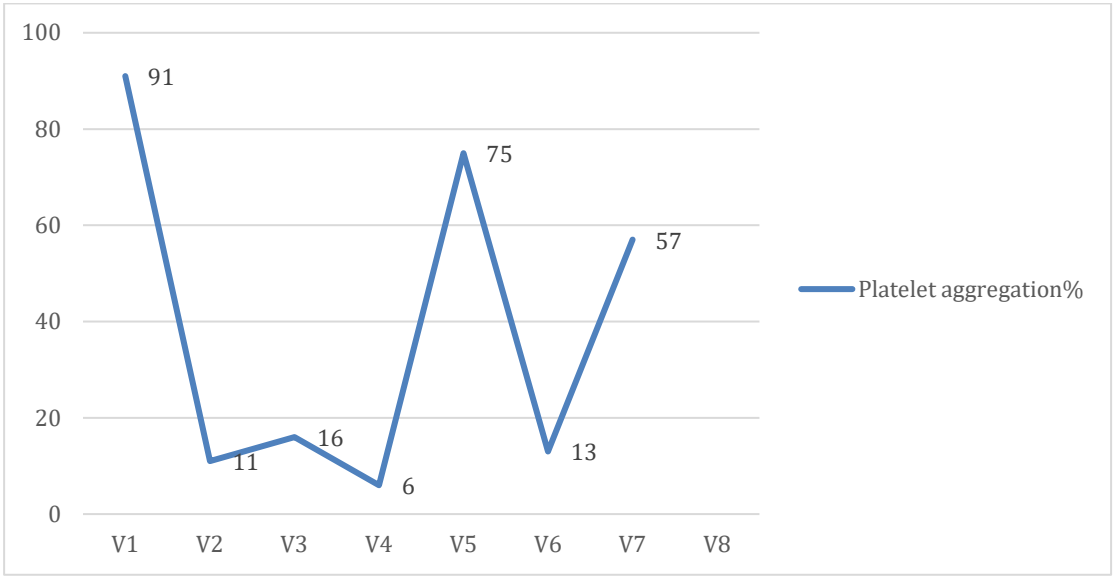

**Figure 3.7. Mean aggregation by treatment and visit**




V=Visit

**Figure 3.8. Platelet aggregation per visit (patient 2), non-aspirin group**

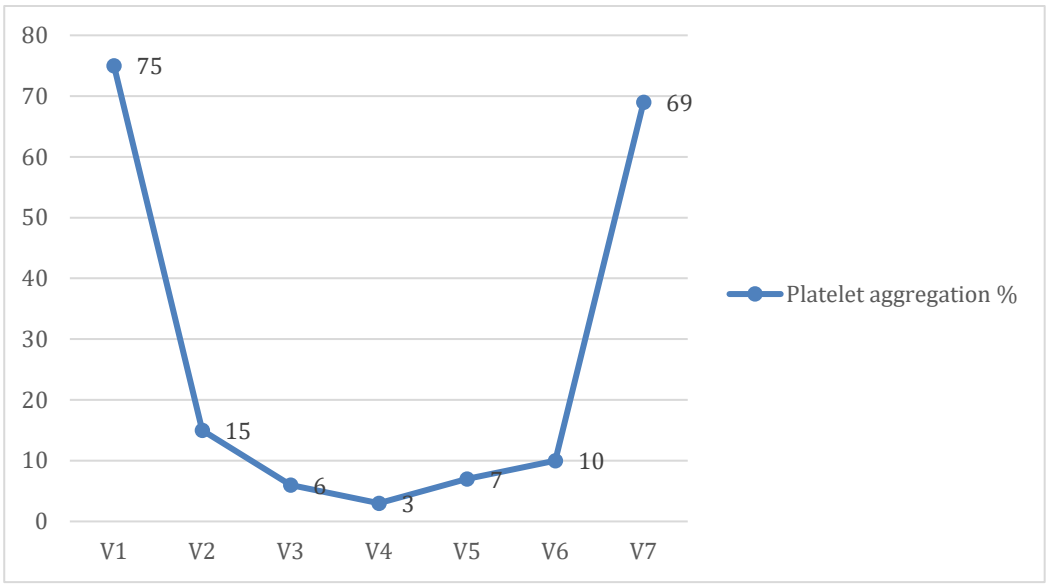
In this patient, the platelet aggregation levels were well in excess of 20% throughout the entire study period.




V=Visit

**Figure 3.9. Platelet aggregation per visit (patient 3), aspirin group**

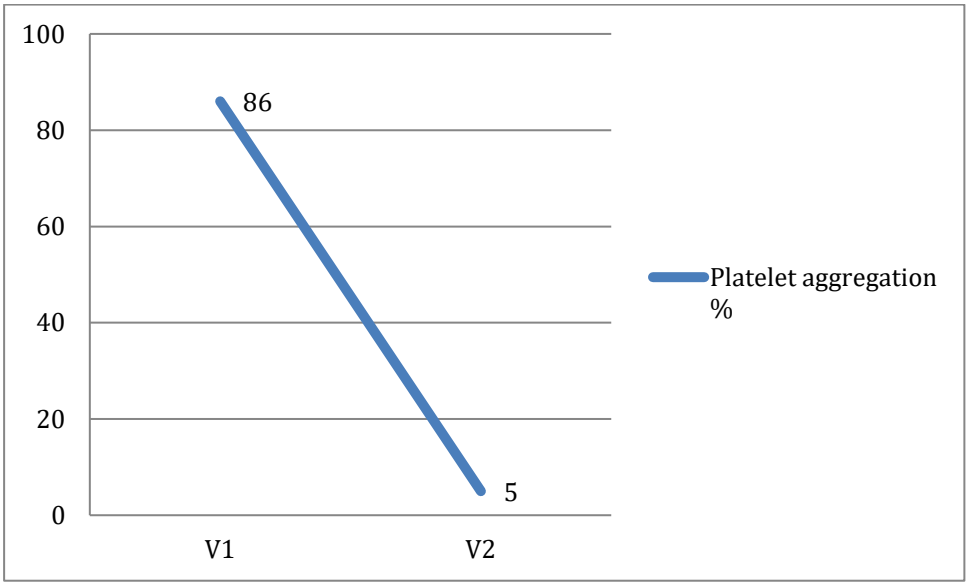
This patient was deemed non-compliant as she missed 10 tablets (over 5% of overall tablets that should have been taken during pregnancy). The level of non-compliance reflected in this participant's pill counts is consistent with the erratic pattern of platelet aggregation objectively demonstrated throughout gestation.




V=Visit

**Figure 3.10. Platelet aggregation per visit (patient 4), aspirin group.**

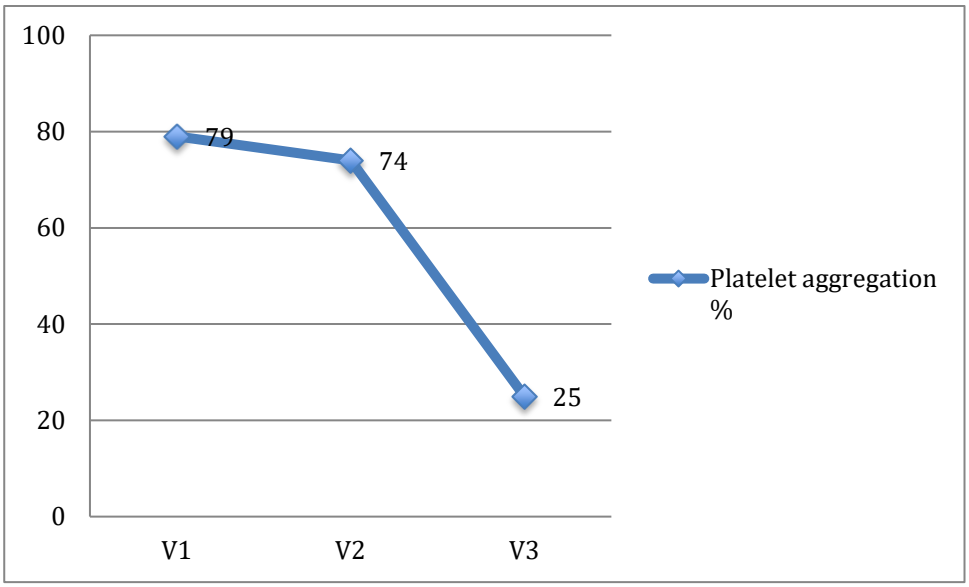
Platelet suppression was achieved until the sixth visit corresponding to $30^{+5}$ weeks' gestation; however; on seventh visit, at $33^{+2}$ weeks' gestation, platelet aggregation was 69% despite the fact patient was deemed compliant with the study protocol as 2 pills were missed on pill count. A sample was taken on the eighth visit, at $36^{+3}$ weeks' gestation, 3 days after stopping aspirin intake, but it was not sufficient for analysis.




V=Visit

**Figure 3.11. Platelet aggregation per visit (patient 5), aspirin group.**

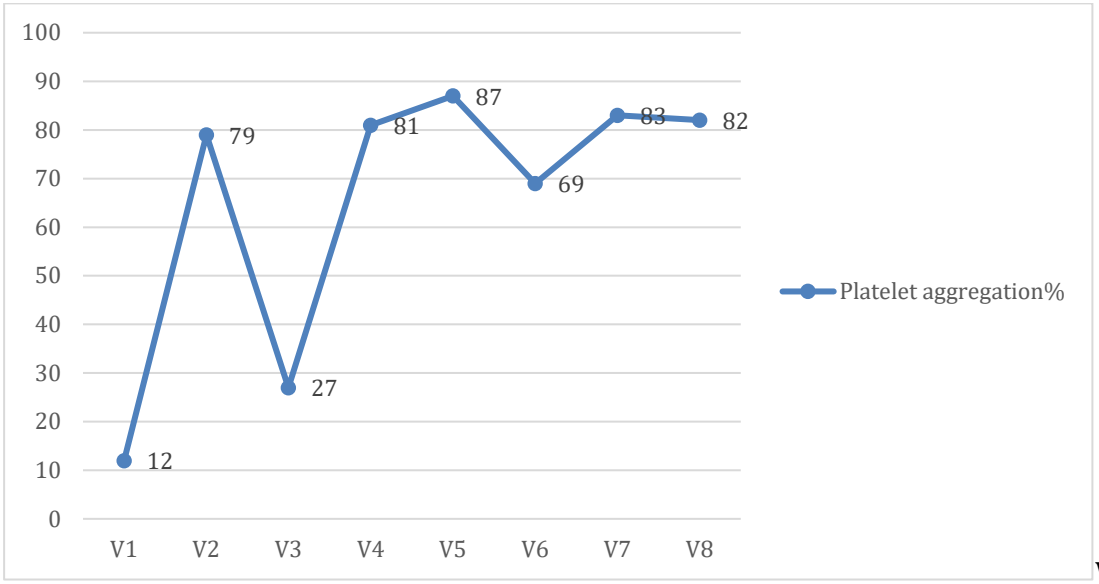
This patient withdrew consent at visit 3 due to difficulty in obtaining blood samples.




V=Visit

**Figure 3.12. Platelet aggregation per visit patient 6, non-aspirin group,**

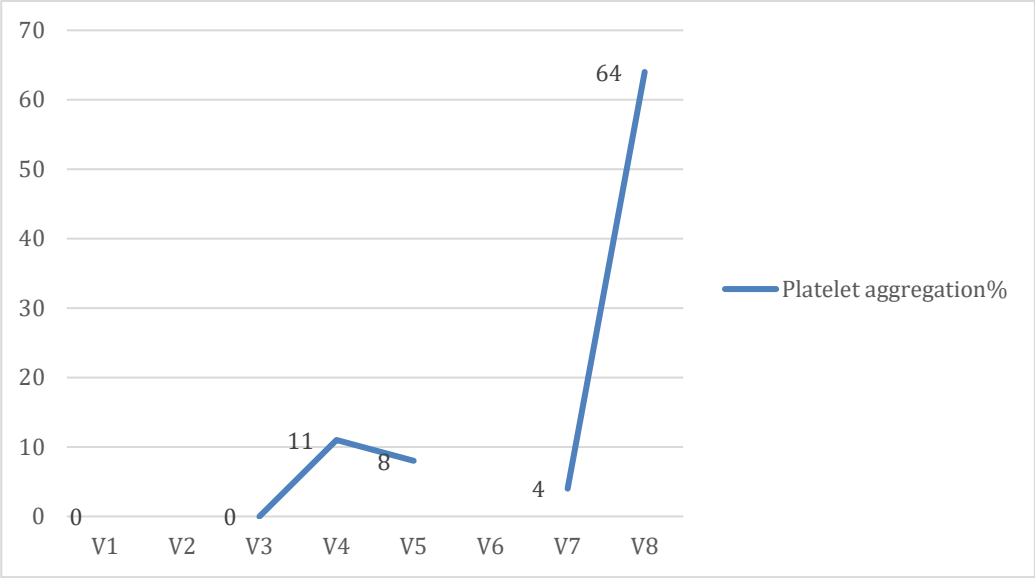
She had a late fetal demise at 20+1 weeks' gestation. Early termination of study and serious adverse event forms were completed.




V=Visit

**Figure 3.13. Platelet aggregation per visit (patient 7), non-aspirin group.**

This patient's first blood test result was below the 20% line at the first visit at 11weeks' gestation, however, nothing in her history suggested she was on antiplatelet therapy. This was evident again on her third visit at 19 weeks' gestation. Those two incidents of suppressed platelet aggregation in this patient who was in the control group and was not on any antiplatelet therapy that would explain the observation, underpins our contention that individual patient suitability for antiplatelet therapy is likely variable and that not all patients may respond similarly to antiplatelet therapy. This will be explored in detail in the Discussion section.




