STUDIES ON DIABETIC AND PREDIABETIC VASCULAR DISEASE AND THE EFFECT OF SELECTED THERAPEUTIC MODALITIES ON ASSOCIATED VASCULOPATHY

SAYEEDA RAHMAN

University Sains Malaysia

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By

SAYEEDA RAHMAN

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Dedication

This thesis is dedicated to the memory of my beloved father Barrister Matiar Rahman (1934-2006). He was not only a loving parent but also my mentor and best friend. His intellectual curiosity and high standard knowledge have been to me a constant source of inspiration and strength. I lost more than I can express with the death of my father, the legacy he left has helped me grow as an individual and as a student. He - who would have been the most happiest of all to see the fulfilment of this thesis, however…..

"…….. Sanctified is He in Whose hand is the control of every thing, and towards Him, you will be returned." Al-Quran: Surah Ya-Sin (Ayat 83)

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VII. List of abbreviations

ACE I= Angiotensin converting enzyme

AGE = Advance glycated end product

AgII = Angiotensin II

AI = Augmentation index

BMI = Body mass index

BP= Blood Pressure

CV= Cardiovascular

CVD = Cardiovascular disease

DBP= Diastolic Blood Pressure

FBS= Fasting blood sugar

FI = Fasting insulin

HDL = High-density lipoprotein

HR = Heart Rate

IFG = Impaired fasting glucose

IGM = Impaired glucose metabolism

IGT= Impaired glucose tolerance

IHD = Ischemic heart disease

IMT= Intima media thickness

LDL = Low-density lipoprotein

NG = Normoglycaemic

NGT = Normal glucose tolerance

OGTT = Oral glucose tolerance test

PPAR = Peroxisome proliferator-activated receptor

PWA = Pulse wave analysis

PWV = Pulse wave velocity

SBP= Systolic Blood Pressure

T2DM= Type 2 diabetes mellitus

2hPPG = 2-hours postprandial glucose

TChol = Total Cholesterol

TG = Triglyceride

TZD= Thiazolidinedione

VSMC= Vascular smooth muscle cell

VIII. ABSTRAK

Pendahuluan

Diabetes jenis 2 (T2DM) sekarang merangkumi 90% dari semua jenis diabetes dan 80% kematian di dalam kumpulan ini adalah berkaitan dengan penyakit kardiovaskular. Risiko kematian akibat penyakit kardiovaskular di kalangan pengidap Gangguan Tolerans Glukosa (IGT) adalah lebih tinggi berbanding mereka yang paras glukosa normal. Perubahan patologi pada salurdarah bermula bebarapa tahun sebelum diabetes jenis 2 dikesan.

Objektif Kajian

Objektif Kajian ini ialah (i) untuk mengkaji epidemiologi klinikal vaskulopati pra klinikal pesakit T2DM dan Gangguan Tolerans Glukosa (IGT) yang baru dikenalpasti dan belum dirawat serta anak-anak mereka yang normoglisemia (Kajian 1), (ii) untuk mengkaji sama ada intervensi farmakologi menggunakan thiazolidinedione dan perencat enzim penukar angiotensin (ACE inhibitor) mampu merubah vaskulopati praklinikal di kalangan pesakit T2DM dan IGT yang baru didiagnosa (Kajian 2).

Metodologi

Saringan awal terhadap 1,620 orang telah dijalankan di mana 644 telah memenuhi syarat kajian. Mereka menjalani Ujian Tolerans Glucosa secara oral (OGTT) dan didapati 70 (10.87%) mengidap T2DM dan 66 (10.25%) IGT. Bagi kajian 1 (bahagian 1), 30 T2DM dan 30 IGT telah dipilih dan dipadankan dari segi jantina dan umur dengan 30 orang yang paras gula normal sebagai kawalan. Data mengenai hemodinamik serta “pulse wave velocity” (PWV) dan ‘Augmentation Index” (AI) telah diukur. Kajian 1 (bahagian 2) melibatkan 30

orang yang paras glukosa normal terdiri dari anak-anak pesakit T2DM dan 30 anak-anak pesakit IGT dibandingkan dengan 30 orang anak-anak dari keluarga yang paras glukosa normal. Data mengenai hemodinamik, “pulse wave velocity” (PWV) dan ‘Augmentation Index” (AI) juga diukur. Bagi Kajian 2 seramai 33 orang T2DM dan 33 IGT yang dipadankan mengikut jantina dan umur telah dirawat dengan menggunakan sama ada rosiglitazone, ramipril atau plasebo selama satu tahun. Data mengenai hemodinamik telah diukur pada tiga fasa rawatan (bulan 1, 7, 12) manakala PWV dan AI diukur sepanjang tempoh rawatan [bulan 1 (minggu 1, minggu 2, minggu 4), 3, 5, 7, 9, 11 dan 12].

Keputusan

Bagi Kajian 1 (bahagian 1), PWV pesakit T2DM lebih tinggi secara bermakna (10.37+2.6 vs 8.70+1.3m/s; p =0.035), dan PWV pesakit IGT melebihi secara sederhana (9.54+1.6 vs 8.70+1.3m/s, p=0.078) berbanding mereka yang paras glukosa normal. Indeks Augmentasi (AI) pesakit T2DM melebihi secara sederhana (134.53+17.3 vs 129.17+11.2%, p=0.055) dan pesakit IGT (132.02+16.11 vs 129.17+11.2%, p=0.059) berbanding mereka yang paras glukosa normal.

Di dalam Kajian 1 (bahagian 2), anak-anak pesakit T2DM dan IGT menunjukkan AI yang lebih tinggi secara bermakna berbanding dengan kawalan (105.62+14.2 vs 96.42+7.7, p=0.001; 104.98+11.1 vs 96.42+7.7, p=0.004). PWV anak-anak pesakit T2DM juga lebih tinggi secara bermakna berbanding dengan kawalan (6.94+0.9 vs 6.33+0.7 m/s, p=0.010). Anak-anak T2DM juga menunjukkan PWV yang lebih tinggi secara bermakna berbanding dengan anak-anak IGT (6.94+0.9 vs 6.43+1.1, p=0.021).

