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LONGITUDINAL ANALYSIS OF FACTORS AFFECTING THE COMPLICATIONS OF TYPE 1 DIABETES: RETINOPATHY, NEPHROPATHY, AND CARDIOVASCULAR OUTCOMES

by
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AN ABSTRACT OF THE THESIS OF

SaadaRiad El Jurdi for Master of Science
Major: Epidemiology

Title: Longitudinal analysis of factors affecting the complications of Type1 Diabetes: Retinopathy, Nephropathy and Cardiovascular Outcomes.

Objective: To assess longitudinally the impact of Kallikrein biomarker along with covariates of interest on three complications of diabetes: Retinopathy, Nephropathy and Cardiovascular complications.

Methods: A longitudinal cohort study design conducted on type 1 diabetic patients chosen from the DCCT/EDIC study. 350 type 1 diabetic subjects were randomly selected out of 1441 total sample. The duration of diabetes at baseline ranged from 1 to 15 years and ages were between 13 and 39 years. Plasma Kallikrein levels for these randomly selected individuals were longitudinally measured at five points in time by Jaffa et al (2003) from 1983 to 2004 across 29 different centers in the United States of Americaand the values of the covariates that correspond to these time points were also recorded. This results in a total number of 1750 observations. Longitudinal analyses along with survival analysis were used in the data analysis mainly. Cross-sectional analysis was also used.

Results: Longitudinally most covariates were significantly associated with kallikrein (P-values <0.05). ETDRS and AER were significantly associated with Log kallikrein over time (P-values: 0.011 &0.039 respectively) (Tables5 and 6). The longitudinal analysis of log IMT internal and common showed no significant association with log Kallikrein both at univariate and multivariate levels. Besides, patients whosekallikrein levels below the 50^{th} percentile had lower risk to develop microvascular complications such as retinipathy and nephropathy. Intensive treatment had played a major role in reducing the risk of microvascular complications such as retinopathy and nephropathy.

Conclusion: Evidently, it is important to continue and expand surveillance for childhood diabetes across the world since it is one of the most potent strategies for understanding the multifactorial etiology of the disease and ultimately preventing it.

CONTENTS

ABBREVIATIONS

ACE	Angiotensin-Converting Enzyme
ACR	Albumin to Creatinine Ratio
AER	Albumin excretion rate
BMI	Body Mass Index
CAD	Coronary Artery Disease
CKD	Chronic Kidney Disease
DBP	Diastolic Blood Pressure
DCCT	Diabetes Control and Complications Trial
DM	Diabetes mellitus
DN	Diabetic Nephropathy
EDIC	Epidemiology of Diabetes Interventions and Complications
ETDRS	Early Treatment of Diabetic Retinopathy Scale
GFR	Glomerular Filtration Rate
HbA1c	Hemoglobin A1c
HDL	High Density Lipoprotein
IDDM	Insulin-Dependent Diabetes Mellitus
IMT	Intima-Media Thickness
LDL	Low Density Lipoprotein
NPDR	Non-Proliferative Diabetic Retinopathy
PDR	Proliferative Diabetic Retinopathy
PK	Plasma Kallikrein
RVP	Retinal Vascular Dysfunction
SBP	Systolic Blood Pressure
T1D	Type1 diabetes
T2D	Type2 diabetes

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CHAPTER I

INTRODUCTION

Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset) is also defined as a disease that is characterized by deficient insulin production and which necessitates daily administration of insulin (WHO, 2013). Insulin, a hormone produced by special cells in the pancreas called beta cells, is needed to move blood glucose into cells to be stored and used for energy later on. These beta cells produce little or no insulin in type1 diabetic patients (Pub Med Health, 2011). Hence, it is advised that everyone with type 1 diabetes must take insulin every day (Pub Med Health, 2011). A person with type1 diabetes can fall into a life-threatening diabetic coma, also known as diabetic ketoacidosis if not diagnosed and treated with insulin (NDIC, 2012).

Studies have shown thatdiabetes is the leading cause of new cases of blindness, kidney failure, and amputations of feet and legs not related to accidents or injury among adults (CDC, 2011). Moreover, Diabetes was the seventh leading cause of death listed on U.S. death certificates in 2007 (CDC, 2011). However, diabetes is likely to be underreported as the underlying cause of death on death certificates (NDIC, 2012). In fact, a person with diabetes has a shorter life expectancy and about twice the risk of dying on any given day as a non-diabetic person of similar age (CDC, 2011).Diabetes is associated with many side effects such as: blindness, heart and blood vessel disease, stroke, kidney failure, amputations, and nerve damage. It can also complicate pregnancy and can result in birth defects in babies born to women with diabetes if not controlled (NDIC, 2012). In 2004, among people aged 65 years or older, heart

disease and stroke were noted on 68 percent and on 16 percent of diabetes-related death certificates respectively (NDIC, 2012). Diabetic retinopathy is a significant cause of blindness, and happens as a consequence of long-term accumulated damage to the small blood vessels in the retina. It is estimated that approximately 2% of people become blind and about 10% develop severe visual impairment after 15 years of diabetes. Moreover, 10-20% of people with diabetes die of kidney failure (WHO, 2012). The burden of diabetes and treatment of its associated complications is costly and could pose financial encumbrance on governments and ministries of health. For instance, in 2007, the cost of treatments for diabetes direct and indirect health related problems was $174 billion in the US alone (NDIC, 2012).

As for demographics, available data have shown that Type 1 diabetes occurs equally among males and females but is more common in whites than in nonwhites. It is rare in most African, American Indian, and Asian populations contrary to some northern European countries, including Finland and Sweden, which have high rates of type 1 diabetes. Thus, a child in Finland for example is 40 times more likely to develop type 1 diabetes than a child in Japan and almost 100 times more likely to get the disease than a child in the Zunyi region of China (BMJ, 2012). In this context, we note that the reasons for these observed demographic disparities are still unknown (NDIC, 2012).

A novel biomarker known as “Plasma Kallikrein (PK)” that is measured in blood was shown to be associated with some diabetic complications. The renal kallikrein-kinin system has been shown to regulate kidney haemodynamics by modulating such parameters as blood pressure, blood flow, renal vascular resistance and capillary permeability (Manto 1993). In this regard, several studies have highlighted the impact of Kallikrein as a risk factor for type1

diabetes and its association with microvascular complications of diabetes such as nephropathy and retinopathy (Phipp&Feener, 2008). PK has also been associated with hypertension which is on its own a major risk factor for the development of macrovascular and microvascular complications of diabetes (Jaffa, 2003). An animal study that was conducted on diabetic rats by Clermont et. al showed that PK contributes to retinal vascular dysfunctions(2011). Moreover, a cross-sectional study conducted by Jaffa et.al (2003) demonstrated the relevance and significance of the PK/ kinin system as a risk factor for the development of vascular complications in diabetic patients. Specifically, their results showed that in type 1 diabetes milieu, PK levels are elevated in association with increased blood pressure, are independently correlated with albumin excretion rate (AER) and significantly elevated in patients with macroalbuminuria. Despite that the detected positive correlation between PK and AER within the subgroups of patients with microalbuminuria suggested that PK could be a marker for progressive nephropathy, longitudinal studies are still needed to confirm this association (Jaffa et. al, 2003). Along the same line, it has also been shown that kallikrein excretion was increased in patients with poorly controlled insulin-dependent diabetes (Mayfield 1983). Hence, all the studies that were conducted thus far suggest a possible association between Kallikrein and diabetes complications that still need to be confirmed by longitudinal studies in humans.

A national longitudinal follow-up epidemiological study was conducted in the United States from the year 1983 to 2004 on 1441 type 1 diabetic patients to examine the relationship among glycemia, other risk factors and long-term complications, and the effects of glycemic therapy (NIH 2005). This study was divided into two parts; the first is the Diabetes Control and Complications Trial (DCCT) which was a multicenter, randomized clinical trial. It aimed at comparing the intensive diabetes treatment to the conventional one with regard to their effects on

the development and progression of the early vascular and neurologic complications of Insulin-Dependent Diabetes Mellitus (IDDM). The intensive-therapy regimen intended to bring blood glucose values (hemoglobin A1C levels) as close to normal values of 6% or less with three or more daily insulin injections or treatment with an insulin pump. On the other hand, conventional therapy offers one or two insulin injections per day. The main advantage of the Intensive therapy for patients with IDDM is that it delays the onset and slows the progression of clinically important retinopathy, including vision-threatening lesions, nephropathy, and neuropathy, by a range of 35 to more than 70 percent (DCCT, 1993).

The second part of the study includes the Epidemiology of Diabetes Interventions and Complications (EDIC). The goal of the EDIC follow-up study was to examine the longer term effects of the original DCCT interventions on diabetes related complications such as cardiovascular complications and more advanced stages of retinal and renal diseases that require a longer period of time to develop (NIH, 2005).

In our study, we conducted secondary data analysis using a randomly selected sample of 350 patients from DCCT/EDIC study chosen randomly from 29 different centers across the United States. The Kallikrein levels were longitudinally measured (5 repeated measures) on these selected patients by Jaffa et al (2003) along with other covariates recorded on these patients during the DCCT/EDIC study. The general aim of our study was to assess the impact of Kallikrein biomarker along with covariates of interest on three complications of diabetes: Retinopathy, Nephropathy and Cardiovascular complications. These associations were examined longitudinally and any potential time effect was also determined.

Significance:

We are proposing here longitudinal study that assessed the contribution of a novel biomarker, plasma prekallikrein on risk to develop microvascular and macrovascular complications of diabetes in a well characterized cohort of type 1 diabetic subjects. The strength of our approach resided in studying diabetes complications on human subjects rather than the commonly conducted animal studies. Moreover, it was the first study to examine the effect of plasma prekallikrein along with the covariates of interest on the three complications (retinopathy, nephropathy and cardiovascular problems) concomitantly and longitudinally for a period of 21 years rather than just simply at a cross sectional level.

In addition, the longitudinal measurements of important covariates such as HDL, LDL, total cholesterol level, BMI, glucose level, SBP, DBP, and Hemoglobin A1c and studying their effects over time on diabetic complications was also of great significance on its own. In this regard having measurements on these factors over a period of 21 years enabled us to monitor over time the progression of the disease and to determine how the change in the levels of these covariates was affecting the severity of the diabetic complications.

Accordingly, this study represented a unique opportunity to perform a longitudinal assessment of the effect of plasma prekallikrein and other factors of interest on the diabetic complications (retinopathy, nephropathy and cardiovascular problems) conducted on human subjects.

Aims:

1- Studied the effect of a novel biomarker "plasma prekallikrein", along with the treatment allocation (intensive versus conventional), and other covariates of interest on Diabetic Retinopathy assessed using the Early Treatment of Diabetic Retinopathy Scale (ETDRS). Specifically, these associations along with the baseline status of retinopathy and progression of disease over time were examined.

Covariates of interest entailed the following clinical determinants: High density lipoprotein (HDL), Low density lipoprotein (LDL), total cholesterol level, Body Mass Index (BMI), glucose level, Systolic and diastolic blood pressure (SBP and DBP), Hemoglobin A1c, all measured longitudinally. Smoking status, duration of diabetes and other demographic factors such as age were also measured at baseline and were considered along with gender.

2- Studied the effect of the novel biomarker "plasma prekallikrein", along with the treatment allocation, and the other covariates of interest (detailed in specific aim 1) on Diabetic Nephropathy which is assessed using the albumin excretion rate (AER). Here we aimed at examining these associations along with the baseline status of nephropathy and progression of diabetic nephropathy over time.

3- Examined the effect of "plasma prekallikrein", treatment allocation, and the determined covariates of interest on surrogate markers of cardiovascular related outcomes by measuring the carotid intima-media thickness (IMT common and internal) at baseline

and overtime. IMT was used to detect the presence of atherosclerosis (vessel wall thickness).

Hypothesis:

1-The higher the kallikrein level the greater the risk to develop the diabetic complications such as retinopathy, nephropathy and cardiovascular outcomes.

2-Type 1 diabetic patients who were on intensive management of blood glucose experienced fewer microvascular and macrovascular complications than patients who were on conventional glucose therapy.

CHAPTER II

LITRATURE REVIEW

A. Definition and classification of Type1 Diabetes:

Diabetes is a disease in which the body has a deficiency of insulin or a decreased ability to make use of it, or both (CDC, 2011). Type 1 diabetes is a chronic lifelong disease in which there are high levels of glucose sugar in the blood (Pub Med Health, 2011). It develops as a result of destruction of pancreatic beta cells, mostly by immune-mediated mechanisms. When patients experience no evidence of autoimmune destruction of pancreatic beta cells then this is called idiopathic type1 diabetes but when patients experience complete insulin deficiency and when antibodies appears to pancreatic beta cells then this is called autoimmune or classical type1 diabetes (BMJ, 2011).

B. Diagnosis of Type1 Diabetes:

Different tests are used to diagnose type1 diabetes. These tests include the Fasting blood glucose test that suggests the presence of diabetes when the blood glucose level is 126 milligrams per deciliter (mg/dL) or higher after an 8-hour fast. To confirm the diagnosis, it is usually necessary to repeat the test a second time on a different day. Another test is the Random (non-fasting) blood glucose test which determines the diagnosis of diabetes if the blood glucose level is higher than 200 mg/dL, and if symptoms such as increased thirst, urination, and fatigue occur. However, this diagnosis must be further confirmed with a fasting test. The Oral Glucose Tolerance test suggests presence of diabetes when the blood glucose level is 200 mg/dL and above 2 hours after drinking a

beverage containing 75 grams of glucose dissolved in water. Finally, the Hemoglobin A1C test indicates a diagnosis of diabetes when the blood glucose level is 6.5 %(48 mmol/mol) and above (NDIC, 2012).

C. Epidemiology of Type1 Diabetes:

Worldwide, 347 million people suffer from Diabetes and 80% of them live in low- and middle-income countries. As projected by the WHO, diabetes deaths will double between 2005 and 2030 (WHO, 2012). In the United States, 25.8 million people (8.3% of the population) have diabetes; 7.0 million of them have undiagnosed diabetes. In the year 2010, about 1.9 million new cases of diabetes were diagnosed in people aged 20 years or older. Globally, the incidence of type 1 diabetes is increasing by 3% every year (BMJ, 2012). This is not to mention that 1 of 3 U.S. adults will have diabetes by 2050 if current trends continue (CDC, 2011). Each year, more than 13,000 young people are diagnosed with type1 diabetes (CDC, 2012). It is estimated that 430,000 people aged 0 to 14 years have type1 diabetes globally (BMJ2012). Type 1 diabetes accounts for about 5 to 10 percent of diagnosed diabetes in the United States. It develops most often in children and young adults but can appear at any age (NDIC, 2012). It has been found that the prevalence per 1000 of type1 diabetes for U.S. residents aged 0-19 years is 1.7 (CDC, 2012). In 1994, the national incidence of type 1 diabetes among African Americans and whites aged 10-19 years is 19 per 100,000 (CDC, 2012). In the US, 15,600 youths were newly diagnosed with type 1 diabetes annually (annual rate for new cases about 19 per 100,000) from 2002 to 2005 (BMJ 2012). The exact cause of type1 diabetes is neither recognized nor preventable (WHO, 2013). Nevertheless, it is most likely an autoimmune disorder that may be caused by genetic mutation whereby an infection or some other trigger causes the body to

mistakenly attack the cells in the pancreas that make insulin. People with type 1 diabetes will suffer from symptoms such as excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue that may occur suddenly (WHO, 2013).

