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Homocysteine levels and some biochemical parameters among type 2 diabetic nephropathy patients in Gaza City

مستويات الهموسيستين وبعض المعايير البيوكيميائية لدي مرضى السكري النوع الثاني المصابين باعتلال الكلي في مدينة غزة

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgement has been made in the text.

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Abstract

Background: Diabetes mellitus is prevalent in the Gaza strip. Recently, hyperhomocysteinemia was linked to diabetic nephropathy in type 2 diabetes.

Objective: To assess homocysteine levels and some biochemical parameters among type 2 diabetic nephropathy patients in Gaza City.

Materials and Methods: This cross sectional study comprised 120 diabetic patients distributed as follows: Group I: 40 normoalbuminuric patients (urinary albumin <30 mg/g), group II: 40 microalbuminuric patients (urinary albumin 30-300 mg/g) and group III: 40 macroalbuminuric patients (urinary albumin >300 mg/g). The control group included 40 non diabetic healthy individuals. A questionnaire interview was applied. Urinary albumin and protein were measured. Body mass index was determined. Serum homcysteine, glucose, urea, creatinine, cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C), and whole blood HbA1c were determined.

Results: The mean urinary albumin concentrations were 15.9±4.8, 22.3±5.2, 146.7±80.7 and 348±37.6 mg/g in control group, groups I, II and III, respectively. The mean ages were 56.9±6.2, 57.7±7.1, 57.8±5.4 and 58.5±6.3 years. Diabetic nephropathy was more prevalent among less educated and unemployment individuals as well as among individuals with family history of diabetes and those who frequently eat meat. The main self-reported complications among patients were retinopathy, cardiovascular diseases and neuropathy. The prevalence of such complications was significantly increased with the development of diabetic nephropathy. The ANOVA test showed a progressive significant increase in the mean levels of serum homocysteine recording the values of 13.4±3.7, 17.1±4.8, 20.3±5.9 and 22.9±5.5 µmol/l in controls, groups I, II and III, respectively (P=0.000). Body mass index was also significantly increase (P=0.000). Serum glucose and blood HbA1c in various diabetic groups exhibited significant gradual increase with the development of diabetic nephropathy (glucose: 225.6±51.1, 251.3±104.3 and 288.7±176.2

mg/dl and HbA1c: 7.6±1.1, 8.6±1.3, 8.8±1.4) in respect to control group (109.7±14.8 mg/dl and 4.7±0.6) with P=0.000. Similar trend was found for urea and creatinine showing values of 26.0±7.5, 48.7±5.38.1, 54.6±40.0 and 72.1±62.6 mg/dl in control group and groups I, II and III for urea, and 0.72±0.16, 0.89±0.62, 1.23±1.25 and 1.68±1.87 mg/dl for creatinine (P=0.000 and P=0.006, respectively). In general, there was gradual significant increase in cholesterol (P=0.001), triglycerides (P=0.000) and LDL-C (P=0.005) whereas HDL-C was significantly decreased (P=0.000) in different diabetic groups towards the development of diabetic nephropathy. Homocysteine levels were higher in less educated and unemployment individuals, individuals with family history of diabetes, and individuals who frequently eat meat and eat less fish. Homocysteine showed significant positive correlations with urinary albumin (r=0.564, P=0.000), serum glucose (r=0.465, P=0.000), blood HbA1c (r=0.517, P=0.000), serum urea (r=0.654, P=0.000), serum creatinine (r=0.561, P=0.000), triglycerides (r=0.320, P=0.001) and significant negative correlation with HDL-C (r=-0.517, P=0.000).

Conclusion: Homocysteine level progressively increased with the development of diabetic nephropathy. Such levels correlated positively with urinary albumin, serum glucose, blood HbA1c, serum urea, creatinine and triglycerides, and negatively with HDL-C.

Keywords: Homocysteine, biochemical parameters, diabetic nephropathy, Gaza city.