Bagi Kajian 2, rosiglitazone menunjukkan penurunan PWV (p=0.039) dan AI (p=0.031) yang bermakna di kalangan pesakit IGT. Ramipril juga menunjukkan penurunan AI (p=0.025) berbanding plasebo semasa rawatan selama 12 bulan. Rosiglitazone dan ramipril tidak menunjukkan penurunan PWV (p=0.962 dan p=1.000) dan AI (p=0.897 dan p=0.677) yang bermakna di kalangan pesakit T2DM berbanding plasebo sepanjang tempoh rawatan.

Kesimpulan

Pesakit T2DM yang baru didiagnosa menunjukkan menifestasi pra klinikal awal penyakit vaskular makro seperti yang ditunjukkan oleh PWV yang tinggi. Anak-anak pesakit T2DM yang normal paras glukosa juga menunjukkan vaskulopati praklinikal di mana PWV dan AI adalah tinggi. Anak-anak pesakit IGT juga menunjukkan pembuluh darah yang tidak normal di mana AI juga tinggi. Rosiglitazone menukarkan keadaan vaskulopati praklinikal di kalangan IGT sebagaimana ditunjukkan oleh penurunan PWV dan AI semasa rawatan selama satu tahun. Selepas satu tahun rawatan ramipril , didapati ketegangan salurdarah besar di kalangan pesakit IGT berkurangan sebagaimana ditunjukkan oleh penurunan AI.

IX. ABSTRACT

Introduction

Type 2 diabetes (T2DM) now accounts for 90% of all diabetes and 80% of deaths in this group are cardiovascular related. The risk of cardiovascular mortality is also substantially higher in individuals with impaired glucose tolerance (IGT) than in those with normal glucose levels and the pathological changes in vascular function begin many years before the diagnosis of overt T2DM.

Aim of studies

The aims of this thesis are (i) to investigate clinical epidemiology of pre-clinical vasculopathy in newly-diagnosed, untreated T2DM and IGT patients and their normoglycaemic offspring (Study 1), and (ii) to examine whether pharmacological interventions with thiazolidinedione and angiotensin converting enzyme (ACE) inhibitors can reverse pre-clinical vasculopathy in newly diagnosed diabetic and IGT individuals (Study 2).

Methodology

Initial screening of 1620 subjects was conducted of which, 644 met study criteria. They had oral glucose tolerance test (OGTT) – 70 (10.87%) were T2DM and 66 (10.25%) IGT individuals. For Study 1 (Part 1), the first 30 T2DM and 30 IGT patients were recruited and compared with age- and sex-matched 30 normoglycaemic controls. Haemodynamic variables, pulse wave velocity (PWV) and augmentation index (AI) were measured. Study-1 (Part-2) involved 30 healthy normoglycaemic offspring of T2DM and 30 healthy

normoglycaemic offspring of IGT patients, compared with 30 age- and sex-matched healthy offspring of normoglycaemic parents. Haemodynamic variables, PWV and AI were measured. For Study-2, age- and sex-matched 33 T2DM and 33 IGT patients were enrolled, comparing one-year treatment with rosiglitazone, ramipril and placebo. Haemodynamic variables were measured at three treatment phases (1^{st}, 7^{th} and 12^{th} month) and PWV and AI were measured through out the treatment period [1^{st} (week 1, week 2, week 4), 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th}, and 12^{th} month].

Result

In Study-1 (Part-1), PWV was significantly higher in T2DM patients (10.37$\pm$2.64vs. 8.70$\pm$1.29m/s; p=0.035) and was of borderline significant in IGT subjects (9.54$\pm$1.56vs.8.70$\pm$1.29m/s, p=0.078) compared to normoglycaemic individuals. Augmentation index was higher of borderline significant in T2DM (134.53$\pm$17.32 vs. 129.17$\pm$ 11.18 %, p=0.055) and IGT patients (132.02$\pm$16.11 vs. 129.17$\pm$ 11.18 %, p=0.059) compared to normoglycaemic individuals.

In Study 1 (Part-2), offspring of T2DM and IGT patients demonstrated significantly higher AI compared to controls (105.62$\pm$14.2 vs. 96.42$\pm$7.7, p=0.001; 104.98$\pm$11.1 vs. 96.42$\pm$7.7%, p=0.004 respectively). Significantly higher PWV was noted in offspring of T2DM compared to offspring of normoglycaemic parents (6.94$\pm$0.9 vs.6.33$\pm$0.7m/s, p=0.010). It was also found to be significantly higher in the offspring of T2DM compared to that of IGT (6.94$\pm$0.9 vs. 6.43$\pm$1.1, p=0.021).

In Study-2, rosiglitazone showed a significant reduction in PWV (p=0.039) and AI (p=0.031) and ramipril demonstrated a significant reduction of AI (p=0.025) in IGT patients in comparison to placebo on the 12th month of treatment. With rosiglitazone and ramipril, no significant difference was observed in PWV (p=0.962 and p=1.000 respectively) and AI (p=0.897 and p=0.677 respectively) in T2DM patients in comparison to placebo during overall treatment period.

Conclusion

Newly diagnosed untreated T2DM patients demonstrated early preclinical manifestations of macrovascular diseases as shown by significantly increased PWV. Normoglycaemic healthy offspring of T2DM patients had demonstrable pre-clinical vasculopathy as assessed by significantly increased PWV and AI. Normoglycaemic healthy offspring of IGT individuals had also large artery abnormalities as shown by significantly increased AI. Rosiglitazone significantly reverses pre-clinical vasculopathy in IGT patients as evident by significant decrease in PWV and AI after one-year treatment. After one-year of ramipril treatment, reduction of large artery stiffness was also observed among IGT patients as shown by significant decrease in AI.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Diabetes and pre-diabetic vascular diseases: An overview

Diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). Diabetes is the fourth or fifth leading cause of death in most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialised nations (IDF, 2003). Patients with type 2 diabetes mellitus (T2DM) have a risk of death from cardiovascular (CV) causes, which is two to six times than that of persons without diabetes (Gæde *et al*., 2003). The CV events associated with T2DM and the high incidence of other macrovascular complications, such as strokes and amputations, are a major cause of illness and an enormous economic burden (O'Brien *et al*., 2003).

