ARTERIAL STIFFNESS, INFLAMMATORY AND ATHEROGENIC MARKERS IN GESTATIONAL DIABETES

SALMI BINTI AB AZIZ

UNIVERSITI SAINS MALAYSIA

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ARTERIAL STIFFNESS, INFLAMMATORY AND ATHEROGENIC MARKERS IN GESTATIONAL DIABETES

by

SALMI BINTI AB AZIZ

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LIST OF ABBREVIATION

PAI-1	plasminogen activator inhibitor- 1
PAI-2	plasminogen activator inhibitor- 2
IDDM	insulin dependent diabetes mellitus
NIDDM	non insulin dependent diabetes mellitus
GDM	gestational diabetes mellitus
OHAs	Oral hypoglycemic agents
HOMA	homeostasis model assessment
HOMA-IR	homeostasis model assessment for insulin resistance
CRP	C-reactive protein
hsCRP	high sensitivity C-reactive protein
TNF-α	tumor necrosis factor alpha
OR	odds ratio
SD	standard deviation
EDRF	endothelium derived relaxing factors
PWV	pulse wave velocity
AI	augmentation index
PWA	pulse wave analysis
CVD	cardio vascular disease
NO	nitric oxide
eNOS	endothelial nitric oxide synthase
AT_1	angiotensin II type 1 receptor
AT_2	angiotensin II type 2 receptor

Ang II	angiotensin II
OGTT	oral glucose tolerance test
MOGTT	modified oral glucose tolerance test
MRI	magnetic resonance imaging
NADPH	nicotinamide adenine dinucleotide phosphate
NSAIDs	non-steroidal anti-inflammatory drugs
m/s	meter per second
rpm	rotations per minute
UV	ultraviolet
PIH	pregnancy induced hypertension
pGDM	previous gestational diabetes mellitus
AD	pulse amplitude of the diameter
MIV	maximum incremental velocity
FMD	flow mediated dilatation
MAP	mean arterial pressure
PP	pulse pressure
aSBP	aortic systolic blood pressure
aDBP	aortic diastolic blood pressure
SBP	systolic blood pressure
DBP	diastolic blood pressure
NGT	normal glucose tolerance
IGT	impaired glucose tolerance
DM2	type 2 diabetes mellitus
IL-6	interleukin 6

IL-1	interleukin 1
BMI	body mass index
SEM	standard error of the mean`
bpm	beats per minute
vs	versus
Dd	diastolic diameter
IV	intravascular
IRS-1	insulin receptor substrate-1
HDL	high density lipoprotein
VLDL	very low density lipoprotein
sPLA2	secretory phospholipase A2
sICAM-1	soluble intercellular adhesion molecule-1
sVCAM-1	soluble vascular cellular adhesion molecule-1
VSMC	vascular smooth muscle cells

LIST OF SYMBOLS

$\geq$	equal or more than
$\leq$	equal or less than
$>$	more than
$<$	less than

KETEGANGAN SALUR DARAH, PENANDA KERADANGAN DAN 'ATHEROGENIC' DALAM PENYAKIT KENCING MANIS SEMASA HAMIL

ABSTRAK

Kencing manis semasa hamil merupakan salah satu penyakit yang biasa dihidapi semasa kehamilan, dengan kadar 1-18% untuk semua kehamilan bergantung kepada populasi. Peningkatan ketegangan salur darah biasa berlaku pada pesakit kencing manis 'Type 1' dan 'Type 2' dan telah dilaporkan dalam banyak laporan kajian. Tahap ketegangan salur darah juga telah ditunjukkan dapat mengesan penyakit-penyakit jantung. Pemeriksaan tahap ketegangan salur darah dapat mengesan lebih awal penyakit-penyakit jantung dan seterusnya dapat membantu dalam megawal penyakit tersebut. Terkini, analisa gelombang denyutan dan halaju gelombang denyutan telah digunakan dan dibuktikan sebagai salah satu cara untuk mengukur tahap ketegangan salur darah yang tidak menyakitkan. Selain itu, penanda keradangan and 'atherogenic' juga boleh memberi kesan kepada fungsi salur darah dan ketegangan salur darah. Oleh sebab itu, kajian ini juga mengukur 'tumor necrosis factor alpha' (TNF-α), 'high sensitivity C-reactive protein' (hsCRP) and 'plasminogen activator inhibitor-1' (PAI-1) sebagai salah satu perimeter.

Tujuan kajian ini dijalankan adalah untuk mengetahui samada pesakit yang menghidapi kencing manis semasa hamil mempunyai kaitan dengan peningkatan tahap ketegangan salur darah dan peningkatan penanda keradangan dan 'atherogenic' berbanding pesakit normal dalam lingkungan umur yang sama.

Kajian ini merupakan kajian secara rentas yang melibatkan 53 wanita hamil pada peringkat trimester ketiga kehamilan. Wanita hamil ini terdiri daripada 31 wanita dengan kehamilan yang normal dan 22 wanita dengan penyakit kencing manis semasa hamil. Pesakit dibahagikan kepada dua kumpulan ini berdasarkan keputusan ujian '75 g Ujian Air Gula yang Dimodifikasi (MOGTT)'. Pesakit diklasifikasikan sebagai penghidap kencing manis semasa hamil sekiranya tahap glukosa selepas mereka puasa sepanjang malam adalah lebih daripada 6.1 mmol/L dan/atau tahap glukosa selepas dua jam pengambilan air gula adalah lebih daripada 7.8 mmol/L. Tahap ketegangan salur darah dalam kajian ini diukur dengan menggunakan prinsipal analisa gelombang denyutan dan halaju gelombang denyutan menggunakan Sphygmocor. Analisa gelombang denyutan juga digunakan untuk mendapatkan bacaan bagi indeks augmentasi (AIx). Tekanan darah aortik, tekanan darah brakial, tekanan denyutan aortik, min bagi tekanan arterial, indeks jisim badan dan paras serum kolesterol secara total juga dicatatkan. Paras penanda keradangan (TNF- α and hsCRP) dan 'atherogenic' (PAI-1) ditentukan menggunakan ELISA.