ملخص الدراسة

مستويات الهموسيستين وبعض المعايير البيوكيميائية لدى مرضى السكرى النوع الثاني المصابين باعتلال الكلى فى مدينة غزة

مقدمة: يعتبر مرض السكري من الأمراض المنتشرة في قطاع غزة والذي يعانى منه الكثير فى عصرنا الحالي. حديثاً, وفى السنوات الأخيرة تم ربط ارتفاع مستوى الهموسيستن في الدم مع مرض اعتلال الكلية السكري في مرض السكري النوع الثانى وهذا يجعل من الهموسيستين احد الكواشف التى تكشف عن تطور مرض السكري إلى مرض اعتلال الكلية السكري.

الهدف: تهدف الدراسة إلى تقييم حالة ومستوى الهموسيستين وبعض المعايير البيوكيميائية لدي مرضى السكري النوع الثاني من المصابين باعتلال الكلية السكري في مدينة غزة.

الطرق والأدوات: منهج هذه الدراسة هو مجموعة مرضية - مجموعة ضابطة، المجموعة المرضية تشمل 120 مريضاً من مرضى السكري موزعون على ثلاث مجموعات كالتالي: المجموعة الأولى تتكون من 40 مريضاً من مرضى السكري العادي (الألبومين في البول اقل من 30 مل جرام/ جرام), المجموعة الثانية 40 مريضاً من مرضى السكري بيلة البومينية الزهيدة (الألبومين في البول من 30–300 مل جرام/ جرام), المجموعة الثالثة 40 مريضاً من مرضى السكري بيلة بروتينية (الألبومين في البول أكبرمن 300 مل جرام/ جرام). وكذلك المجموعة الضابطة تتكون من 40 شخصاً من الأصحاء الغير مصابين بمرض السكر. تم الحصول على معلومات هذه الدراسة من خلال الإجابة على الاستبيان, وفحوصات الدم والبول لكل من المرضى والأصحاء وحساب معامل كتلة الجسم.

النتائج : تم تحديد متوسط تراكيز الألبومين فى البول وكانت (4.8±15.9 مليجرام/جرام) في المجموعة الضابطة, بينما المجموعة الاولى كانت (5.2±22.3مليجرام/جرام), والمجموعة الثانية (80.7±146.7 ملجرام/جرام), والمجموعة الثالثة (37.6±348 مليجرام/جرام). كذلك متوسط الأعمار كانت بالترتيب من المجموعة الضابطة حتى المجموعة الثالثة كالتالي (6.2±56.9 مليجرام/جرام, 7.1±57.7 مليجرام/جرام, 5.4±57.8 مليجرام/جرام, 6.3±58.5 مليجرام/جرام). ووجد أن مرض اعتلال الكلية السكري أكثر انتشاراً بين الأشخاص الأقل فى المستوى التعليمي والعاطلين عن العمل وكذلك الأشخاص ذو تاريخ عائلي بمرض السكري وأيضاً الأشخاص الذين يكثرون من أكل اللحم. كما أن المضاعفات الرئيسية التي سجلت على لسان المرضى أوضحت وجود اعتلال الشبكية، وأمراض القلب واعتلال الأعصاب. وكانت هناك زيادة في المضاعفات مع زيادة تطور مرض اعتلال الكلية السكري. وأوضحت النتائج بازدياد معنوى في متوسط مستويات الحمض الأميني الهوموسيستين 3.7±13.4, 4.8±17.1, 5.9±20.3, 5.5±22.9 ميكرومول/ لتر في المجموعة الضابطة والمجموعات الأولى والثانية والثالثة على التوالي. وأيضاً كانت هناك زيادة فى مؤشر كتلة الجسم مع تطور المرض (P=0.000). أظهرت الدراسة أن مستوى سكر الدم و البروتين المحمل بالسكر فى مجموعات مرضى السكرى توضح وجود زيادة تدريجية ذات دلالة إحصائية مع تطورمرض اعتلال الكلية السكرى ومسجلة القيم التالية لسكر الدم فى المجموعات الاولى والثانية والثالثة على التوالى (51.1±225.6, 104.3±251.3, 176.2±288.7 مليجرام/ ديسليتر) مقارنة مع المجموعة الضابطة التى اعطت القيمة (14.8±109.7 مليجرام/ ديسليتر) وكذلك البروتين المحمل بالسكر أعطى فى مجموعات مرضى السكرى على الترتيب هذه القيم (1.1±7.6 ,1.3±8.6, 1.4±8.8) مقارنة مع المجموعة الضابطة التى اعطت القيمة (0.6±4.7). وفى نفس الاتجاه وجد أن مستوى البولينا والكرياتينين هناك زيادة ذات دلالة احصائية فى مجموعات مرضى السكر الثلاثة مقارنة مع المجموعة الضابطة وتزداد كلما اتجهنا الى مجموعتي مرضى اعتلال الكلية السكري (المجموعة الثانية والثالثة). كما أنه وجد أن مستوى كل من الكولسترول والدهون الثلاثية والكولسترول منخفض الكثافة فى زيادة تدريجية ذات دلالة إحصائية في مجموعات مرضى

