



UNIVERSITI PUTRA MALAYSIA

***SINGLE NUCLEOTIDE POLYMORPHISM (SNP45) AND PLASMA
ADIPONECTIN LEVEL AMONG ANTENATAL WOMEN IN SELECTED
POLYCLINICS IN SELANGOR***

LOW CHEN FEI

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By

LOW CHEN FEI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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December 2009**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

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ADIPONECTIN LEVEL AMONG ANTENATAL WOMEN IN SELECTED
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December 2009

Chairman: Dr. Eusni Rahayu Mohd Tohit

Faculty: Faculty of Medicine and Health Science

Gestational diabetes mellitus according to World Health Organization definition is the carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first diagnosed during pregnancy. Chronic insulin resistance appears to be the core of pathophysiology of gestational diabetes mellitus that actuates during mid-pregnancy and progresses through third trimester. Adiponectin which is one of the adipocyte-secreted hormones was recently been reported to be associated with insulin resistance, and accumulating literature is included gestational diabetes mellitus. Adiponectin has been identified to possess anti-atherogenic, insulin-sensitizing as well as lipid-oxidation enhancing properties. Single nucleotide polymorphisms in adiponectin gene had shown significant correlation with its plasma concentration and to the extent of risk of

metabolic disorder. However, dissimilar findings had been reported from different populations and ethnics group. This present study aimed to identify the association of adiponectin single nucleotide polymorphism (SNP45) with gestational diabetes mellitus; and compare various parameters between gestational diabetic and normal patients during early trimester of pregnancy. A total number of 104 antenatal patients from three different polyclinics; Polyclinic Bangi, Polyclinic Kajang and Polyclinic Seri Kembangan; participated in this study, and patients were in early trimester of pregnancy at first visits to antenatal clinic. Subsequently, the respondents were followed up and 79 patients retained in the following study, comprised of 53 normal and 26 gestational diabetic patients. All biochemical tests were conducted using blood plasma and patients' deoxyribonucleic acid was extracted from whole blood. Adiponectin SNP45 was screened using restriction enzyme SmaI. Findings from this present study revealed that gestational diabetic patients exhibit a significantly lower plasma adiponectin level as compared to normal patients ($P < 0.05$) during early trimester. Besides, no significant difference was observed in patients' plasma fructosamine level and hemoglobin A_{1c} percentage between gestational diabetic and normal patients during the early trimester. Analysis of adiponectin SNP45 revealed a significant association with gestational diabetes mellitus ($P = 0.044$); and that G allele in SNP45 is significantly associated with gestational diabetes ($P = 0.038$). A significant lower level of plasma adiponectin in gestational diabetic patients that carry TG/GG genotype in adiponectin

SNP45 as compared to normal patients that carry TT genotype was found, revealing a possible role of adiponectin SNP45 in circulating plasma adiponectin concentrations. In normal patients, patients that carry TG/GG genotype exhibit significant higher BMI compared to patients that carry TT genotype. However, this observation was not found in gestational diabetic patients. Therefore, it has been concluded that gestational diabetic patients exhibit lower level of plasma adiponectin during early trimester; and adiponectin SNP45 is associated with gestational diabetes mellitus in the sample population from this present study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Sarjana Sains

**POLIMORFISME NUKLEOTIDA (SNP45) DAN PARAS ADIPONECTIN DI
KALANGAN ANTENATAL DI POLIKLINIK TERPILIH DALAM
SELANGOR**

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Gestasi diabetes mellitus mengikut definasi Pertubuhan Kesihatan Sedunia adalah ketidaktoleransi terhadap karbohidrat justeru mengakibatkan hiperglisemia dengan permulaan atau pertama kali didiagnosis semasa kehamilan. Rintangan terhadap insulin yang kronik telah menjadi teras dalam patofisiologi kepada gestasi diabetes mellitus semasa pertengahan kehamilan dan rintangan terhadap insulin terus meningkat serta berlanjutan sehingga trimester ketiga. Adiponectin merupakan salah satu jenis hormon yang dirembeskan oleh tisu adipose, dan telah dikenali berhubung-kait dengan rintangan terhadap insulin. Laporan-laporan saintifik telah menunjukkan perkaitan antara adiponectin dengan gestasi diabetes mellitus. Adiponectin telah dikenalpasti dapat meningkatkan tahap sensitiviti terhadap insulin dan