Purata umur dan minggu kehamilan antara wanita hamil yang normal dan wanita yang menghidapi kencing manis semasa hamil tidak menunjukkan sebarang perbezaan yang signifikan (31.1±5.68 *vs.* 32.9±8.46 tahun; 29.0±2.43 *vs.* 29.6±1.54 minggu untuk masing-masing). Tahap glukosa semasa berpuasa dan selepas dua jam minum air gula adalah tinggi secara signifikan pada wanita yang menghidapi kencing manis semasa hamil (3.94±0.44 vs. 5.27±1.19 mmol/L, $p<0.001$; 5.68±1.10 vs. 9.66±1.76 mmol/L, $p<0.001$). Prinsipal halaju gelombang dan indeks augmentasi tidak berbeza secara signifikan antara wanita hamil secara normal dan wanita yang menghidapi kencing

manis semasa hamil. Walau bagaimanapun, tahap TNF-α, PAI-1 and hsCRP adalah tinggi secara signifikan pada wanita yang menghidapi kencing manis semasa hamil [TNF-α; 0.81 (0.15) *vs.* 0.72 (0.13) pg/ml, PAI-1; 54.48 (13.07) *vs.* 36.16 (15.58) ng/ml, hsCRP; 7.91 (1.16) *vs.* 6.70 (1.45) mg/L]. Tiada perbezaan yang signifikan antara kedua-dua kumpulan kajian bagi bacaan tekanan darah aortic dan brakial, min bagi tekanan darah arterial, indeks jisim badan dan paras serum kolesterol secara total.

Paras penanda keradangan TNF-α dan hsCRP, dan penanda 'atherogenic' PAI-1 meningkat pada wanita yang menghidapi kencing manis semasa hamil berbading wanita hamil secara normal yang mempunyai purata umur yang sama. Tiada perbezaan secara signifikan pada tahap ketegangan salur darah, iaitu prinsipal halaju gelombang dan indeks augmentasi, yang dapat dilihat antara kedua-dua kumpulan kajian.

ARTERIAL STIFFNESS, INFLAMMATORY AND ATHEROGENIC MARKERS IN GESTATIONAL DIABETES

ABSTRACT

Gestational diabetes mellitus (GDM) is one of the common medical complications occurring during pregnancy, affecting approximately 1-18% of all pregnancies; depending on the population. Increased arterial stiffening has been demonstrated consistently in both Type 1 and Type 2 diabetes in a number of studies. Arterial stiffness has been shown to precede cardiovascular diseases. The assessment of arterial stiffness allows early detection of cardiovascular disease and may help in preventive strategies. Pulse wave velocity (PWV) and pulse wave analysis (PWA) are non invasive method used to assess arterial stiffness. Inflammatory and the atherogenic markers may influence vascular function and arterial stiffness. The objectives of this thesis were to determine whether gestational diabetes mellitus (GDM) is associated with increased arterial stiffness, inflammatory and atherogenic markers compared to age and gestational age matched controls. Inflammatory and atherogenic markers measured in this study include tumor necrosis factor alpha (TNF-α), high sensitivity C-reactive protein (hsCRP) and plasminogen activator inhibitor-1 (PAI-1) as a parameters.

This is a cross sectional study involving 53 pregnant women in the early third trimester of pregnancy consisting of 31 women with normal pregnancy and 22 women with GDM. Subjects were divided based on the 75 g Modified Oral Glucose Tolerance Test (MOGTT) results, and considered as GDM if the fasting glucose level after an overnight

fast ≥ 6.1 mmol/L and/or the level after 2 hours of glucose intake ≥ 7.8 mmol/L. Arterial stiffness was assessed non invasively using the principles of PWA and carotid femoral PWV using the Sphygmocor device. PWA was used to obtain the parameter percent augmentation index (AI). Aortic blood pressure, brachial blood pressure, aortic pulse pressure, aortic mean arterial pressure, body mass index (BMI) and serum total cholesterol were also recorded. The inflammatory (TNF- α and hsCRP) and atherogenic (PAI-1) markers were measured using the principle of Enzyme-linked Imunosorbent Assay (ELISA).

Mean ages and gestational ages for the control and GDM groups showed no significant differences (31.1±5.68 *vs.* 32.9±8.46 years; 29.0±2.43 *vs.* 29.6±1.54 weeks respectively). Fasting and two hours glucose levels were significantly higher in GDM; (3.94±0.44 vs. 5.27±1.19 mmol/L, $p<0.001$; 5.68±1.10 vs. 9.66±1.76 mmol/L, $p<0.001$). PWV and AIx were not significantly different between normal and GDM pregnancies. However, TNF-α, PAI-1 and hsCRP levels were significantly higher in GDM compared to controls [TNF-α; 0.81 (0.15) *vs.* 0.72 (0.13) pg/ml, PAI-1; 54.48 (13.07) *vs.* 36.16 (15.58) ng/ml, hsCRP; 7.91 (1.16) *vs.* 6.70 (1.45) mg/l]. There were no significant differences between them for aortic and brachial BP, MAP, BMI and serum total cholesterol.

In conclusion, the inflammatory markers TNF-α and hsCRP, and the atherogenic marker PAI-1 were elevated in GDM compared to age and gestational age matched controls. This study found no association between arterial stiffness and GDM.