D. Complications of Type1 Diabetes:

Type1 diabetes can result in microvascular complications such as retinopathy and nephropathy as well as in macrovascular complications such as cardiovascular diseases.

1. Diabetic retinopathy:

There are approximately 93 million people with Diabetic Retinopathy (DR), 17 million with proliferative DR worldwide (Yau et al. 2012).DRis the leading cause of blindness among working aged adults around the world (Yau et al. 2012) and it is the most common microvascular complication of diabetes which has an increased risk at levels of HbA1c above the non-diabetic range that is 48 mmol/mol and above. On the basis of the data from all 35 studies on more than 20,000 participants with diabetes, it has been estimated that among individuals with diabetes, the overall prevalence of any DRwas 34.6%, PDRwas 7.0% (YAU 2012).The incidence is 1 per 100 person-years for a mean HbA1c value of 37 mmol/mol (5.5%) and 9.5 per 100 person-years for a mean HbA1c value of 91 mmol/mol (10.5%) (BMJ,2012). More than 95% patients have evidence of retinopathyafter 20 years of being diagnosed with Type 1diabetes (Funk 2010). It results as a retinal consequence of chronic progressive diabetic microvascular leakage and occlusion and eventually occurs to some degree in all patients with diabetes mellitus (DM). It appears in two different forms: non-proliferative and proliferative.

Non-proliferative diabetic retinopathy (NPDR) is a consequence of fluid leakage from the blood vessels in the eye into the retina resulting in a blurry vision. This type happens at an early stage of the disease and is less severe. On the other hand, proliferative diabetic retinopathy (PDR) is a more advanced form of the disease whereby new fragile blood vessels start to grow in the eye (neovascularisation) causing possible haemorrhage, vision loss and scarring of the retina (BMJ 2012). It has been shown that the risk factors for retinopathy were diabetes duration, HbA_1c >7.0% (53 mmol/mol), smoking and male sex (Hammes et. al 2011). Moreover, the risk factors for advanced retinopathy were duration, male sex, HbA_1c greater than 7.0% (53 mmol/mol), triacylglycerol higher than 1.7 mmol/l and blood pressure more than 140/90 mmHg (Hammes et. al 2011). Screening is essential when dealing with retinopathy given that it is usually asymptomatic until its late stages. It is also important to focus on primary prevention such as strict glycaemic and blood pressure control which helps in delaying the progression of retinopathy into the non-proliferative stage. In advanced stages of the disease, photo-coagulation and vitrectomy can be done to prevent blindness (BMJ 2012).

2. Nephropathy:

Diabetic nephropathy (DN) is defined as micro-albuminuria when albumin to creatinine ratio (ACR) is 3.4 to 34 mg/mmol. It associated with diabetic retinopathy whether type 1 or type 2 diabetes and/or with duration of type 1 diabetes mellitus above 10 years. This type of DN occurs at an early stage and acts as a marker of much increased cardiovascular risk. Thus this demands the need for cardiovascular screening through a yearly testing in diabetic patients aged 10 years or older who have been diabetic for more

than 5 years. DN is defined as macroalbuminuria when ACR level exceeds 34 mg/mmol. So if these criteria are met, chronic kidney disease (CKD) can be attributable to diabetes. Moreover, CKD should also be considered in the absence of diabetic retinopathy, in the presence of active urinary sediment (e.g., cellular casts in urine), during a rapid decrease in Glomerular Filtration Rate (GFR) or if any signs or symptoms of other systemic disease existed. Clinical presentation is characterized by progressive albuminuria, hypertension, and decline in GFR in a long-standing diabetic patient who has been suffering of diabetes for more than 10 years. Certain risk factors contribute to the development of diabetic nephropathy such as: genetic susceptibility, sustained hyperglycemia, hypertension, glomerular hyperfiltration, smoking, dyslipidemia, proteinuria levels and dietary factors, such as the amount and source of protein and fat in the diet. Diagnosis is most conclusively made by kidney biopsy, though it is rarely necessary (BMJ 2012).Worldwide, diabetes mellitus is the most common cause of chronic kidney disease (CKD). Mostly, 40% of diabetic patients develop diabetic nephropathy despite the fact the glucose levels are maintained for long periods of time (Gross 2005).

Around 20% to 30% of diabetic patients will have microalbuminuria 15 years after being diagnosed with DM. The prevalence of nephropathy is 2.2% and 7.7% at 20 years and at 30 years of onset of type1 diabetes respectively. In developed countries, diabetic nephropathy is considered the most common cause of end-stage renal disease. The onset and progression of the disease could be delayed by controlling the blood pressure and the glycaemic level with an angiotensin-converting enzyme (ACE) inhibitor, or angiotensin-

II receptor blocker.Protein restriction may also be useful in selected patients with overt nephropathy and decreased glomerular filtration rate (BMJ 2012).

3. Cardiovascular disease:

Cardiovascular disease is divided into 4 major categories: coronary artery disease; cerebrovascular disease; peripheral vascular disease; and aortic atherosclerosis. Diabetes is an important risk factor for all forms of cardiovascular disease with the exception of aortic disease. Many risk factors are responsible for cardiovascular diseases in diabetic patients. These factors include: high levels of LDL and triglycerides, low levels of HDL**,** high blood pressure (hypertension), smoking, obesity, lack of physical activity, and high blood sugar levels. Cardiovascular disease is the major cause of death and a major cause of morbidity for diabetic patients. The incidence of macrovascular disease in type 1 diabetes could be decreased by an intensive glycaemic control. Lifestyle and behavioural therapy are essential components for treatment of the cardiovascular disease. Moreover to decrease the risk of cardiovascular disease, the primary goal is to lower LDL and triglycerides to less than 70 mg/dL and 150 mg/dL respectively and to raise HDL to more than 40 mg/dL. It is also recommended that blood pressure should be treated to less than 130/80 mm Hg with an ACE inhibitor or angiotensin-II receptor blocker; most patients will require 2 or 3 drugs to reach goal. Furthermore, treatment with aspirin (75-162 mg/day) and have smoking-cessation counseling and treatment are needed for adult diabetic patients who have cardiovascular disease. This is not to mention that there are no evidence-based guidelines that exist for screening asymptomatic patients for coronary heart disease (BMJ 2012).

E. Economic Impact of Type1 Diabetes:

It has been found that the total yearly medical expenditure attributable to T1D accounts for $6.9 billion compared to the medical expenditures of a matched group of non-diabetic individuals. In addition to that, the per capita expenditures amount to $6,288 per year given that hospital in-patient visits and prescription drugs including medical supplies account for over 75 percent of the yearly cost attributable to T1D (Tao et. al 2010).

Each year T1D costs the US $14.4 billion in medical costs and lost income. In terms of lost income, type 1 patients earn a disproportionate share of type 1 and type 2 costs. Moreover, an estimated $10.6 billion incurred by a new cohort and $422.9 billion incurred by the existing number of type 1 diabetic patients would be avoided over their lifetime if the disease were eliminated by therapeutic intervention. So, it is not appropriate to combine T1D and T2D when estimating costs since the costs attributed to T1D are disproportionately higher than the number of type 1 patients compared with type 2 patients (Tao et. al 2010).

F. Summary:

Diabetes –characterized by high blood glucose (blood sugar) - occurs when the body either does not produce enough insulin or is unable to use its own insulin effectively. When glucose builds up in the blood, it causes a condition that, if not controlled, can lead to serious health complications and even death. A person with diabetes has twice the risk to die than a person of similar age who does not have diabetes (CDC 2012).

Not only diabetes can cause complications, such as vision loss, kidney failure, and amputations of legs or feet but it is a major cause of heart disease and stroke. Adults with diabetes have 2–4 times higher rates of dying from heart disease and risk of stroke than those without diabetes.Moreover, 67% of U.S. adults who report having diabetes also report having high blood pressure. In fact, high blood pressure levels, high cholesterol levels, and smoking increase the risk of heart disease and stroke for people with diabetes but this risk can be reduced by controlling blood pressure and cholesterol levels and quitting smoking. This is not to mention that effective glucose control, as measured by A1c levels, and blood pressure control can prevent or delay these complications (CDC 2012).

T1D-an autoimmune disease- is often diagnosed early in life and characterized by the destruction of the insulin-secreting beta cells in the pancreas. As a result, patients become insulin-dependent and they must follow a rigid, daily regimen of exogenous insulin replacement. According to the American Diabetes Association (ADA), it is estimated that there are 17.9 million individuals diagnosed with diabetes in the U.S. with 5 to 10 percent representing those with T1D. Worldwide, the incidence rate of T1D has been growing especially amongst young children. Moreover, patients with T1D typically suffer from the disease for a longer period of time which requires regular maintenance of T1D with daily insulin shots and constant monitoring. Thus, this represents a significant lifelong cost and time requirement (Tao 2010).

Despite the known risk factors which are responsible for T1D such as autoimmune, genetic, or environmental, but, there are no recognized ways to prevent it (CDC 2012). This is not to mention that the novel biomarker “Plasma Kallikrein (PK)”

that is measured in blood was shown to act as a risk factor for T1D and was found to be associated with its microvascular and macrovascular complications.

To examine the effects of attempted glucose normalization (tight or intensive diabetic control) on the incidence of complications, a paradigm shift in diabetes treatment as a first major trial occurred in 1993 with publication of the results of the Diabetes Control and Complications Trial (DCCT). This study of individuals with Type 1 DM showed that intensive (vs. conventional) treatment reduced microvascular complications (retinopathy, nephropathy, neuropathy) by 60% and hence improved health outcomes of patients (Funk 2010).

Given all these facts, the significance of this study lies in studying the effect of the novel biomarker “plasma prekallikrein”, along with the treatment allocation (intensive versus conventional), and other covariates of interest on Diabetic complications: Retinopathy, Nephropathy and Cardiovascular complications at cross sectional points and over time.

CHAPTER III

METHODOLOGY

A. Study Design

A longitudinal cohort study design was conducted on type 1 diabetic patients. Our study was based on a randomly selected sample of 350 type 1 diabetic patients chosen from the DCCT/EDIC study. The Kallikrein levels were longitudinally measured (5 repeated measures) on these selected patients by Jaffa et al (2003) and the other covariates were recorded on these patients during the DCCT/EDIC study.

B. Sample Size and Selection:

1. Sample size:

Sample size calculation for our study was determined using the formula by Hedeker D et. al (1999) for longitudinal data. In this study we assumed 80% power, significance of 5%, and 5 measurement time points. In Table 1 we present the different sample sizes that were needed in every group assuming different correlations between the repeated measures ranging from medium to high correlation in a longitudinal setting, and various levels of effect size ranging from low, medium to high effect size. Groups were defined as being above the 50^{th} percentile of kallikrein levels and below it. Based on this power calculation, a total sample size of 350 subjects resulted in a correlation of about 0.65 and effect size of 0.25.

Table1: Sample Size Correlation

Sample Size Correlation of 0.4	Sample Size Correlation of 0.5	Sample Size Correlation of 0.6	Sample Size Correlation of 0.7	Effect Size
130.64	150.74	170.84	190.94	0.25
90.73	104.68	118.64	132.60	0.3
51.03	58.88	66.74	74.59	0.4
32.66	37.69	42.71	47.74	0.5
22.68	26.17	29.66	33.15	0.6
16.66	19.23	21.79	24.35	0.7

2. Sample selection:

From the DCCT/EDIC study conducted from 1983 to 2004 across 29 different centers in the United States of America, 350 type 1 diabetic subjects were randomly selected out of 1441 total sample. The duration of diabetes at baseline ranged from 1 to 15 years and ages were between 13 and 39 years. Plasma Kallikrein levels for these randomly selected individuals were measured at five points in time and the values of the covariates that correspond to these time points were also recorded. This resulted in a total number of 1750 observations.

C. Measures

1. Dependent variables:

1-The Intima-media thickness (IMT) internal and external was used to detect the presence of atherosclerosis thickness of heart artery. The IMT is a continuous variable and is normally distributed.

2-The Early Treatment of Diabetic Retinopathy Scale (ETDRS) was used to assess status of retinopathy. The ETRDS scores are measured on a continuous scale with values ranging from 1 to 23 (American Academy of Ophthalmology, 2003):

a. 1-3: it is considered normal

b. 3-9: it is considered mild

c. > 9: it is considered abnormal

3-The albumin excretion rate (AER) was used to assess the development of diabetic nephropathy. The AER is measured on a continuous scale with values ranging from 1 to >300 in mg/24 hrs (BMJ, 2004):

a. 1-39: it is considered normal

b. 40-299: it is considered microalbuminuria

c. ≥ 300: it s considered macroalbuminuria

2. Independent variables:

Independent variables include: Kallikrein levels and type of treatment (intensive versus conventional).

Covariates: HDL, LDL, total cholesterol level, BMI, glucose level, SBP, DBP, Hemoglobin A1c, Smoking at baseline, duration of diabetes at baseline, and other demographic factors that encompassed age at baseline and gender.

D. Data Collection

In the DCCT-EDIC study, assessment of carotid intima-media thickness was performed by certified technicians at the clinical centers, recorded on videotapes, and read in a central unit by a single reader, who was unaware of the subjects' diagnostic groups, treatment assignments and the time of the studies. The assessment included a

single longitudinal lateral view of the distal 10 mm of the right and left common carotid arteries. In addition to that, three longitudinal views in different imaging planes of each internal carotid artery were obtained. The latter defined as including both the carotid bulb and the 10-mm segment distal to the tip of the flow divider that separates the internal from the external carotid artery (DCCT 2003).

Assessment of Retinopathy was done by 7-field stereo fundus photography. If a patient had previously undergone panretinal photocoagulation in both eyes, then the photography was not conducted. Using ETDRS scores and DCCT methods, all photographs were graded centrally, with graders masked to therapy assignment. The time to the first occurrence of further retinopathy progression during EDIC- defined as a 3-step or more progression from the level of retinopathy at DCCT closeout- was considered the primary outcome. This represents a reproducible measure of clinically important worsening. Whereas the time to the first occurrence of proliferative diabetic retinopathy (PDR) or worse during EDIC was considered as the secondary retinopathy outcome. Some other retinopathy outcomes includes the prevalence of a 3-Step or more progression from DCCT entry, severe NPDR (ETDRS level53/<53) or worse, clinically significant macular edema (CSME), and photocoagulation therapy (focal or scatter). If patients received panretinal scatter photocoagulation (laser) therapy in either eye, then they were counted as having worsened retinopathy for all of these outcomes thereafter but if patients received focal photocoagulation for macular edema, then they were counted as having CSME thereafter. Visual acuity was assessed by ETDRS methods. During EDIC, inter-reader reliability was evaluated by having different graders reread the same 50 fundus photographs at each EDIC year and comparing the results with the

primary double reading at DCCT closeout. The ranges for the individual weighted k measure of interrater agreement beyond chance varied between 0.82 and 0.92 for ordinal ETDRS scores and from 0.71 to 0.90 for ordinal CSME scores over 10 years of measurements. Whereas the overall weighted k stratified for EDIC year was 0.91for ETDRS scores and 0.84 for CSME scores (DCCT 2008).