V=Visit

**Figure 3.14. Platelet aggregation per visit (patient 8), aspirin group.**

This patient was fully compliant with the study medications. The patient's baseline platelet function profile is similar to patient 7. Platelet aggregation was not possible to obtain in 2 visits because of a low volume blood sample. In addition, this patient platelet aggregation was 0% even before antiplatelet therapy was commenced despite the fact that she denied use of aspirin/ antiplatelet medications prior to commencement of the study.

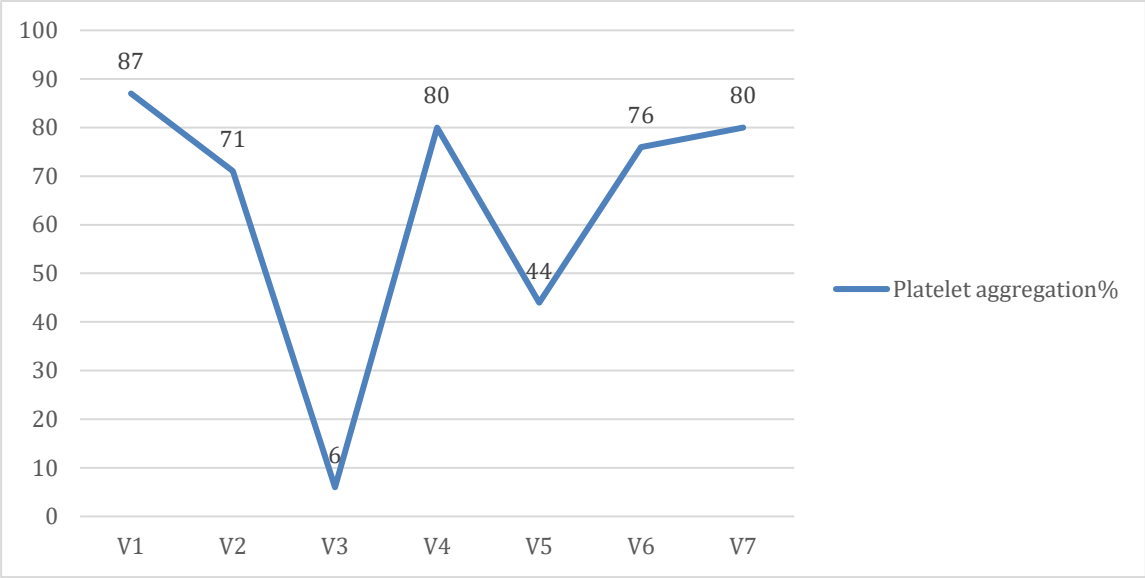



**Figure 3.15. Platelet aggregation per visit (patient 9), non-aspirin group.**

Platelet aggregation levels were above the threshold of 20%, except at visit 3, as there was an unexplained decline in platelet aggregation to below 20%.

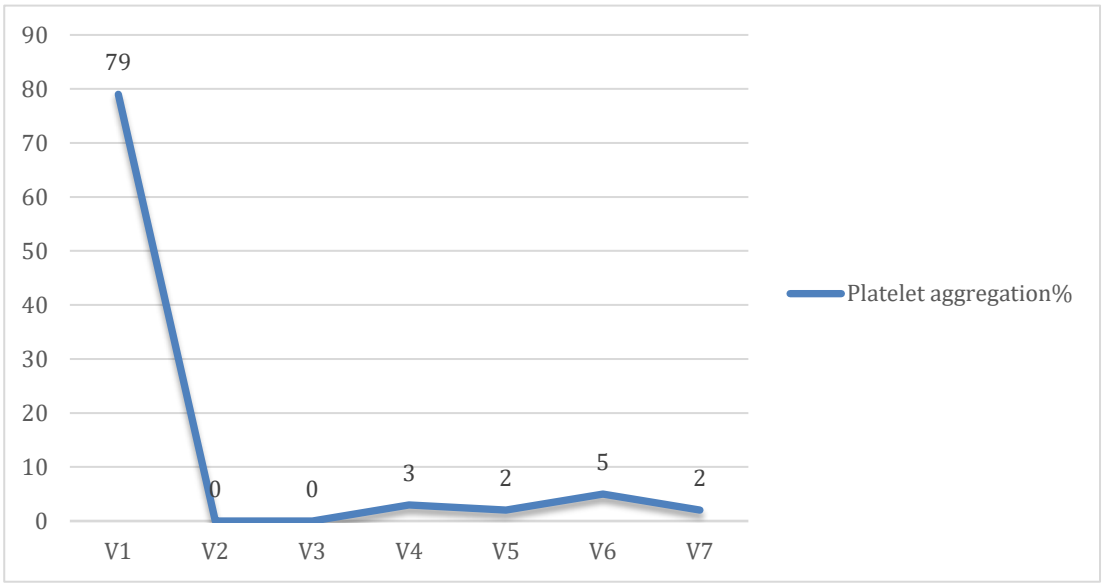



**Figure 3.16. Platelet aggregation per visit (patient 10), aspirin group.**

This patient was compliant all through the study period. The platelet aggregation results were consistent with this. Patient had her last study visit at $37^{+4}$ and a blood sample was obtained but was not enough to be processed for analysis, so it was difficult to confirm the return of platelet aggregation to normal baseline post cessation of aspirin intake at $36^{+0}$ weeks' gestation.

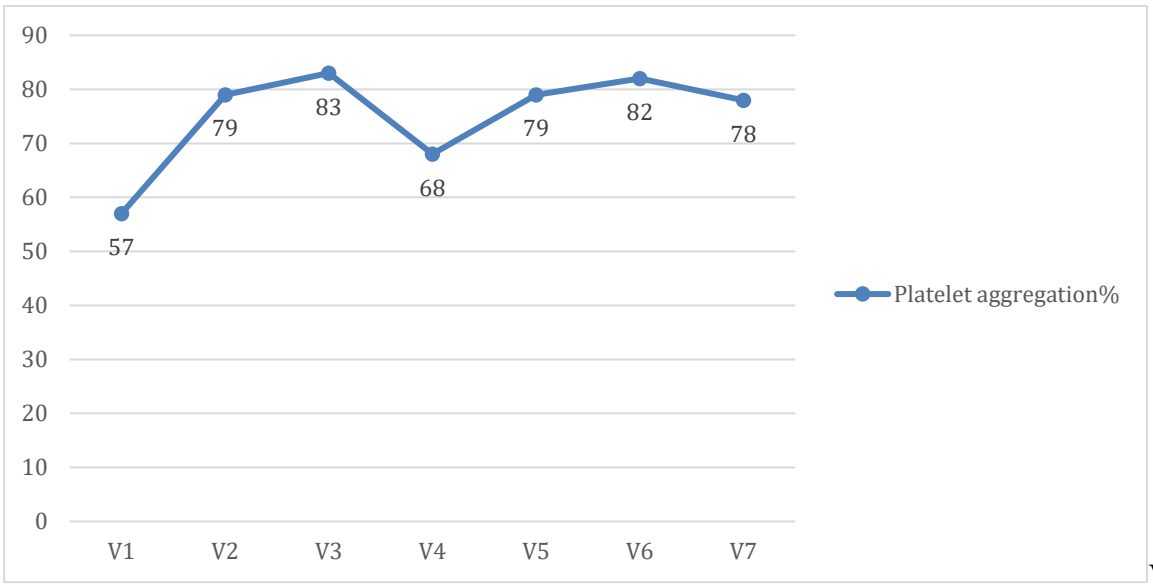
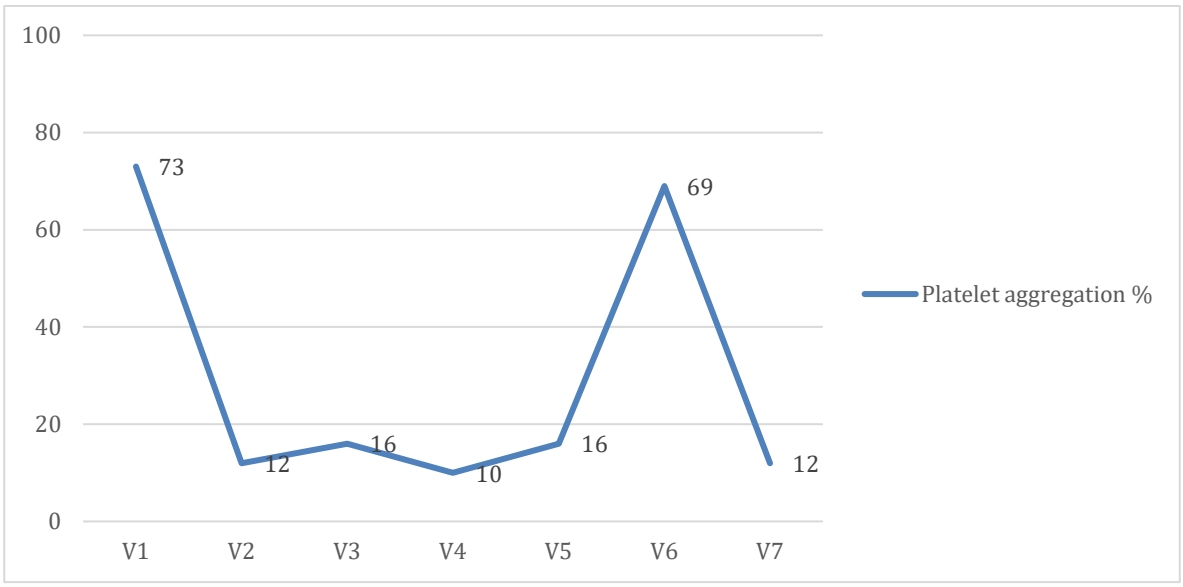



**Figure 3.17. Platelet aggregation per visit (patient 11), non-aspirin group.**

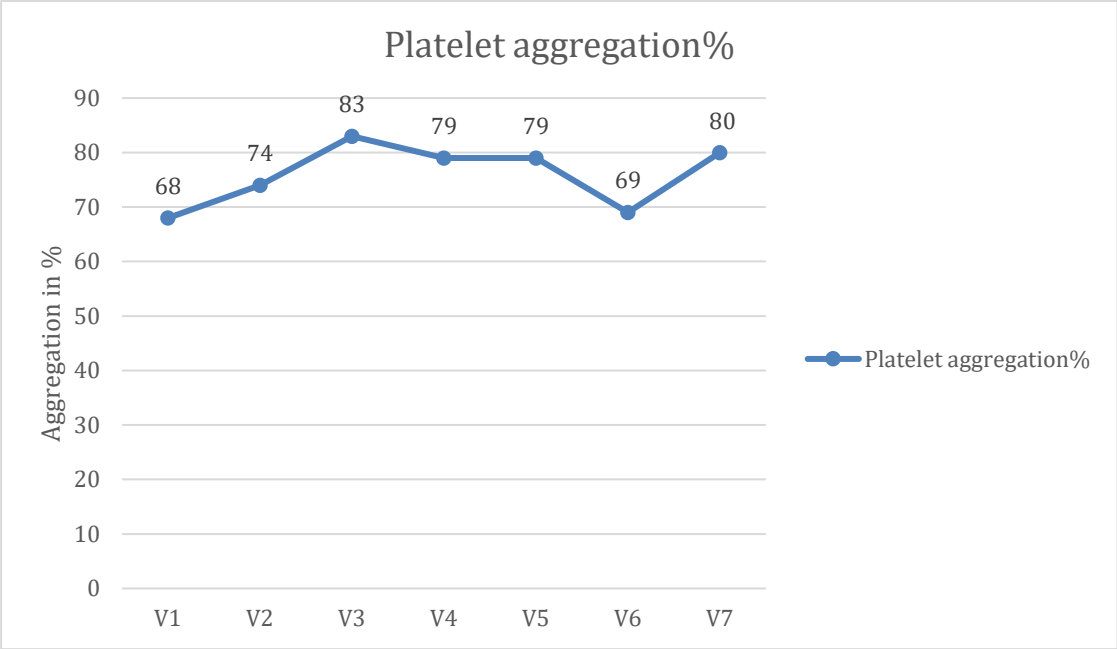
The results are consistent with non-use of antiplatelet therapy.




V=Visit

**Figure 3.18. Platelet aggregation per visit (patient 12), aspirin group.**

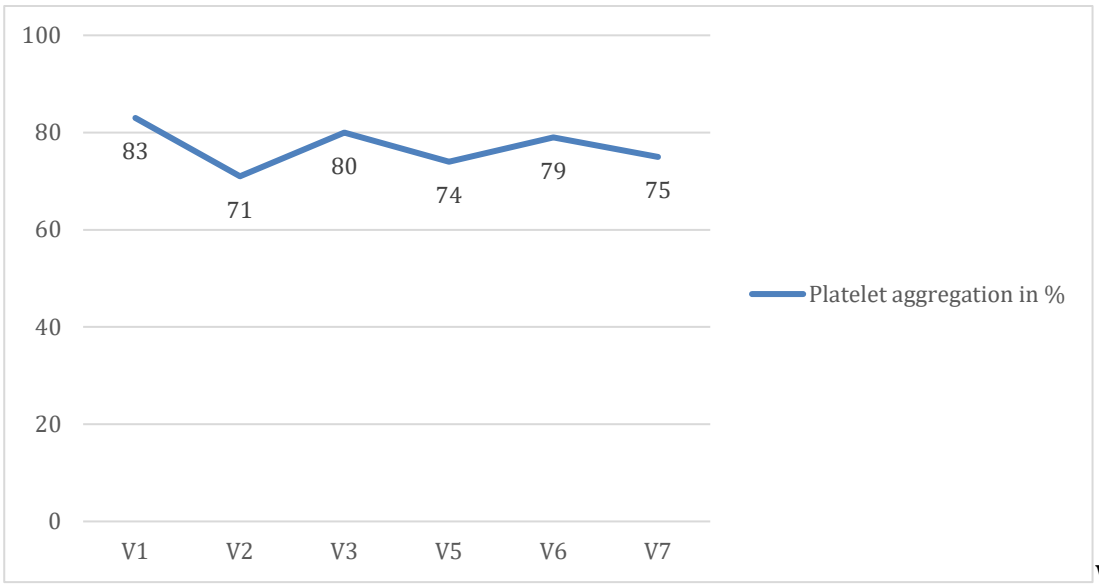
This patient was judged to be compliant with the study protocol throughout the study period. There was no deviation identified to account for the platelet aggregation results of visit 6.