CHAPTER 1

LITERATURE REVIEW

CHAPTER 1

LITERATURE REVIEW

Pregnancy

Pregnancy is the period during which a woman carries a developing fetus, normally in the uterus (Oxford University Press, 2007). Gestation is the period during which a fertilized egg cell develops into a baby that is ready to be delivered (Oxford University Press, 2007). Gestational period normally lasts for 40 weeks and is divided into three trimester that are trimester I from 1^{st} to 12^{th} weeks, trimester II from 13^{th} to 28^{th} weeks and trimester III from 29^{th} to 40^{th} weeks. During this period, many changes and adaptations occur in the woman's body such as cardiovascular, respiratory and endocrine systems to accommodate the needs of the mother and fetus.

1.1 Physiological changes during pregnancy

1.1.1 Cardiovascular changes

a) Heart rate

Maternal heart rate may increase as early as four weeks of gestation, with an approximate 20% increase seen by seven weeks of gestation (Campbell, 2000). This heart rate increase occurs as a response to falling systemic vascular resistance (Duvekot *et al.*, 1993).

b) Blood pressure

Blood pressure falls approximately 10% by seven to eight weeks of gestation (Clapp *et al.,* 1988) due to peripheral vasodilatation (Phippard *et al.,* 1986). Systemic vascular resistance decreases by 20% in the second trimester because of the hormonal influences of oestrogen and progesterone and it remains low until term (Campbell, 2000). In the supine position, the gravid uterus may compress the inferior vena cava leading to significant reduction in maternal mean arterial pressure.

c*) Central Haemodynamics*

Due to increase in blood volume during pregnancy, stroke volume increases by 20% as early as five weeks of gestation and continue to increase to 30% at the end of the second trimester (Campbell, 2000). Cardiac output begins to increase from five weeks of gestation because of increased heart rate and stroke volume. The cardiac output continues to increase to 40% by the end of first trimester and reaches 50% by term (Mabie *et al.,* 1994).

1.1.2 Hematological changes

a) Blood volume

Plasma volume increases up to 15% as early as the first trimester to as high as 50% in the second trimester. This condition is the direct result of maternal oestrogen (which increases plasma rennin) and progesterone (which increases aldosterone production) as well as fetal hormones such as dehydroepiandrosterone. These hormonal influences lead to sodium and water retention. During pregnancy, red cell volume decreases in the first trimester but increases to 30% above pre pregnancy levels by term (Campbell, 2000).

The increase in red cell volume meets the extra oxygen demands of the mother and fetus. The normal range of hemoglobin in pregnancy is between 11- 12 g/dL. World Health Organization recommends iron supplementation if the level of hemoglobin is less than 11.0 g/dL. Hematocrit decreases by 15% at the second trimester of pregnancy. White cell count increases in pregnancy and the normal range of white cell count for pregnancy is between 5000- 15000/mm^3 (Carlin and Alfirevic, 2008). The platelet count usually falls in pregnancy (O'Brien, 1976). Mild thrombocytopenia (100000-150000/mm^3) is present in approximately 8% of pregnancies and has been termed 'gestational thrombocytopenia' (Burrows and Kelton, 1990).

b) Coagulation system

In pregnancy, the circulating levels of factors VII, VIII, IX, X and XII, fibrinogen and von Willibrand factor increases, factor XI decreases, while prothrombin and factor V remain unchanged (Hellgren, 1996). The natural anticoagulants antithrombin III and protein C levels are unchanged or increase, and protein S levels falls (Hellgren, 1996). Fibrinolytic activity is known to decrease, mainly due to the marked increase in plasminogen activator inhibitors, PAI-1 and PAI-2 (Davis, 2000). The combination of all these changes increases the risk of thrombosis during pregnancy and puerperium (Carlin and Alfirevic, 2008).

1.1.3 Respiratory changes

a) Mechanics of ventilation

As the pregnancy progresses, the uterus expands upwards and changes the chest shapes. The lower ribs 'flare' due to looser ligaments under the influence of progesterone. The thoracic circumference increases by 8% (Contreras *et al.*, 1991), and both the transverse and the anteroposterior diameters increase by 2 cm. These changes increase the chest excursion and lead to a 5 cm elevation of the diaphragm (Elkus and Popovich J, 1992).

b) Ventilation

During pregnancy, oxygen consumption increases by 30- 50 mL/min. Two- third of the increase meets additional maternal requirements (mainly the kidney) and one- third is for the developing fetus (Alaily and Carrol, 1978). The increase in oxygen consumption is associated with a 40% increase in ventilation secondary to a progressive rise in tidal volume of 200 ml (from 500 ml to 700 ml) (Cugell *et al.,* 1953). Respiratory rate increases between 14- 15 breaths/min (Pernoll *et al.,* 1975). Minute ventilation increases by approximately 40%, with tidal volume, from 7.5 L/min to 10.5 L/min (Spatling *et al.,* 1992). However, the reason for the ventilation increase is still unclear. Progesterone may play a role by lowering the threshold (Wilbrand *et al.,* 1952) and/or increase the sensitivity (Skatrud *et al.,* 1978) of the respiratory centre to carbon dioxide, or act independently as a primary stimulant of these two mechanisms (Lunell *et al.,* 1978).