Assessment of the renal function was performed using a 4-hour urine collection for albuminuria, with approximately half of the EDIC participants evaluated at odd EDIC study years and half at even years. At the time of their annual assessments, participants were not asked to discontinue any medications, including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, or other anti-hypertensive medications. The results for those participants evaluated during years 1 and 2, years 3 and 4, years 5 and 6, and years 7 and 8 are combined. By a variation of the Jaffe method, creatinine levels in serum and urine were measured. The fluoroimmuno assay was used to measure the urine albumin level. Throughout the EDIC study, coefficients of variation and coefficients of reliability were, respectively, 2.3% and 94% for serum creatinine concentration; 2.3% and 100% for urine creatinine concentration; 9.4% and 94% for urine albumin concentration; and 14% and 95% for the 4-hour excretion rate of albumin. Adjusting for body surface area, glomerular filtration rates were determined by timed clearance of Iiothalamate at DCCT closeout (EDIC 2003).

E. Statistical Analysis:

Data cleaning was first conducted to ensure that no errors in data collection or entry were transpired. This was achieved by graphical representation that enabled us to

detect outliers and by a numerical summary of the variable values. A frequency analysis was then conducted to look at the distribution of age, gender, smoking habits, type of treatment, HDL, LDL, total cholesterol level, BMI, glucose level, SBP, DBP, Hemoglobin A1c, duration of diabetes at baseline. Specifically, mean, median, standard deviations, proportions along with confidence intervals were all reported. Longitudinal analysis along with profile analysis was conducted on the repeated measures at five different points in time to assess progression of diabetic complications over time and to determine any possible time effect. Auto-regressive variance covariance matrix was assumed in our longitudinal data analysis. Survival analysis was used to analyze data in which the time until the event is of interest by conducting the cox proportional hazard function which was mainly utilized to explore the relationship between the survival of a patient and several explanatory variables.Analysis was also performed at the various cross-sectional points in time to study the effect of the different covariates on the 3 different outcomes (retinopathy, nephropathy and cardiovascular problems) at every time point. Analysis of this project was conducted using SAS, STATA and SPSS as statistical packages.

F. Ethical Considerations:

This study considered the three principles of ethics throughout. As for autonomy, subjects were given an informed consent from which they had the free choice to decide whether or not to participate. Subjects were also given the freedom to withdraw from the study at any point in time. Beneficence was not breached since no harm was done to the participants. Justice was fulfilled by including the 29 medical centers across

the different states. No one center was preferred over the other. Subjects were all offered equal opportunity to participate in the study.

An explanation of the study was comprehensively presented. All participants were ensured confidentiality of data and that no identifying information was ever used or revealed to ensure that our participants were all de-identified. One of the important ethical issues was that after the beneficiary effects of the intensive treatment were shown, the DCCT study was stopped and all participants were informed of the benefits of the intensive treatment in controlling glucose levels. The DCCT follow-up study recommended the intensive treatment for all patients after it was shown that it was highly effective in reducing long-term complications of diabetes at the end of DCCT in 1993(NIH 2007).

We conducted in this research project a secondary data analysis using a subset of the study that was already granted IRB exemption from AUB.

CHAPTER IV

RESULTS

A. Descriptive analysis:

Data cleaning was first conducted to ensure that no errors in data collection or entry were transpired. This was achieved by graphical representation that enables us to detect outliers and by a numerical summary of the variable values.

A frequency analysis was conducted to look at the distribution of the study outcomes: AER, ETDRS, IMT common and IMT internal (Table3) as well as other continuous variables such as kallikrein, BMI, DBP, SBP, duration of diabetes at baseline, Hemoglobin A1c, HDL, LDL, total cholesterol level, glucose level, and age (Table1)

Other covariates were also considered such as gender, smoking habits, type of treatment (intensive versus conventional) and whether or not a patient is on ace inhibitor or not (Table3). Specifically, mean and standard deviation, min, and max, along with P-values obtained from longitudinal analysis of the covariates as a function of kallikrein were all reported. Results were shown in (tables 1, 2 &3).

As evident in Tables 1 and 2, all patients that were recruited in the study consisted of men and women aged 13-39 years with 1-15 years of diabetes at study entry. Fifty-one percent of the cohorts were males. Almost half of the patient sample was randomly assigned to conventional diabetes treatment, and the other half was assigned to intensive diabetes treatment. Thirteen percent of the patients were smokers. The mean BMI was 25.990 which was almost close to normal for most patients [normal level: 18.5

– 24.9 kg/m^2 (CDC 2011)]. The average SBP and DBP for the patients was found to be 117 mmHg and 75 mmHg respectively. These values were also close to normal levels [SBP <120 mm Hg and DBP <80 mm Hg (CDC 2008)]. The mean cholesterol level for the good cholesterol HDL was 56 mg/dl (Normal>40mm Hg).Whereas, the mean cholesterol level for the bad cholesterol was 110 mg/dl. On the other hand, the average total cholesterol level was found to be 183 mg/dl (Normal<200mm Hg). The LDL was the only type of cholesterol which was higher than the normal value which should be less than 100 mm Hg (CDC 2012). Moreover, most patients had a mean HbA1c of 8% which exceeded the normal range that varied between 4% and 6 % (Edelman et al. 2004). According to Fasting blood glucose test, a patient was diagnosed as having diabetes if the blood glucose exceeded 126 mg/dL on two consecutive times. As shown in table 1, the mean glucose was equal to 212mg/dl which exceeded the normal value (NDIC 2012). Only 8% of the recruited subjects have used angiotensin-converting enzyme (ACE) inhibitors – medicines that treat heart, blood vessel, and kidney problems (NIH 2013).

Our longitudinal data analysis showed that most covariates were significantly associated with Log kallikrein (P-values <0.05) (Table4). With respect to the outcomes, Log ETDRS and Log AER were significantly associated with Log kallikrein over time (P-values: 0.011 &0.039 respectively) (Tables5&6). On average, patients suffered from microalbumineria (AER=47 mg/24hrs) and proliferative retinopathy (ETDRS=4) (Table3).

Our data analysis had three fold; the descriptive part that we have just discussed; the longitudinal data analysis that assesses over time the effect of the different covariates on the rate of change in the levels of the outcomes (ETDRS, AER, IMT common and

IMT internal). In addition, the survival data analysis at a univriate and multivariable levels was conducted to determine the factors that could impact the time to micro and macro albumineria, and non proliferative as well as proliferative retinopathy. In this regard we have conducted the Cox proportional hazard for longitudinal data whereby we investigated the effect of being above and below the 50^{th} percentile in kallikrein levels at baseline on the time to develop these complications. Moreover, we have also examined the time to these complication when the longitudinal repeated measures of kallikrein were incorporated in the survival. Last but not least we conducted longitudinal analysis to relate the different covariates with kallikrein itself and same analysis was conducted at cross sectional levels.

B. Longitudinal analysis:

Longitudinal analysis was conducted on the repeated measures at five different points in time to assess progression of diabetic complications over time and to determine any possible time effect. Auto-regressive variance covariance matrix was assumed in our longitudinal data analysis.

Longitudinal analysis was done for the four outcomes at univariate level and at a multivariate one to study the association with log kallikrein and other covariates over time. Only covariates with P-values less than or equal to 0.2 at a univariate level were included in the multivariable longitudinal analysis. Covariates that have resulted in collinearity at the multivariate levels were excluded from the model. The log function was used for the four outcomes to normalize the distribution of the data. Longitudinal analysis was also conducted on log kallikrein to assess its relationship with the different

covariates (Table 4). In this regard, our longitudinal analysis showed that log kallikrein is significantly positively associated with LDL, Hb1Ac, total cholesterol, glucose and BMI which indicates that these covariates act as risk factors for the outcome, and negatively significantly associated with HDL(all P-values <0.05). With respect to the outcomes of interest, log ETDRS was highly significantly associated with log kallikrein, BMI, DBP, SBP, duration of diabetes, LDL, total cholesterol, glucose and treatment group at a univariate level (all with P-values <0.05) (Table 5). All of these covariates acted as risk factors for ETDRS except for the intensive treatment that appeared to be protective for this diabetic complication. No significant association was found between log ETDRS and HDL, HbA1c, smoking status, age, gender and ace inhibitor. At multivariate level, all parameters that showed significance at univariate remained significant except for SBP and total cholesterol. No significant association has been observed between log ETDRS and HDL, SBP, smoking and HbA1c at multivariate level (Table9). On the other hand, the relation between log AER and other covariates such as kallikrein, DBP, duration of diabetes, HbA1c, LDL, total cholesterol, smoking, treatment group, gender and ace inhibitor showed high significance when studied longitudinally at a univariate level (P-values <0.05) (Table6). The variables that didn't show significance at a univariate level included: BMI, HDL, glucose, age and SBP. At a multivariate level, some covariates that showed to be significant at a univariate level, have become non-significant. Those include DBP, HbA1c, total cholesterol and ace inhibitor intake. Moreover, no significant association was found between log AER neither with HDL nor with SBP (Table10). As evident by our results, being on intensive treatment vs. conventional and being a female

are considered protective factors against the development of log AER both at a univariate and multivariate levels.

The longitudinal analysis of log IMT internal and common showed no significant association with log Kallikrein both at univariate and multivariate levels. With respect to the other covariates, IMT common showed significant association with BMI, duration of diabetes, HbA1c, HDL, smoking, age, gender, ace inhibitor at a univariate level (P-values <0.05) (Table7). On the other hand, no significant association with log IMT internal was detected at the levels of DBP, SBP, LDL, total cholesterol, glucose and treatment Group (Table8). At multivariate level BMI and ace inhibitor lost their significance. No significant association was shown between log IMT common and the following covariates: BMI, LDL, SBP, total cholesterol and ace inhibitor at a multivariate level (Table11). Moreover, IMT internal appeared to be significantly associated at a univariate level with duration of diabetes, HDL, smoking, age, gender, ace inhibitor; but not associated with BMI, DBP, SBP, LDL, HbA1c, total cholesterol, glucose, and treatment group (Table 8). At a multivariate level LDL and SBP were found to be significantly associated with IMT internal, in addition to Smoking, age and gender (P-value < 0.05). No significant relationship was found between IMT internal and duration of diabetes, HbA1c, HDL and ace inhibitor (Table 12).

C. Survival analysis:

Survival analysis was conducted to analyze the time to event of interest. In this respect, cox proportional hazard regression analysis for longitudinal data was implemented to explore the relationship between the time to event (survival or censored)

of a patient and several explanatory variables at both univariate and multivariate levels. Only variables with P-values <0.2 at univariate levels were includedin the multivariate model. Baseline kallikrein measures were used in the analysis and they were dichotomized by above or below the 50^{th} percentile to assess whether or not baseline measures of kallikrein levels could predict the occurrence of diabetic complications

Our results suggested that baseline levels of kallikrein were not significantly associated with time to develop microalbumineria as well as macroalbumineria (P-values 0.291 and 0.575 respectively). Nonetheless, duration of diabetes, BMI, DBP, HbA1c, SBP and total cholesterol were all found to be associated with time to develop microalbumeria at a univariate level (Table 13). Specifically the hazard ratio to develop microalbumineria was 2 fold for HbA1c. Nevertheless, no association was found at the levels of HDL, LDL, glucose, smoking, treatment group, age, gender and ace inhibitor (Table 13).

Regarding, macroalbumineria, most variables exhibited significant hazard ratio (P-values<0.05) at a univariate level. Smokers appeared to have double the risk of developing macroalbumineria compared to non smokers, and a 1mmol/mol increase in HbA1c also doubled the risk of developing macroalbumineria. No significant association was detected with BMI, duration of diabetes, HDL, LDL, glucose, age and gender (table 14).

Non-Proliferative and proliferative Retinopathy have shown significant association with kallikrein at a univariate level. The hazard increased by 1.42 and 2.79 for non-proliferative and proliferative retinopathy respectively for subjects who had their kallikrein levels at baseline above the 50^{th} percentile compared to those below this

percentile. For non-proliferative Retinopathy, covariates such as duration of diabetes, HbA1c, SBP and smoking have all contributed to an increase in the hazard of the disease except for the treatment group whereby being on intensive treatment decreased the hazard non-proliferative retinopathy by 43%. No significant association was found between Non-Proliferative Retinopathy and the following covariates: BMI, DBP, HDL, LDL, total cholesterol, glucose, age, gender and ace inhibitor(P-values>0.05) (Table 15). With respect to proliferative retinopathy the hazard increased as a function of duration of diabetes, DBP, HbA1c, SBP but had no effect with BMI, HDL, LDL, total cholesterol, glucose, smoking, age, gender and ace inhibitor. Besides, being on intensive treatment also decreased the hazard of proliferative retinopathy by 69% (Table 16).

At a multivariate level, Microalbumineria and Macroalbumineria have not been associated with kallikrein (P-values: 0.495 and 0.346 respectively). HbA1c was associated with increased hazard for both outcomes by 2 fold. Intensive treatment showed to be associated with a decrease in hazard to develop microalbumineria by half (P-value=0.028). LDL and Smoking showed no association with time to develop microalbumineria (Table 17). HbA1c appeared to be strongly associated with an increased risk for macroalbumineria (HR = 1.6 and P-value = 0.039) Duration of diabetes, smoking, age, gender and total cholesterol have all shown no association (Table 18).

The hazard ratios for non-proliferative retinopathy at a multivariate level indicated that those with kallikrein levels above the 50^{th} percentile had an increased risk of 31% to develop non-proliferative retinopathy (P-value=0.047) compared to those below the 50^{th} percentile. Moreover, the duration of diabetes and HbA1c increased the

hazard by 18 and 17% respectively (P-values=0.000) but no significant association was found at the levels of the following parameters: BMI, HDL, SBP, smoking and gender. Moreover, being on intensive treatment reduces the risk to develop non-proliferative retinopathy by 41 % (P-value=0.00) (Table 19).

Proliferative retinopathy has been highly associated with kallikrein wherein the hazard ratio for those who are above the 50^{th} percentile for kallikrein at baseline had 4 times the risk of developing proliferative retinopathy compared to those who are below the 50^{th} percentile (P-value=0.000). HbA1c also increased the risk of the outcome by 40% (P-value=0.004). Nonetheless, Kallikrein is shown to be the main covariate to impact the risk of proliferative retinopathy. There was no association between proliferative retinopathy and DBP, SBP, glucose and total cholesterol. On the other hand; subjects who were on intensive treatment had a 76% decrease in hazard to develop this complication compared to those who were on the conventional treatment P-value=0.001) (Table 20).

Analysis of the cox proportional hazard for the association between the outcomes and clinical parameters was also conducted using measures of kallikrein over time. At univariate level, only microalbumineria and proliferative retinopathy have revealed significant association with kallikrein. The latter being minimally associated with the risk of developing microalbumineria (hazard ratio=2.174with P-value=0.024) and highly associated with proliferative retinopathy (hazard ratio= 6.069with P-value=0.001) (Table21)

At a multivariate level, results showed that kallikrein was not associated with neither microalbumineria nor macroalbumineia (P-values>0.05). For microalbumineria, the risk increased by 54% as a result of a single unit increase in HbA1c (P-value=0.000). Duration of diabetes and BMI had a minimal effect on increasing hazard (6 to 7 %) while LDL and Smoking showed no significance (P-values>0.05). Nonetheless, this risk decreased if the patient was in the intensive treatment group by 43% compared to conventional treatment (Table 22). The only covariate that was associated with macroalbumineria was SBP with minimal hazard of 3%. Other covariates such as: duration of diabetes, HbA1c, smoking, age, gender and total cholesterol were not related to the outcome. In contrast, being on intensive treatment has proven a 90% significant decrease in the risk of macroalbumineria (P-value=0.035) (Table23).