V=Visit

**Figure 3.19. Platelet aggregation per visit (patient 13), non-aspirin group.**

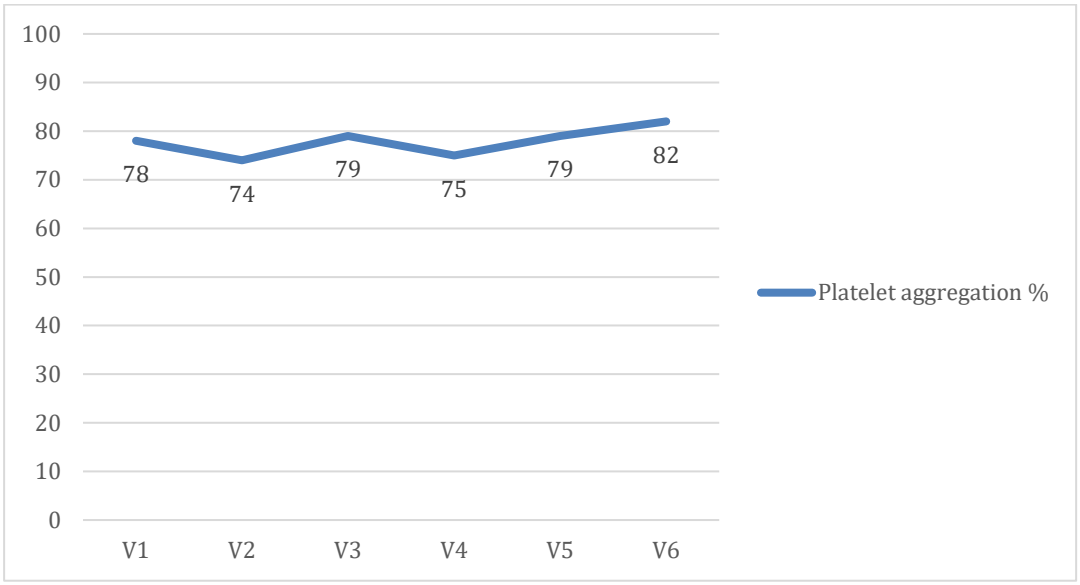
The results are consistent with non-use of antiplatelet therapy.




V=Visit

**Figure 3.20. Platelet aggregation per visit (patient 14), non-aspirin group.**

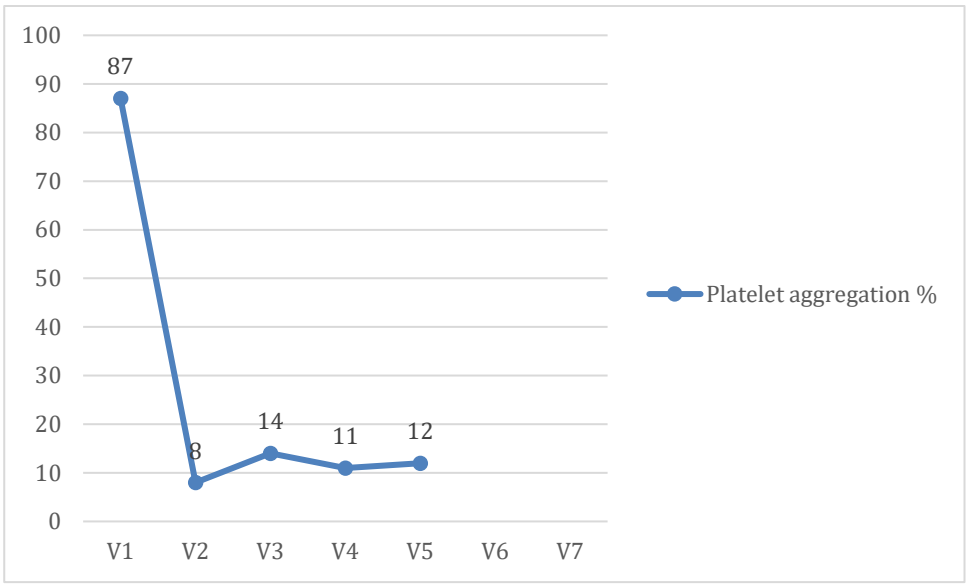
The results are consistent with non-use of antiplatelet therapy.




V=Visit

**Figure 3.21. Platelet aggregation per visit (patient 15), aspirin group.**

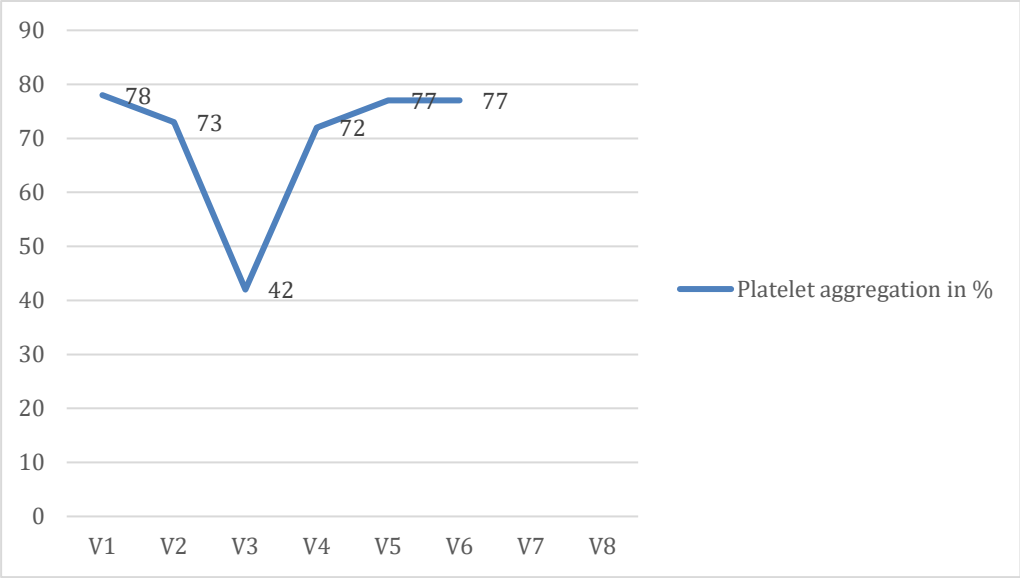
This patient was fully compliant with the study protocol, as judged by diary card and pill count; however, her platelet aggregation profile was not consistent with an expected antiplatelet therapy effect.




V=Visit

**Figure 3.22. Platelet aggregation per visit (patient 16), aspirin group.**

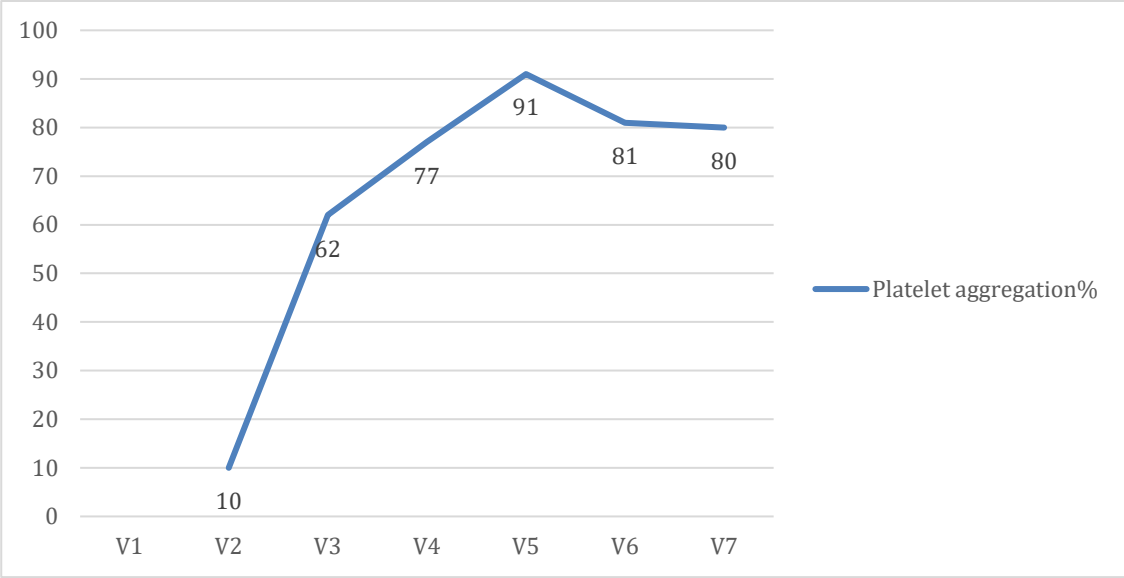
The patient was compliant with study protocol. However, this patient had no blood results for the last 3 visits of the study. Pill count and diary card showed full compliance.




V=Visit

**Figure 3.23. Platelet aggregation per visit (patient 17), non-aspirin group.**

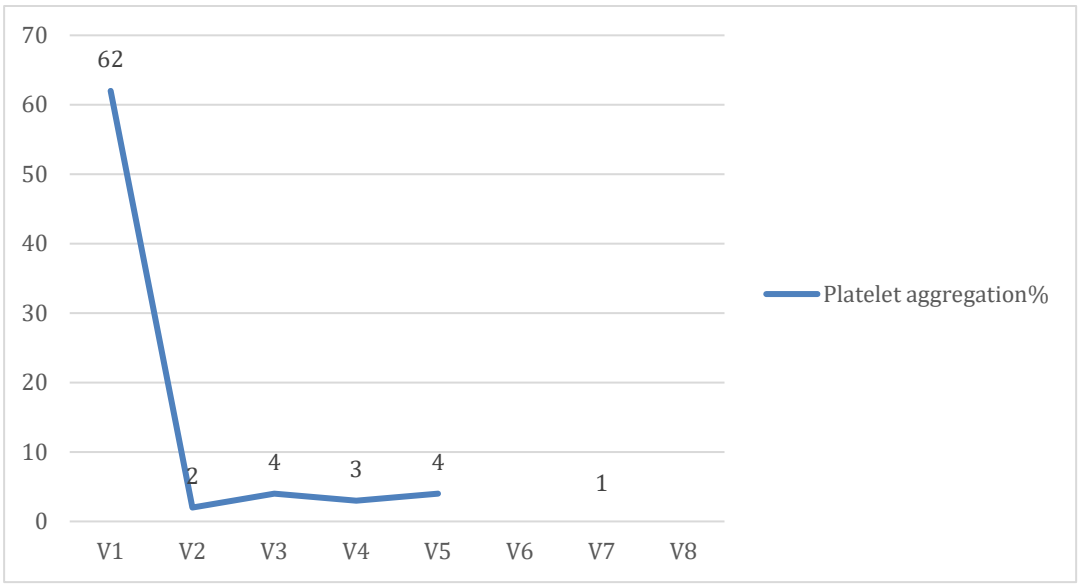
The line graph demonstrating platelet aggregation activity in excess of 20% throughout. This patient had no results for the last 2 visits because of insufficient samples.




V=Visit

**Figure 3.24. Platelet aggregation per visit (patient 18), non-aspirin group.**

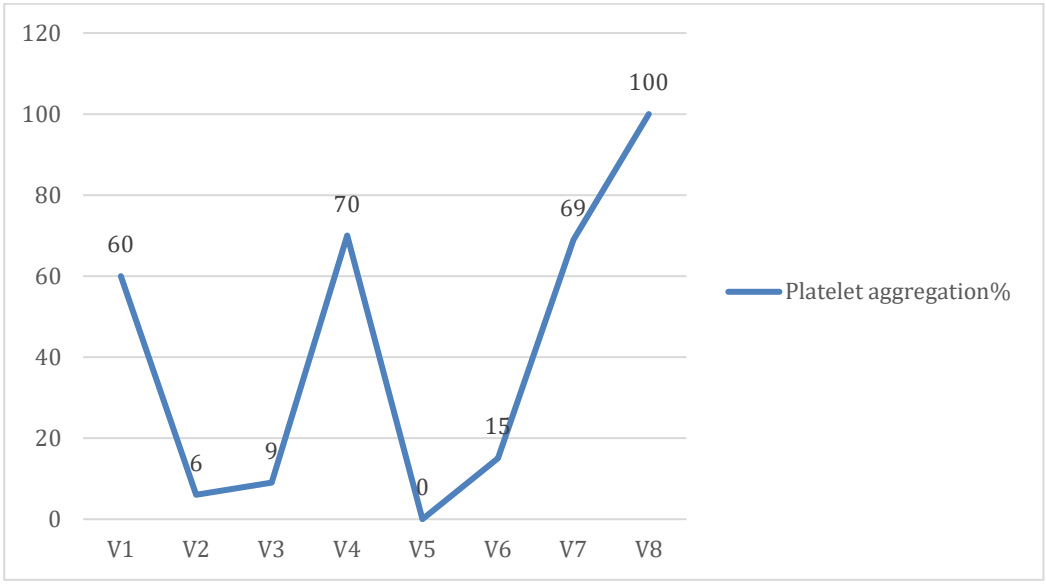
Once again, in early gestation at $8^{+3}$ weeks' gestation, this participant was noted to have a suppressed platelet aggregation picture, which recovered to normal platelet aggregation activity thereafter. The patients' only declared medications in the first trimester were oral folic Acid and subcutaneous insulin injections.