1.2 Diabetes: Definition, diagnosis and classification

Diabetes is diagnosed if the (venous) fasting plasma glucose (FPG) value is $\geq$ 7.0 mmol/L (126 mg/dl), or if the casual plasma glucose value is $\geq$ 11.1 mmol/L (200 mg/dl), or if the plasma glucose value 2 hours after a 75g oral load of glucose $\geq$ 11.1 mmol/L (200 mg/dl) (Table 1.1). Impaired glucose regulation (Impaired glucose tolerance and impaired fasting glycaemia) refers to a metabolic state intermediate between normal glucose homeostasis and diabetes (WHO, 1999).

Table 1.1: Biochemical criteria (venous plasma) for the diagnosis of diabetes, impaired glucose tolerance and fasting glucose or impaired fasting glucose

	Glucose concentration mmol/L (mg/dl) (Venous plasma)
Diabetes mellitus:	
Fasting ***and/or***	≥ 7.0 (≥ 126)
2-hrs post glucose load	≥ 11.1 (≥ 200)
Impaired glucose tolerance (IGT)	
Fasting	< 7.0 (< 126)
and 2-hrs post glucose load	≥ 7.8 (≥ 140) ***and*** < 11.1 (<200)
Impaired fasting Glycaemia or impaired glucose (IFG)	
Fasting	≥ 6.1 (≥ 110) ***and*** <7.0 (<126)
and 2-hrs post glucose load	< 7.8 (<140)

Source: (WHO, 1999; Unwin *et al*., 2002)

Impaired glucose tolerance (IGT) is categorised as a stage in the natural history of disordered carbohydrate metabolism. Impaired fasting glycaemia (IFG) refers to fasting glucose concentrations, which are lower than those required to diagnose diabetes but higher than the 'normal' reference range. Impaired fasting glycaemia and IGT are not interchangeable and represent different abnormalities of glucose regulation, one in the fasting state and one post–prandial; however, both are risk categories for the future development of T2DM and cardiovascular disease (CVD).

1.2.1 Type 1 Diabetes

Type 1 diabetes results from cellular-mediated autoimmune destruction of pancreatic islet beta-cells causing the loss of insulin production (Atkinson & Maclaren, 1994). It ranks as the most common chronic childhood disease in developed nations (LaPorte *et al*., 1995) but occurs at all ages (Molbak *et al*., 1994) and the clinical presentation can vary with age (Gleichmann *et al*., 1984; Groop *et al*., 1986). The global incidence of type 1 diabetes in children and adolescents is increasing with an estimated overall annual increase of around 3% (Onkamo *et al*., 1999; EURODIAB ACE Study Group, 2000; Green & Patterson 2001). It is estimated that on an annual basis some 65,000 children aged less than 15 years develop the disease worldwide. Of the estimated total of approximately 430,000 prevalent cases of type 1 diabetes in childhood, more than a quarter come from the South-East Asian (SEA) Region, and more than a fifth from the European (EUR) Region. Despite having the largest childhood population, the Western Pacific (WP) Region has the lowest number of type 1 cases.

The predominant cause of hyperglycaemia in type 1 diabetes is the autoimmune destruction of the beta cells, which leads to absolute dependence on insulin treatment and a high rate of complications typically occurring at relatively young ages. Type 1 diabetes, therefore, places a particularly heavy burden on the individual, the family and the health services.

1.2.2 Type 2 Diabetes (T2DM)

Type 2 diabetes is characterized by insulin resistance and relative insulin deficiency, either of which may be present at the time that diabetes becomes clinically manifest (Reaven *et al*., 1976; Tuomilehto *et al*., 2001). The specific reasons for the development of these abnormalities are not yet known. The diagnosis of T2DM usually occurs after the age of 40 years although the age of onset is often a decade earlier in populations with high diabetes prevalence (Zimmet *et al*., 1990b). People with T2DM may not show any symptoms for many years and the diagnosis is often made from associated complications or incidentally through an abnormal blood or urine glucose test.

Type 2 diabetes is often, but not always, associated with obesity, which itself can cause insulin resistance and lead to elevated blood sugar levels. It is strongly familial, but major susceptibility genes have not yet been identified. In contrast to type 1 diabetes, persons with T2DM are not dependent on exogenous insulin and are not ketosis-prone, but may require insulin for control of hyperglycaemia if this is not achieved with diet alone or with oral hypoglycaemic agents.

1.2.3 Impaired Glucose Tolerance (IGT)

Impaired glucose tolerance is an intermediate category between normal glucose tolerance and overt diabetes (Harris, 1989; WHO, 1994). Several previous prospective studies have shown that subjects with IGT are at high risk of progression to T2DM (King *et al*., 1984; Saad *et al*., 1988; Schranz, 1989; Motala *et al*., 1993; Nijpels *et al*., 1996). And it is estimated that about 40-70% of people with IGT would progress to T2DM (Alberti, 1998; IDF, 2003). People with IGT are known to be at significantly increased risk of CVD than those with normal glucose levels and the pathological changes in vascular function begin many years before the diagnosis of overt T2DM (Tominaga *et al*., 1999). The Whitehall study (Fuller *et al*., 1980) and the Bedford survey (Jarrett *et al*., 1982) mentioned that IGT is associated with increased risk of macrovascular disease. The DECODE (Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe) study (2003) found that people with IGT were 50% more likely to die of CV complications during follow-up than people with normal blood glucose control. The most alarming conclusion of the study was that as there were four times as many people with IGT as with diabetes, there were more premature deaths attributable to IGT than to diabetes.