1.1.4 Endocrine changes

a) Progesterone

Progesterone is mainly produced by the placenta. The level of progesterone increases during pregnancy. Progesterone mainly inhibits uterine smooth muscle contraction and decreases prostaglandin formation. These functions allow the fetus to grow with the expanding uterus. As progesterone levels increase, other smooth muscle in the body may also be affected, such as the lower esophageal sphincter, which results in increased heartburn and acid reflux, especially in the later stages of pregnancy. Progesterone also softens the cartilage and may be responsible for the commonly occurring hip and pubic bone pain. This hormone also causes tenderness in the breasts and the bloated feeling experienced by many women throughout pregnancy (Catalano, 1991).

b) Oestrogen

Oestrogen level also increases during pregnancy and are produced primarily by the placenta. Oestrogen is crucial to the reproductive success of woman. Oestrogen has a major role during pregnancy of building tissue. One role of oestrogen during pregnancy is to regulate the production of progesterone over the full term. As oestrogen is produced by the placenta, progesterone production is stimulated and regulated. Oestrogen is a major factor in the increased size of the uterus and thickening of the uterine wall. During the pregnancy, the uterus increases in size to accommodate the developing pregnancy. Oestrogen is responsible for increased blood, lymphatics and nerve supply to the uterus, and throughout the body. The considerable increase in the number and size of vessels in the uterine vascular bed causes a decrease in the uterine vascular resistance. As a result, blood flows to and from the uterus more freely. Prolactin, the hormone that allows for

postpartum lactation, also increases throughout pregnancy, and its production is thought to be stimulated by the increasing levels of oestrogens (Sembulingam *et al.,* 2001).

c*) Insulin*

Insulin is a natural hormone produced by the beta cell of the islets of Langerhans in the pancreas and plays a role in controlling the level of glucose in the blood. Cells cannot utilize glucose without insulin. The failure of insulin production or reduced insulin sensitivity leads to diabetes mellitus. The diminished ability of cells to respond to the action of insulin in glucose uptake from the bloodstream into muscle and other tissues is called insulin resistance. During pregnancy, insulin sensitivity declines by 50% in the third trimester (Catalano, 1991), which may be mediated by increases in various hormones such as oestrogen, progesterone, β-human chorionic gonadotrophin, human placental lactogen, growth hormone and cortisol (Ryan *et al.,* 1988).

1.2 Diabetes mellitus

Diabetes mellitus affects over 150 million people worldwide and this number is expected to double by the year 2025 (Genuth *et al.,* 2003). Diabetes mellitus is a chronic disease characterized by insulin deficiency, resulting in glucose intolerance. Other than diabetes mellitus, the word diabetes is also used for another disease, which is diabetes insipidus. As diabetes insipidus is rare, diabetes mellitus is usually known simply as diabetes. Diabetes mellitus can be divided into insulin dependent diabetes mellitus (IDDM) or Type 1 diabetes mellitus, and non insulin dependent diabetes mellitus (NIDDM), also known as Type 2 diabetes mellitus. Type 1 diabetes mellitus is the result of an absolute deficiency in insulin secretion due to autoimmune destruction of the β

cells of the pancreas. Type 2 diabetes mellitus is most commonly due to tissue resistance to insulin action and relative insulin deficiency. Gestational diabetes mellitus (GDM) is impaired glucose tolerance that occurs during pregnancy.

Insulin is a protein that regulates the transport of glucose from the plasma into the cytoplasm of cells to provide energy for body use. Usually after a large meal, insulin level in the blood increases, this induces the tissues to take up and store glucose. Without enough insulin, glucose builds up in the bloodstream instead of going into the cells. Cells of the body are unable to use this glucose for energy despite high levels in the bloodstream. This is detected by the central satiety centre, leading to a sense of hunger. In addition, the high level of glucose in the renal tubule causes osmotic diuresis and manifest as increase in urination. This leads to decrease in extracellular volume and excessive thirst.

The symptoms of diabetes mellitus include frequent urination, excessive thirst, extreme hunger, increased fatigue, unusual weight loss, irritability, and blurred vision. Diabetes mellitus is often linked to other chronic diseases such as atherosclerosis, which leads to cardiovascular diseases such as stroke and hypertension, retinopathy, glaucoma, cataracts, gangrene, and kidney failure.

1.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is also known as juvenile or insulin dependent diabetes mellitus. It begins more commonly in childhood or adolescence. Only approximately 10% of type 1 diabetes patients were above 60 years old.

For type 1 diabetes, patients usually depend on exogenous insulin. These patients may require several injections of different types of insulin during the day to keep their blood sugar levels within a normal range. There are two conditions that may lead to Type 1 diabetes that are firstly, insufficient insulin production because of pancreas damage particularly at beta cell of Islet of Langerhans and secondly, abnormal form of insulin produced.

1.2.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is the more common type of diabetes, accounting for 85-90% of all those diagnosed with diabetes mellitus. This form of diabetes normally occurs in adults and is also known as non insulin dependent diabetes mellitus (NIDDM). Type 2 diabetes mellitus is a chronic metabolic syndrome that occurs due to insulin resistance. This leads to hyperglycaemia (increased blood sugar levels) and deranged metabolism of carbohydrate, fats and proteins. These abnormalies result in decreased glucose transport into muscle, increased liver glucose production, and increased breakdown of fat.

Obesity and aging are common risk factors for type 2 diabetes. The incidence of type 2 diabetes increases with age. Most patients develop type 2 diabetes mellitus after 40 years of age. Genetics is another risk factor of type 2 diabetes. Genetic predisposition plus unhealthy lifestyle such as excess intake of fat and sugar or inadequate physical activity increase the risk of diabetes mellitus.

1.2.3 Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is defined as glucose intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy (Metzger *et al.,* 1998). GDM usually appears between the 24th to 28th gestational weeks. The blood glucose level usually will return to normal after delivery. GDM is one of the most common medical complications occurring during pregnancy (Metzger *et al.,* 1998). The prevalence of GDM may range from 1%-4% worldwide depending on the population and the method of screening (ADA, 2000). Malaysian prevalence of GDM was reported to be approximately 18.3% (Idris *et al.,* 2007).

Gestational diabetes mellitus occurs when the body is unable to make effective use of insulin during pregnancy (Chandrasoma *et al.,* 1995). According to Chandrasoma *et al.* (1995), gestational diabetes is pathophysiologically similar to non insulin dependent diabetes mellitus. GDM carry risks for both the fetus and the mother. The high blood glucose levels pass through the placenta, causing the baby to also have high blood glucose levels. The baby's pancreas will respond by producing additional insulin to lower the blood glucose. More glucose will be converted to energy than the fetus' needs, and the extra energy is stored as fat, causing macrosomia (large babies), which is one of the common effects of GDM. The newborn may also experience hypoglycemia (shortly after birth), breathing problems and prolonged jaundice. These babies experience hypoglycemia occurring shortly after birth because of the high insulin levels of the fetus.