V=Visit

**Figure 3.25. Platelet aggregation per visit (patient 19), aspirin group.**

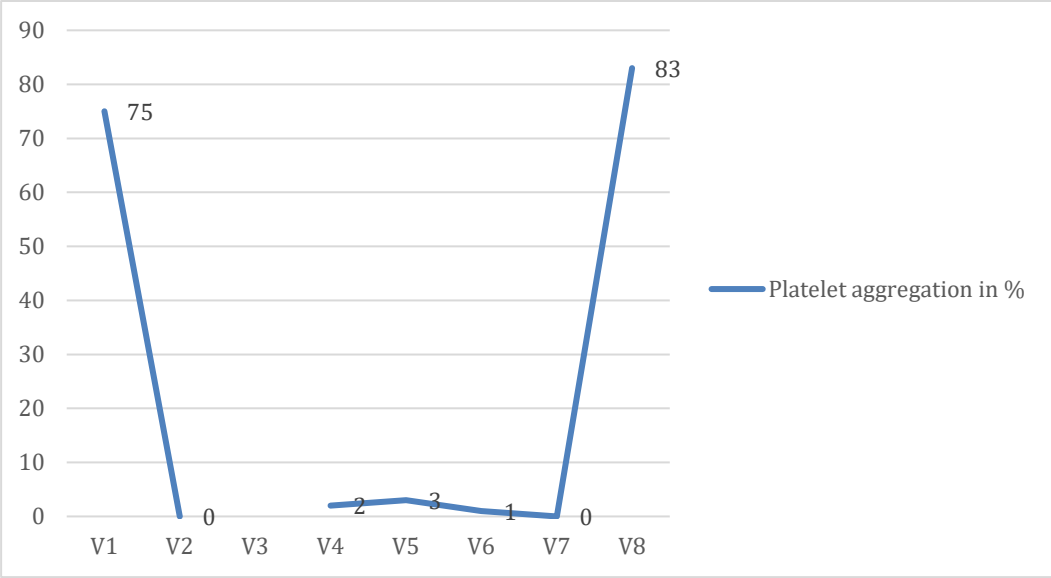
This patient was compliant objectively and subjectively, judged by diary card, pill count and platelet aggregation. Post-treatment return to baseline platelet activity was not demonstrable due to non-attendance to obtain a blood sample for analysis.




V=Visit

**Figure 3.26. Platelet aggregation per visit (patient 20), aspirin group.**

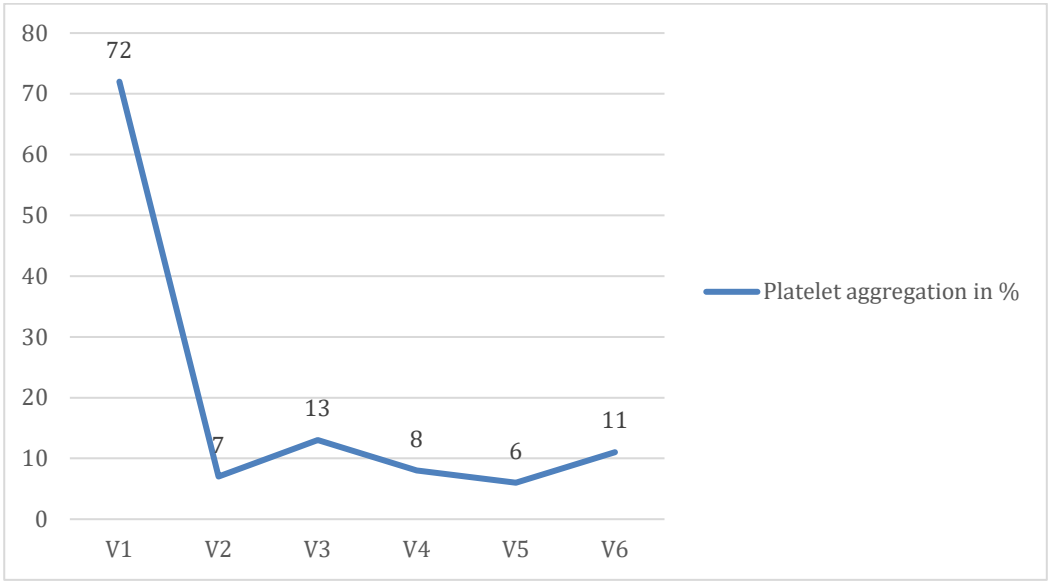
This patient was deemed non-compliant as she missed over 5% of total aspirin pills that should have been administered through the study period. Poor compliance is reflected in the erratic line of platelet aggregation results through the study.




V=Visit

**Figure 3.27. Platelet aggregation per visit (patient 21), aspirin group.**

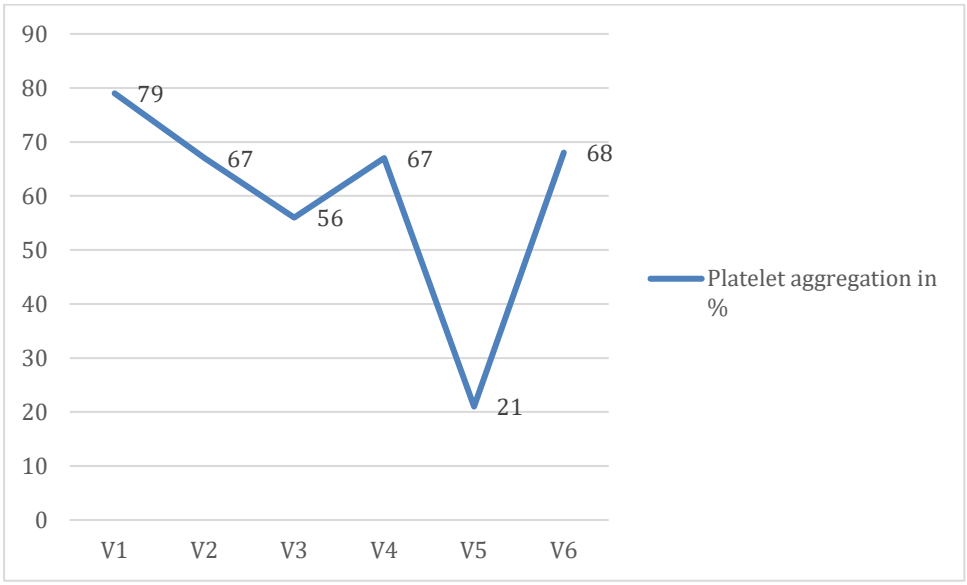
This patient was a compliant and a responder throughout the study period.




V=Visit

**Figure 3.28. Platelet aggregation per visit (patient 22), aspirin group.**

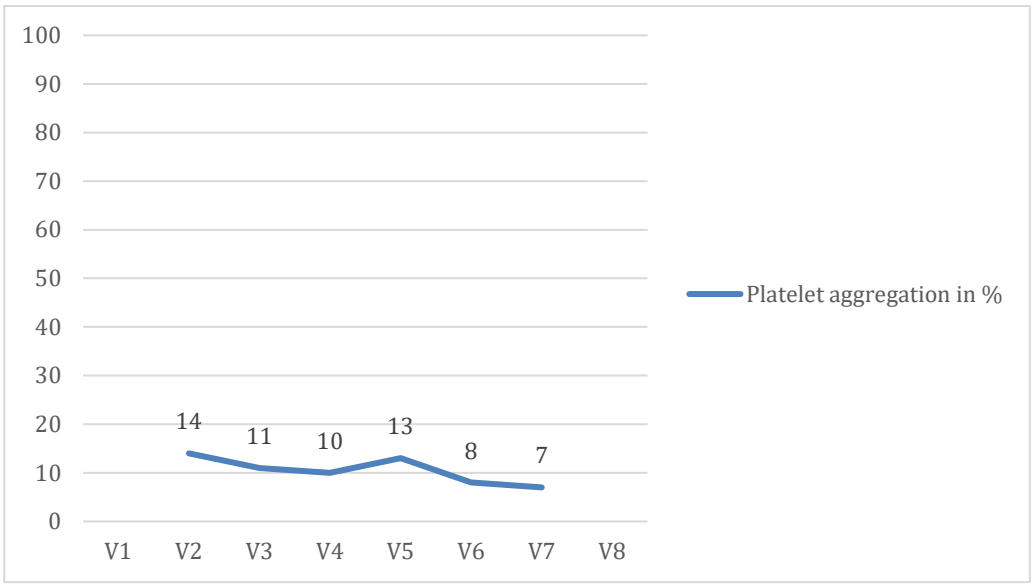
This patient was compliant throughout the study period; however, aspirin was stopped at $33^{+6}$ weeks' gestation (around visit 6) in anticipation of preterm delivery as the patient developed obstetric cholestasis.




V=Visit

**Figure 3.29. Platelet aggregation per visit (patient 23), non-aspirin group.**

This line chart represents platelet aggregation results which are above the 20% cut-off line.




V=Visit

**Figure 3.30. Platelet aggregation per visit (patient 24), aspirin group.**

In this patient, bloods failed to be drawn on visits 1 and 8.

**Table 3.2. Summary of patients' narratives**

| patient No. | Aspirin/non aspirin group | Type of diabe-tes | Microvascular Complications | Parity | BMI | Ethnicity | Medications | Compliance | SAE | Delivery | Neonatal admission |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 01 | Withdrew prior to randomisation | | | | | | | | | | |
| 02 | Non-aspirin | 2 | No | M | 29.2 | East European | Metformin<br>FA<br>Insulin at 7 weeks | NA | No | SVD | No |
| 03 | Aspirin | 2 | No | M | 36.8 | Irish | Metformin<br>Insulin at 7 weeks | No | 0 | SVD | No |
| 04 | Aspirin | 1 | No | P | 21.7 | Irish | Thyroxine<br>FA<br>Insulin | Yes | 1 | EmLSCS | No |
| 05 | Aspirin | 2 | No | M | 36 | African | Metformin<br>FA<br>Insulin at 6 weeks | Yes | 0 | Withdrew | |
| 06 | Non-aspirin | 1 | No | M | 29 | Irish | FA<br>Insulin | NA | 1 | IUD | NA |
| 07 | Non-aspirin | 2 | No | P | 22.7 | Asian | Metformin<br>Insulin at 6 weeks | NA | 2 | OVD | No |
| 08 | Aspirin | 2 | No | P | 30 | Irish | Amlodipine<br>FA<br>Insulin at 6 weeks | Yes | 8 | OVD | No |

| patient No. | Aspirin/non aspirin group | Type of diabe-tes | Microvascular Complications | Parity | BMI | Ethnicity | Medications | Compliance | SAE | Delivery | Neonatal admission |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 09 | Non-aspirin | 1 | No | M | 20.7 | Irish | Thyroxine<br>FA<br>Insulin | NA | 0 | El.LSCS | No |
| 10 | Aspirin | MODY | No | P | 30 | Irish | Thyroxine<br>FA<br>Insulin | Yes | 1 | SVD | No |
| 11 | Non-aspirin | 1 | No | P | 25 | Irish | Thyroxine<br>FA<br>Insulin | NA | 1 | Em. LSCS | No |
| 12 | Aspirin | 2 | No | M | 34 | Irish | Cabergoline<br>Thyroxine<br>Metformin<br>Insulin at 7 weekss | Yes | 0 | SVD | No |
| 13 | Non-aspirin | 1 | No | P | 24 | Irish | FA<br>Insulin | NA | 1 | Em.LSCS | No |
| 14 | Non-aspirin | 2 | No | M | 26 | Irish | Thyroxine<br>Insulin at 7 weeks | NA | 0 | El.LSCS | No |
| 15 | Aspirin | 1 | No | P | 24 | European | FA<br>Insulin | Yes but non responder | 1 | Em.LSCS | No |
| 16 | Aspirin | T2 | No | M | 32 | Asian | FA<br>Metformin<br>Insulin at 6 weeks | Compliant | 1 | El.LSCS | No |
| 17 | Non-aspirin | 1 | Yes | P | 28 | Irish | FA<br>Insulin | NA | 1 | El.LSCS | No |

| patient No. | Aspirin/non aspirin group | Type of diabe-tes | Microvascular Complications | Parity | BMI | Ethnicity | Medications | Compliance | SAE | Delivery | Neonatal admission |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | Non-aspirin | 2 | No | M | 23 | Irish | FA | NA | 1 | Em LSCS | No |
| 19 | Non-aspirin | 2 | Retinopathy | M | 27 | Asian | Metformin Labetalol Insulin at 6 weeks | NA | 1 | El.LSCS | No |
| 20 | Aspirin | 1 | No | P | 26 | White American | FA Insulin at 7 weeks | No | 2 | OVD | No |
| 21 | Aspirin | 2 | No | M | 34 | Black | Metformin FA Insulin at 6 weeks | Yes | 2 | SVD | No |
| 22 | Aspirin | 1 | Retinopathy | P | 32 | Irish | FA Insulin | Yes | 1 | SVD | yes |
| 23 | Non-aspirin | 2 | No | P | 23 | Asian | FA Insulin at 6 weeks | NA | 1 | OVD | Yes |
| 24 | Aspirin | 1 | No | M | 25 | Irish | FA Insulin | Compliant | 1 | El.LSCS | No |