meningkatkan oksidasi terhadap lipid. Polimorfisme dalam gen adiponectin telah dikenal pasti berhubung kait dengan paras adiponectin dalam plasma darah, justeru risiko kepada permasalahan metabolisme. Walaubagaimanapun, terdapat perbezaan dalam hasil kajian dari pelbagai populasi dan kumpulan etnik telah dilaporkan. Kajian ini bertujuan mengenal pasti perhubungan antara polimorfisme (SNP45) dalam gen adiponectin dengan gestasi diabetes mellitus; serta membandingkan pelbagai parameter antara pesakit gestasi diabetes dan pesakit yang normal pada awal tempoh kehamilan. Seramai 104 pesakit antenatal dari 3 poliklinik; iaitu Poliklinik Bangi, Poliklinik Kajang dan Poliklinik Seri Kembangan bersetuju untuk mengambil bahagian dalam kajian ini. Pesakit-pesakit adalah dalam awal tempoh kehamilan yang menjiarahi klinik antenatal untuk kali yang pertama pada kehamilan tersebut. Seterusnya, hanya 79 pesakit antenatal yang meneruskan kajian susulan; yang mana terdiri daripada 53 pesakit normal dan 26 pesakit gestasi diabetik. Semua ujian biokimia dijalankan dengan menggunakan sampel plasma darah dan asid deoksiribonukleik telah diekstrak dari darah pesakit. Saringan adiponectin SNP45 dijalankan dengan menggunakan enzim SmaI. Hasil daripada kajian ini menunjukkan pesakit gestasi diabetik mempunyai paras adiponectin yang lebih rendah berbanding dengan pesakit normal ($P < 0.05$) pada awal tempoh kehamilan. Di samping itu, tiada perbezaan dalam paras fruktosamin dan peratusan hemoglobin A_{1c} dapat dilihat di antara dua kumpulan pesakit tersebut. Analisis adiponectin SNP45 menunjukkan perhubungan dengan

gestasi diabetes mellitus ($P = 0.044$); dan allele G dalam SNP45 dapat dikaitkan dengan gestasi diabetes mellitus ($P = 0.038$). Pesakit gestasi diabetik yang membawa genotip TG/GG dalam adiponectin SNP45 mempunyai paras adiponectin yang rendah berbanding dengan pesakit normal yang membawa genotip TT. Ini menunjukkan bahawa adiponectin SNP45 berkemungkinan mempengaruhi paras adiponectin dalam peredaran darah. Di antara para pesakit normal, pesakit yg membawa genotip TG/GG dalam adiponectin SNP45 mempunyai BMI yg lebih tinggi berbanding dengan pesakit yang bergenotip TT. Kesimpulannya, pesakit gestasi diabetik mempunyai paras adiponectin yang lebih rendah pada awal tempoh kehamilan ; dan adiponectin SNP45 dapat dikaitkan dengan gestasi diabetes mellitus dalam populasi sampel daripada kajian ini.

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I certify that an Examination Committee has met on **14 December 2009** to conduct the final examination of **Low Chen Fei** on his **Master of Science** thesis entitled **“Single Nucleotide Polymorphism (SNP45) And Plasma Adiponectin Level Among Antenatal Patients In Selected Polyclinic In Selangor”** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science degree.