السكري مقارنة مع المجموعة الضابطه، وبالمقابل الكولسترول عالي الكثافة كان في نقصان واضح في مجموعات مرضى السكري ويزداد النقصان مع تطور مرض اعتلال الكلية السكرى.
ولوحظ أن مستوى الهوموسيستين أعلى فى الأشخاص الأقل تعليما، والأشخاص العاطلين عن العمل، والأشخاص ذوي تاريخ عائلي لمرض السكري، وكذلك مع الأشخاص الذين يكثرون أكل اللحوم والذين يقللون أكل الأسماك, وكانت هناك علاقة إيجابية بين الهوموسيستين مع الألبومين البولى وسكر الدم والبروتين المحمل بالسكر و البولينا والكرياتينين والدهون الثلاثية ، بينما كانت علاقة الهموسيستين سلبية مع البروتين الدهني عالى الكثافة.
الاستنتاج: نستنتج من دراستنا ان مستوى الهموسيستين يزداد تدريجيا مع تطور مرض اعتلال الكلية السكري, كما أن ارتفاع مستويات الهموسيستين لها علاقة وارتباط ايجابي مع الألبومين البولى وسكر الدم والبروتين المحمل بالسكر و البولينا والكرياتينين والدهون الثلاثية وعلاقة سلبية مع البروتين ألدهني عالى الكثافة.
الكلمات المفتاحية: الحمض الأميني الهوموسيستين, العوامل البيوكيميائية, اعتلال الكلية السكرى, مدينة غزة.

Dedication

This thesis is dedicated to:

My beloved parents who have always supporting me

My brothers and sister,

My friends,

Dedication is almost expressed to the Palestinian people who have suffered and will be struggling with the persistence to have a free Palestine.

Mahmoud Alnajjar

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At the end, I am very grateful to those who participated and helped me to complete this study.

List of abbreviations

ADA	American Diabetes Association
AER	Albumin excretion rate
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CKD	Chronic kidney disease
CVD	Cardiovascular disease
ESRD	End stage renal disease
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
HbA1c	Glycated Haemoglobin
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
MOH	Ministry of Health-Palestine
tHCy	Total homocysteine
TG	Triglycerides
WHO	World Health Organization

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Chapter 1

Introduction

1.1 Overview

Diabetes mellitus is a combination of metabolic disorders due to deficiency in insulin secretion, insulin action, or both. Two major forms of diabetes were identified; type 1 and type 2. Lack of or severe reduction in insulin secretion due to autoimmune or viral destructions of β cells is responsible for type 1 diabetes, which accounts for 5-10% of diabetic patients. The more prevalent form, type 2 diabetes, accounts for more than 90% of cases (Olefsky, 2001). Type 2 diabetes usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce it (Cohen, 2006).