SVD: Spontaneous Vaginal Delivery
OVD: Operative Vaginal Delivery
El.LSCS: Elective Lower Uterine Caesarean Segment
Em. LSCS: Emergency Lower Uterine Caesarean Segment
MODY: Maturity Onset Diabetes of theYoung
IUD: Intra Uterine Death
SAE: Serious Adverse Events
FA: Folic Acid
P: Primiparous
M: Multiparous

## Table 3.3. Serious adverse events per patient

| Subject Number | No. of SAE | GA (Weeks) | SAE Event | Further Description Of event | Treatment Allocation | Related To IMP | Outcome | Serious Criteria | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | 1 | $37^{+3}$ | PE | Confirmed PE at 37 weeks' gestational age | Aspirin | Not Related | Resolved | Hospitalisation and delivery | |
| 7 | 2 | $23^{+5}$ | Allergic reaction to Novorapid | Reaction in the form of a skin rash and facial swelling. | Non-aspirin | Not Related | Resolved | Hospitalisation | |
| 7 | | $23^{+5}$ | Allergic reaction to Humalin S & I | | | Not Related | Resolved | Hospitalisation | |
| 8 | 8 | 17 | 6 | 1 | $20^{+1}$ | Intrauterine death/late miscarriage | Presented at $20^{+1}$with PV bleeding. Late fetal demise was confirmed. No cause identified on post-mortem examination. | Non-aspirin | Not Related |
| 8 | | $27^{+2}$ | Reduced Fetal Movement | Reports reduced fetal movement for 2-3 days. Admitted for CTG which showed normal fetal activity | | Not Related | Resolved | Hospitalisation | |
| 8 | | $27^{+5}$ | Abdominal Pain | Admitted with nonspecific abdominal pain.Admit for observation. | | Not Related | Resolved | Hospitalisation | |
| 8 | | $31^{+1}$ | Vaginal Spotting | Unclassified non-substantial antepartum haemorrhage. | | Not Related | Resolved | Hospitalisation | |
| 8 | | $33^{+2}$ | Reduced Fetal Movement | Admitted for CTG and fetal scan | | Not Related | Resolved | Hospitalisation | |
| 8 | | $33^{+5}$ | Vaginal Spotting | Unclassified non-specific antepartum haemorrhage | | Not Related | Resolved | Hospitalisation | |
| 8 | | $34^{+6}$ | High Blood Pressure | Non-proteinuric pregnancy-induced hypertension | | Not Related | Resolved | Hospitalisation | |

| Subject Number | No. of SAE | GA (Weeks) | SAE Event | Further Description Of event | Treatment Allocation | Related To IMP | Outcome | Serious Criteria |
|---|---|---|---|---|---|---|---|---|
| 8 | | $37^{+4}$ | Vomiting and diarrhoea | Gastroenteritis | | Not Related | Resolved | Hospitalisation |
| 10 | 1 | Post-natal | PPH | Primary PPH due to atonic uterus. Managed conservatively. | Aspirin Stopped at 36 weeks and patient delivered at 39 weeks | Not Related | Resolved | Hospitalisation |
| R11 | 1 | Postnatal | PPH | Primary PPH due to uterine atony<br>EBL 700 ml | Non-aspirin | | | |
| R12 | 1 | $36^{+4}$ | Upper respiratory tract infection (URTI) | Viral URTI | Aspirin | Not Related | Resolved | Hospitalisation |
| R13 | 1 | Post-natal | | Primary PPH due to uterine atony<br>EBL10000 ml | Non-aspirin | Not Related | Resolved | Hospitalisation |
| R15 | | $37^{+4}$ | Possible pyelonephritis | Presented with flank pain and temperature of 38.2. WBC 16.2 and CRP 86mg/L. Patient's condition improved with intravenous co-amoxiclav and gentamycin. Urine culture was negative | Aspirin | Not Related | Resolved | Hospitalisation |
| R15 | 2 | $37^{+5}$ | Pre-eclampsia. | Confirmed preeclampsia at 37 weeks' gestational age | | Not Related | Resolved | Hospitalisation |
| R17 | 1 | 34+2 | Vitreous Haemorrhage | Deterioration on ophthalmological examination | Non-aspirin | Not Related | Resolved | Hospitalisation |
| R18 | 1 | Post-natal | PPH | Uterine atony-induced postpartum haemorrhage (1000 ml) | Non-aspirin | Not Related | Resolved | Prolonged hospitalisation |
| R19 | 1 | Post-natal | PPH | Postpartum haemorrhage. LSCS followed by primary PPH of 1400cc. Misoprostol 1gm PR and 40 IU oxytocin IV infusion. No blood transfusion | Aspirin Stopped at 36+0 and patient delivered at 39+3 | Not Related | Resolved | PProlonged hospitalisation |
| R20 | 2 | 35+3 | Gastroenteritis | Viral gastroenteritis | | Not Related | Resolved | Hospitalisation |

| Subject Number | No. of SAE | GA (Weeks) | SAE Event | Further Description Of event | Treatment Allocation | Related To IMP | Outcome | Serious Criteria | |
|---|---|---|---|---|---|---|---|---|---|
| R20 | | Post-natal | PPH | Primary PPH due to uterine atony. EBL 700 ml. Hb dropped from 11.2 to 7.4 g/L | Aspirin Stopped at 36+0 and patient delivered at 39+3 | Not Related | Resolved | Prolonged hospitalisation | |
| R21 | 2 | 29+5 | Pneumonia | Admitted with shortness of breath and cough for intravenous antibiotics. Chest x-ray was normal | Aspirin | Not Related | Resolved | R16 | 1 |
| R21 | | Post-natal | PPH | Postpartum haemorrhage (600 ml) secondary to atonic uterus. misoprostol PR 1gm given | Aspirin Stopped at 36+0 and patient delivered at 37+6 | Not Related | Resolved | Prolonged hospitalisation | |
| R22 | 1 | 31+6 | Obstetric Cholestasis | Confirmed obstetric cholestasis | Aspirin | Not Related | Resolved | Hospitalisation | |
| R23 | 1 | 29+3 | Intrauterine growth restriction (IUGR) | Asymmetrical IUGR AC< 5$^{th}$ centile with normal Umbilical artery Doppler (assumed placental insufficiency) | Non-aspirin | Not Related | Resolved | Hospitalisation | |
| R24 | 1 | 9+5 | URTI | Admitted with cough and fever. Throat swabs confirmed Influenza B virus. | Aspirin | Not Related | Resolved | Hospitalisation | |

PE: Preeclampsia
PV: Per Vaginum
DVT: Deep Vein Thrombosis
CTG: Cardiotocogram
EBL: Estimated Blood Loss
LSCS: Lower Segment Caesarean Section
PPH: Post-Partum Haemorrhage
IUGR: Intra Uterine Growth Restriction
URTI: Upper Respiratory Tract Infection
IMP: Investigational Medicinal Product

Table 3.3 Represents the number of SAEs and a brief description of each SAE and it is relatedness to aspirin intake.

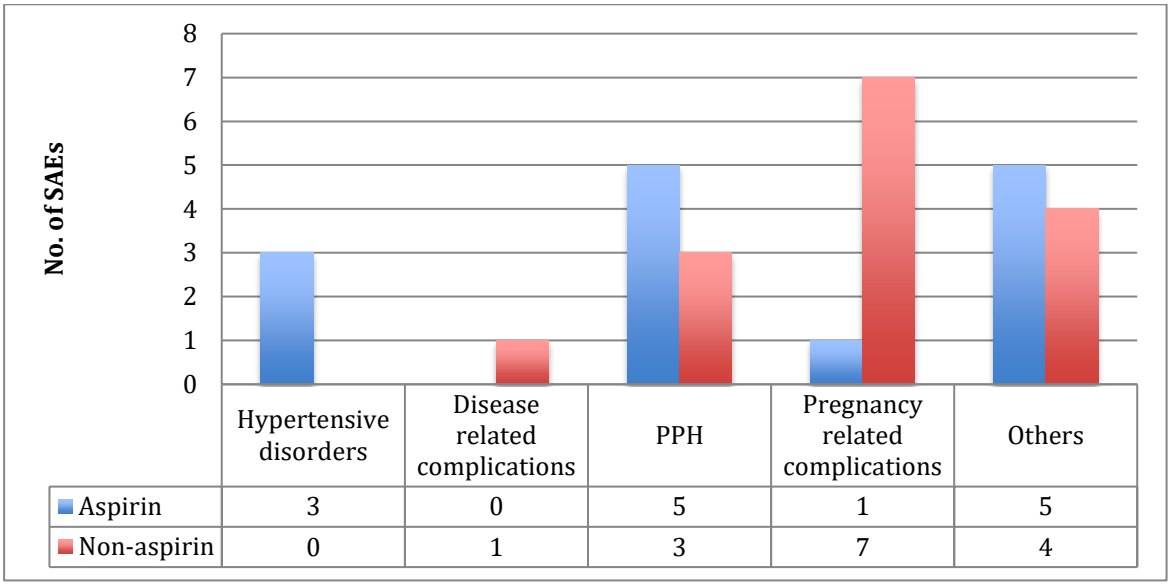



**Figure 3.31. Serious adverse events in aspirin vs non- aspirin groups**

This column chart represents the frequency of SAEs between aspirin and no-aspirin group.

| Characteristic | | Non- aspirin (N=9) | aspirin (N=12) | P-value |
|---|---|---|---|---|
| GA at delivery (weeks) | | 38 ± 1 | 38 ±1 | 0.625 |
| GA at Aspirin cessation (weeks) | | n/a | 35.5 ± 1.3 | n/a |
| PE | | 0 | 2 (17%) | 0.198 |
| Mode of Delivery | SVD | 1 (11%) | 6 (50%) | 0.238 |
| | OVD | 2 (22%) | 2 (17%) | |
| | Elective CS | 3 (33%) | 3 (25%) | |
| | Emergency CS | 3 (33%) | 1 (8.3%) | |
| Labour onset | IOL | 5 (56%) | 6 (50%) | 0.239 |
| | SOL | 0 | 3 (25%) | |
| | n/a | 4 (44%) | 3 (25%) | |
| PPH | | 3 (33%) | 5 (42%) | 0.697 |
| NICU admission | | 1 (11%) | 1 (9.1%) | 0.881 |

PE: Preeclampsia
SVD Spontaneous Vaginal Delivery
OVD: Operative Vaginal Delivery
IOL: Induction of Labour
SOL: Spontaneous Labour
PPH: Postpartum Haemorrhage
NICU: Neonatal intensive Care Unit

**Table 3.4. Delivery and neonatal outcomes**

It demonstrates the delivery and neonatal outcome between aspirin and no aspirin groups.

### 3.6 Study Acceptability

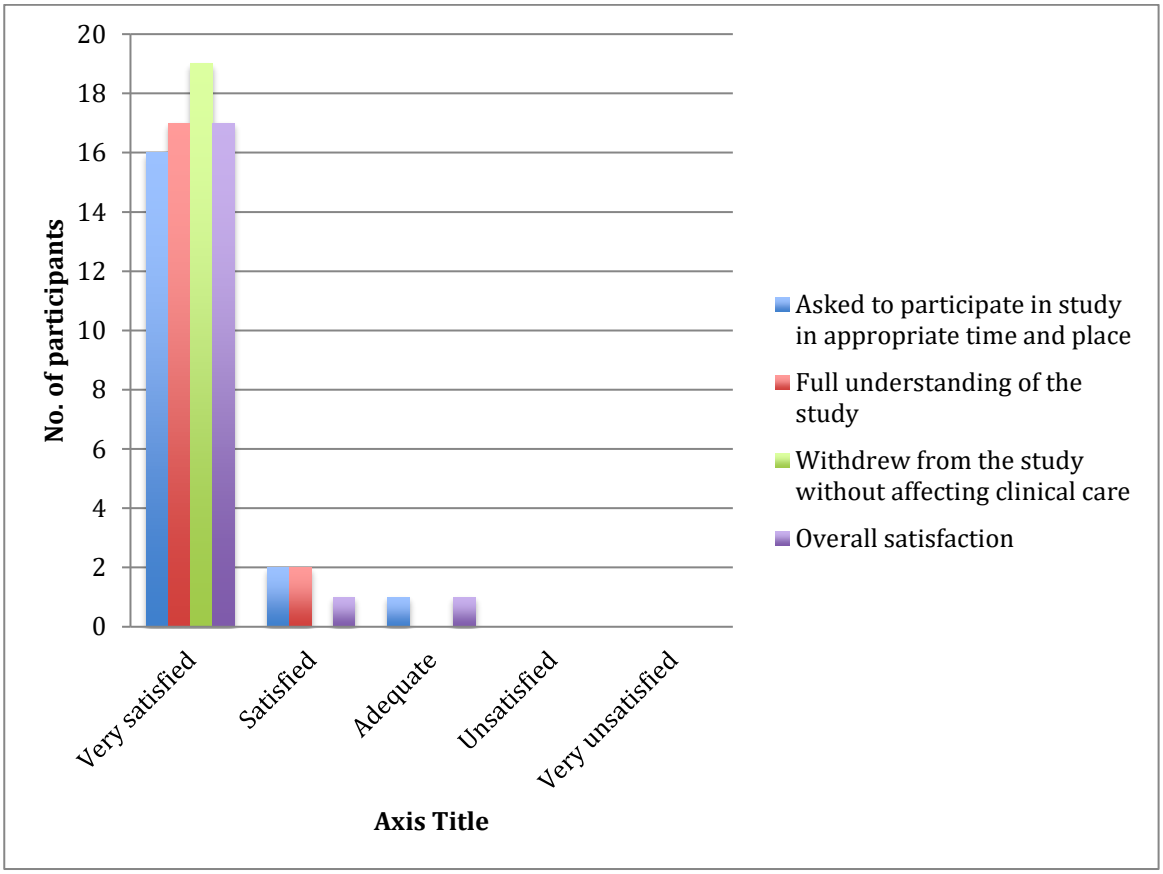



Figure 3.**32. Patient satisfaction questionnaire**

This column chart shows 19 patients out of 24 participated in a questionnaire relating to their overall satisfaction with the study.