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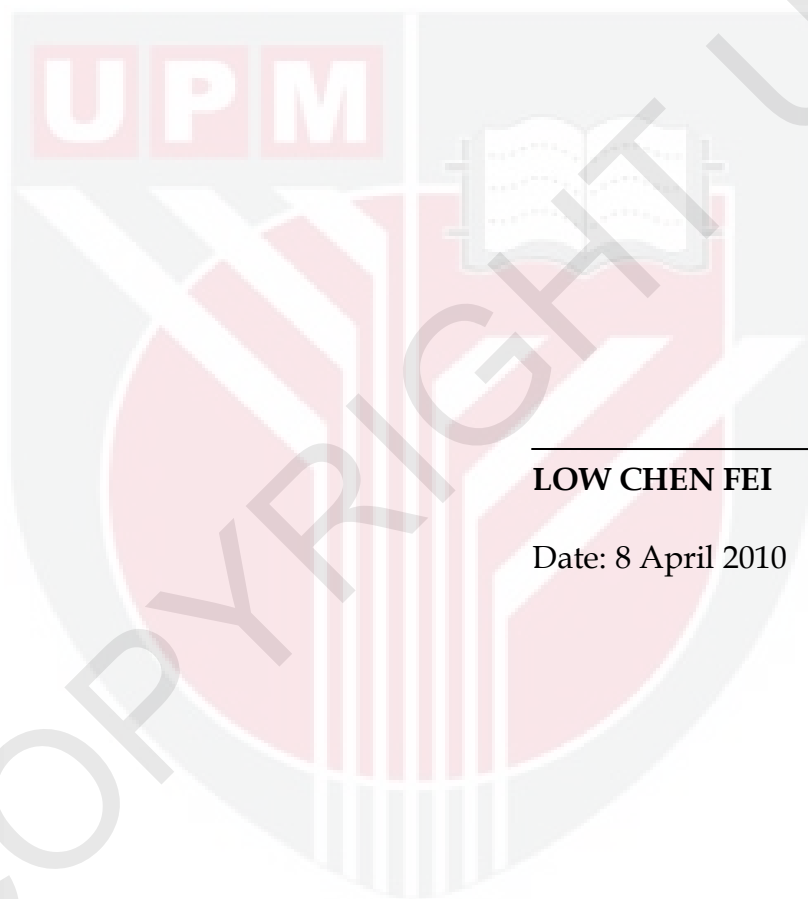
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



LOW CHEN FEI

Date: 8 April 2010

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LIST OF ABBREVIATIONS

WHO	World Health Organization
ASP	acylation-stimulating protein
TNF- α	tumor necrosis factor-alpha
PAI-1	plasminogen activator inhibitor-1
SNP	single nucleotide polymorphism
mRNA	messenger ribonucleic acid
GDM	gestational diabetes mellitus
BMI	body mass index
Hb	hemoglobin
HbA _{1c}	hemoglobin A _{1c}
ADA	American Diabetes Association
ACOG	American College of Obstetricians and Gynecologist
CDA	Canadian Diabetes Association
ADIPS	Australasian Diabetes in Pregnancies Society
EASD	European Association for the Study of Diabetes
LMW	low molecular weight
HMW	high molecular weight
T2DM	type 2 diabetes mellitus
OGTT	oral glucose tolerance test
PCR	polymerase chain reaction
DNA	deoxyribonucleic acid

CHAPTER ONE

INTRODUCTION

1.1 Definition of gestational diabetes mellitus

Diabetes and pregnancy can be associated in two ways: pregnancy that occurs in woman who is already diabetic, which is referred to as diabetes of pregestational origin; and diabetes that occur in woman who is already pregnant. The latter was termed as gestational diabetes. Gestational diabetes is a condition that was noted over 100 years ago, but it was not until 1961 when O'Sullivan coined the term and thus became an entity. O'Sullivan in 1961 explained that gestational diabetes is referred to an asymptomatic condition indicated by a range of blood glucose concentration that lies between borderline area at upper reaches of normality on one hand and admitting of no doubt of diabetes on the other. World Health Organization defines gestational diabetes mellitus as "carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy" (WHO 1999). It has always been the fundamental problem in defining the indistinctive state of "normality", and it took time to accomplish and coming to agreement about definition and classification in studying this phenomenon, and even now is not fully resolved. The uncertainty was then dissipated by the soon well established findings that during uncomplicated pregnancy, a trace amount of glucose could

be found in the urine frequently, realized that it was unrelated to the disease of gestational diabetes mellitus.