On the mother's side, women with GDM have a problem of obstructed labor due to large babies. Obstructed labor leads to other consequences which include ceasareans delivery, birth injuries to the mother and newborn and low apgar scores. Cardiovascular complications such as pregnancy induced hypertension and preeclampsia also occur more frequently in GDM compared to non GDM pregnancies (Paradisi *et al*., 2002).

Women with GDM have increased risk of subsequently developing diabetes mellitus late in life. According to O'Sullivan *et al.* (1984), one–third to one-half of women with a history of GDM will develop type 2 diabetes mellitus within three to five years, and 70% will develop type 2 diabetes mellitus if followed up for more than ten years. Metzger *et al.* (1993) also reported that women with history of GDM have a risk between 18-50% of developing type 2 diabetes within 5 years following pregnancy.

Management of GDM can be divided into four main aspects, which include monitoring, non-pharmacological interventions, pharmacological intervention and obstetrics management. Essentially, maternal metabolic progression should be monitored. Daily self-monitoring of blood glucose is very important prior to the follow up visits (ADA, 2000). Glucose level should be as near as possible to normal range: fasting plasma glucose level should be < 5.3 mmol/L; post-prandial levels at first and second hour should be < 7.8 mmol/L and < 6.7 mmol/L respectively (Metzger and Coustan, 1998). Fetal monitoring should be performing routinely which include maternal monitoring of fetal movement, cardiotocography and ultrasonography. The presence of fasting hyperglycemia >5.8 mmol/L may be associated with an increased risk of intrauterine

fetal death during the last 4-8 weeks of pregnancy (ADA, 2000), thus the importance of monitoring maternal blood glucose levels and fetal condition.

Secondly, non pharmacological therapy includes diet and exercise. Nutritional counseling is very important in patients with GDM. An experienced and qualified dietician should perform the counseling. It is important to provide adequate calories for maternal needs, fetal growth and adequate weight gain while avoiding hyperglycemia and ketosis (ADA, 2000). Exercise is one of the important steps in the management of GDM. Exercise has been reported to improve glycaemic control in patient with GDM (Bung *et al.,* 1991).

Pharmacology therapies may include use of insulin and oral hypoglycemic agents (OHAs). Insulin should be considered when the diet and exercise are inadequate to maintain optimal level of blood glucose. Insulin therapy should be initiated if the fasting blood sugar is more than 5.3 mmol/L or two-hour post prandial level is more than 6.7 mmol/L (ADA, 2000). Human insulin should be the insulin of choice. OHAs is not recommended in pregnancy because of the potential side effects to the fetus, particularly that of hypoglycemia and fetal anomalies (ADA, 2000).

Another component to GDM management is obstetric management. GDM patients should be reassessed after at least 6 weeks post delivery (ADA, 2000). If sugar levels are normal post partum, these patients should be monitored at least 3 yearly. They should also be informed of their risk of developing type 2 diabetes and the potential symptoms (Gabbe *et al.,* 1998).

Most research that study the effect of GDM on vascular function, inflammatory and metabolic markers focused on women with previous history of GDM and compare them to those with history of uncomplicated pregnancy. Normoglycemic women with a history of GDM have been reported to have increased insulin resistance and decreased endothelium-dependent vasodilatation compared to women who had a history of uncomplicated pregnancy (Kousta *et al.*, 2003). Heitritter *et al.* (2005) reported that 25 nondiabetic women with previous gestational diabetes showed increased biochemical and hemodynamic markers of cardiovascular disease compared to 23 nondiabetic women without a history of gestational diabetes. Subjects with a history of GDM also had significantly higher diastolic blood pressure and mean arterial pressure compared to women with no history of GDM. Metabolic parameters such as fasting blood glucose, triglyceride, and homeostasis model assessment (HOMA) were also significantly higher in women with history of GDM. Compared to the control group, women with a history of GDM had higher levels of inflammatory markers (C-reactive protein, interleukin-6, and plasminogen activator inhibitor-1) and lower levels of adiponectin. These indicate that women with prior GDM have subclinical inflammation, are hypoadiponectinemia, and display early vascular dysfunction, all of which may increase their risk of developing cardiovascular diseases.

1.3 Arterial compliance and arterial stiffness

1.3.1 Structure of the arteries

The circulation of the blood from the heart passes through elastic arteries, muscular arteries, arterioles, capillaries, venules and veins. All arteries comprised of three distinct layers, which are the tunica intima, tunica media and tunica externa. The outermost layer is known as the tunica externa or tunica adventitia, and is composed of connective tissue. Inside this layer is tunica media, which is made up of smooth muscle cells and elastic tissues. The tunica intima, or innnermost layer, consists of a layer of endothelial cells separated from the inner layer by a narrow layer of connective tissues. This layer is in direct contact with the blood flow. Lastly, the hollow internal cavity in which the blood flows is called the lumen.

The aorta and arteries have more elastic tissues while the arterioles have more smooth muscles. The arterial branches become narrower and their walls become thinner when reaching the periphery from the heart. The diameters of arteries arising from the aorta are about 25 mm and in the arterioles is 0.3 mm. The capillaries have diameter of about five to eight micrometer (Sembulingan *et al.,* 2000).