1.3 Global diabetes catastrophe

Diabetes is a global health problem and has been described as the epidemic of the 21st century (Ramachandran & Snehalatha, 1999). It accounts for about 85% to 95% of all diabetes in developed countries, with even higher percentage in developing countries (IDF, 2003). The number of people with diabetes is escalating

both in the developed and developing countries. Diabetes currently affects 5% of the world's population (Marso, 2003) and its prevalence is doubling every generation (Gerdes, 2003).

1.3.1 Global estimates of diabetes

In 1994, the International Diabetes Federation (IDF, 1994) estimated that over 100 million people worldwide had diabetes. The recent statistics of the International Diabetes Federation (IDF, 2003) are alarming – approximately 194 million people worldwide have diabetes. This estimate is expected to increase to some 333 million, or 6.3% of the adult population, by 2025 and 366 million by 2030 (IDF, 2003). Table 1.2 shows the worldwide prevalence of diabetes and IGT.

The number of people with diabetes in Malaysia is increasing alarmingly (NDS/MOH, 2003) (Table 1.3), as the country is 'seeing a major shift in lifestyles and longevity of the population' (Zaini, 2000). In the National Health and Morbidity Survey I the prevalence of T2DM and IGT were estimated to be 6.3% and 4.8% respectively (NHMSI, 1986). However, the second National Health and Morbidity Survey showed that the national prevalence is estimated to be 8.3% with 7% of adult population had blood glucose in the diabetic range and 5% in the IGT (NHMSII, 1996). The survey revealed variations in the observed prevalence by states and ethnicity. Highest observed prevalence occurred in the more developed states and in Indians ethnic.

Table 1.2: Diabetes and IGT: Prevalence and projections

All diabetes and IGT	**2003**	**2025**
Total world population (billions)	6.3	8.0
Adult population (millions) (20-79 years)	3.8	5.3
Number of people with diabetes (millions) (20-79 years)	194	333
World diabetes prevalence (%)(20-79 years)	5.1	6.3
Number of people with IGT (millions) (20-79 years)	314	472
IGT prevalence	8.2	9.0

Source: IDF (2003)

Table 1.3: Projection of diabetes in Malaysia

Burden of diseases	**1996 NHMSII**	**2002**	**2006**	**2010**	**2020**
Diabetes Mellitus	608,000 (8.3%)	836,200 (9.5%)	983,650 (10.3%)	1,109,200 (11.1%)	1,558,660 (13.1%)

Note: Based on NHMSII 1996. Prevalence rate increase proportionately.

Source: NDS/MOH (2003)

The prevalence and projections of T2DM and IGT in Malaysia, according to *Diabetes Atlas* (IDF, 2003), are shown in Table 1.4. Diabetes prevalence and risk factors reported in national surveys are also shown in Table 1.5. A study conducted in Kelantan (n=2508; ≥30 years) found prevalence of diabetes at 10.5% and IGT at 16.5% (Mafauzy *et al*., 1999). Khebir *et al*. (1996) studied the changing prevalence of T2DM amongst rural Malays in Kuala Selangor and represented a marked increment of 212.8 per cent over a 10-year period (1984-1994). Statistics from the Ministry of Health (MOH) records also showed increased number of admissions and deaths in government hospitals for diabetes (NDS/MOH, 2003) (Figure 1.1). Admission increased from 19,629 cases in 1991 to 30,661 cases in 2001, an increase of 56% over a span of 10 years and mortality also increased from 254 deaths in 1991 to 380 deaths in 2001, which is an increase of 50%.

1.4 Type 2 Diabetes and cardiovascular disease: Double jeopardy

Cardiovascular disease is the major cause of morbidity and mortality in people with T2DM (Stemmer, 1997; Ness et *al.,* 1999) and coronary heart disease is the most common cause of death. It is estimated that up to 80% of the 200 million people with diabetes globally will die of CVD (UKPDS, 1996; IDF, 2003). Multiple modifiable risk factors for late complications in patients with T2DM, including hyperglycaemia, hypertension, and dyslipidaemia, increase the risk of a poor outcome (Stamler *et al*., 1993). Up to 70% of people with T2DM have raised BP and over 70% have raised cholesterol levels. Management of blood lipid, hypertension and blood glucose levels can contribute to the reduction in CV risk in people with T2DM (Table 1.6).

Table 1.4: Diabetes and IGT: Prevalence and projections in Malaysia

	2003		2025	
Population (20-79) (million)	13,280		21,032	
Diabetes prevalence (%)	9.4		12.4	
IGT prevalence (%)	17.7		18.4	
Rural (million)	323.5		449.0	
Urban (million)	928.1		2153.1	
	Diabetes	**IGT**	**Diabetes**	**IGT**
Male	527.3	1173.7	1088.4	1933.5
Female	724.3	1174.3	1513.7	1926.4
20-39 years	134.6	919.9	205.9	1299.5
40-59 years	688.4	1131.3	1207.1	1802.8
60-79 years	428.6	296.9	1189.1	757.7
Total	1251.6	2348.0	2602.1	3860.6