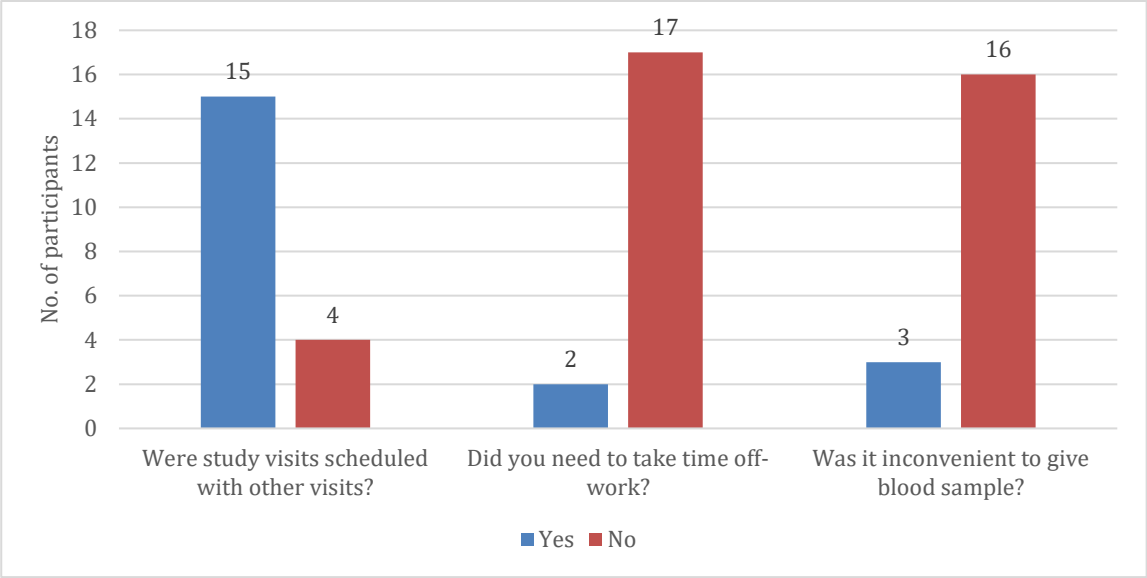



**Figure 3.33. Assessment of convenience**

It explores the inconvenience that participants might have encountered during the study period.

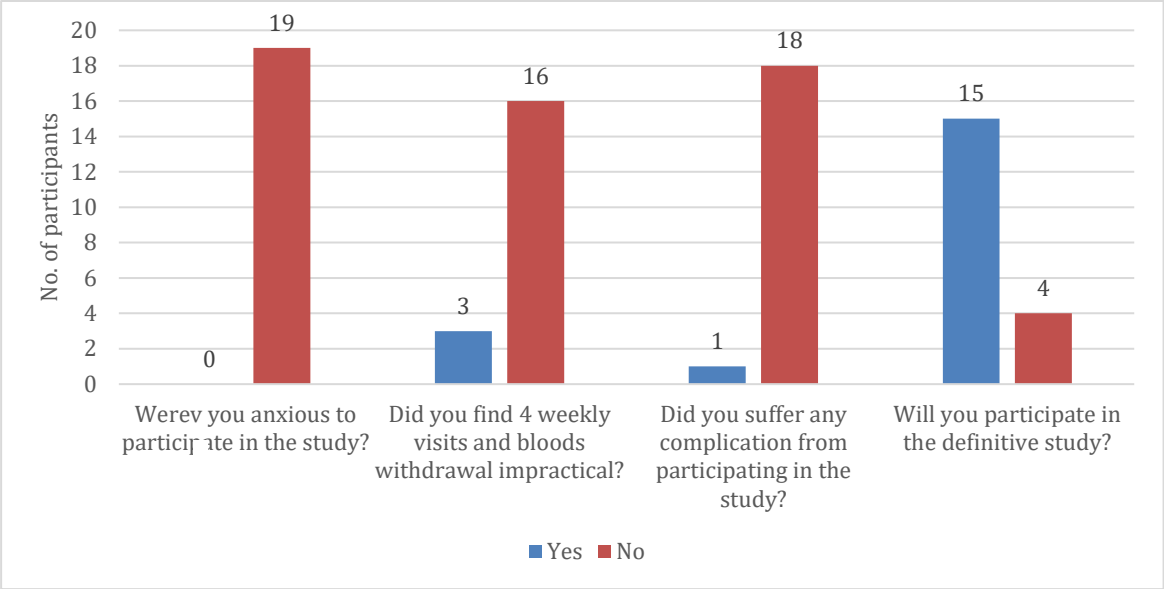



**Figure 3.34. Anxiety and future participation in definitive study**

It shows that no patients experienced any level of anxiety regarding study participation; however, only one patient reported that she suffered a complication secondary to the usage of aspirin as she had a postpartum haemorrhage. Fifteen declared that they will be happy to participate in a definitive study, should one start, and they were pregnant again.

# Chapter 4: Discussion

## 4.1 Patient population, demographics and observations

In this chapter I provide a brief description of the results of each section followed by a discussion of the significance of these results and their context within the current literature. I will also highlight the inherent flaws and strengths in the study design. As explained before, this pilot study is a precursor for a more definitive randomised controlled study on a wider scale in terms of number of centres involved and number of patients planned for recruitment. A comment on the clinical utility of these findings will also be presented.

For women with PGDM, an elevated glycosylated haemoglobin (HbA1C) is associated with increased adverse pregnancy events including miscarriage, congenital anomalies and stillbirth(226). James et al (1988) described the increased risk of spontaneous fetal loss among women with T1 DM; however, the risk was significantly higher in poorly controlled diabetes. Dunne et al (227), showed a more favourable outcome in terms of miscarriage among women with T2 DM in an observational study over 12 years period, which reported a miscarriage rate of 8.8 % among the study cohort. Pearson DWM et al documented a 13.4% pregnancy loss in the first trimester in women with PGDM(228). Our study showed a high incidence of miscarriage among those screened for eligibility (17%), which led to a significant number of patients being ineligible for study entry. However, the overall miscarriage rate was not dissimilar to other international diabetes units with similar patient profiles.

The observed significant first trimester pre-recruitment pregnancy loss rate in the screened cohort has been very pertinent for informing the planned definitive clinical trial in this field.

The annual report of the Rotunda Hospital for 2016 showed that 1029 of patients attended the diabetes service of which, 54 had PGDM(229). The prevalence of PGDM in the Rotunda Hospital increased from 4.2 per 1000 births in 1998 to 6.1 per 1000 in 2016, driven mainly by a sharp increase in TII DM, (test for linear trend, $P < 0.0001$).

Table 3.1 describes the maternal characteristics between the aspirin and non-aspirin (control) groups. Twenty-three patients' data were available for analysis, as one withdrew consent on the first visit before randomisation. Eleven patients were T1 diabetics, 6 in the aspirin group and 5 in the non-aspirin group. Twelve patients were T2 diabetics, 7 in the aspirin group and 5 in the non-aspirin group. Generally speaking, patients were equally distributed between both groups when describing the type of PGDM. There was no statistical difference between groups when baseline characteristics were compared (ethnicity, gravidity, parity, smoking status, onset of conception or gestational age at recruitment, body mass index, systolic and diastolic blood pressure readings.

Figure 3.3 shows the distribution of body mass indices of the cohort. Only 9 out of 23 (39%) were normal weight (BMI of ≥ 18.5 and <25 kg/m$^2$). 7 (30%) were in the overweight category (BMI of ≥ 25 and <30 kg/m$^2$), 4 (17.3%) were obese (BMI of ≥ 30 and <40 kg/m$^2$). There were none in the underweight category (BMI of <18.5 kg/m$^2$) and 3 (13%) were morbidly obese (BMI of ≥ 40 kg/m$^2$). O'Dwyer et al described the body mass indices of 6000 pregnant women booking for antenatal care in Ireland in 2010. They found that 54% were normal weight (BMI of ≥ 18.5 and <25 kg/m$^2$), 28% were overweight (BMI of ≥ 25 and <30 kg/m$^2$), 13% were obese (BMI of ≥ 30 and <40 kg/m$^2$), 3% were underweight (B.MI of <18.5 kg/m$^2$), and 2% were morbidly obese (BMI of ≥ 40 kg/m$^2$).(230) Our population has a larger proportion of overweight, obese and morbidly obese patients at 30%, 17.3% and 13%, respectively.

Multiple studies have explored the effect of high BMI on platelet responsiveness to aspirin. Bryan et al,(231) conducted a study that was performed as part of the Genetic Study of Aspirin Responsiveness (GeneSTAR), which was designed to examine the genetic determinants of platelet responsiveness to low-dose aspirin therapy. It demonstrated that baseline platelet activation is higher among patients with high BMI compared to normal BMI patients and that platelet aggregation suppression to aspirin is less among the same group. In our study, and considering the small number of participants, there was one patient (patient 15) who had a suboptimal response to aspirin based on platelet aggregation testing with evidence of compliance to aspirin intake based on other assessment tools (diary card and pill count). She had a normal BMI with no known medication intake that can interfere with aspirin function. In the absence of alternate evidence of non-compliance with the study medication, non-

response in this case remains unexplained, but the definitive study will be well placed to ascertain the prevalence of non-response to aspirin and to explore potential contributory factors.

Different studies have suggested that low dose aspirin is insufficient for complete suppression of platelet activation(232, 233).

The dose of aspirin used in randomised trials ranges from 50mg to 150mg. For some women, particularly those with TII diabetes-related obesity, a 60mg dose may be too low to exert a full effect on $ThA_2$ production. Recent work by one of the collaborators for this submission(191) indicates that 20% of patients in Ireland with established coronary artery disease are inadequately 'protected' by aspirin, as evidenced by a thromboxane B2 level of >2.2 (indicating platelet aggregation of greater than 20%). Age, hypertension and weight were identified as risk factors for an inadequate aspirin response. Furthermore, randomised trials of this nature are potentially compromised by an inability to confidently ensure patient compliance.

The National Pregnancy in Diabetes (NPID) Audit published a paper in 2014(82) and included the range of BMI among patients with pre-existing diabetes in England and Wales and the results were not dissimilar from our population (normal BMI 31.4%, overweight 31.6% and obese categories 8.7%). .

Figure 3.6 shows a comparison between patients who attended the preconception clinic and compares them between types 1 and 2 diabetes groups. Nine out of 11 patients with T1 DM attended the clinic compared to 3 out of 12 in the T2 DM groups. The CEMACH report on pregnancy in women with type I and type 2 diabetes in 2003/2005 stressed the importance of consistent preconception care of a high standard(234).

### 4.2 Patients' narratives and compliance

The statistical reporting of randomised clinical trials usually focuses on individual recorded outcomes/variables, which are subsequently analysed in isolation (with the exception of multivariate modelling of prognostic variables and outcomes). However, this rarely reflects the full picture of a complicated medical situation. For this reason, patient safety narratives are also a key element in clinical study reporting, according to the International Conference on Harmonisation (ICH) tripartite guideline on the

Structure and Content of Clinical Study Reports (CSRs)(235). A CSR should contain brief narratives describing, for example, each death, serious adverse event or other events that are judged to be of special interest because of their clinical importance.

A patient narrative (case-report) therefore provides a full, clinically relevant, chronological account of the progression of a patient throughout the clinical study. Pilot studies are particularly suitable to narratives of individual patients by virtue of their small sample size. Case reporting can detail many different aspects of the patient's medical situation (e.g. patient history, physical examination, diagnosis, psychosocial aspects, follow up), which are not discernible, otherwise, from aggregated statistical analyses. Case reporting is particularly important for reporting on high-risk populations, such as those in our pilot study, providing a more comprehensive evaluation of the patients and but also informing us on subsequent larger randomised controlled trial.

Medication compliance issues are widespread, multifactorial and difficult to accurately quantify with traditional methods. In cardiology were the majority of aspirin trials were conducted, aspirin non-compliance and/ or non-responsiveness is known to be associated with worse clinical outcomes. In obstetrics, the ASPRE study demonstrated a significant reduction in preterm PE with 150 mg aspirin also described high aspirin compliance. Adherence was considered to be good if the reported intake of tablets was 85% and that was evident in 1294 of 1620 participants (79.9%)(134).

For this study compliance was judged based on the percentage of pills missed during the study period. A 15% cut off for missed pills was selected, above which any patient would be deemed non-compliant. Two patients were deemed non-compliant with the study protocol, judging by diary card and pill count. This was evident with the non-consistent pattern of platelet aggregation objectively demonstrated throughout gestation.

There is currently poor agreement on what constitutes acceptable aspirin compliance, the acceptable number of missed doses defined in trial reports use arbitrary cut-offs, not underpinned by a pharmacological basis. This is even more confusing in a pregnant population and the proportion of immature platelets is increased than in the non-pregnant population.

Aspirin has proven efficacy, but reliable compliance information is required for accurate assessment of effectiveness at least research context.
There are clear limitations in the information that can be gleaned from a study that subjectively assesses compliance without an objective mean to support it is validity and that was the purpose of using multiple testing in our study.