Results showed that kallikrein when measured over time and when studied at a multivariate level, was significantly associated with proliferative retinopathy (P-value=0.003) but not with non-proliferative retinopathy (P-value=0.927).With respect to non-proliferative retinopathy, BMI, HbA1c and treatment group were the only covariates that were found to be significant. HDL, SBP, smoking and gender had no impact on this hazard (P-values>0.05) (Table24). Regarding proliferative retinopathy, covariates that showed significance include duration of diabetes and HbA1c with P-values<0.05. Intensive treatment was a significant determinant in decreasing the hazard of this outcome by 74 %(P-value=0.002). DBP, LDL, SBP and glucose showed no significance (Table 25).

Comparing the hazard of developing microalbumineria, macroalbumineria, proliferative and non-proliferative retinopathy for those with baseline kallikrein greater than the 50^{th} percentile with those with baseline kallikrein lower than the 50^{th} percentile revealed the following results. The hazard of developing microalbumineria for those with baseline kallikrein greater than the 50^{th} percentile was higher than those with baseline kallikrein lower than the 50^{th} percentile. But as shown from the log rank test that there was no significant association between kallikrein at baseline and microalbumineria (Figure1). With respect to Non-proliferative and proliferative Retinopathy, the hazard for those with baseline kallikrein greater than the 50^{th} percentile was higher than those with baseline kallikrein lower than the 50^{th} percentile. As shown from the log rank tests, significant association was observed between kallikrein at baseline and non-proliferative and proliferative retinopathy(P-values=0.002 &0.001 respectively) (Figures 2 and3).

- Proportionality assumption:

Since the Cox proportional hazards model relies on the hazards to be proportional, i.e. that the effect of a given covariate does not change over time, it is very important to verify that the covariates satisfy the assumption of proportionality. The tests for both non-proliferative (P-value=0.377) and proliferative retinopathy (P-value=0.977) were not significant (P-values over 0.05) then we couldn't reject proportionality and it is assumed that there was no violation of the proportional assumption. The conclusion was that all of the time-dependent variables were not significant either collectively or individually thus supporting the assumption of proportional hazard.

D. Cross-sectional analysis:

Analysis was also performed at the various cross-sectional points in time to study the effect of log kallikrein on the 3 different outcomes (retinopathy, nephropathy and cardiovascular problems) at every time point. Time1 correspond to year 1986, time 2 to year 1993, time 3 to 1998, time 4 to year 2002, and time 5 to year 2004.

Log AER was associated with log kallikrein only at baseline (year 1986) (P-value=0.038). Log ETDRS showed significance association with Log kallikrein in the years: 1986, 1993, and 1998 and lost its significance in the year 2002 and 2004. When looking at the association between Log IMT common and log IMT internal with log kallikrein at cross sectional levels studied on three different years (1998, 2002, &2004), no association with log kallikrein has been observed (Table 26).

CHAPTER V

DISCUSSION

In the United States, diabetes prevalence has been increasing over the last decade and has reached 10% in 2007 in those aged 20 years or older (Funk et al. 2010). Incidence rates were marked low to intermediate in South America to high in North America (Karvonen et al.2000). Type 1 DM is usually less common than Type 2, accounting for 5-10% of cases of primary diabetes. It is characterized by autoimmune destruction of pancreatic βcells which results in severe insulin deficiency. Yet, the cause of Type 1 DM is still unknown in the minority of patients. Type1 DM commonly affects individuals younger than 30 years and the highest incidence occurs around age 5–7 years as well as at puberty. Patients usually suffer acute clinical symptoms such as polyuria, polydipsia, weight loss and an elevation in serum glucose concentrations. Type1 diabetic patients may also suffer a severe life-threatening acidosis also called diabetic ketoacidosis. Treatment with insulin is necessary for Type1 diabetic patients (Funk et al. 2010). Factors such as genetic and environmental could contribute to the development of the disease (Karvonen et al.2000). The incidence of Type1 diabetes is usually high in males and in the age group 10-14 years (Karvonen et al.2000).

In our study, we assessed longitudinally the contribution of plasma prekallikrein biomarker as well as other clinical parameters on the risk to develop over time microvascular and macrovascular complications of diabetes in a well characterized cohort of type 1 diabetic subjects. Our longitudinal study also compared the intensive

diabetes treatment to the conventional one with regard to their effects on the development and progression over time of the early vascular and neurologic complications of Type1 diabetes.

A. ETDRS:

Our study proved that longitudinally Log ETDRS was highly significantly associated with log kallikrein, BMI, DBP, duration of diabetes, LDL, glucose and treatment group at a multivariate level (P-values <0.05). This is in agreement with what has been established regarding the contribution of the Kallikrein-Kinin system to DR. Increased levels of plasma KKS components, including plasma kallikrein (PK) were found in vitreous fluid obtained from people with advanced stages of DR (Liu et al. 2013). Moreover, an animal study has proved that the activation of the intraocular KKS induces retinal vascular permeability, vasodilation, and retinal thickening, and these responses were worsened in diabetic rats. Another animal study has shown that intravitreal injection of PK increased retinal thickness compared with baseline to a greater extent (P-value=0.017) in diabetic rats from (193±10µm to 223±13µm) compared to non-diabetic rats (from 182±8µm to 193±9µm) (Clermont et al. 2011). This is in concordance with our study whereby the cox proportional hazard analysis showed that hazard is higher for those with baseline kallikrein level higher than the 50^{th} percentile (>80units/ml) compared to those with baseline kallikrein levels below the 50^{th} percentile for both non-proliferative (Figure2) and proliferative retinopathy(Figure3). On the other hand, the administration of PK inhibitors to diabetic rats had been shown to ameliorate

retinal vascular hyper-permeability and inflammation as tested by preclinical studies (Liu et al. 2013).

Duration of diabetes and HbA1c were found to play a major role in the development of non-proliferative and proliferative retinopathy (Hammes et al. 2011; Klein et al.1998; Yau et al. 2012). This was also demonstrated in our study wherein we were able to show that duration of diabetes was significantly associated with both non-proliferative and proliferative retinopathy (P-values<0.05). This was achieved by conducting longitudinal and survival analysis both at univariate and multivariate analysis. Along the same line, a study conducted by Hammes et al. (2011) where he employed data from the prospective German Diabetes Documentation System survey aimed to analyze the risk profile for diabetic retinopathy under real-life conditions in a large cohort of patients (n=18,891) with type 1 diabetes. This study showed that advanced retinopathy which includes both non-proliferative and proliferative retinopathy has been associated with duration (1.124 per year, P-value<0.0001).

Moreover, our study proved that HbA1c increases the hazard of non-proliferative and proliferative retinopathy by 17% (P-value=0.000) (Tabe19) and 40% (P-value=0.004) (Table 20) respectively. Another study that generated results complementary to ours is the population-based incidence by Kein et al (1998) conducted in 11 counties in southern Wisconsin on 634 diabetic patients diagnosed before age 30 years and who are on insulin treatment. This study showed that the 14-year rate of progression of retinopathy was 86% and that increased risk of proliferative retinopathy was associated with severe baseline retinopathy, higher glycosylated hemoglobin at

baseline, an increase in the glycosylated hemoglobin between the baseline and 4-year follow-up examination. The study by Hammes et al. (2011) also showed that advanced retinopathy which includes both non-proliferative and proliferative retinopathy was associated with HbA_{1c}>7.0% (53 mmol/mol) (1.499, P-value <0.0001) (Hammes et al. 2011).

In our study, hypertension has been also related to the risk of developing proliferative and non-proliferative retinopathy when hazard is studied at a univariate survival analysis (Table 15). This is what was also observed by Hammes et al. wherein blood pressure>140/90 mmHg was associated with advanced retinopathy (1.911, P-value <0.0001).

In the present study, the longitudinal analysis showed a significant association between total cholesterol and retinopathy at a univariate longitudinal level. Similarly there was a significant cross sectional relationship between triacylglycerol>1.7 mmol/l and advanced retinopathy (1.398, P-value =0.0013) (Hammes et al. 2011).

Moreover, gender showed significance in our study when we did longitudinal analysis of retinopathy at a multivariate level thus being a male was a risk factor for developing retinopathy (P-value=0.028) (Table7). This was also shown in Hammes et al.'s cross sectional study (P-value=0.0020).

Our longitudinal survival analysis showed that smoking was associated with increasing the risk of developing retinopathy by 19% (P-value=0.022) (Table 15).

Moreover, a systematic review showed that the overall prevalence for any DR was 34.6% and 6.96% for proliferative DR. All DR prevalence end points increased with diabetes duration, HbA1c, and blood pressure levels and were higher in people with type 1 compared with type 2 diabetes (Yau et al. 2012).This is in line with our study which proved thatdiabetes duration, HbA1c, and blood pressure levels behave as risk factors for type1 diabetes. These data highlight the substantial worldwide public health burden of DR and the importance of modifiable risk factors in its occurrence (YAU et al. 2012). Thuslower glycosylated hemoglobin, hypertension control and non-smoking were proved to be protective factors that prevent progression to severe levels under real-life conditions (Klein 1998 &Hammes et al. 2011).

B. AER:

In the present study, it was shown that log AER was significantly associated with log kallikrein at both a univariate level (P-value=0.039) (Table 6) and at a multivariate one when studied over time (P-value=0.018) (Table10). These findings are in concordance with a study done by Jaffa et al. on microalbumineric patients (AER=40-300 mg/24hrs), the results of which suggested that at cross-sectional level PK was positively, significantly and independently correlated with AER at both univariate and multivariate levels (P-value<0.03) (Jaffa et al. 2003). In specific, PK levels have been significantly higher in patients with macroalbumineria than in patients with normoalbumineria (P-value<0.01) (Jaffa et al. 2003).In our study we also assessed the relationships of the outcomes with Kallikrein at a cross sectional level. Specifically we conducted a cross-sectional analysis to see how kallikrein was related to nephropathy at

the 5 different time points but we found a significant association only at time 1(year=1986) (P-value=0.038) (Table26). Although previous cross-sectional studies showed that there was a positive correlation between PK and AER that suggest that PK could be a marker for progressive nephropathy, but it was necessary to confirm this relationship via longitudinal analysis to show how the outcome is changing overtime rather than studying it only in one point in time. Cox proportional hazard analysis of the association between Microalbumineria and kallikrein also demonstrated significant association at a univariate level (P-value=0.024).

Our longitudinal study showed that duration of diabetes, LDL, and smoking were significantly associated with nephropathy and that female and intensive treatment group were both protective factors against increases in AER levels (P-values<0.05) (Table10). Moreover, intensive treatment was also shown to be protective against AER both at a univariate (P-value=0.001) and multivariate levels (P-value=0.002). Our result is in line with the study by Nathan et al (2003) in which it was shown that during EDIC study, new cases of microalbumineria occurred in 39 of the 1349 participants originally assigned to the intensive treatment group versus 87 of those assigned to the conventional treatment group. This result highlights that those who were on intensive treatment had a lower risk to develop microalbumineria compared to those who were not. Moreover, this study also showed that AER levels were significantly lower in the group receiving intensive treatment compared to those receiving conventional one during the six years follow-up of the EDIC study. This reflects the long-lasting beneficial effects of intensive therapy on diabetic nephropathy. Since intensive treatment has been shown to be a protective factor against diabetic complications, it should be highly

recommended to type1 diabetic patients. This intensive treatment allocation is crucial especially that the number of interventions such as intensive glycemic control, and others like blood pressure regulation and treatment with ace inhibitors that aim at slowing the progression of renal disease in diabetic patients are still small in number (Jaffa et al. 2003).

C. IMT common and internal

Our longitudinal analysis also showed that IMT common is significantly associated with HbA1c, HDL, smoking, both at a univariate level (Table7) and at multivariate one (P-values <0.05) (Table 11). This association was also supported by another cohort study that proved that at year six of EDIC, IMT common was found to be associated smoking status, and mean glycosylated hemoglobin level during DCCT (Nathan et al. 2003). On the other hand, in our study, IMT internal was found to be associated with smoking and SBP when studied longitudinally at a multivariate level. HDL has also been shown to be associated with the outcome at a univariate level. The study by Nathan at al. also showed the association of IMT internal with smoking, SBP and HDL (2003).

A study conducted on 2,329 type 1 diabetic patients without prior CHD in Europe to examine risk factors in the prediction of coronary heart disease (CHD) and differences in men and women for a period of seven years, showed that 151 patients developed CHD, and the 7-year incidence rate was 8.0 and 10.2 (per 1,000 person-years) in men and in women respectively. This study also showed but after adjustment for age and/or duration of diabetes that age, HDL, cholesterol, smoking were associated with

CHD in men. Whereas, age, SBP, and fasting triglycerides showed association with CHD in women. These results are also in agreement with our results.

Another study to examine the recent trends in the prevalence of selected biological CVD risk factors using a cross-sectional, stratified, multistage probability sample survey of the US civilian, non-institutionalized population (NHANES) was conducted on 3383 participants aged 12 to 19 years from the 1999 through 2008. Results showed that among the US adolescents aged 12 to 19 years, hypertension, HDL, LDL, and diabetes were associated with CHD during the survey period from 1999 to 2008 (May 2012). Moreover, the prevalence of prediabetes/diabetes increased from 9% to 23% from 1999–2000 to 2007–2008 thus suggesting a higher risk of developing CHD events. The significance of our study lies in that it studied the association of these variables as well as others over time rather than cross-sectionally to assess their effects on the progression of CVD.

No significant association was found between treatment group and IMT common and internal. On the other hand, the study conducted by Nathan et al. (2003) also showed that the IMT for the common and internal carotid arteries was significantly greater in diabetic patients than non-diabetic ones for both sexes and after adjustment for the smoking status during year 6 of DCCT. Moreover, the progression of IMT for the common carotid artery whether measured alone or combined with the internal carotid artery, was less in the group of patients who received intensive treatment rather than conventional one (Nathan et al. 2003). No significant association between treatment group and IMT common and internal was detected in our study. This was expected since the randomly selected patients were young (ages between 13 and 39 years) and the incidence of cardiovascular disease outcome was low.

D. Concluding Remark:

Despite that the above mentioned studies some of which are cross-sectional and others that are longitudinal were centered on diabetic complications and associated covariates, but none of them examined the association of these covariates with diabetic outcomes in the presence of the biomarker kallikreinwhich is the novelty of our proposed study.

E. Strengths and limitations:

Our study was based on a randomly selected sample of 350 type 1 diabetic patients chosen from the DCCT/EDIC study - anational longitudinal follow-up epidemiological study that was conducted in the United States from the year 1983 to 2004 on 1441 type 1 diabetic patients. It aimed to examine the relationship among glycemia, other risk factors and long-term complications, and the effects of glycemic therapy (NIH 2005). Due to the longitudinal nature of the original study, we were able to draw significant associations between the dependent variables: retinopathy, nephropathy and cardiovascular diseases and the independent variables especially with the biomarker kallikrein. Random selection of patients is important to prevent selection bias and to avoid results that could be overestimated or underestimated.

The strength of our approach was in studying diabetes complications on human subjects rather than the commonly conducted animal studies. Moreover, it was the first study to examine the effect of plasma prekallikrein along with the covariates of interest on the three complications (retinopathy, nephropathy and cardiovascular problems) concomitantly and longitudinally for a period of 21 years rather than just simply at a cross sectional level.