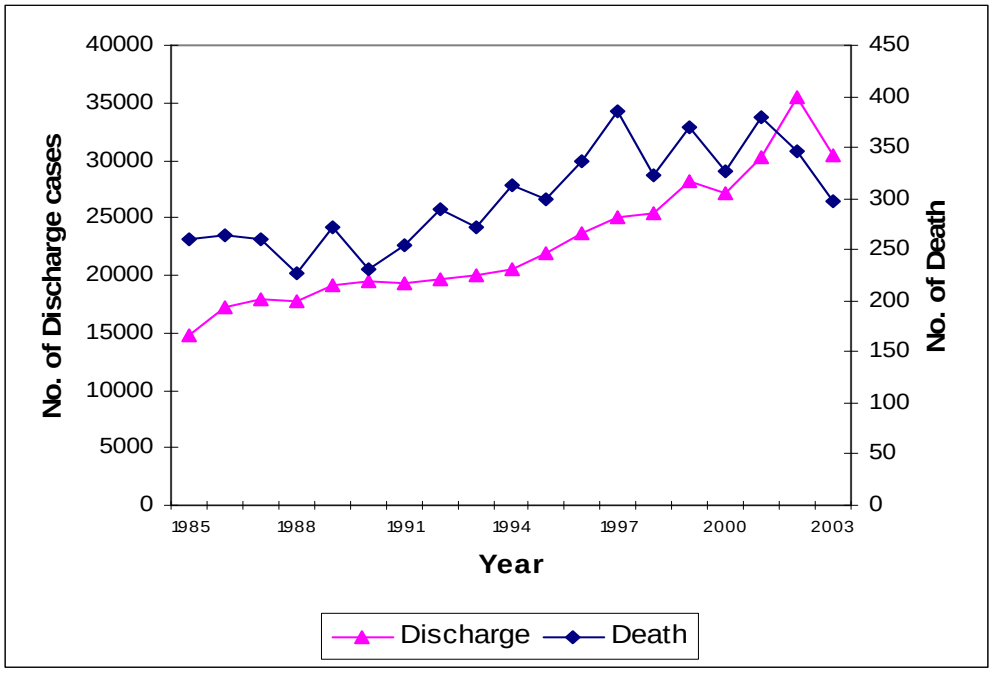
Source: IDF (2003).

Table 1.5: Diabetes prevalence and risk factors in Malaysia

	1986 NHMS1	1996 NHMS2
Diabetes Prevalence	6.3%	8.3%
Impaired Glucose Tolerance	4.8%	4.3%
Prevalence of undiagnosed diabetes	1.8%	2.5%
Hypertension		29.9%
Smoking		24.8%
Overweight		21%.
Adequate physical activity		11.6%.

Source: NDS/MOH (2003).

Figure 1.1: Admission and death of diabetes cases in Govt. Hospitals (1998-2003)



Source: NDS/MOH (2003).

Table 1.6: Reduction of risk of complications in people with T2DM

Strategy	Complications	Reduction of complications
Lipid control	Coronary heart disease mortality	36%[1]
	Major coronary heart disease event	55%[1]
	Any atherosclerosis event	37%[1]
	Cerebrovascular disease event	62%[1]
Blood pressure control	Cardiovascular disease	51%[2]
	Heart failure	56%[3]
	Stroke	44%[3]
	Diabetes related deaths	32%[3]
Blood glucose control	Heart attack	37%[3]

Source: [1]The 4S Study (Pyörälä *et al*., 1997)
[2]Hypertension Optimal Treatment (HOT) Randomised Trial (Hansson *et al*., 1998)
[3]UKPDS Group (1996).

1.5 Cardiovascular risk factors in diabetic patients

Among the traditional risk factors, smoking, hypercholesterolemia, hypertriglyceridaemia, fasting plasma glucose concentration and hypertension are probably the most important in diabetic patients. However, several factors, unique to diabetes, may confound the impact of these traditional factors and independently influence the atherosclerotic process, thereby increasing the morbidity and mortality in this group. These additional risk factors include chronic inflammation (C-reactive protein and interlukin-6), endothelial dysfunction, advance glycated end products (AGEs), plasminogen activator 1, fibrinogen, and genetic susceptibility. Risk factors and possible mechanisms of the excess risk of CVD in T2DM are shown in Table 1.7.

Serum total cholesterol is a powerful predictor of ischemic heart disease morbidity and mortality in both diabetics and non-diabetics (Pyörälä *et al*., 1997). Besides total cholesterol, elevated triglycerides, increased low-density lipoprotein (LDL) cholesterol, and decreased high-density lipoprotein (HDL) cholesterol have also been shown to independently increase CV mortality in diabetic patients (Resnick & Howard, 2002). The Scandinavian Simvastatin Survival Study (Pyörälä *et al*., 1997) found that lowering cholesterol with HMG-CoA reductase inhibitors reduced the risk of major macrovascular events in patients with diabetes. Similarly, subgroup analysis of the Helsinki Heart Study, a 5-year ischemic heart disease primary prevention trial using gemfibrozil, provided additional evidence for the potential benefit of lipid-lowering agents for both non-diabetics and diabetics (Koskinen *et al*., 1992).

Table 1.7: Risk factors and possible mechanisms of the excess risk of CVD in T2DM

Risk factors	Possible mechanisms
Hyperglycaemia	Glycation/oxidation – lipoproteins; vessel wall matrix/collagen Increased protein kinase C – altered growth factors permeability and other vascular changes
Hyperinsulinaemia and Insulin resistance	Increased vascular matrix; Proliferation of arterial smooth muscle; Increased plasminogen-activator inhibitor-1 concentrations; and increased dense LDL-cholesterol concentration.
Dyslipidaemia High triglyceride concentration Low serum HDL cholesterol Small dense LDL cholesterol	 Lowers serum HDL cholesterol Atherogenesis Atherogenesis
Increased central adiposity	Insulin resistance; dyslipidaemia
Increased plasminogen-activator inhibitor-1	Decreases fibrinolysis
Increased platelet aggregation/ adhesiveness	Increases thrombosis
Increased fibrinogen concentrations	Thrombogenesis, atherogenesis
Increased plasma oxidative state	Endothelial damage; lipoprotein oxidation/ atherogenesis
Renal dysfunction	Atherogenesis, hypertension, oxidation
Cardiovascular autonomic neuropathy	Sudden death
Abnormal vascular reactivity	Vasoconstriction, ischaemia, hypertension

Source: Nathan *et al.,* 1997.