In our study there was a patient in the aspirin group (patient 15) who showed evidence of non-response to aspirin in which platelet aggregation was above the 20% mark all through pregnancy despite subjective tests to check for medication compliance indicating that she was taking aspirin as prescribed. Two patients (patient 4 and 12) showed a sub-optimal response to aspirin on two different visits based on platelet aggregation testing results,

The idea of increasing the aspirin dose in order to overcome resistance has been assayed in many studies, since there is some evidence that response to aspirin may be dose-dependent(38, 153, 236).

The dose of 75 mg of aspirin per day was selected on the basis of previous evidence of a dose-dependent benefit to therapy.(192) As mentioned previously in section (1.9.2) a low dose aspirin is considered to represent a dosage between between 60-150 mg/day.

### *4.2.1 Low platelet aggregation in patients not on antiplatelet therapy.*

Acquired platelet dysfunction is a frequent topic for discussion since the early 1970s. George & Shattil (1991) published a paper in the New England Journal of Medicine and explained the variables that can contribute to low platelet aggregation in patients not on antiplatelet therapy(237). This paper explored how pre-analytical variables such as patients' sex, age, race, mental stress, smoking and caffeine intake, in addition to food intake such as herbal remedies, garlic and alcohol, can affect platelet activity. Specimen collection is another factor mentioned in many papers(108, 238, 239). Blood collection using a standardised, less traumatic protocol, from the antecubital fossa, by clean venipuncture using minimum tourniquet pressure with a needle between 19–21 gauge was described and applied to minimise blood collection related laboratory errors(240). Samples was tested between 30 minutes and no more than 4 hours from blood collection(241).

The concentration of antagonist needed to sufficiently enhance platelet activity differs from one patient to another and in fact the same person may have different responses on repeated determinations(242).

Different medication groups have caused low platelet activity. A list of medications has been published by Kottke-Marchant and Corcoran (2002)(243); and included cardiovascular agents such as b-adrenergic blockers (propranolol), vasodilators (nitroprusside, nitroglycerin), diuretics (furosemide), calcium channel blockers, antimicrobials b-lactams (penicillins, cephalosporins), antifungal (amphotericin), antimalarial (hydroxychloroquine), chemotherapeutic agents (asparaginase, plicamycin, vincristine), psychotropics and anaesthetics, tricyclic antidepressants (imipramine), Phenothiazines (chloropromazine), local and general anaesthesia (halothane) and antidepressants, specifically Selective Serotonic- Releasing Inhibitors (SSRIs). In our study, we came across 3 patients (patients 7, 8 and 18) who had a low platelet activity initially at early gestation without being on antiplatelet therapy prior to study recruitment and randomisation. All three patients were not on any medications that were suggested to interfere with platelet activity according to Kottke-Marchant and Corcoran's list.

Careful consideration of the issue of potential platelet-function altering medications has led this group to exclude from recruitment for the definitive study those patients taking antidepressants in the form of SSRIs, a relatively common concomitant medication in pregnancy. The remaining listed medications are not typically used in pregnancy.

### 4.3 Serious adverse events (SAEs)

An important aspect to this thesis was to highlight the measures taken to ensure safety and wellbeing of subjects involved in the study and that was evident.

Reporting of SAEs is fundamental to detecting subject safety issues. Our trial protocol clearly stated the methods by which SAEs were monitored and reported, and provisions to ensure proper care for those experiencing unfavourable and unintended signs or symptoms associated with their participation in the clinical trial were a necessary component as well.

Accurate, complete, and on-time reporting of these events from the investigator to the sponsor was essential to the sponsor's effective evaluation and management of the study's safety data.

### 4.4 Delivery and neonatal outcome

There were more spontaneous vaginal deliveries in the aspirin group compared to the non-aspirin group (6/12 vs 1/9). We propose no biological explanation for this, as the impact of aspirin on mode of delivery cannot be determined by pilot data, but this highlights the importance of looking into delivery outcomes in the definitive study. The same applies to the number of emergency caesarean section deliveries (3/12 in the aspirin group compared to 1/9 in the non-aspirin group). Villa (2012)(232) published results of a randomised trial along with conducting meta-analysis to strengthen the power of his trial. It included all randomised controlled trials of women who had abnormal uterine artery Dopplers and they were on aspirin for that before 16 weeks' gestation. It demonstrated no difference in mode of delivery when aspirin-treated women with abnormal uterine artery dopplers was compared to those not treated with aspirin.

There were two cases of term PE that necessitated intervention and delivery. It is interesting to note that the two cases occurred in the aspirin group and were evident a few days after cessation of aspirin. It is not possible to draw any conclusion from that observation, since the study is not powered to do so, but this should be highlighted for the purpose of looking into the effect of aspirin on PE incidence in the definitive study.

There were 8 cases of PPH, 5 in the aspirin group and 3 in the non-aspirin group. Importantly, aspirin was discontinued at least 2-3 weeks before onset of labour and the platelet function testing demonstrated no ongoing antiplatelet effect 10 days post-cessation of aspirin. All patients who had PPH in both groups had mild to moderate loss (500-1000 ml post vaginal delivery, 1000-1500 post caesarean section), And no participant required blood transfusion. Askie (2007) and colleagues have(185), published a metaanalysis concluding that, antiplatelet therapy including aspirin was not associated with significant bleeding, maternal or neonatal adverse events.

There were two admissions to neonatal units, one in each group, and it was for 24-48 hours for management of transient tachypnea of the newborn in one case and management of low birth weight in the other.

### 4.5 Acceptability of the study

In this section we examine the acceptability and impact of the study on the participants. Healthcare quality has moved increasingly towards involving patients in their own care and different methods have been proposed for measuring patients' satisfaction. Crispin (2002) launched a national health survey in which all workers in Trust hospitals in England surveyed their patients on an annual basis and send the results to their regulators(244).

A potential criticism of our study is the fact that it is a pilot study and the number of patients returning the questionnaire was even smaller (19 patients). It is possible that the questionnaire was subject to selection bias as the small numbers of patients were quite familiar with the researcher and possibly wanted to provide a positive overview of their experience during the study. It is difficult to assess the extent of this potential bias.

### 4.6 Study strengths and weaknesses

A pilot study is, "A small-scale test of the methods and procedures to be used on a larger scale" (Porta, 2008)(245).

Pilot studies represent a fundamental phase of the research process. The purpose of conducting a pilot study initially before a larger scale randomised study is to examine the feasibility of an approach that is intended. It is important to bear in mind that a pilot study is not a hypothesis testing study.

The strength of our study relies on the fact it was a randomised open labelled, controlled study, which gives it a more realistic examination of recruitment, randomisation and implementation of interventions. It provided valuable feedback about the practices used to enhance data integrity, refinement of source documentation, informed consent procedure, data collection tools, SAEs reporting to ensure patients' protection and adherence to good clinical practice rules. There was a full complement of procedures involved in this study from clinical governance, to quality reassurance oversight to pharmacovigilance and safety reporting.

There is no doubt that this pilot study provided pivotal data in the ability to recruit the desired number required for the definitive study and the timing needed for that. The data gleaned from this feasibility work has proven highly informative in designing the definitive double-blinded RCT. We would anticipate a high rate of participation at this key site (77% of those eligible consented to participation) with demonstrable high compliance levels with study protocol and medication.

A weakness of this study lies in the fact that we used LTA to assess platelet aggregation and while the methods that measure directly the capacity of platelets to synthesise $ThA_2$ are certainly preferable, of these, the urinary levels of the $TxB_2$ metabolite, our laboratory resources could only offer LTA as a method of detection of platelet aggregation.
However, we have demonstrated in the Introduction chapter that platelet function tests are not equally effective in measuring aspirin's antiplatelet effect and that they correlate poorly amongst themselves.

### 4.7 Future study

This work has served as a pivotal contribution to a successful grant awarded by the Health Research Board for the purposes of conducting a definitive double-blinded placebo-controlled study (IRELAnD; Investigating the Role of Low Dose Aspirin iN Pregestational Diabetes). The definitive trial will be a phase III multicentre randomised double blinded placebo controlled trial in 8 large maternity units across Republic and Northern Ireland (Rotunda Hospital, Coombe for Women and Infants University Hospital, National Maternity Hospital, Cork University Maternity Hospital, University Hospital Galway, Our Lady of Lourdes Hospital, University Hospital in Limerick and Queen's University Hospital in Belfast). HRB funding has been secured for this study early in 2017 and the plan to start is in December 2018 as the pilot study provided a very valuable data on patient compliance and feasibility of recruiting patients with PGDM at a larger scale.

Participants are pregnant women with a background history of pre-pregnancy type I or type II diabetes of at least 6 months duration. Women will be randomized to one of 2 study groups: low-dose aspirin (150mg) or placebo once daily from the first trimester (initiated between 8+0 weeks' gestational age and 11+6 weeks) until 36 weeks or

sooner if early delivery is planned. Blinding will be achieved through use of active and matching placebo treatment.

The decision to opt for a 150mg dose of aspirin for the definitive trial was taken on foot of the recently published ASPRE trial (Rolnik et al NEJM 2017) which randomised women at high-risk for preterm PE to aspirin 150mg versus placebo. The 150mg dose is still considered ‘low dose’, but the authors make a convincing case for the observed dose-dependent response to 150mg noted in their pilot data.(193)

The primary objective of the IRELAnD study is to investigate the effect of aspirin therapy initiated in the first trimester of pregnancy in women with pregestational type I or type II diabetes on a composite clinical measure of placental dysfunction (PE, preterm birth less than 34 weeks, birth weight below the 10th centile or perinatal mortality), and the secondary objectives are to ascertain whether aspirin therapy may be effective in reducing the risk of PE among women with PGDM and microalbuminuria. It will also review the potential effect of aspirin therapy on maternal outcomes unrelated to hypertension such as mode of delivery and neonatal outcomes and lastly, it will assess the differences between the intervention and control groups with regard to histopathological evidence of microvascular disease within the placenta. All women with type 1 and 2 diabetes of more than 6 months duration, who attend the diabetic service in those 8 maternity units, will be invited to participate in the study. It is estimated that around 350 eligible candidates will be attending per year and our pilot study showed that 66% will be eligible for entry criteria which means the duration of study will be around 36 months for completion.

Power calculation is based upon the assumptions that PE has an incidence of approximately 20% in the study population, and that the incidence of preterm delivery before 34 weeks is 4%, birth weight below the 10th centile is 9% and perinatal mortality 3%. Given that outcomes may overlap or co-occur, we assume the baseline rate for the composite outcome of placental dysfunction to be 30% (PE incidence plus approximately 50% of the remaining outcome rates). A sample size of 283 participants in each arm of the study would be required to demonstrate a 35% reduction in the composite clinical measure of placental dysfunction with 80% power (two-sided type I error 0.05). We plan to enrol 566 research participants in total - (283 in each arm). The target will be 600 to allow for drop-out and loss-to follow-up.

Enrollment is expected to take up to 36 months. The duration of subject participation is a maximum of 39 weeks (28 weeks study medication and maximum 11 weeks' follow-up thereafter).

Owing to the theoretical concern that relates to the risk of peripartum haemorrhage, antiplatelet therapy in pregnancy is generally discontinued at 36 weeks' gestational age in the setting of anticipated term delivery. Guidelines recommend stopping aspirin 7 to 10 days before surgery. This represents a conservative approach, as it has been demonstrated that platelet aggregation recovers within 4 days of stopping aspirin.(244) If preterm delivery is anticipated, study medication will be discontinued to allow a 7-day aspirin-free interval between discontinuation and delivery, where achievable. No specific measures are mandated in the event that a patient has an emergency/ unplanned preterm delivery without having discontinued their study medication within 7 days.

Following enrollment, baseline (pre-treatment) assessments and randomisation, 4-weekly study visits will be scheduled until 32 weeks' gestation. A visit at 34 weeks will then be scheduled, followed by weekly prenatal study visits from 36 weeks' gestation until delivery. The end-of-study visit will take place within 5 days of delivery (while the patient is still in hospital). The follow-up visit will be scheduled for 6 weeks postpartum. Individual study participants will therefore participate in the study for a maximum of 39 weeks, in the event of recruitment at (minimum) 8+0 weeks, discontinuation of study medication at 36 weeks, delivery at (maximum) 41+0 weeks and final study visit 6 weeks postpartum.

Data will be contemporaneously entered onto an eCRF and uploaded onto a central consolidated database.

Placental histopathological examination will be performed at all sites according to a standardised study protocol.

### 4.8 Sub-studies

Platelet function testing will be performed on study participants at the Rotunda hospital only at 5 time points (pre-treatment, 20, 28 and 34 weeks' gestation and 3-5 days postpartum).

Dynamic platelet function testing will allow the following aspects of antiplatelet therapy in pregnancy to be studied:

- Assessment of participant compliance with the study medication in this subgroup
- Evaluation of dynamic changes that may be observed in platelet aggregation with respect to patient-specific variables that may affect platelet biology, such as BMI, age, concurrent illness or concomitant medication.

Recruitment: Based in the pilot study data we collected we expect to identify 4 potentially eligible participants per year with a projected recruitment rate of 36 per annum at the Rotunda site. Identification of 31 eligible patients in an 8-month period, 77% of whom were willing to participate. This will be comfortably achieved over a 36 months period. Applying the same adjustment to all sites, anticipated recruitment figures are:

**Table 4.1. Anticipated recruitment in IRELaND study per site per annum**

| Study Site | Expected Eligible per annum* | Expected Participants per annum | Expected Participants over 36 months |
|---|---|---|---|
| Rotunda | 45 | 36 | 108 |
| Coombe Womens | 54 | 45 | 135 |
| National Maternity | 37 | 31 | 93 |
| CUMH | 44 | 37 | 111 |
| Limerick | 22 | 18 | 54 |
| UCHG | 30 | 25 | 75 |
| Belfast | 60 | 50 | 150 |
| OLLH Drogheda | 13 | 11 | 33 |
| **TOTAL** | **305** | **283** | **759** |

*Expected number eligible per annum obtained from annual maternity reports. Adjustments made based on pilot data from the Rotunda hospital.