1.3.2 Function of the arteries

The blood flows through two divisions of the circulatory system which is the systemic circulation and pulmonary circulation. Systemic circulation starts when the oxygenated blood is pumped from the left ventricle and passes through a series of blood vessels of the arterial system until it reaches the tissues. The blood vessels of the arterial system

are the aorta, arteries (large and small arteries) and arterioles. The arterioles branch into capillaries. The capillaries are responsible for exchange of various substances between blood and the tissues. The deoxygenated blood enters the venous system and returns to the right atrium of the heart. The venous system consists of venules, veins and the vena cava. Pulmonary circulation is the circulatory of blood from the right ventricle to the lungs through the pulmonary artery. Exchange of gases occurs in the lungs between blood and the alveoli. The oxygenated blood then returns to left atrium through the pulmonary veins (Sembulingam *et al.,* 2000).

In both circulatory systems, arteries play an important role for blood distribution. Large arteries branching off the aorta distribute blood flow to specific organs. Examples of large arteries are the carotid, mesenteric and renal arteries. Once a large artery reaches the organ it branches into smaller arteries that distribute blood flow within the organ. Small arteries and arterioles are involved in the regulation of arterial blood pressure as well as blood flow within the organ (Chandrasoma *et al.,* 1991).

The arterial tree also function to cushion the pulsation generated by the heart so that the capillary blood flow is continuous. In the arterial system, the cushioning and conduit functions are combined. This combination of functions leads to pressure wave travel and reflection (O'Rourke, 1982).

1.3.3 Arterial compliance and arterial stiffness

Arterial compliance is an index of vascular health and is defined as the ability of the arterial to expand due to a given change in the arterial pressure (O'Rourke and Mancia, 1999). Therefore arterial compliance is pressure dependent and varies inversely with the level of mean arterial pressure within the physiological range. The measurement of arterial compliance has received increased attention because of its association and predictor value for cardiovascular diseases (Havlic *et al.,* 2003, Laurent *et al.,* 2001).

Arterial stiffness is a dysfunctional property of the arterial circulation that precedes the development of clinical cardiovascular disease (Laurent *et al.,* 2001). Arterial stiffness is determined by functional and structural components related to the intrinsic elasticity of the artery. Elastic property is the ability of the artery to stretch when a stress is given and cope with a given stress to return to its original shape when excess stress is removed. The stiffer the artery, the lesser its compliance and vice versa.

Arterial stiffness is an independent predictor of cardiovascular morbidity and mortality (Hansen *et al.,* 2006, Laurent *et al.,* 2001). Arterial stiffness is a major contributor to atherosclerotic and small vessel disease and thus to occurrence of stroke, myocardial infarction, and renal failure. Laurent *et al.,* (2000) reported a significant association of aortic stiffness assessed using the the parameter pulse wave velocity with cardiovascular mortality in patients with cardiovascular diseases [odd ratio (OR) of 1.34 with every 5 m/s increment in PWV]. Hansen *et al.* (2006) stated that for each 1-SD increment in aortic pulse wave velocity, the risk of cardiovascular disease increases by 16 % to 20 %.

Arterial stiffness has been shown to be increased in pregnant women with pregnancy induced hypertension, preeclampsia, and diabetic patients (Tihtonen *et al.,* 2006, Macaedo *et al.* 2008, Khalil *et al.,* 2009, Robb et al., 2009, Veikko *et al.,* 1995, Lehmann *et al.,* 1992). When this study was planned and started, there were no studies conducted to determine if GDM is associated with increased arterial stiffness, thus this is one of the objectives of this study. In our study, arterial stiffness will be measured using the parameters carotid femoral pulse wave velocity and augmentation index derived from pulse wave analysis.

1.3.4 Factors affecting arterial stiffness

Arterial compliance and arterial stiffness has been reported to be affected by several factors such as age (Anette *et al.,* 2008), gender (Maxwell, 1998), physical activity (Tanaka *et al.,* 2000), obesity (Satoru *et al.,* 2009), smoking (Nirandeep *et al.,* 2006), hypertension (Benetos *et al.,* 1997), diabetes mellitus (Lehmann *et al.,* 1992), hypercholesterolemia (Wilkinson *et al.,* 2002) and genetic factors (Mitchell *et al.,* 2005). Some of the stated factors are described below.

a) Age

Increased stiffness of the arteries is commonly associated with advancing age (Anette *et al.,* 2008). With ageing, the aorta and central elastic arteries dilate and stiffen. Dilatation and stiffening are most marked in the proximal aorta and its major branches such as the brachiocephalic, carotid and subclavian arteries. Increased arterial stiffening with age is apparent as an increase in pulse wave velocity (O'Rourke, 2007). A typical value of carotid femoral PWV in a 20 years old is approximately 5 m/s while in 80 years old is

approximately 12 m/s (2.4 fold increase over 60 years) (Laurent *et al.*, 2006). Structural and functional changes associated with aging may impair the compliance of the arterial circulation. In terms of vascular functional changes, altered regulation of vascular tone due to reduced presence or production of endothelium derived relaxing factors (EDRF) could increase arterial stiffness. Endothelial release of nitric oxide has been shown to be impaired with aging (Gerhard *et al.*, 1996). Reduced nitric oxide would not only produce vasoconstriction, which increases arterial stiffness, but would also promote vascular smooth muscle growth that can lead to the increase of arterial stiffness.