1.2 Pathophysiology of gestational diabetes mellitus

Apart from difficulty in defining gestational diabetes mellitus, and classifying patients, the pathophysiology of this metabolic disorder is not fully resolved yet. Chronic insulin resistance became the core of the pathophysiology of gestational diabetes given the fact that in women with previous history of gestational diabetes, insulin resistance exists before pregnancy, and worsen during gestation. This progressive insulin resistance is normally present during pregnancy period that actuates near mid-pregnancy and progresses through the third trimester to the levels that resemble insulin resistance in individuals with type 2 diabetes. The inadequacy of insulin secretion to countervail insulin resistance, leads to hyperglycemia that is detected by routine glucose screening in pregnancy (Barbour *et. al.* 2007). Insulin sensitivity is comparably lower in normal women than in women with gestational diabetes during pregnancy, but slightly greater insulin resistance was revealed in women with gestational diabetes than normal pregnant women when precise insulin sensitivity measurements were applied in third trimester. Buchanan *et. al.* (2007) reviewed an increased in insulin sensitivity in advanced to mitigation of physiological insulin resistance of pregnancy after delivery, but the increment in insulin

sensitivity is found to be greater in normal women than in women with gestational diabetes, revealed a separate chronic form of insulin resistance in women who had gestational diabetes. Thus, women who develop gestational diabetes mellitus commonly presented two form of insulin resistance. The first is insulin resistance that is triggered by the normal physiology of late pregnancy; and the second is chronic insulin resistance that is present before pregnancy, and aggravated by the physiological changes of pregnancy.

1.3 Risk of diabetes mellitus following gestational diabetes mellitus

It was reviewed by Kim *et. al.* (2002) that most of the women with previous gestational diabetes history, subsequently develop diabetes outside of pregnancy especially during the first decade after the index pregnancy in long-term follow-up studies. Thus, in most women, gestational diabetes appears to be a stage in the evolution of diabetes in their later life time. It was thus recommended that soon after delivery, women with prior gestational diabetes history to be tested for diabetes periodically. The types of diabetes that occur after gestational diabetes have not been investigated. However, type 2 diabetes appears to predominate, given the fact that gestational diabetes and type 2 diabetes shared almost the same extent of risk factors such as obesity and weight gain, but not to be neglected that immune and monogenic forms of diabetes occur as well but in a smaller group of patients who do not appear to

be insulin resistant. Women who had gestational diabetes tend to be overweight or obese, thus mechanisms that promote obesity as well as mechanisms that link obesity to insulin resistance apparently play an important role.

1.4 Adipocyte and its cytokines

Obesity itself is a complex disorder, and apparently a rapid growing health problem worldwide. Increased in adiposity conferring excess risk for the development of other disorder, particularly, type 2 diabetes and atherosclerotic cardiovascular disease. Adipose tissue that was once known as the storage of excess energy is currently being considered as an endocrine system as well, that actively secretes cytokines that mediate in the control of metabolism. Adipocytokines is the term that has been coined to refer to the biologically active molecules that derived from adipocyte. Extensive studies on these adipocytokines showed that several of these secreted proteins emerge to play an important role in the pathophysiology of several diseases. Among the adipocytokines, acylation-stimulating protein (ASP) was found to increase adipocyte glucose uptake and subsequently increases triglyceride synthesis; tumor necrosis factor- α (TNF- α) was found to be involved in insulin resistance in obesity through its overproduction; plasminogen activator inhibitor-1 (PAI-1) was found to be the factor that causes vascular thrombosis; and resistin that has

been discovered lately to possess property to impair insulin sensitivity and glucose tolerance in murine models (Juan and Pedro 2003).

1.5 Adiponectin and metabolic syndrome

Adiponectin which is one of the adipocyte-secreted hormones, is also known as Acrp30 (30 kDa adipocyte complement-related protein), GBP28 (gelatin-binding protein), AdipoQ, and apM1 (adipose most abundant gene transcript 1). The concentration of adiponectin in blood circulation ranges from 5 to 30 $\mu\text{g}/\text{ml}$ that accounts for about 0.01% of total plasma protein. Experimental data suggested that adiponectin possesses multivalent functions that included anti-atherogenic, insulin-sensitizing, lipid-oxidation enhancing and vasodilatory properties (Ryo *et. al.* 2004). However, the physiological role of adiponectin has not been fully established yet. The implication of low or reduced plasma adiponectin level in the pathogenesis of obesity and type 2 diabetes has been studied extensively (Statnick *et. al.* 2000), conducted in experimental lab animal models as well as in humans. In experimental lab animal models, Hotta *et. al.* (2001) reported that decreased in plasma adiponectin levels is parallel to the occurrence of insulin resistance and diabetes in rhesus monkeys. Berg *et. al.* (2001) reported that serum glucose level in normal and diabetic rodents reduces when treated with recombinant adiponectin without prior stimulating of insulin secretion. Genetic study on adiponectin encoding gene in mice conducted by Kubota *et. al.* (2002)