There is considerable debate concerning appropriate target levels for LDL lowering among diabetics (as well as non-diabetics). The National Cholesterol Education Program ATP III (NCEP Expert Panel, 2001) and American Diabetes Association guidelines indicate a goal of 100 mg/dl (2.6mmol/L) for individuals with diabetes because of their known high risk for CVD and tendency to have multiple risk factors. High LDL levels in diabetic patients are thought to be particularly atherogenic because of altered composition, glycation, and susceptibility to oxidation. However, more recent study suggested that an even lower level (<2mmol/L) confers better benefits (Colhoun *et al*., 2004). Obesity and central obesity are important contributors to the insulin resistance syndrome and predictors of coronary heart disease in non-diabetic individuals. According to several studies, they are not independently associated with coronary heart disease or other CV complications in patients with T2DM (Turner *et al*., 1998). Abnormal lipids, smoking, hypertension, abdominal obesity, psychosocial factors, less consumption of fruits, vegetables, more alcohol intake and sedentary life style all these are account for most of the risk of CV complication (Yusuf *et al*., 2004). Hypertension develops early in the course of the disease the prevalence is twice as high in patients with IGT as compared to normal controls. The association of hypertension with T2DM is ominous; mortality is increased by a factor of 4 to 7 in patients with T2DM and hypertension when compared to normotensive non-diabetic matched controls (The Hypertension in Diabetes Study Group, 1993).

The UKPDS clearly demonstrated the link between hypertension and the high risk for CV complications in T2DM patients (Adler *et al*., 2000). The 10/5mmHg

difference in BP was associated with a 15% decrease in CVD, a 32% decrease in death due to diabetes, and a 44% reduction in the incidence of cerebrovascular attack. Although the optimum target BP for hypertensive with diabetes has not yet been determined with certainty, completed trials indicated that reducing systolic blood pressure (SBP) to at least 130 mmHg provides substantial reduction in both macro- and microvascular disease progression. Clinical trials have also demonstrated that treatment of BP even in the so-called normotensive range of T2DM is associated with prevention of BP elevation and increased urinary protein excretion, and would likely reduce the risk of CVD and the progression of renal disease (Viberti *et al*., 1994). The HOT study (Hansson *et al*., 1998) showed that optimum of diastolic blood pressure (DBP) for diabetic patients is <80mmHg. The ABCD-2 study (Schrier *et al*., 2002) showed that <130/80 mmHg is the optimum BP to reduce CV events.

Researchers are already beginning to focus on the involvement of other non-traditional, or diabetic-specific, risk factors in the development of CVD in diabetic patients. It has been proposed that markers of inflammation – such as interleukin-6 (IL-6), C-reactive protein (CRP) – are part of a complex of pro-CVD risk factors characterising the insulin resistance syndrome. These markers contribute to CVD risk independently of established metabolic abnormalities commonly observed in insulin resistance. Studies have shown that T2DM patients with an elevated CRP have a high risk for coronary heart disease and an up to 8.5-fold increase in morbidity and mortality (Mojiminiyi *et al*., 2002).

Cardiovascular risk factors are found to be prevalent among normal population and T2DM patients in Malaysia. Study (Mafauzy *et al*., 2003) conducted in the state of Kelantan in North-East Peninsular Malaysia showed that hypertension is a common disease in this area and is associated with multiple risk factors for CVD. Subjects with hypertension also had a higher prevalence of T2DM (19.0%), obesity (39.4%) and hypercholesterolaemia (70.7%) than non-hypertensive subjects. Of the hypertensive subjects, 83.3% had one other risk factor for CVD, 66.7% had 2 other risk factors and 16.7% had more than two risk factors. Mohamad *et al*. (1996) studied prevalence of obesity and overweight in North-East Peninsular Malaysia and their relationship with CV risk factors and found that high prevalence of overweight and obesity was associated with adverse lipid and glucose metabolism as well as poor BP control.

Prevalence of risk factors was high in the rural population of Malaysia. Global risk assessment conducted by Nawawi *et al*. (2002) showed a high-risk profile with two-thirds being at risk, and one-third being categorised into the high-risk group. The prevalence of hypercholesterolaemia for total cholesterol concentrations of >= 5.2, >=6.5 and >=7.8 mmol/l were 67.3, 30.5 and 11.8% respectively. There was a high prevalence of low serum high-density lipoprotein cholesterol (13.1%), hypertension (30.3%), smokers (24.4%), diabetes (6.4%), IFG or glucose tolerance (13.9%), overweight or obesity (44.7%) and increased waist-hip ratio (WHR) (48.5%).

A study (Mafauzy *et al.*, 1999) in Kelantan studied the T2DM and associated CV risk factors and found that T2DM patients were more obese (38.4%) and had a

higher prevalence of hypertension (12.9%) and hypercholesterolaemia (71.9%). Subjects with IGT also had a higher prevalence of obesity (35.5%), hypertension (9.0%) and hypercholesterolaemia (63.0%). Ismail *et al*. (2000) found high prevalence of dyslipidaemia in T2DM patients and concluded that ethnicity and glycaemic control are major determinants of diabetic dyslipidaemia in Malaysia. A retrospective study (n= 302) (Chiam *et al*., 2002) done on patients, who had undergone coronary artery bypass grafting (CABG) in Hospital Universiti Kebangsaan Malaysia, found the prevalence of T2DM (45.7%), hypertension (78.8%) and hyperlipidaemia (89.1%) among this cohort. The Indians had the highest prevalence of the three risk factors. The Chinese and the Malays most frequently presented with the combination of hypertension and hyperlipidaemia. From the studies it is established that the prevalence of obesity and overweight, hypertension and other risk factors and their relationship with CVD and T2DM in Malaysia is likely to increase in the near future with increasing affluence and becoming a major health problem.

1.6 Diabetic vasculopathy

Diabetes is associated with a number of microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (ischemic heart disease, cerebrovascular disease, and peripheral vascular diseases) complications (Cooper *et al*., 2001; Clark & Lee, 1995). Diabetic associated vascular complications usually involve large, medium and small size vessels. Macrovascular complications are the most common long-term problems and affect the heart and large blood vessels. In the following sections, pathophysiology and pathogenesis of these factors will be discussed.