Lack of insulin action and/or secretion in type 2 diabetes induces hepatic glucose output by inhibiting glycogen synthesis and stimulating glycogenolysis and gluconeogenesis then increased rates of hepatic glucose production result in the development of overt hyperglycemia, especially fasting hyperglycemia (Defronzo et al., 1992 and Michael et al., 2000). In such conditions, lipolysis in adipose tissue is promoted leading to elevated circulating levels of free fatty acids. Ketones are produced, and are found in large quantities in ketosis, the liver converts fat into fatty acids and ketone bodies which can be used by the body for energy (Botion and Green, 1999). In addition, excess fatty acids in serum of diabetics are converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins (Jaworski et al., 2007). In addition, impairment of urea and creatinine was reported in type 2 diabetes (Yassin et al., 2011).

One of the most severe chronic complications of diabetes is the development of diabetic nephropathy (Katalin, 2003 and Cohen et al., 2010). By convention, diabetic nephropathy is defined as the appearance of persistent clinical albuminuria in an individual with diabetes for more than 5 years (Richard et al., 2000). The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels ≥30 mg/day of albumin in the urine, referred to as microalbuminuria, and patients with microalbuminuria are referred to as having incipient nephropathy (Jha et al., 2010). Without specific interventions, about 80% of subjects with type 1 diabetes who develop sustained microalbuminuria have their urinary albumin excretion increase at a rate of ~10-20% per year to the stage of overt nephropathy (macroalbuminuria) or clinical albuminuria (≥300 mg/24 h) over a period of 10-15 years (American Diabetes Association, 2002).

Homocysteine is a sulphur containing intermediary amino acid which is derived by the demethylation of methionine (Shipchandler et al.1995). The primary source of methionine is animal protein (Hankey and Eikelboom,1999). The normal range of serum homocysteine is 5-15 µmol/L (Ueland et al. 1993, Graham et al. 1997). Elevated serum homocysteine beyond the normal range (>15 µmol/L) is traditionally referred as hyperhomocysteinemia.

Hyperhomocysteinemia may be due to genetic insufficiencies of the enzymes needed for its metabolism, to nutritional deficits in vitamin cofactors, or to medical conditions such as cardiovascular disease (Humphrey et al., 2008 and Coldea-Agoston et al., 2011). Similarly, low intake and plasma concentrations of folate and vitamins B6 and B12 have been associated with increased plasma homocysteine levels (Lee and Prasad, 2002). In addition, hyperhomocysteinemia has been linked to diabetic nephropathy in type 2 diabetic patients (Okumura and Aso, 2003; Li et al., 2007 and Cho et al., 2010).

Although diabetes mellitus is prevalent in the Gaza strip and the mortality rate of diabetes mellitus among Palestinians constituted 8.5 per 100,000 population in the year 2010 (Ministry of Health, MOH, 2010), there is under-diagnosis and under-reporting of the disease. Therefore diseases will

progress in many cases towards diabetic nephropathy. Biochemical studies conducted on diabetic nephropathy in Gaza strip are few and recent and handled some enzymes, lipid abnormalities and leptin hormon (Shubair, 2008, Abu Mustafa, 2011 and Abu Snayma, 2012). However, no previous study investigates homocysteine status in diabetic nephropathy. Therefore, this study is the first to assess homocysteine level in type 2 diabetic patients with diabetic nephropathy in Gaza City, and whether this level varies with different stages of diabetic nephropathy. Understanding the status of homocysteine and other parameters could be useful in the management of the disease.

1.2 General objective

The general objective of the present study is to assess homocysteine levels and some biochemical parameters among type 2 diabetic nephropathy patients with diabetic nephropathy in Gaza City.

1.3 Specific objectives

1. To study socio-demographic and clinical data and body mass index of type 2 diabetic patients with and without diabetic nephropathy.

2. To determine serum homocysteine levels in different stages of diabetic nephropathy (micro- and macroalbuminuric patients) and compare them with diabetic patients (normoalbuminurics) and controls.