reported that heterozygous adiponectin-deficient mice showed mild insulin resistance, and homozygous adiponectin-deficient mice showed moderate insulin resistance with glucose intolerance. Delayed in clearance of free fatty acid from plasma was observed in adiponectin knock-out mice in another study (Maeda *et. al.* 2002). Meanwhile, studies conducted in humans found a clear relationship between plasma adiponectin and adiposity. To date, among the known adipose-specific protein, adiponectin appears to be the only that is negatively regulated in obesity (Juan and Pedro 2003). Arita *et. al.* (1999) reported mean plasma adiponectin level of 3.7 μ g/ml in a group of obese patients, and a significant higher level of mean plasma adiponectin of 8.9 μ g/ml in non-obese patients. Hotta *et. al.* (2000) reported a lower level of plasma adiponectin in patients diagnosed with type 2 diabetes mellitus as compared to non-diabetic patients. From another perspective, patients with lower concentrations of plasma adiponectin level are predisposed to the development of type 2 diabetes than patients with higher plasma adiponectin concentration (Lindsay *et. al.* 2002). It was found that the adiponectin gene (apM1) is located at chromosome 3q27 where diabetes susceptibility locus is identified. Single nucleotide polymorphism in adiponectin gene which also known as SNP45 in exon 2 (Hara *et. al.* 2002; Menzaghi *et. al.* 2002) was reported to be associated with type 2 diabetes. It has been reported again recently by Li *et. al.* (2007) that the SNP45 polymorphism is closely correlated with the prevalence of type 2 diabetes. However, limited data was reported on the association of plasma

adiponectin level with the occurrence of gestational diabetes mellitus, though, Ranheim *et. al.* (2004) and Thyfault *et. al.* (2005) reported a decreased in plasma adiponectin level in gestational diabetic patients. In the same study by Ranheim *et. al.* (2004), reduced in mRNA expression of adiponectin in adipose tissue biopsies from gestational diabetic patients was also observed. Yet, the association between single nucleotide polymorphism in adiponectin gene with gestational diabetes mellitus warrant further investigation.

1.6 Hypotheses

Single nucleotide polymorphism (SNP45) of adiponectin gene was frequently reported in type 2 diabetes mellitus as well as its low plasma level contributes to the occurrence of metabolic syndrome. Thus, it was hypothesized in this present study that, this frequently reported SNP could associated with the occurrence of gestational diabetes mellitus. Also, it was hypothesized that patients who develop gestational diabetes mellitus have lower level of plasma adiponectin as compared to normal patients during early pregnancy period. In addition, hemoglobin A1c percentage and plasma fructosamine level are hypothesized to be higher in gestational diabetic patients as compared to normal patients.

1.7 Objectives of the study

In this present study, the association of single nucleotide polymorphism in adiponectin encoding gene with gestational diabetes mellitus was determined. One of the frequently found single nucleotide polymorphism of adiponectin gene in type 2 diabetes mellitus which is SNP45 was studied to determine its association with the occurrence of gestational diabetes mellitus. Plasma adiponectin level was determined and compared between different group of genotype in gestational diabetic patients and normal patients. Besides, hemoglobin level was also been determined as literature evidence is increasing that high maternal hemoglobin level is associated with adverse pregnancy outcome. In addition, hemoglobin A_{1c} percentage and plasma fructosamine level, which both served to monitor the control of glycemia were also been examined.

1.8 Specific objectives

- To identify the correlation between plasma adiponectin and hemoglobin level with BMI in antenatal patients
- To identify the correlation between plasma adiponectin and hemoglobin level with BMI in gestational diabetic patients

- To compare mean plasma adiponectin, fructosamine, hemoglobin, BMI and hemoglobin A_{1c} percentage in gestational diabetic and normal patients
- To identify the association of SNP45 with gestational diabetes mellitus
- To compare mean plasma adiponectin, fructosamine, hemoglobin, BMI and hemoglobin A_{1c} percentage in gestational diabetic and normal patients with different genotype of adiponectin SNP45

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