1.6.1 Pathophysiology of diabetic vasculopathy

There is much physiological impairment that plausibly link diabetes with a marked increase in atherosclerotic vascular disease. These include endothelial dysfunction, smooth muscle cell dysfunction, platelet hyper-reactivity, impaired fibrinolysis coupled with a tendency for thrombosis and coagulation, and increased inflammation (Luscher *et al.*, 2003; Creager *et al.*, 2003). However, endothelial dysfunction is considered to be an important nexus of dysfunction in diabetes mellitus, linking each of these pathological manifestations (Beckman, 2004).

1.6.1.1 Endothelial dysfunction

Endothelial dysfunction may be functionally defined as the failure of the vascular endothelium to sub-serve its normal role in vasodilation and/or vascular homeostasis. The predominant effect of endothelium stimulation is vasodilatation. Other regulatory functions involve vasorelaxation, vasoconstriction, anti-platelet and anticoagulant effects (Kadirvelu *et al*., 2002). It emerges as a key component in the pathophysiology of diverse cardiovascular abnormalities associated with atherosclerosis, diabetes, hypertension, and aging (Drexler & Hornig 1999; Van der Loo *et al*., 2000). Endothelial dysfunction, present at disease onset, is the prime focus of atherosclerotic lesions that present throughout the course of diabetes and associated with late-stage adverse outcomes. Diabetes related endothelial dysfunction precedes morphologic and structural vascular changes (Taylor & Poston, 1994), which includes accelerated disappearance of capillary endothelium (Tooke, 1995), weakening of intercellular junctions (Rattan *et al.*, 1997), altered protein synthesis and altered expression/production of adhesion glycoproteins on

endothelial cells (Tooke, 1995; Rattan *et al.*, 1997; Tesfamariam *et al.*, 1991; Paston & Taylor, 1995), promoting attachment of monocytes and leukocytes, as well as their trans-endothelial migration (Rattan *et al.*, 1997).

Endothelial dysfunction results from the imbalance between endothelium-derived contracting and relaxing factors. Unlike normal endothelium it produces abnormal response when exposed to endogenous or exogenous vasodilators. Due to endothelium's strategic location between the circulating blood and vascular smooth muscle, it is a primary target and mediator of cardiovascular disease. Endothelium modulates the activity of vascular smooth muscle and therefore it regulates vascular tone (Furchgott & Zawadzki, 1980). Endothelial cells (EC) release humoral factors that control relaxation and contraction, thrombogenesis and fibrinolysis, platelet activation and inhibition (Kadirvelu *et al*., 2002). These cells provide metabolically active interface between blood and tissue that modulates blood flow, nutrient delivery and leukocyte diapedesis (Cines *et al*., 1998). The endothelial cells also synthesize important bioactive substances e.g. nitric oxide (NO), other reactive oxygen species, prostaglandins, endothelin and angiotensin II, which regulate blood vessel structure and function. Nitric oxide potently dilates vessels and mediates much of the endothelium's control of vascular relaxation (Verma & Anderson, 2001).

Endothelial cell damage, with loss of the vascular protective effects of NO, is likely the early step in atherosclerosis. Impaired endothelial-dependent vasodilatation has been described in patients with diabetes and the degree of impairment may correlate

with glycaemic control (Storey *et al.,* 2001). This is also seen with other associated conditions, which lead to endothelial dysfunction e.g. hypertension, dyslipidaemia (Storey *et al.,* 2001). This may be an effect of hyperglycaemia itself leading to an increase oxidative stress and release of mediators (e.g. cytokines) by adipocytes in the presence of insulin resistance (Storey *et al.,* 2001). High glucose concentration is associated with increased oxidative stress (Baynes, 1991), enhanced leukocyte endothelial interaction (Morigi *et al*., 1998), and glycosylation of protein in the body, including lipoproteins, apolipoproteins, and clotting factors. Hyperglycaemia also enhances endothelial cell matrix production, which may contribute to basement membrane thickening (Cagliero *et al*., 1991). It also increases enzymes involved in collagen synthesis (Cagliero *et al*., 1991) and specifically enhances endothelial cell collagen IV and fibronectin synthesis (Cagliero *et al*., 1991).

Over time, through a complex series of dehydrogenation and oxidation reactions, hyperglycaemia enhances the formation of advanced glycation end products (AGEs) (Vlassara, 1997). These end products are found in plasma, vessel wall, and tissues and are linked to the development of diabetic complications (Tesfamariam *et al.,* 1991; Johnstone *et al.,* 1993; Parving *et al*., 1996; Deckert *et al*., 1989; Zeiher *et al*., 1991; Wautier *et al*., 1996). These end products can also induce excessive cross-linking of collagen and extracellular matrix proteins in the vascular wall, which in turn could lead to accumulation of low-density lipoprotein (LDL) particles of prolonged half-life. Such particles are more susceptible to oxidative modification, impairing endothelial cell function, stimulating inflammation and adhesion, and promoting vascular smooth muscle cell (SMC) changes (Lyons, 1993).

Atherosclerotic vascular disease is the leading cause of death in patients with diabetes, which mainly occurs due to endothelial dysfunction (Beckman *et al.,* 2002; Seligman *et al*., 2000). Improved metabolic control in diabetic patients, whatever the treatment used, is associated with near normalisation or restoration of normal endothelial function (Guerci *et al.*, 2001).

1.6.1.2 Smooth muscle cell dysfunction

Progression of atherosclerotic lesion or alteration of vasculature is the characteristic feature of diabetic complications (Ruderman & Haudenschild, 1984). Diabetes accelerates these processes by stimulating the atherogenic activity of vascular SMC - the integral part in the development of atherosclerosis (Beckman *et al.,* 2002). The process begins as a response to chronic minimal injury to the endothelium leading to it being dysfunctional.