In addition, the significance of this study resided in looking at the longitudinal measurements of important covariates such as HDL, LDL, total cholesterol level, BMI, glucose level, SBP, DBP, and Hemoglobin A1c and studying their effects over time on diabetic complications. In this regard having measurements on these factors over a period of 21 years enabled us to monitor over time the progression of the disease and to determine how the change in the levels of these covariates was affecting the severity of the diabetic complications.

Accordingly, this study represented a unique opportunity to perform a longitudinal assessment of the effect of plasma prekallikrein and other factors of interest on the diabetic complications (retinopathy, nephropathy and cardiovascular problems) conducted on human subjects.

Moreover, we had sufficient statistical power to determine that intensive treatment decreased the frequency and severity of diabetic microvascular and neurologic complications over time.

This study had some limitations. First of all, the cohort age group ranges between 13 and 39 years so probably for the decrease in the progression of IMT with intensive diabetes therapy to be translated into a clinically meaningful reduction in cardiovascular disease events, longer follow-up is needed. Another limitation is that the percentage of smokers was minimal with respect to non-smokers (13% vs.82%). Hence this could lead to an underestimated relationship between smoking and AER, IMT common and internal.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

In our study, we used secondary data analysis to assess the impact of Kallikrein biomarker along with covariates of interest on the three complications of diabetes: Retinopathy, Nephropathy and Cardiovascular complications. These associations were examined longitudinally and any potential time effect was also determined.

Data from the 2011 National Diabetes Fact Sheet showed that in the United States, 25.8 million children and adults in the United States (8.3% of the population) have diabetes in 2011. Only 18.8 million were diagnosed and the remaining 7 million were undiagnosed. In 2010, 1.9 million new cases of diabetes were diagnosed in people aged 20 years and older in 2010. The total costs of diagnosed diabetes accounted for $245 billion in 2012 2008 (ADA 2013). Moreover, heart disease was noted on 68% of diabetes-related death certificates among people aged 65 years or older in 2004. Diabetes is known as the leading cause of new cases of blindness among adults aged 20–74 years as well as the leading cause of kidney failure, accounting for 44% of new cases in 2008 (ADA 2013). All these data highlight the importance of discovering associations between diabetic complications and determinants that are either harmful or protective in order to take preventive measures against such complications. This will help alleviate theburden of this disease whether health wise or economic wise on patients and on governments.

Our study results showed that longitudinally most covariates were significantly associated with kallikrein (P-values <0.05). ETDRS and AER were significantly associated with Log kallikrein over time (P-values: 0.011 &0.039 respectively) (Tables5 and 6). The longitudinal analysis of log IMT internal and common showed no significant association with log Kallikrein both at univariate and multivariate levels. Besides, we were able to show that those whose kallikrein levels below the 50^{th} percentile had lower risk to develop microvascular complications such as retinipathy and nephropathy.

As shown by our study, intensive treatment had played a major role in reducing the risk of microvascular complications such as retinopathy and nephropathy. Hence, knowing that intensive treatment has many beneficial effects on type1 diabetic complications, it should be recommended as a therapeutically regimen that needs to be implemented as early as possible in type1 diabetic patients.

Evidently, it is important to continue and expand surveillance for childhood diabetes across the world since it is one of the most potent strategies for understanding the multifactorial etiology of the disease and ultimately preventing it.

Table 1: Percent distribution for clinical characteristics by Log kallikerin

Variable	N*	n**	Min	Max	Mean±SD	P-value***
Log Kallikrein(units/ml)	1745	349	16	261	88.580±33.860	
BMI(kg/m^2)	1711	349	16	47	25.990±4.320	0.839
DBP(mm Hg)	1708	349	40	119	74.780±8.641	0.178
SBP(mm Hg)	1708	349	74	180	116.950±13.428	0.199
Duration of diabetes(yr)	1745	349	1	15	5.530±4.022	0.334
HbA1c (mmol/mol)	1711	349	4	14	8.230±1.471	0.000
HDL(mg/dl)	1691	349	19	116	55.890±14.562	0.029
LDL(mg/dl)	1682	349	29	223	109.630±28.672	0.000
Total Cholesterol(mg/dl)	1692	349	83	323	182.700±32.936	0.000
LogGlucose(mg/dL)	1745	349	0	506	212.360±86.916	0.045
Age(yr)	1745	349	13	39	26.360±6.860	0.262

*N: number of observations (repeated measures over five points in time).

**n: number of subjects recruited in the study

***P-value: obtained from univariate longitudinal analysis for the continuous variables as a function of Logkallikrein.

Table 2: Percent distribution of gender, treatment group, smoking and ace inhibitor by log kallikrein

Variable		n (%)	P-value**
Gender	**Male**	177(51)	0.060
	Female	155(44)	
Smoking	**Yes**	46(13)	0.781
	No	286(82)	
Treatment group	**Conventional**	167(48)	0.499
	Intensive	165(47)	
Ace inhibitor	**Yes**	27(8)	0.724
	No	321(92)	

*n: number of subjects recruited in the study

**P-value: obtained from univariate longitudinal analysis for gender, treatment group, smoking and ace inhibitor as a function of Log kallikrein

Table 3: Descriptive analysis of Log ETDRS, Log AER, Log IMT common and Log IMT internal

Variable	Total observations	n	Min	Max	Mean±SD	P-value*
Log ETDRS	1599	349	1	17	3.71±2.737	0.011
Log AER(mg/24hr)	1697	348	1	7357	46.54±330.23	0.039
Log IMT common	934	313	0	2	0.95±0.241	0.197
Log IMT Internal	923	308	0	3	0.99±0.371	0.591

*P-value: obtained from univariate longitudinal analysis for studied outcomes as a function of Logkallikrein

Table 4: Longitudinal analysis of the association between Log Kallikrein and Clinical Parameters over time

Log Kallikrein	Coef.	SE	P –value	95% CI for Coef.
LDL	0.001	0.000	0.000	[0.001;0.002]
HDL	-0.002	0.000	0.029	[-0.003;-0.001]
HbA1c	0.033	0.005	0.000	[0.022;0.044]
Total Cholesterol	0.001	0.000	0.000	[0.001;0.002]
Log Glucose	0.067	0.033	0.045	[0.002;0.132]
Age	0.003	0.002	0.262	[-0.002;0.007]
BMI	0.001	0.002	0.839	[-0.004;0.005]
DBP	-0.001	0.000	0.178	[-0.003;0.001]
SBP	-0.001	0.000	0.199	[-0.002;0.001]
Duration of Diabetes	0.004	0.003	0.334	[-0.004;0.011]
Gender	0.100	0.030	0.060	[-0.003; 0.118]
Smoking	-0.010	0.019	0.781	[-0.043;0.032]
Treatment Group	0.021	0.031	0.499	[-0.039;0.081]
Ace Inhibitor	0.010	0.027	0.724	[-0.044;0.064]

Table 5: Longitudinal analysis of the association between log ETDRS and clinical parameters at a univariate level

Log ETDRS	Coef.	Std. Err.	P-value (unadjusted)	95% CI for Coef.
Log kallikrein	0.106	0.041	0.011	[0.024;0.187]
BMI	0.020	0.004	0.000	[0.010;0.030]
DBP	0.004	0.001	0.007	[0.001;0.010]
SBP	0.002	0.001	0.006	[0.001;0.004]
Duration of Diabetes	0.100	0.005	0.000	[0.072;0.100]
LDL	0.002	0.001	0.000	[0.001;0.003]
Total Cholesterol	0.001	0.000	0.000	[0.001;0.002]
Log glucose	0.159	0.063	0.013	[0.033;0.284]
Treatment Group	-0.314	0.057	0.000	[-0.426;-0.202]

Non-significant variables: HDL, HbA1c, smoking status, Age, Gender, Ace inhibitor were not shown in Table 5 (P-values > 0.05)

Table 6: Longitudinal analysis of the association between log AER and clinical parameters at a univariate level

Log AER	Coef.	Std. Err.	P-value(unadjusted)	95% CI for Coef.
Log Kallikrein	0.145	0.070	0.039	[0.010;0.282]
DBP	0.010	0.002	0.000	[0.004;0.014]
Duration of Diabetes	0.031	0.010	0.002	[0.011;0.051]
HbA1c	0.100	0.016	0.000	[0.050;0.112]
Log LDL	0.275	0.102	0.007	[0.075;0.475]
Log Total Cholesterol	0.396	0.148	0.008	[0.104;0.687]
Smoking	0.117	0.051	0.023	[0.020;0.219]
Treatment Group	-0.256	0.081	0.002	[-0.415;-0.096]
Gender	-0.242	0.081	0.003	[-0.402;-0.082]
Ace inhibitor	0.396	0.083	0.000	[0.233;0.561]

Non-significant variables: BMI, HDL, glucose, Age, SBP were not shown in Table 6(P-values > 0.05)

Table 7: Longitudinal analysis of the association between IMT common and clinical parameters at a univariate level.

Log IMT common	Coef.	Std. Err.	P-value(unadjusted)	95% CI for Coef.
Log Kallikrein	0.020	0.014	0.197	[-0.010;0.047]
BMI	0.004	0.002	0.021	[0.001;0.007]
Duration of Diabetes	0.010	0.002	0.008	[0.001;0.010]
HbA1c	0.010	0.004	0.029	[0.001;0.017]
HDL	-0.001	0.000	0.000	[-0.002;-0.001]
Smoking	0.044	0.009	0.000	[0.025;0.063]
Age	0.010	0.001	0.000	[0.007;0.011]
Gender	-0.100	0.015	0.000	[-0.102;-0.042]
Ace inhibitor	0.040	0.013	0.005	[0.011;0.065]

Non-significant variables: DBP, SBP, LDL, Total Cholesterol, Glucose,Treatment Group were not shown in Table 7 (P-values > 0.05)

Table 8: Longitudinal analysis of the association between Log IMT internal and clinical parameters at a univariate level

Log IMT internal	Coef.	Std. Err.	P-value(unadjusted)	95% CI for Coef.
Kallikrein	-0.020	0.028	0.591	[-0.100;0.041]
Duration of Diabetes	0.010	0.003	0.024	[0.001;0.015]
Log HDL	-0.110	0.043	0.014	[-0.194;-0.022]
Smoking	0.078	0.019	0.000	[0.041;0.116]
Age	0.014	0.002	0.000	[0.011;0.018]
Gender	-0.146	0.029	0.000	[-0.204;-0.088]
Ace inhibitor	0.079	0.026	0.003	[0.027;0.132]

Non-significant variables: BMI, DBP, SBP, LDL, HbA1c, Total cholesterol, Glucose, Treatment group were not shown in Table 8(P-values > 0.05)

Table 9: Longitudinal analysis of the association between log ETDRS and clinical parameters at a multivariate level

Log ETDRS	Coef.	Std. Err.	P-value(adjusted)	95% CI for Coef.
Log Kallikrein	0.800	0.039	0.045	[0.002;0.160]
BMI	0.013	0.004	0.003	[0.005;0.022]
DBP	0.004	0.001	0.005	[0.001;0.007]
Duration of Diabetes	0.083	0.005	0.000	[0.072;0.093]
LDL	0.002	0.000	0.002	[0.001;0.003]
Log Glucose	0.150	0.046	0.001	[0.060;0.240]
Treatment group	-0.306	0.042	0.000	[-0.390;-0.222]
Gender	-0.100	0.045	0.028	[-0.200;-0.011]

All parameters with P-values <=0.2 at a univariate level were included in this model.

Non-significant variables: HDL, SBP, Smoking, HbA1c were not shown in Table 9 (P-values > 0.05)

Table 10: Longitudinal analysis of the association between log AER and clinical parameters at a multivariate level

Log AER	Coef.	Std. Err.	P-value (adjusted)	95% CI for Coef.
Log Kallikrein	0.170	0.070	0.018	[0.030;0.310]
Duration of Diabetes	0.030	0.009	0.005	[0.010;0.050]
LDL	0.002	0.000	0.031	[0.001;0.004]
Smoking	0.101	0.050	0.044	[0.003;0.200]
Treatment group	-0.251	0.078	0.001	[-0.406;-0.096]
Gender	-0.201	0.084	0.017	[-0.370;-0.040]

All parameters with P-values <=0.2 at a univariate level were included in this model.

Non-significant variables: HDL and SBP were not shown in Table 10 (P-values > 0.05).

Table 11: Longitudinal analysis of the association between log IMT common and clinical parameters at a multivariate level

Log IMT common	Coef.	Std. Err.	P-value(adjusted)	95% CI for Coef.
Log Kallikrein	-0.001	0.013	0.931	[-0.027;0.025]
Duration of Diabetes	0.003	0.001	0.035	[0.001;0.006]
HbA1c	0.010	0.003	0.028	[0.001;0.016]
HDL	-0.001	0.000	0.015	[-0.002;-0.001]
Smoking	0.031	0.008	0.000	[0.015;0.050]
Age	0.010	0.000	0.000	[0.010;0.011]
Gender	-0.060	0.013	0.000	[-0.090;-0.032]

All parameters with P-values <=0.2 at a univariate level were included in this model.

Non-significant variables: BMI, LDL, SBP, Total Cholesterol, Ace inhibitor were not shown in Table 11(P-values > 0.05)

Table 12: Longitudinal analysis of the association between log IMT internal and clinical parameters at a multivariate level

Log IMT internal	Coef.	Std. Err.	P-value(adjusted)	95% CI for Coef.
Log Kallikrein	-0.050	0.027	0.090	[-0.100;0.007]
LDL	0.001	0.000	0.014	[0.0008;0.001]
SBP	0.001	0.000	0.028	[0.0009;0.003]
Smoking	0.066	0.017	0.000	[0.031;0.100]
Age	0.012	0.002	0.000	[0.008;0.016]
Gender	-0.126	0.027	0.000	[-0.181;-0.071]

All parameters with P-values <=0.2 at a univariate level were included in this model.