Compliance: As discussed previously before, compliance was assessed using patient diaries, pill counts and platelet function testing. Compliance was inferred from the continuous objective suppression of platelet aggregation observed for 10/13 (85%)

participants taking aspirin. Two subjects demonstrated variable aggregation patterns throughout gestation that were deemed to reflect non-compliance rather than non-response to aspirin, owing to consistency between the aggregometry patterns and periods of poor compliance demonstrated with pill counting and diary review. There was an unexplained case of non-responder.

### 4.9 Summary

This was an open-label study (low-dose aspirin versus no aspirin) at the Rotunda Hospital that served to inform the IRELAnD definitive intervention trial. The full complement of 24 participants was recruited within 8 months. Compliance was objectively measured through platelet function testing. Key inclusion and exclusion criteria were consistent with the definitive trial. The stated objectives were to determine:

1. Proportion of eligible women who agreed to participate in the pilot phase of a randomised trial of low dose aspirin therapy.

2. Compliance, as judged by diary card, pill count and platelet function monitoring.

3. Analysis of adverse events occurring after randomisation.

4. Logistical issues relating to recruitment, data collection and procurement, transport and storage of serum samples were tested.

A total of 47 women were screened for eligibility over an 8-month period. The proportion that presented prior to the gestational age cut-off of 12 weeks was 45/47 (96%). Of 47 women screened for eligibility, 31 were deemed eligible (66%). The most common criterion for ineligibility was miscarriage (17%) and only 2/47 (4%) of women screened were already taking aspirin. The participation rate among eligible women was 77% (24/31).

Platelet function testing was deemed to be informative for reflecting patient compliance with aspirin therapy, and for demonstrating non-response to aspirin among women judged to be compliant through other compliance-assessment methods. The pilot data presented in this thesis have set the scene for a large-scale clinical trial due to commence recruiting in 2019, the aim of which is to determine whether antiplatelet

therapy in pregnancy can reduce obstetric and perinatal morbidity among arguably the most medically vulnerable obstetric women.

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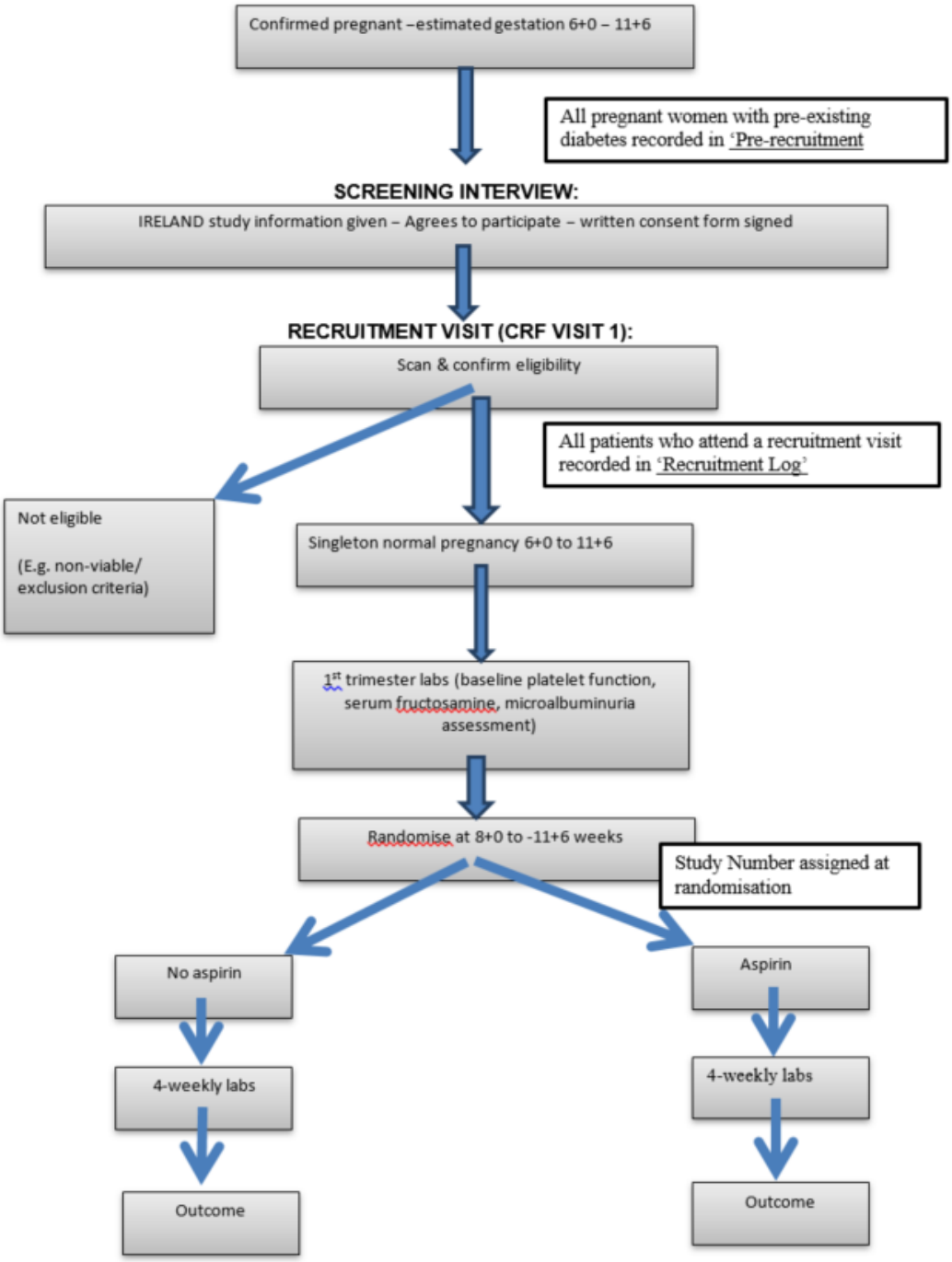
# Appendices

## Appendix 1: Flow diagram demonstrating study design



## Appendix 2: Patient Consent Form


**Lay title** **Aspirin in Diabetes Pregnancy (IRELAnD Study)**

**Principal investigator(s)** **Dr Fionnuala Breathnach, Rotunda Hospital**
**Prof Sean Daly, Coombe Hospital**

**Brief outline** of the project incl. benefits, possible risks, inconveniences and discomforts

This study involves investigating whether low-dose aspirin treatment, started early in pregnancy and continued until close to delivery, may reduce the risk of complications in pregnancies of women with pre-existing type 1 or type 2 diabetes. Taking part in this study would involve a selection process whereby you may be asked to take one low-dose aspirin tablet every night from early pregnancy until either 36 weeks or until you are instructed to stop. Alternatively, you may be asked to participate in this study but not to take aspirin. Some additional blood tests would be obtained from you, but would be timed to be taken only when you are having blood drawn for your routine prenatal tests. Additional urine collections would be required from you up to 3 times during the pregnancy. Aspirin has an excellent safety record in pregnancy. Further details are provided in the Patient Information Leaflet.

**I** (Patient's name) _____

**Voluntarily consent to taking part in this project which was explained to me by**
Mr / Ms / Dr _____

**I have received a Patient information sheet to keep and I fully understand the purpose, extent and possible effects of my involvement. I have been asked if I would like to have a family member or friend present while this project was explained to me.**

**I consent to the storage of blood samples for testing for the effect of aspirin on my blood during the study period and following completion of the study.**

**I understand that if I refuse to consent or withdraw myself from the study at any time without explanation, this will not affect my access to the best available treatment and care from this hospital.**

**I understand I will receive a copy of this consent form.**

Patient's signature _____

Date _____

Witness (not a project investigator) _____
Witness signature _____
Date _____

Researcher's signature _____
Date _____

## Appendix 3: Patient Information Leaflet



**THE IRELAND STUDY: INVESTIGATING the ROLE OF EARLY LOW-dose ASPIRIN IN DIABETES**

We are conducting a study in all the major obstetric centres in Ireland, aimed at investigating whether low-dose aspirin therapy started early in pregnancy and continued until close to delivery, may improve pregnancy outcomes in women with pre-existing diabetes. The role of aspirin in reducing complications such as preeclampsia has been investigated in many trials world-wide, involving over 30,000 women to date. Some trials have shown a benefit, while others have not. These trials have tended to start aspirin therapy relatively late in pregnancy, which may explain the fact that truly beneficial results have not yet been demonstrated in women with diabetes. Importantly, these large trials in pregnancy have not demonstrated any adverse effect from aspirin therapy. We wish to investigate whether starting aspirin earlier in pregnancy (before 12 weeks) may reduce pregnancy complications in women with diabetes. The first phase of this study ('Pilot Phase') is aimed at simply investigating how willing patients are to participate, how likely they are to take aspirin every day and to perform laboratory-based tests to examine the effect of aspirin on blood cells responsible for clotting.

**Eligibility:** You are being asked to participate in this study because you have type 1 or type 2 diabetes. Therefore, your pregnancy is considered at 'high-risk' for complications. Provided you have a single on-going pregnancy, are not already taking aspirin, have not had severe early-onset preeclampsia in a previous pregnancy, and have no known aspirin allergy or peptic ulcer disease, you may be considered eligible for participation in this study. If you are taking any medication, other than folic acid or prenatal vitamins, you should inform a member of the study team prior to enrolment.

**What will happen if I volunteer?**

If you volunteer to participate in this study, you will be randomly selected (by a computer programme) to either take low-dose aspirin or no aspirin, every night with your night-time insulin dose, starting between 8 and 12 weeks of pregnancy, and stopping the medication at 36 weeks, or sooner if instructed by your doctor. This is an 'open' study, meaning that both you and your medical team will be aware that you have been selected for the 'aspirin' group, or the 'no aspirin'

group. It is expected that a larger study that follows on from this initial phase will involve taking aspirin or 'placebo' (inactive tablet) in a blinded way.

As a study participant, the schedule of your prenatal visits to the Diabetes clinic will not be altered in any way, but when you are having your routine blood tests drawn in the Diabetes clinic, every 4 weeks, an additional sample will be taken along with the other bloods. It is not anticipated that an extra needle would be needed. The purpose of this extra test will be to confirm that aspirin is having an effect in slightly thinning your blood. It is hoped that this testing will allow the research team to determine whether women in the 'aspirin group' were continuing to take aspirin throughout pregnancy.

All women with pre-existing diabetes, whether study participants or not, are asked to provide a 24-hour urine collection at the beginning of pregnancy to test for kidney function. Study participants will be asked to provide 2 more samples, later in pregnancy.

Volunteering to participate in the study also involves allowing the research team to record the details of your medical and pregnancy history and the outcome of this pregnancy (See *Data Collection and Storage, below*).

**Antenatal Care:**

Participation in this study will not affect the schedule of your antenatal consultations, which will be provided in the Combined Obstetric Endocrine clinic. Women who have been taking aspirin therapy as part of the study will be advised to stop the aspirin at 36 weeks. If you develop obstetric complications, such as vaginal bleeding or preeclampsia, your doctor may decide to stop the aspirin treatment early. After delivery the afterbirth or placenta from your pregnancy will be examined under the microscope.

**Labour and delivery**

This study will not, in any way, affect your labour and delivery. **Aspirin therapy does not increase the risk of needing a Caesarean section. All options for pain relief, including epidural, will be available to women who participate in this study.** Care of your baby will be unchanged by your participation in this study and your baby will have a routine physical examination by a paediatric doctor prior to discharge. The placenta will be stored for later analysis to look for evidence of placental problems related to pre-eclampsia and growth restriction.

**Data Collection and Storage**

The data generated by this study will be stored on a central database located at the Royal College of Surgeons Ireland for statistical analysis. Your details will be recorded under a special study number, rather than your name, and access to the database will be password-protected to ensure access only by the study investigators. The research team may require access to your medical records and to those of your baby. The team that oversees the safety of this clinical trial may also require access to your medical records.

The blood and urine samples taken at the initial visit and later in pregnancy will form part of your medical record and be available to your doctors, except for the platelet function test, which tests how effective aspirin is at 'thinning' your blood. The results of this testing are not part of routine prenatal care and will be available to the researchers only. The afterbirth will be examined also. A coded study number will identify your sample - your name and any other information that allows you to be identified will not be used. These samples will be stored securely in the laboratory at the Coombe Womens and Infants' Maternity Hospital (for Coombe patients) and the Rotunda

Hospital (for Rotunda patients). The platelet function test will be done in an RCSI research laboratory. All samples will be destroyed once all study tests are completed.

**Are there any risks involved in participating in the study**?

While use of aspirin in non-pregnant people may be associated with a slight increased risk of bleeding, the use of a low dose as in this study has not been associated with increased risks for mum or for baby. None or very few side effects for mother or baby have been seen in large clinical trials to date. It is regarded as a safe drug to take in pregnancy up until 36 weeks at a low dose and is routinely used in 'high-risk' women without any problems. We will withdraw you from the study if you develop a severe medical complication or if your safety is at risk.

**If you agree to participate in this study, we will ask you to sign a consent form. This study is not compulsory and deciding not to participate will not affect your care in any way.** Furthermore, you are free to withdraw from the study at any stage. You will not have to justify your decision to anyone involved in the research. The more women that participate, the more valid the findings will be. We feel that the findings of this study will help us to decide whether we should be offering aspirin therapy for all women with pre-existing diabetes. We will keep your study details for up to 10 years once the study ends.

This study has been funded by Perinatal Ireland and the research protocol has been approved by the Institutional Ethics Board of each participating hospital.