A dysfunctional endothelium is found to be more porous, which allows macrophages and LDL to penetrate to the medial layer of arteries heralding the formation of foam cells. The vessels are then on the way to develop atheroma. Once the macrophage-rich fatty streak forms, vascular SMCs in the medial layer of the arteries migrate into the nascent intimal lesion. Here vascular SMCs replicate and lay down a complex extracellular matrix, important steps in the progression to advanced atherosclerotic plaque (Beckman *et al.,* 2002). These cells being the source of collagen, strengthen the atheroma, making it less likely to rupture and cause thrombosis. Lesions that have disrupted and caused fatal thrombosis tend to

have few vascular SMCs (Libby, 2001). Fewer vascular SMCs are also found in diabetic patients with advanced atherosclerotic lesions (Fukumoto *et al*., 1998). Hyperglycaemic lipid modifications of LDL regulate the increased migration and cell death of vascular SMCs in atherosclerotic lesions. Low-density lipoprotein that has undergone non-enzymatic glycation induces vascular SMC migration in vitro, while oxidized glycated LDL can induce cell death of vascular SMCs (Taguchi *et al*., 2000). High glucose concentrations promote necrotic cell death through hydrogen-peroxide (H_2O_2) formation, which may participate in the development of diabetic vasculopathy (Chaturvedi *et al*., 2001). Thus, diabetes alters vascular smooth muscle function in ways that promote atherosclerotic lesion formation, plaque instability and clinical events.

1.6.1.3 Impaired platelet function

Platelet aggregation and adhesion are characteristically highlighted in people with diabetes (Walsh *et al.*, 1995; Stein *et al.*, 1995; Winocour *et al*., 1990; Davi *et al.*, 1990). Diabetes increases intrinsic platelet activation and decrease endogenous inhibitors of platelet activity (Beckman *et al*., 2002). Platelets from patients with diabetes exhibit enhanced platelet aggregation activity in the early disease state that may precede the development of CVD (Vlassara, 1997; Parving *et al.*, 1996; Chaturvedi et *al*., 2001; Walsh *et al.*, 1995; Stein *et al.*, 1995; Winocour *et al*., 1990; Davi *et al.*, 1990). Numerous biochemical abnormalities have been found that correlate with platelet hyper-reactivity. Vascular diseases are characterised by abnormal vascular homeostasis manifest as impaired vasodilation and the promotion of platelet aggregation. Abnormalities in platelet function may exacerbate the

progression of atherosclerosis and the consequences of plaque rupture. The functional abnormalities appear to be related to exaggerated elevations in platelet intracellular calcium mobilization (Davi *et al.*, 1990; Standley *et al.*, 1993; Levy *et al.*, 1994; Fakuda *et al.*, 1997). Intra-platelet glucose concentration reflects the extracellular concentration, but glucose entry into the platelet does not depend on insulin (Vinik *et al*., 2001). Platelets can modulate vascular function and participate significantly in thrombus formation. Platelets from people with diabetes have reduced membrane fluidity that is thought to be related to membrane cholesterol-to-phospholipid ratio (Winocour *et al*., 1990). Another process that likely contributes to enhanced platelet aggregation is an increase in glycation of platelet membrane proteins (Sampretro *et al.*, 1986).

Dyslipidaemia contributes directly and indirectly to platelet aggregation (Sowers & Epstein, 1999). Besides, platelet-derived growth factor (PDGF) stimulates migration and proliferation of SMC and extracellular matrix (ECM) production. These contribute to the formation of subendothelial 'fibrointimal' lesion and possibly the formation of the outer capsule of predominantly fatty lesions, which induce the development of atherosclerosis (Myllärniemi *et al*., 1997). Increased production of PDGF by the vascular endothelium in response to high glucose concentration and angiotensin II has been reported in vitro (Okuda *et al.*, 1996). The PDGF-β-receptor expression has also been demonstrated to be stimulated by hyperglycaemia via protein kinase C (PKC) activation in vascular SMCs, decreased production of platelet-derived NO and increased formation of superoxide (O^{-2}). This suggests a possible involvement of PDGF in the development of diabetic vasculopathy (Assert

et al., 2001; Inaba *et al.,* 1996). These abnormalities may result from decreased endothelial production of the antiaggregants NO and prostacyclin, decreased antioxidant levels, increased production of fibrinogen and platelet activators such as thrombin and von Willebrand factor (vWF) (Vinik *et al*., 2001). Moreover, diabetic patients have increased expression of activation-dependent adhesion molecules e.g., glycoprotein Ib (GpIb-IIIa) and P-selectin (Vinik *et al*., 2001).

Among diabetic individuals, increased platelet aggregation and adhesiveness (Jokl & Colwell, 1997; Halushka *et al.*, 1981; Mayfield *et al.*, 1985; Watala *et al.,* 1999; Martina *et al.,* 1998; Trovati *et al.*, 1997; Sarji *et al.*, 1979; Tschoepe *et al*., 1997) are caused by reduced membrane fluidity, increased arachidonic acid metabolism, increased thromboxane A_2 (TXA_2) synthesis, and altered Ca^{2+} and Mg^{2+} homeostasis (increased intracellular Ca^{2+} mobilization and decreased intracellular Mg^{2+}) (Li *et al.,* 2001). Nitric oxide is a principal mediator of vascular homeostasis. These features of abnormal vascular homeostasis are due to impaired bioactivity of endothelium- and platelet-derived NO. Disordered calcium regulation may contribute significantly to abnormal activity; since intra-platelet calcium regulates platelet shape change, secretion, aggregation and thromboxane formation (Vinik *et al*., 2001).

1.6.1.4 Coagulation abnormalities in diabetes

Diabetes also brings about some changes in coagulation of blood. A procoagulant state has been demonstrated in people having diabetes (García *et al*., 1987; Ford *et al*., 1991; Carmassi *et al.,* 1992). There is increase in the number of coagulation