Structurally, the thickening of the intima and media layers of the vascular wall play a role in the increased stiffness seen with ageing. Nagai *et al.* (1998) reported increased thickness of intima-media with increasing age. With advancing age, the distensibility of an artery decrease in proportion to the increase in collagen in the media, and to a lesser extent in the intima. Increased pulse wave velocity has been linked to structural alterations in the vascular media which include increased collagen, reduced elastin content, elastin fractures and calcifications (Elliott *et al.*, 1994). In large arteries, aging results in progressive deposition of calcium salts, fraying and fragmentation of elastin, an increase in the number and cross linking of collagen fibers that alter the compliance characteristics of the vessel wall (Robert *et al.*, 1996).

b) Gender

Arterial compliance is greater in females compared to males of the same age before the onset of menopause (Maxwell, 1998). Oestrogen receptors were demonstrated in aorta of dogs (Horowitz and Horowitz, 1982), rats (Lin and Shain, 1985), rabbits (Perrot-Applanat *et al.*, 1988), baboons (Lin *et al.*, 1986), in smooth muscle cells isolated from the rat (Lavigne *et al.*, 1995) and human (Losordo *et al.* 1994) coronary arteries. Limited- capacity, high- affinity binding sites for oestrogen were also reported in cytosols isolated from endothelium of rabbit (Colburn and Buonassisi, 1978), and bovine aorta (Bayard *et al.*, 1995), suggesting that the vascular endothelium is also oestrogen-sensitive. The presence of oestrogen receptors showed the ability of oestrogen binding in the endothelium, which is essential to a change in the loading conditions on the elastin and collagen fibers. Oestrogen may alter vessel wall loading conditions through vasodilatation by mechanisms that include potentiation of endothelial- dependent vasodilatation mediated by nitric- oxide (Lieberman *et al.*, 1994, Sudhir *et al.*, 1995). Vascular dilations transfers vascular wall stress from the collagen fibers, resulting in a more compliant vasculature.

c) Physical activity

Regular aerobic exercise is proven to reduce central arterial stiffness associated with ageing (Tanaka *et al.*, 2000). Regular physical activity such as aerobic exercise is known to be an important component of prevention and treatment of cardiovascular diseases (Hodes *et al.*, 1995). Boreham *et al.* (2004) reported that cardiorespiratory fitness and sports- related physical activity were inversely associated with arterial stiffness in young adults. Sports related physical activity score in men and women was shown to be

inversely and significantly associated with pulse wave velocity (PWV) of the aortodorsalis pedis, meaning improved aortic stiffness with increasing physical activity. Adjustment for other lifestyle variables (age, sex, height, weight, mean arterial pressure, smoking, and alcohol consumption) and body fat did not attenuate the strength of association between physical activity and arterial stiffness.

d) Obesity

Obesity is associated with increased CVS risk and arterial stiffness. Kodama *et al.* (2009) reported that increased body mass index and adiposity are associated with arterial stiffening. However, the pathophysiology mechanisms that link obesity to arterial stiffening is still largely unknown. One of the mechanisms that could be involved is increased circulating proinflammatory cytokines and leptin (Singhal *et al.,* 2002). Leptin has been shown to induce oxidative stress in endothelial cells (Oda *et al.,* 2001). Oda *et al.* (2001) showed that activator of leptin receptors stimulates smooth muscle cell proliferation and migration, whereas prolonged treatment with leptin has been shown to increase vascular cell calcification (Parhami *et al.,* 2001). Reduced nitric oxide would not only produce vasoconstriction, which increases arterial stiffness, but would also promote vascular smooth muscle growth which can lead to increase in arterial stiffness.

e) Smoking

Smoking leads to alterations in hemostatic factors, endothelial function and blood lipids. There is evidence that compliance of both large and medium- sized arteries decrease immediately after the smoking of one cigarette (Kool *et al.,* 1993). Nirandeep *et al.* (2006) showed significantly increased systemic augmentation index [(median 17.25 %

vs. 11.75 %)] (a parameter for arterial stiffness) using the SphygmoCor device in 50 smokers (who smoked at least ten cigarettes per day) compared to 50 non smokers (p= 0.004). Higher percent augmentation index indicates increased arterial stiffness. This suggested that smoking ten cigarettes per day is enough to induce marked endothelial dysfunction. This finding is supported by Barua *et al.* (2002), who found that nitric oxide (NO) production and eNOS expression and activity were all impaired both in light and heavy smokers. Reduced NO would promote vascular smooth muscle growth which leads to increased arterial stiffness.

f) Hypercholesterolemia

It has been reported that asymptomatic hypercholesterolemia substantially alters endothelial function, leading to decreased relaxation of arteries (Wilkinson *et al.*, 2002). Wilkinson *et al.* (2002) showed significant rises of augmentation index (24.8% ± 11.3% *vs.* 15.6% ± 12.1%; p<0.001) and central pulse pressure (37 ± 11 mm/Hg *vs.* 33 ± 10 mm/Hg; p=0.028) in 68 subjects with hypercholesterolemia (≥6.5 mmol/L) compared to 68 normocholesterolemic patients. The same authors, had previously shown hypercholesterolemia to be strongly associated with endothelial dysfunction and reduced nitric oxide bioavailability (Wilkinson *et al.*, 1998), which leads to arterial stiffening.

g) Hypertension

Stiffening of large arteries may be both a cause and a consequence of hypertension. As arterial pressure rises, arterial compliance decreases. Hypertension may produce arterial stiffening by both functional and structural mechanisms (Nichlos *et al.*, 1998). Elevated blood pressure over time can lead to structural changes which influence mainly the

elastic arteries, responsible for the cushioning function of the arterial tree. These structural changes are due to vascular remodeling and hypertrophy associated with hypertension itself (Nichlos *et al.,* 1998). Functionally, an initial elevation in blood pressure may establish a feedback mechanism. This increases arterial stiffness by increased cylic acids without any structural change. Elevation of blood pressure may consequently lead to vascular hypertrophy and hyperplasia, collagen deposition and atherosclerosis, thus resulting into elevation of arterial stiffness (Arnett *et al.,* 2000).

h) Diabetes mellitus

Increased arterial stiffening has been demonstrated consistently in both Type 1 and Type 2 diabetes mellitus (Aoun *et al.,* 2001). The study by Aoun showed significantly increased pulse wave velocity and aortic pulse pressure in diabetic patients compared to non-diabetic patients. Lehmann *et al.* (1992) showed increased aortic stiffness in both Type 1 (n=25) and Type 2 (n=25) diabetes mellitus subjects using the non invasive Doppler ultrasound technique. Fujii *et al.* (1997) showed that hyperinsulinemia as assessed by an oral glucose tolerance test (OGTT) is associated with early atherosclerosis in normotensive, nondiabetic men. High insulin resistance causes upregulation of angiotensin II type 1 receptor (AT_1) (Nickening *et al.,* 1998) and may be the cause of stiffness in diabetes mellitus. They also reported that elevated levels of insulin result in overexpression of vascular AT_1 receptor (Nickening *et al.,* 1998). Dubey *et al.* (1998) reported that angiotensin II (Ang II) is a potent mitogen for smooth muscle cells in the presence of insulin. These actions were inhibited by AT_1 but not AT_2 receptor antagonists. Thus in conditions where insulin levels are increased, the

exaggerated effects of Ang II on the vessel could aid in the development of arterial stiffness.