Non-significant variables: Duration of Diabetes, HbA1c, HDL, Ace inhibitor were not shown in Table 12 (P-values > 0.05)

Table 13: Cox Proportional hazard analysis of the association between Microalbumineria** and clinical parameters at a univariate level

Time to Microalbumineria	Hazard Ratio for Microalbumineria	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	1.291	0.312	0.291	[0.803;2.073]
Duration of Diabetes	1.074	0.028	0.008	[1.018;1.132]
BMI	1.061	0.028	0.029	[1.010;1.118]
DBP	1.045	0.014	0.001	[1.020;1.074]
HbA1c	1.550	0.109	0.000	[1.349;1.781]
SBP	1.026	0.008	0.002	[1.010;1.043]
Total cholesterol	1.010	0.003	0.034	[1.009;1.014]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50th percentile

Microalbumineria: AER=40-299 mg/24hrs

Non-significant variables: HDL, LDL, Glucose, Smoking, Treatment Group, Age, Gender, Ace inhibitor were not shown in Table 13 (P-values > 0.05)

Table 14: Cox Proportional hazard analysis of the association between Macroalbumineria**and clinical parameters at a univariate level

Time to Macroalbumineria	Hazard Ratio for Macroalbumineria	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	1.389	0.813	0.575	[0.441;4.376]
DBP	1.081	0.034	0.014	[1.016;1.149]
HbA1c	1.733	0.288	0.001	[1.251;2.401]
SBP	1.065	0.018	0.000	[1.029;1.102]
Total cholesterol	1.022	0.007	0.006	[1.010;1.037]
Smoking	2.048	0.599	0.014	[1.155;3.634]
Treatment group	0.088	0.092	0.020	[0.011;0.681]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50^{th} percentile

**Macroalbumineria: AER level>300mg/24hrs

Non-significant variables: BMI, Duration of Diabetes, HDL, LDL, Glucose, Age, Genderwere not shown in Table 14 (P-values > 0.05)

Table 15: Cox Proportional hazard analysis of the association between Non-proliferative** Retinopathy and clinical parameters at a univariate level

Time to Non-proliferative Retinopathy	Hazard Ratio for Non-proliferative Retinopathy	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	1.421	0.184	0.007	[1.103;1.832]
Duration of Diabetes	1.168	0.017	0.000	[1.134;1.204]
HbA1c	1.181	0.047	0.000	[1.091;1.277]
SBP	1.012	0.005	0.020	[1.010;1.022]
Smoking	1.188	0.089	0.022	[1.025;1.378]
Treatment Group	0.579	0.074	0.000	[0.451;0.744]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50^{th} percentile

**Non-proliferative Retinopathy: ETDRS: 3-9

Non-significant variables: BMI, DBP, HDL, LDL, Total cholesterol, Glucose, Age, Gender,

Ace inhibitor were not shown in Table 15(P-values > 0.05)

Table 16: Cox Proportional hazard analysis of the association between Proliferative** Retinopathy and clinical parameters at a univariate level

Proliferative Retinopathy	Hazard Ratio for Proliferative Retinopathy	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	2.791	0.943	0.002	[1.438;5.414]
Duration of Diabetes	1.170	0.044	0.000	[1.087;1.259]
DBP	1.044	0.019	0.022	[1.010;1.084]
HbA1c	1.402	0.139	0.001	[1.153;1.704]
SBP	1.026	0.011	0.018	[1.010;1.048]
Treatment group	0.312	0.119	0.002	[0.146;0.663]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50^{th} percentile

Proliferative Retinopathy: ETDRS >9

Non-significant variables: BMI, HDL, LDL, Total cholesterol, Glucose, smoking, Age, Gender, Ace Inhibitor were not shown in Table 16 (P-values > 0.05)

Table 17: Cox Proportional hazard analysis of the association between Microalbumineria** and clinical parameters at a multivariate level

Time to Microalbumineria	Hazard Ratio for Microalbumineria	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	1.188	0.301	0.495	[0.722;1.955]
Duration of Diabetes	1.072	0.031	0.015	[1.013;1.133]
BMI	1.063	0.031	0.037	[1.010;1.127]
HbA1c	1.560	0.118	0.000	[1.344;1.811]
Treatment Group	0.568	0.146	0.028	[0.343;0.940]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50th percentile

**Microalbumineria: AER=40-299 mg/24hrs

All parameters with p-values <=0.2 at the univariate level were included in this model.

Non-significant variables: LDL and Smoking were not shown in Table 17 (P-values > 0.05

Table 18: Cox Proportional hazard analysis of the association between Macroalbumineria and clinical parameters at a multivariate level

Time to Macroalbumineria	Hazard Ratio for Macroalbumineria	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	1.945	1.373	0.346	[0.487;7.762]
HbA1c	1.559	0.335	0.039	[1.023;2.376]
SBP	1.041	0.019	0.032	[1.011;1.081]
Treatment Group	0.092	0.099	0.028	[0.011;0.771]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50th percentile

**Macroalbumineria: AER level>300mg/24hrs

All parameters with p-values <=0.2 at the multivariate level were included in this model.

Non-significant variables: Duration of Diabetes, Smoking, Age, Gender and Total Cholesterol were not shown in Table 18 (P-values > 0.05)

Table 19: Cox Proportional hazard analysis of the association between Non-proliferative** Retinopathy and clinical parameters at a multivariate level

Time to Non-proliferative Retinopathy	Hazard Ratio for Non-proliferative Retinopathy	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	1.311	0.178	0.047	[1.010;1.711]
Duration of Diabetes	1.181	0.019	0.000	[1.144;1.221]
HbA1c	1.174	0.051	0.000	[1.077;1.279]
Treatment Group	0.596	0.081	0.000	[0.456;0.780]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50^{th} percentile

**Non-proliferative Retinopathy: ETDRS: 3-9

All parameters with p-values <=0.2 at the univariate level were included in this model.

Non-significant variables: BMI, HDL, SBP, Smoking and Gender were not shown in Table 19(P-values > 0.05)

Table 20: Cox Proportional hazard analysis of the association between Proliferative** Retinopathy and clinical parameters at a multivariate level

Time to Proliferative Retinopathy	Hazard Ratio for Proliferative Retinopathy	Std. Err.	P-value (unadjusted)	95% CI for HR
Kallikrein*	3.651	1.314	0.000	[1.804;7.394]
HbA1c	1.397	0.161	0.004	[1.114;1.753]
Treatment Group	0.245	0.101	0.001	[0.108;0.553]

*Kallikrein is measured at baseline and is dichotomized by above or below the 50^{th} percentile

**Proliferative Retinopathy: ETDRS >9

All parameters with p-values <=0.2 at the univariate level were included in this model.

Non-significant variables: DBP, SBP, Glucose, Total Cholesterol were not shown in Table 20 (P-values > 0.05).

Table 21: Cox proportional hazard analysis of the association between Microalbumineria, Macroalbumineria, Non-proliferative Retinopathy and Proliferative Retinopathy with Log Kallikrein at a univariate level

Outcome		Hazard Ratio	Std. Err.	P-value (unadjusted)	95% CI for HR
Microalbumineria	**Log Kallikrein***	2.174	0.750	0.024	[1.106;4.276]
Macroalbumineria		2.135	1.809	0.370	[0.405;11.237]
Non-proliferative Retinopathy		1.234	0.221	0.241	[0.868;1.754]
Proliferative Retinopathy		6.069	3.153	0.001	[2.192;16.802]

*Log Kallikrein is measured over time

Table 22: Cox Proportional hazard analysis of the association between Microalbumineria** and clinical parameters at a multivariate level

Time to Microalbumineria	Hazard Ratio for Microalbumineria	Std. Err.	P-value (unadjusted)	95% CI for HR
Log Kallikrein*	1.395	0.479	0.333	[0.711;2.737]
Duration of Diabetes	1.071	0.030	0.014	[1.014;1.133]
BMI	1.062	0.031	0.043	[1.002;1.126]
HbA1c	1.542	0.118	0.000	[1.326;1.794]
Treatment Group	0.576	0.146	0.030	[0.349;0.948]

*Log Kallikrein is measured over time

**Microalbumineria: AER=40-299 mg/24hrs

All parameters with p-values <=0.2 at the univariate level were included in this model.

Non-significant variables: LDL and Smoking were not shown in Table 22 (P-values > 0.05).

Table 23: Cox Proportional hazard analysis of the association between Macroalbumineria and clinical parameters at a multivariate level

Time to Macroalbumineria	Hazard Ratio for Macroalbumineria	Std. Err.	P-value (unadjusted)	95% CI for HR
Log Kallikrein*	2.963	3.032	0.288	[0.398;22.019]
SBP	1.037	0.019	0.051	[0.999;1.075]
Treatment Group	0.107	0.114	0.037	[0.013;0.871]

*Log Kallikrein is measured over time

**Macroalbumineria: AER level>300mg/24hrs

All parameters with P-values <=0.2 at the multivariate level were included in this model.

Non-significant variables: Duration of Diabetes, HbA1c, Smoking, Age, Gender and Total Cholesterol were not shown in Table 23(P-values > 0.05)

Table 24: Cox Proportional hazard analysis of the association between Non-proliferative** Retinopathy and clinical parameters at a multivariate level

Time to Non-proliferative Retinopathy	Hazard Ratio for Non-proliferative Retinopathy	Std. Err.	P-value (unadjusted)	95% CI for HR
Log Kallikrein*	1.015	0.173	0.927	[0.726;1.420]
Duration of Diabetes	1.182	0.019	0.000	[1.144;1.221]
BMI	1.036	0.018	0.039	[1.010;1.100]
HbA1c	1.177	0.051	0.000	[1.100;1.282]
Treatment Group	0.597	0.081	0.000	[0.456;0.780]

*Log Kallikrein is measured over time

**Non-proliferative Retinopathy: ETDRS: 3-9

All parameters with P-values <=0.2 at the univariate level were included in this model.

Non-significant variables: HDL, SBP, Smoking and Gender, were not shown in Table 24 (P-values > 0.05)

Table 25: Cox Proportional hazard analysis of the association between Proliferative** Retinopathy and clinical parameters at a multivariate level

Time to Proliferative Retinopathy	Hazard Ratio for Proliferative Retinopathy	Std. Err.	P-value(unadjusted)	95% CI for HR
Log Kallikrein*	5.879	3.564	0.003	[1.791;19.293]
Duration of Diabetes	1.212	0.048	0.000	[1.120;1.312]
HbA1c	1.354	0.155	0.008	[1.081;1.695]
Treatment Group	0.263	0.111	0.002	[0.115;0.604]

*Log Kallikrein is measured over time

**Proliferative Retinopathy: ETDRS >9

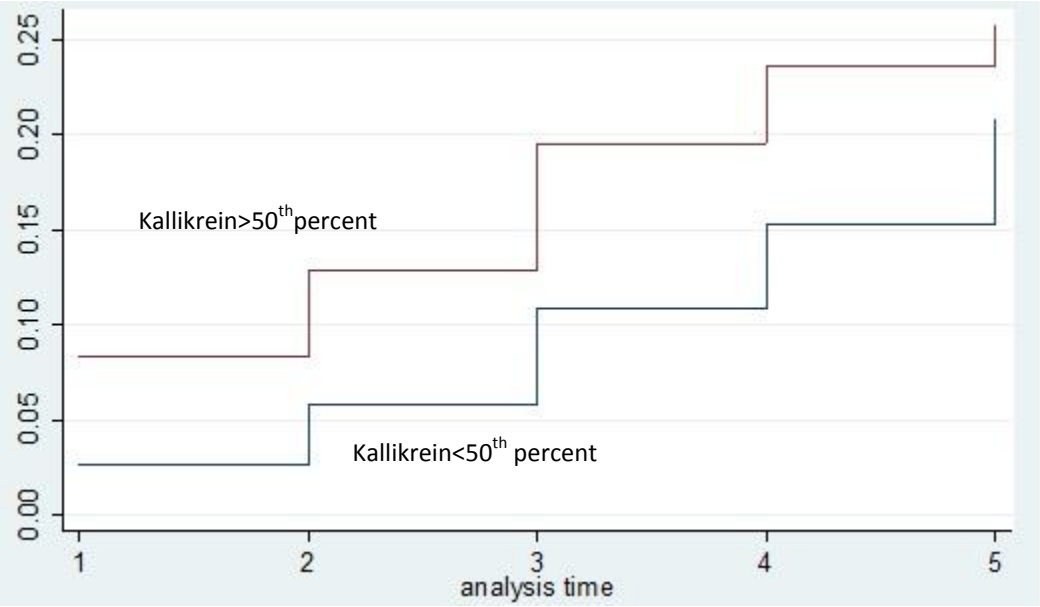
All parameters with p-values <=0.2 at the univariate level were included in this model.

Non-significant variables: DBP, LDL, SBP, Glucose were not shown in Table 25 (P-values > 0.05)

Table 26: Association between Log AER, Log ETDRS, Log IMT common, Log IMT internal and Log kallikrein at cross sectional levels

Outcome	Parameter	T	Coef.	Std. Err.	P- value	95%CI for Coef.
Log AER	**Log Kallikrein**	1	0.241	0.116	0.038	[0.013;0.469]
		2	-0.068	0.149	0.649	[-0.362;0.226]
		3	0.260	0.141	0.066	[-0.017;0.538]
		4	0.297	0.179	0.099	[-0.056;0.651]
		5	0.076	0.180	0.673	[-0.279;0.432]
Log ETDRS		1	0.254	0.093	0.007	[0.070;0.438]
		2	0.238	0.117	0.043	[0.010;0.469]
		3	0.183	0.089	0.041	[0.010;0.359]
		4	0.149	0.114	0.192	[-0.075;0.374]
		5	0.186	0.099	0.061	[-0.010;0.381]
Log IMT common		3	0.022	0.018	0.227	[-0.014;0.058]
		4	0.023	0.025	0.356	[-0.026;0.074]
		5	0.012	0.028	0.676	[-0.044;0.070]
Log IMT internal		3	-0.044	0.030	0.159	[-0.105;0.017]
		4	0.001	0.049	0.995	[-0.097;0.097]
		5	0.093	0.059	0.118	[-0.024;0.210]

Figure 1: The cumulative hazard of Microalbumineria** as a function of Kallikrein*

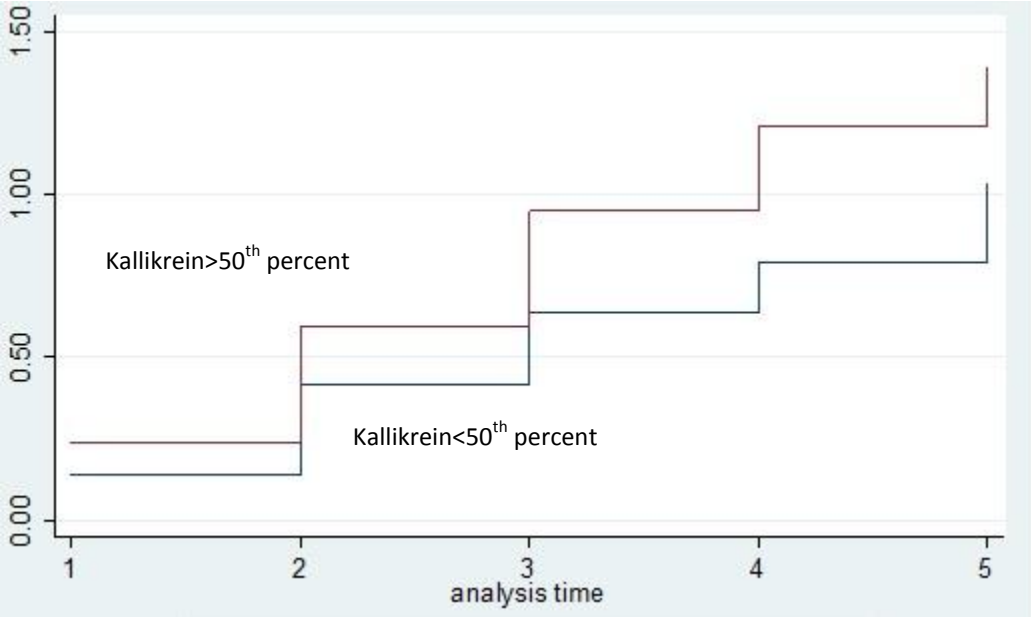


*Kallikrein is measured at baseline and dichotomized by above or below50th percentile.