1.4 Measurement of arterial stiffness

There are several methods that can be used to assess arterial stiffness. Common non invasive parameters used to assess arterial stiffness include performing the pulse wave velocity (PWV), augmentation index (AI) and magnetic resonance imaging (MRI). In this study, PWV and AI were used to assess arterial stiffness in the study subjects. The MRI was not used as it is a very expensive equipment and not available for us to use.

1.4.1 Pulse Wave Velocity (PWV)

The velocity of pulse waveform along a stretch of artery is dependent on the stiffness of the artery along which the pulse is travelling. The velocity is slower in a compliant stretch of artery. Higher values of PWV correspond to a higher arterial stiffness. Serial measurements of PWV in a section of artery will indicate the magnitude of change in arterial compliance / stiffness in that section of artery. Carotid femoral PWV is a well established and non- invasive method to assess arterial stiffness (Lim *et al.,* 2004, Pannier *et al.,* 2002 and Wilkinson *et al.,* 1998). It is recommended as one of the best methods for measuring arterial stiffness (O'Rourke *et al.,* 2002). Pulse wave velocity can be measured in various segments of the arterial circulation such as carotid-femoral, carotid- radial and brachial- ankle. Carotid femoral pulse wave velocity was used in the current study. Carotid femoral pulse wave velocity gives the velocity of the pulse wave travel along the carotid- femoral segment by calculating the time delay between the pulse pressure waves at the two sites. The time taken for the arterial pulse to propagate

from the carotid to the femoral artery is divided from the distance between the two sites, which is determined by superficially by measuring the distance between the carotid and femoral pulsations.

Pulse wave velocity and pulse wave analysis has been recently used to assess arterial stiffness in pregnancy. Macedo *et al.* (2008) showed significant difference in PWV between pregnant women (n=193), with gestational ages between 11-41 weeks, and nonpregnant controls (n=23), matched for age and height. Applanation tonometry was used to assess PWV. They found that normal pregnancy is associated with a reduction in central blood pressure and arterial stiffness. Compared to nonpregnant controls, pregnant women had lower mean arterial pressure (85±8.9 mmHg *vs.* 81.1±7.2 mmHg), central systolic blood pressure (103±11 mmHg *vs.* 96±8 mmHg), central diastolic blood pressure (71±9 mmHg *vs.* 67±7 mmHg), and augmentation index (19±11% *vs.* 4±12%). The augmentation index decreased significantly with gestation reaching its nadir at midpregnancy (p=0.001). The difference in augmentation index (between pregnant and non pregnant women) was present even after adjusting for known determinants of augmentation index such as maternal age (p<0.001), heart rate (p<0.001) and mean arterial blood pressure (p<0.001). However in this study, pulse wave velocity (carotid-radial and carotid- femoral) became not significant between pregnant and non pregnant women after adjustments for maternal age and mean arterial pressure.

Mersich *et al.* (2005) showed a reducing trend of aortic PWV throughout pregnancy. Aortic PWV was gradually reduced during pregnancy from 6.2 m/s at first trimester to 5.4 m/s at third trimester, then increased in the post partum period to 6.7±0.2 m/s. PWV

has also been used to assess the effect of diseases on arterial stiffness in pregnancy. In a study using 16 patients with pregnancy induced hypertension (PIH), PWV was shown to increase proportionally to the gestational age (Oyama-Kato *et al.,* 2006). In his study, the predictive value of both PWV and blood pressure for developing PIH is higher than the value of blood pressure alone, suggesting the usefulness of measuring PWV to predict PIH. The trend of increasing PWV with gestational ages in PIH as shown by Oyama *et al.* (2006) was in contrast to the PWV changes in normal pregnancy as reported by Macaedo *et al.* (2008).

Hu *et al.* (1998) stated that women with previous gestational diabetes mellitus (pGDM) (n=17) showed evidence of increased wall stiffness in the common carotid artery and a lower maximum incremental velocity of the pulsatile vessel diameter change in both aorta and carotid artery compared with controls (n=20). That study assessed the elastic properties in the large arteries using ultrasonic scanner. They found that the elastic modulus and the stiffness index of the carotid artery were higher in the pGDM. In aorta; pulse amplitude of the diameter (AD) [0.91 (0.47-1.58) mm *vs.* 1.06 (0.87-1.659) mm] and maximum incremental velocity of the diameter during the systolic phase (MIV) [32 (146-58.6) mm *vs.* 45.2 (31.4-77.3) mm] were significantly lower in pGDM. In the common carotid artery; MIV [11.8 (5.5-19.3) mm *vs.* 13.9 (8.1-24.8) mm], elastic modulus [0.83 (0.58-1.77) *vs.* 0.70 (0.47-0.96)] and stiffness index [6.6 (54-12.7) *vs.* 6.2 (4.48.3)] were significantly different between pGDM and controls. Stiffness index of the aorta was significantly higher in pGDM. Thus, this study showed asymptomatic women with history of GDM have abnormal vascular function compared to women without history of GDM (Hu *et al.,* 2005).