**Microalbumineria: AER=40-299mg/24hrs

Log rank test: P-value=0.279

Figure 2: The cumulative hazard of Non-proliferative** Retinopathy as a function of Kallikrein*

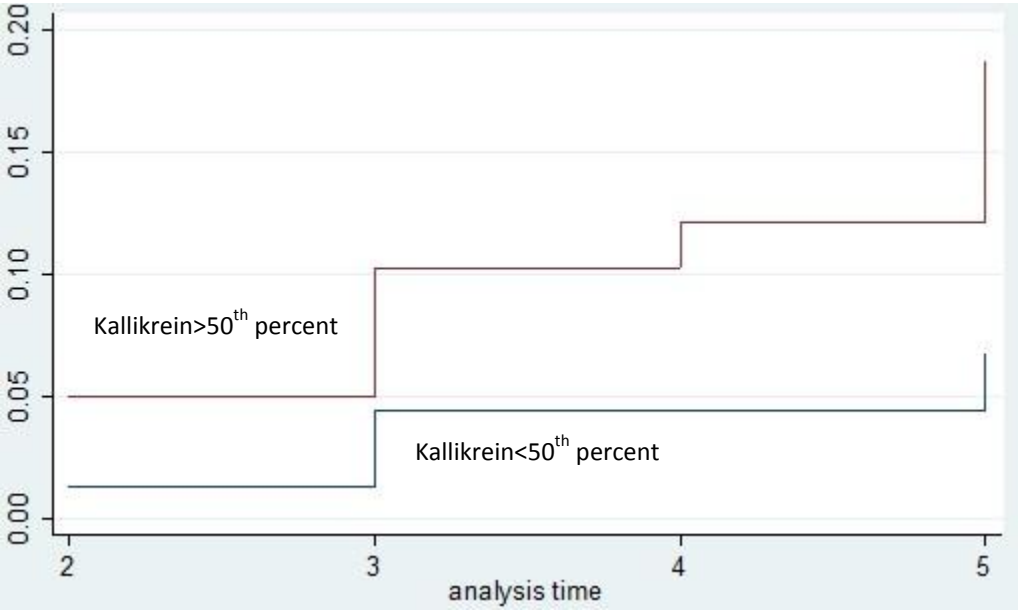


*Kallikrein is measured at baseline and dichotomized by above or below 50th percentile.

**Non-proliferative Retinopathy: ETDRS: 3-9

Log rank test: P-value=0.002

Figure 3: The cumulative hazard of Proliferative** Retinopathy as a function of Kallikrein*



*Kallikrein is measured at baseline and dichotomized by above or below 50th percentile.

Proliferative Retinopathy: ETDRS: >9

Log rank test: P-value=0.001

REFERENCES

American Academy of Ophthalmology. (2003). *Proposed international clinical diabetic*. Retrieved from http://www.v2020la.org/pub/PUBLICATIONS_BY_TOPICS/Diabetic Retinopathy/Proposed...pdf

BMJ(2012). Type1 Diabetes. Retrieved March 8, 2013, from Evidence-Based Medicine.

BMJ. (2004, May 6). *Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study*. Retrieved from http://www.bmj.com/content/328/7448/1105

CDC. (2008, 12 12). *Blood pressure categories by race/ethnicity, united states*. Retrieved from http://www.cdc.gov/features/dsbloodpressure/index.html

CDC. (2011, 9 13). *Healthy weight - it's not a diet, it's a lifestyle!*. Retrieved from http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/

CDC. (2012, 3 14). *Cholesterol*. Retrieved from http://www.cdc.gov/cholesterol/what_you_can_do.htm

CDC (2012). Diabetes. Retrieved from http://www.cdc.gov/diabetes/projects/cda2.htm

Centers for Disease Control and Prevention. (2011). Retrieved from http://www.cdc.gov/chronicdisease/resources/publications/AAG/ddt.htm

Centers for Disease Control and Prevention. (2012). Retrieved from http://www.cdc.gov/diabetes/projects/cda2.htm

Chapman, K. (n.d.). *Cholesterol*. Retrieved from http://web.aces.uiuc.edu/vista/pdf_pubs/CHOLSTRL.PDF

Clermont et al. (2011). Plasma Kallikrein Mediates Retinal Vascular Dysfunction and Induces Retinal Thickening in Diabetic Rats. *Diabetesjournals(60)*. 1590-1598.

DCCT (1993). The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. *The New England Journal of Medicine 329*:977-986

DCCT (2008). Prolonged Effect of Intensive Therapy on the Risk of Retinopathy Complications in Patients with Type 1 Diabetes Mellitus.*Arch Ophthalmol 126(12):1707-1715.*

DCCT(2003). Intensive Diabetes Therapy and Carotid intima-Media Thickness in Type1 Diabetes Mellitus. The New England Journal of Medicine384 (23): 2294-2303.

Eckman, A. (2011, 6 28). *Type 1 diabetes*. Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000305.htm

Edelman, D. (2004). Utility of hemoglobin a1c in predicting diabetes risk. *J Gen Intern Med.*, *19*(12), Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles

EDIC (2003). Sustained Effect of Intensive Treatment of Type 1 Diabetes Mellitus on Development and Progression of Diabetic Nephropathy. *JAMA 290*(16): 2159-2167.

Funk, J. L. (2010). *Pathophysiology of Disease*. United States: The McGraw-Hill Companies. Retrieved March 9, 2013, from Access Medicine Database.

Gross, J. L. (2005). Diabetic Nephropathy: Diagnosis, Prevention, and Treatment. *Diabetes Care 28* (1), 176–188. Retrieved March 10, 2013, from *care.diabetesjournals.org/content/28/1/164.full.pdf*

Hammes et al. (2011), Diabetic retinopathy in type 1 diabetes—a contemporary analysis of 8,784 patients. *Diabetologia 54*(8), pp 1977-1984. Retrieved March 10, 2013 from http://www.ncbi.nlm.nih.gov/pubmed/21638132

Hedeker D, Gibbons R, Waternaux C (1999). Sample size estimation for longitudinal designs with attrition: comparing time-related contrasts between two groups. Journal of Educational and Behavioral Statistics*24*: 70–93

Jaffa et al. (2003). Plasma Prekallikrein:A Risk Marker for Hypertension and Nephropathy in Type 1 Diabetes. *DIABETES (52),* 1216-1221.

Jaffa, A., et al. (2003). A risk marker for hypertension and nephropathy in type1 diabetes. *Diabetes Journal*, *52*, 1215-1221. Retrieved from http://care.diabetesjournals.org/content/28/1/164.full

Jaffa, A., et al. (2008). Connective tissue growth factor and susceptibility to renal and vascular disease risk in type1 diabetes. *Endojournals*, *93*(5), 1893-1900. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2386274/

Karvonen, M., at al. (2000). Incidence of childhood type1 diabetes worldwide. *Diabetes Care*, *23*, 1516-1526. Retrieved from http://care.diabetesjournals.org/content/23/10/1516.long

Klein , R., et al. (1998). The wisconsin epidemiologic study of diabetic retinopathy: Xvii. the 14-year incidence and progression of diabetic retinopathy and associated risk

factors in type 1 diabetes. *Ophthalmology*, *105*(10), 1801-1815. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9787347

Liu, J., et al. (2013). Plasma kallikrein-kinin system and diabetic retinopathy. *Biological Chemistry*, *394*(3), 319-328. Retrieved from http://www.degruyter.com/view/j/bchm.2013.394.issue-3/hsz-2012-0316/hsz-2012-0316.xml

Manto, A. (1993). Urinary kallikrein excretion in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*, *36*, 423-427. Retrieved from www.ncbi.nlm.nih.gov/pubmed/8314446

May, A., Kuklina, E., & Yoon, P. (2012). Prevalence of cardiovascular disease risk factors among us adolescents, 1999−2008. *American Academy of Pediatrics*.

Mayfield et. al(1983). Urinary Kallikrein Excretion in Insulin-Dependent Diabetes Mellitus and Its Relationship to Glycemic Control.*JCEM(59)* 2: 278-286

Nathan, D., et al. (2005). Sustained effect of intensive treatment of type1 diabetes mellitus on development and progression of diabetic nephropathy. *JAMA*, *290*(16), 2159-2167. Retrieved from http://jama.jamanetwork.com/article.aspx?articleid=197530

Nathan, D., et al. (2003). Intensive diabetes therapy and carotid intima-media thickness in type1 diabetes mellitus. *NEJM*, *348*(23), 2294-2303. Retrieved from http://www.nejm.org/doi/full/10.1056/NEJMoa022314

Nathan, D., et al. (2005). Intensive diabetes treatment and cardiovascular disease in patients with type1 diabetes. *NEJM*, *353*(25), 2643-2653. Retrieved from http://www.nejm.org/doi/full/10.1056/NEJMoa052187

National Diabetes Information Clearinghouse (NDIC). (2012). Retrieved from http://diabetes.niddk.nih.gov/dm/pubs/overview/#what

National Institutes of Health 2005. Retrieved from www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/GetPdf.cgi?id

.

NIH (2007).Long-Term Effect of Diabetes and Its Treatment on Cognitive Function: *The New England Journal of Medicine 356*(18): 1842–1852.

Phipps, J.A &Feener, E.P(2008). The kallikrein–kinin system in diabetic retinopathy:Lessons for the kidney. *Kidney International (73)*, 1114–1119.

Pub Med Health. (2011). Retrieved from http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001350/

Soedamah-Muthu, S. (2004). Risk factors for coronary heart disease in type 1 diabetic patients in europe. *DIABETES CARE*, *27*(2), 530-537. Retrieved from http://care.diabetesjournals.org/content/27/2/530.long

Tao et al. (2010). Estimating the Cost of Type 1 Diabetes in the U.S.: APropensity Score Matching Method. *Plos one5*(7), 1-11. Retrieved March 11, 2013 from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2901386/pdf/pone.0011501.pdf

White, N., et al. (2008). Prolonged effect of intensive therapy on the risk of retinopathy comlications in patients with type1 diabetes mellitus. *Arch Ophthalmol*, *126*(12), 1707-1715. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19064853

World Health Organization (2012). Diabetes. Retrieved from http://www.who.int/mediacentre/factsheets/fs312/en/index.html

World Health Organization (2012). Diabetes. Retrieved from http://www.who.int/mediacentre/factsheets/fs312/en/index.html

World Health Organization (2012). World Diabetes Day 2012. Retrieved on January 3, 2013 from http://www.who.int/diabetes/en/

World Health Organization. (2013). Retrieved from http://www.who.int/mediacentre/factsheets/fs312/en/index.html

Yao et al. 2012. Global Prevalence and Major Risk Factors of Diabetic Retinopathy. *Diabetes Care 35:*556–564. Retrieved from http://care.diabetesjournals.org/content/35/3/556.long

Appendices

Table 27: Association between Log Kallikrein and Clinical Parameters at cross sectional levels

Log Kallekrein	T	Coef.	Std. Err.	P- value	95%CI for coef.
LDL	1	0.002	0.000	0.003	[0.001;0.003]
	2	0.002	0.00	0.013	[0.001;0.003]
	3	0.002	0.000	0.002	[0.001;0.004]
	4	0.002	0.000	0.003	[0.001;0.003]
	5	0.002	0.000	0.031	[0.001;0.003]
HDL	1	0.002	0.001	0.228	[-0.001;0.005]
	2	0.001	0.001	0.381	[-0.001;0.003]
	3	-0.001	0.001	0.806	[-0.004;0.003]
	4	-0.001	0.001	0.737	[-0.003;0.002]
	5	0.001	0.001	0.794	[-0.001;0.003]
HbA1c	1	0.052	0.012	0.000	[0.030;0.080]
	2	0.030	0.010	0.004	[0.010;0.050]
	3	0.040	0.015	0.018	[0.010;0.070]
	4	0.040	0.013	0.005	[0.011;0.064]
	5	0.030	0.013	0.062	[-0.001;0.053]
Total Cholesterol	1	0.002	0.000	0.000	[0.001;0.003]
	2	0.002	0.000	0.001	[0.001;0.003]
	3	0.002	0.000	0.005	[0.001;0.003]
	4	0.002	0.000	0.009	[0.001;0.003]
	5	0.002	0.000	0.003	[0.001;0.003]
Log Glucose	1	0.080	0.041	0.052	[-0.001;0.161]
	2	0.090	0.036	0.014	[0.018;0.162]
	3	0.078	0.048	0.108	[-0.017;0.172]
	4	0.035	0.040	0.398	[-0.046;0.115]
	5	0.053	0.041	0.200	[-0.028;0.133]
Ace Inhibitor	3	0.104	0.083	0.210	[-0.060;0.267]
	4	0.100	0.052	0.070	[-0.010;0.198]
	5	0.040	0.045	0.412	[-0.052;0.127]
Age	1	0.002	0.002	0.408	[-0.003;0.010]
	2	0.002	0.002	0.499	[-0.003;0.010]
	3	0.005	0.003	0.159	[-0.002;0.011]
	4	0.003	0.002	0.280	[-0.002;0.010]
	5	0.001	0.002	0.678	[-0.004;0.010]
BMI	1	0.022	0.007	0.002	[0.010;0.036]
	2	0.010	0.003	0.006	[0.003;0.017]
	3	0.016	0.005	0.004	[0.005;0.026]
	4	0.020	0.004	0.000	[0.011;0.030]

	5	0.020	0.004	0.000	[0.012;0.028]

Kallekrein	T	Coef.	Std. Err.	P- value	95%CI for coef.
DBP	1	0.003	0.002	0.229	[-0.002;0.010]
	2	0.003	0.001	0.101	[-0.001;0.010]
	3	0.003	0.002	0.308	[-0.002;0.010]
	4	0.005	0.002	0.036	[0.001;0.010]
	5	-0.003	0.002	0.296	[-0.010;0.003]
SBP	1	-0.001	0.001	0.816	[-0.004;0.003]
	2	0.002	0.001	0.168	[-0.001;0.004]
	3	0.004	0.002	0.026	[0.001;0.010]
	4	0.002	0.001	0.125	[-0.001;0.010]
	5	0.001	0.001	0.742	[-0.002;0.003]
Duration of Diabetes	1	0.011	0.004	0.020	[0.002;0.020]
	2	0.001	0.004	0.895	[-0.010;0.010]
	3	0.010	0.005	0.202	[-0.004;0.017]
	4	-0.001	0.004	0.756	[-0.010;0.010]
	5	0.001	0.004	0.761	[-0.010;0.011]
Smoking	1	0.011	0.023	0.657	[-0.040;0.060]
	2	-0.022	0.021	0.307	[-0.064;0.020]
	3	0.022	0.028	0.442	[-0.034;0.080]
	4	-0.021	0.024	0.385	[-0.070;0.030]
	5	-0.017	0.023	0.474	[-0.063;0.029]
Treatment Group	1	0.073	0.037	0.054	[-0.001;0.150]
	2	-0.060	0.033	0.086	[-0.124;0.010]
	3	0.010	0.044	0.829	[-0.078;0.100]
	4	0.010	0.037	0.831	[-0.100;0.100]
	5	0.100	0.037	0.057	[-0.002;0.145]
Ace Inhibitor	3	0.104	0.083	0.210	[-0.100;0.267]
	4	0.100	0.052	0.070	[-0.010;0.198]
	5	0.040	0.045	0.412	[-0.100;0.127]

Table 28:Association between Log AER and Clinical Parameters at cross sectional levels

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
Log Kallikrein	1	0.241	0.116	0.038	[0.013;0.469]
	2	-0.068	0.149	0.649	[-0.362;0.226]
	3	0.260	0.141	0.066	[-0.017;0.538]
	4	0.297	0.179	0.099	[-0.056;0.651]
	5	0.076	0.180	0.673	[-0.279;0.432]
LDL	1	0.002	0.001	0.086	[-0.001;0.010]
	2	0.002	0.001	0.197	[-0.001;0.010]
	3	0.003	0.002	0.131	[-0.001;0.010]
	4	0.010	0.002	0.008	[0.002;0.010]
	5	0.004	0.002	0.107	[-0.001;0.010]
HDL	1	-0.010	0.003	0.081	[-0.012;0.001]
	2	-0.005	0.003	0.127	[-0.011;0.001]
	3	-0.010	0.004	0.055	[-0.016;0.001]
	4	-0.003	0.004	0.428	[-0.011;0.005]
	5	-0.004	0.004	0.333	[-0.013;0.005]
HbA1c	1	0.053	0.028	0.061	[-0.003;0.109]
	2	0.061	0.028	0.030	[0.010;0.116]
	3	0.221	0.040	0.000	[0.141;0.300]
	4	0.190	0.043	0.000	[0.104;0.276]
	5	0.187	0.047	0.000	[0.095;0.280]
Total Cholesterol	1	0.002	0.001	0.083	[-0.001;0.005]
	2	0.001	0.001	0.604	[0.002;0.004]
	3	0.002	0.001	0.297	[-0.002;0.005]
	4	0.010	0.001	0.003	[0.002;0.010]
	5	0.005	0.002	0.022	[0.001;0.010]
Log Glucose	1	0.056	0.090	0.533	[-0.121;0.233]
	2	0.087	0.102	0.394	[-0.114;0.289]
	3	0.018	0.127	0.882	[-0.232;0.269]
	4	0.026	0.137	0.846	[-0.243;0.296]
	5	-0.066	0.140	0.635	[-0.342;0.209]
BMI	1	0.010	0.015	0.560	[-0.022;0.040]
	2	0.003	0.010	0.781	[-0.017;0.022]
	3	0.010	0.014	0.657	[-0.021;0.034]
	4	0.011	0.014	0.416	[-0.016;0.040]
	5	0.010	0.015	0.555	[-0.020;0.038]
Age	1	-0.010	0.005	0.170	[-0.020;0.004]
	2	-0.005	0.006	0.493	[-0.018;0.010]
	3	-0.010	0.008	0.412	[-0.023;0.010]
	4	0.005	0.009	0.605	[-0.013;0.023]
	5	-0.001	0.009	0.901	[-0.019;0.017]

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
DBP	1	0.010	0.004	0.054	[-0.001;0.018]
	2	0.010	0.004	0.170	[-0.003;0.016]
	3	0.017	0.006	0.010	[0.004;0.030]
	4	0.027	0.007	0.000	[0.012;0.041]
	5	0.030	0.009	0.001	[0.012;0.048]
SBP	1	0.005	0.003	0.093	[-0.001;0.012]
	2	0.010	0.003	0.047	[0.001;0.013]
	3	0.013	0.004	0.004	[0.004;0.021]
	4	0.025	0.004	0.000	[0.015;0.033]
	5	0.015	0.004	0.001	[0.010;0.024]
Duration of Diabetes	1	0.054	0.009	0.000	[0.035;0.073]
	2	0.039	0.011	0.001	[0.016;0.062]
	3	0.029	0.014	0.041	[0.001;0.058]
	4	0.010	0.015	0.515	[-0.020;0.041]
	5	0.011	0.015	0.457	[-0.019;0.042]
Gender	1	-0.057	0.082	0.488	[-0.219;0.104]
	2	-0.214	0.093	0.023	[-0.397;-0.029]
	3	-0.166	0.116	0.155	[-0.396;0.063]
	4	-0.420	0.123	0.001	[-0.662;-0.177]
	5	-0.319	0.128	0.013	[-0.572;-0.067]
Smoking	1	0.163	0.051	0.002	[0.062;0.264]
	2	0.110	0.059	0.063	[-0.010;0.227]
	3	0.047	0.074	0.527	[-0.099;0.193]
	4	0.083	0.078	0.290	[-0.071;0.238]
	5	0.161	0.082	0.050	[-0.001;0.323]
Treatment Group	1	0.102	0.082	0.215	[-0.059;0.263]
	2	-0.214	0.093	0.022	[-0.398;0.031]
	3	-0.418	0.114	0.000	[-0.644;-0.193]
	4	-0.404	0.123	0.001	[-0.646;-0.162]
	5	-0.310	0.127	0.016	[-0.562;0.059]
Ace Inhibitor	3	0.618	0.219	0.005	[0.187;1.050]
	4	1.181	0.160	0.000	[0.865;1.497]
	5	0.714	0.146	0.000	[0.426;1.002]

Table 29: Association between Log ETDRS and Clinical Parameters at cross-sectional levels

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
Log Kallikrein	1	0.254	0.093	0.007	[0.070;0.438]
	2	0.238	0.117	0.043	[0.010;0.469]
	3	0.183	0.089	0.041	[0.010;0.359]
	4	0.149	0.114	0.192	[-0.075;0.374]
	5	0.186	0.099	0.061	[-0.010;0.381]
LDL	1	0.002	0.001	0.086	[-0.001;0.004]
	2	0.001	0.001	0.452	[-0.002;0.004]
	3	0.002	0.001	0.055	[-0.001;0.005]
	4	0.003	0.001	0.028	[0.001;0.010]
	5	0.002	0.001	0.093	[-0.001;0.005]
HDL	1	-0.004	0.002	0.113	[-0.010;0.001]
	2	-0.010	0.002	0.052	[-0.010;0.001]
	3	-0.004	0.003	0.133	[-0.010;0.001]
	4	-0.010	0.002	0.000	[-0.014;-0.005]
	5	-0.010	0.002	0.012	[-0.011;-0.001]
HbA1c	1	0.042	0.022	0.069	[-0.003;0.087]
	2	0.075	0.022	0.001	[0.030;0.119]
	3	0.118	0.026	0.000	[0.066;0.170]
	4	0.081	0.030	0.008	[0.021;0.141]
	5	0.132	0.024	0.000	[0.085;0.180]
Total Cholesterol	1	0.002	0.001	0.096	[-0.001;0.004]
	2	-0.001	0.001	0.818	[-0.003;0.002]
	3	0.002	0.001	0.137	[-0.001;0.004]
	4	0.002	0.001	0.122	[-0.001;0.005]
	5	0.001	0.001	0.371	[-0.001;0.003]
Log Glucose	1	0.068	0.073	0.352	[-0.075;0.212]
	2	0.241	0.080	0.003	[0.082;0.398]
	3	0.194	0.081	0.018	[0.034;0.354]
	4	0.165	0.082	0.047	[0.002;0.328]
	5	0.155	0.076	0.043	[0.010;0.306]
BMI	1	0.028	0.012	0.023	[0.004;0.053]
	2	0.012	0.008	0.132	[-0.004;0.028]
	3	0.015	0.009	0.089	[-0.002;0.033]
	4	0.021	0.009	0.026	[0.002;0.039]
	5	0.017	0.008	0.037	[0.001;0.034]

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
DBP	1	0.010	0.003	0.057	[-0.001;0.014]
	2	0.010	0.003	0.141	[-0.002;0.014]
	3	0.010	0.004	0.052	[-0.001;0.016]
	4	0.010	0.004	0.041	[0.001;0.019]
	5	0.011	0.005	0.025	[0.001;0.022]
SBP	1	0.002	0.002	0.460	[-0.004;0.010]
	2	0.010	0.002	0.003	[0.003;0.014]
	3	0.004	0.002	0.186	[-0.002;0.010]
	4	0.010	0.002	0.004	[0.003;0.015]
	5	0.010	0.002	0.007	[0.002;0.012]
Duration of Diabetes	1	0.125	0.004	0.000	[0.116;0.135]
	2	0.103	0.007	0.000	[0.090;0.117]
	3	0.078	0.008	0.000	[0.061;0.094]
	4	0.054	0.009	0.000	[0.036;0.072]
	5	0.043	0.008	0.000	[0.026;0.060]
Gender	1	0.068	0.066	0.308	[-0.063;0.199]
	2	-0.060	0.074	0.448	[-0.203;0.089]
	3	-0.180	0.073	0.016	[-0.325;-0.034]
	4	-0.170	0.078	0.031	[-0.324;-0.016]
	5	-0.184	0.069	0.009	[-0.322;-0.047]
Smoking	1	0.055	0.042	0.187	[-0.030;0.138]
	2	0.091	0.046	0.052	[-0.001;0.184]
	3	0.083	0.047	0.079	[-0.010;0.175]
	4	0.032	0.049	0.513	[-0.064;0.129]
	5	0.082	0.043	0.061	[-0.004;0.169]
Treatment Group	1	-0.050	0.066	0.469	[-0.179;0.083]
	2	-0.288	0.072	0.000	[-0.431;-0.145]
	3	-0.460	0.070	0.000	[-0.598;-0.322]
	4	-0.429	0.074	0.000	[-0.575;-0.283]
	5	-0.356	0.067	0.000	[-0.489;-0.223]
Ace Inhibitor	3	0.333	0.139	0.017	[0.059;0.608]
	4	0.257	0.118	0.031	[0.023;0.490]
	5	0.156	0.083	0.060	[-0.010;0.320]

Table 30: Association between Log IMT common and Clinical Parameters at cross sectional levels

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
Log Kallikrein	3	0.022	0.018	0.227	[-0.014;0.058]
	4	0.023	0.025	0.356	[-0.026;0.074]
	5	0.012	0.028	0.676	[-0.044;0.070]
LDL	3	0.001	0.000	0.001	[0.001;0.002]
	4	0.001	0.000	0.001	[0.001;0.002]
	5	0.001	0.000	0.010	[0.001;0.002]
HDL	3	-0.001	0.000	0.093	[-0.002;0.001]
	4	-0.001	0.000	0.059	[-0.002;0.001]
	5	-0.001	0.000	0.055	[-0.003;0.001]
HbA1c	3	0.015	0.005	0.009	[0.004;0.026]
	4	0.020	0.006	0.002	[0.010;0.033]
	5	0.010	0.007	0.322	[-0.010;0.023]
Total Cholesterol	3	0.001	0.000	0.001	[0.001;0.002]
	4	0.001	0.000	0.001	[0.001;0.002]
	5	0.001	0.000	0.053	[0.001;0.002]
Log Glucose	3	0.004	0.017	0.826	[-0.030;0.037]
	4	0.037	0.020	0.064	[-0.002;0.077]
	5	0.010	0.022	0.736	[-0.037;0.052]
BMI	3	0.010	0.001	0.001	[0.002;0.010]
	4	0.003	0.002	0.159	[-0.001;0.010]
	5	0.005	0.002	0.047	[0.001;0.010]
Age	3	0.010	0.001	0.000	[0.006;0.010]
	4	0.010	0.001	0.000	[0.010;0.012]
	5	0.011	0.001	0.000	[0.010;0.014]

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
DBP	3	0.002	0.000	0.012	[0.001;0.004]
	4	0.004	0.001	0.000	[0.002;0.010]
	5	0.002	0.001	0.155	[-0.001;0.010]
SBP	3	0.002	0.001	0.000	[0.001;0.004]
	4	0.004	0.000	0.000	[0.003;0.010]
	5	0.002	0.000	0.001	[0.001;0.004]
Duration of Diabetes	3	0.004	0.001	0.055	[-0.001;0.010]
	4	0.010	0.002	0.023	[0.001;0.010]
	5	0.010	0.002	0.012	[0.001;0.011]
Gender	3	-0.060	0.015	0.000	[-0.090;-0.030]
	4	-0.063	0.018	0.001	[-0.099;-0.028]
	5	-0.093	0.019	0.000	[-0.132;-0.054]
Smoking	3	0.037	0.009	0.000	[0.018;0.057]
	4	0.042	0.011	0.000	[0.018;0.064]
	5	0.053	0.012	0.000	[0.028;0.078]
Treatment Group	3	0.027	0.015	0.078	[-0.003;0.060]
	4	-0.005	0.018	0.802	[-0.041;0.032]
	5	-0.001	0.020	0.983	[-0.041;0.040]
Ace Inhibitor	3	0.100	0.028	0.053	[-0.001;0.112]
	4	0.113	0.025	0.000	[0.064;0.163]
	5	0.073	0.023	0.002	[0.030;0.120]

Table 31: Association between Log IMT internal and Clinical Parameters at cross sectional levels

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
Log Kallikrein	3	-0.044	0.030	0.159	[-0.105;0.017]
	4	0.001	0.049	0.995	[-0.097;0.097]
	5	0.093	0.059	0.118	[-0.024;0.210]
LDL	3	0.002	0.000	0.000	[0.001;0.003]
	4	0.002	0.000	0.000	[0.001;0.003]
	5	0.002	0.000	0.031	[0.001;0.003]
HDL	3	-0.001	0.001	0.256	[-0.003;0.001]
	4	-0.003	0.001	0.031	[-0.010;-0.001]
	5	-0.003	0.001	0.065	[-0.010;0.001]
HbA1c	3	0.010	0.009	0.339	[-0.010;0.028]
	4	0.019	0.013	0.143	[-0.010;0.045]
	5	0.024	0.016	0.151	[-0.010;0.100]
Total Cholesterol	3	0.001	0.000	0.000	[0.001;0.002]
	4	0.001	0.000	0.008	[0.001;0.003]
	5	0.001	0.000	0.170	[-0.001;0.002]
Log Glucose	3	-0.020	0.028	0.547	[-0.074;0.040]
	4	-0.010	0.039	0.877	[-0.083;0.071]
	5	0.028	0.047	0.543	[-0.064;0.122]
BMI	3	0.010	0.003	0.081	[-0.001;0.012]
	4	0.002	0.004	0.565	[-0.010;0.011]
	5	-0.001	0.005	0.945	[-0.011;0.010]
Age	3	0.010	0.001	0.000	[0.010;0.014]
	4	0.020	0.002	0.000	[0.010;0.020]
	5	0.020	0.002	0.000	[0.013;0.024]

Variables	T	Coef.	Std. Err.	P- value	95%CI for coef.
DBP	3	0.003	0.001	0.032	[0.001;0.006]
	4	0.005	0.002	0.034	[0.001;0.010]
	5	0.002	0.003	0.603	[-0.005;0.010]
SBP	3	0.003	0.001	0.004	[0.001;0.005]
	4	0.010	0.001	0.000	[0.004;0.010]
	5	0.010	0.001	0.001	[0.002;0.010]
Duration of Diabetes	3	0.004	0.003	0.185	[-0.002;0.010]
	4	0.010	0.004	0.023	[0.001;0.020]
	5	0.012	0.005	0.022	[0.002;0.022]
Gender	3	-0.110	0.025	0.000	[-0.160;-0.100]
	4	-0.140	0.034	0.000	[-0.210;-0.720]
	5	-0.191	0.041	0.000	[-0.273;-0.110]
Smoking	3	0.044	0.016	0.009	[0.011;0.077]
	4	0.067	0.022	0.003	[0.023;0.112]
	5	0.127	0.026	0.000	[0.075;0.180]
Treatment Group	3	0.015	0.026	0.552	[-0.035;0.067]
	4	0.010	0.035	0.776	[-0.060;0.080]
	5	0.026	0.043	0.543	[-0.058;0.111]
Ace Inhibitor	3	0.140	0.047	0.003	[0.047;0.233]
	4	0.122	0.050	0.016	[0.023;0.221]
	5	0.165	0.051	0.001	[0.065;0.265]