3. To measure serum glucose, urea, creatinine, cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) as well as blood HbA1c in micro- and macroalbuminurics and compared them with normoalbuminurics and controls.

4. To verify possible relations between homocystiene and the previous parameters.

1.4 Significance

1. Diabetic nephropathy represents one of the major problems developed in diabetes mellitus patients as they have five times greater risk for developing nephropathy than healthy persons, which is a common cause for end-stage renal disease (ESRD).

2. In the Gaza strip only two studies assessed some enzymes, lipid abnormalities and leptin hormone in type 2 diabetic patients with diabetic nephropathy (Shubair, 2008, Abu Mustafa, 2011 and Abu Snayma, 2012). However, homocysteine status in diabetic nephropathy has never been investigated in Palestinian diabetic patients. This is the first study to assess homocystiene level and some biochemical parameters among type 2 diabetic nephropathy patients in Gaza City.

3. Early detection of microalbuminuria and monitoring of blood glucose level delay diabetic nephropathy progression before onset of clinical symptoms, thereby leading to increased survival and lower treatment costs.

4. Understanding the status of homocysteine in diabetic nephropathy may be of diagnostic and therapeutic values in the course development of the disease.

Chapter 2

Literature Review

2.1 Definition of diabetes mellitus

Diabetes mellitus is metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both**.** It is a chronic disease that affects the lives of millions around the world (International Diabetes Federation, IDF, 2006).

2.2 Types of diabetes

The most common types of diabetes mellitus are:

2.2.1 Type 1 diabetes

It was previously called insulin-dependent diabetes mellitus or juvenile-onset diabetes. Type 1 diabetes develops when the body's immune system destroys pancreatic beta cells resulting in failure of insulin production. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age. Type 1 diabetes accounts for 5-10% of all diagnosed cases of diabetes (Olefsky, 2001).

2.2.2 Type 2 diabetes

It was previously called non insulin-dependent diabetes or adult-onset diabetes. Type 2 diabetes results from insulin resistance, a condition in which the body fails to properly use insulin, combined with relative insulin deficiency (Robbins and Cotran, 2004). This form of diabetes accounts for about 90-95% of all diagnosed cases of diabetes. Type 2 diabetes is associated with older age, obesity, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity (Olefsky, 2001).

2.3 Prevalence and mortality rate of diabetes mellitus in Palestine

The prevalence of diabetes mellitus in Palestine was examined in a study conducted in 2000 in cooperation with Al-Quds University and Ministry of Health. The results indicated that the prevalence was about 9% (Ministry of Health, 2002). It is around the reported prevalence rate in Egypt and Tunisia (9%) and less than in Saudi Arabia (12%) and Oman (13%). However, in Palestine, there is under-diagnosis and under-reporting of the disease. This is due to lack of proper hospital and clinic recording system (Ministry of Health, 2005). Mortality rate of diabetes mellitus among Palestinians constituted 3.6% of total population deaths. The mortality rate of diabetes mellitus among Palestinians constituted 5.9 per 100,000 population in the year 2009 (Ministry of Health 2009), and this figure rose to 8.5 per 100,000 population in the year 2010 (Ministry of Health 2010).

2.4 Type 2 diabetes

2.4.1 Metabolism in type 2 diabetes

The circulating glucose is derived from 1) intestinal absorption during the fed state in which the rates of gastric emptying determine how quickly glucose appears in the circulation during the fed state, and from 2) hepatic processes including glycogenolysis and gluconeogenesis. Renal gluconeogenesis contributes substantially to the systemic glucose pool only during periods of extreme starvation. Although most tissues have the ability to hydrolyze glycogen, only the liver and kidneys contain glucose-6-phosphatase, the enzyme necessary for the release of glucose into the circulation.

The rate of glucose entering the circulation balanced by the rate of glucose removal from the circulation. The glucoregulatory hormones of the body are designed to maintain circulating glucose concentrations in a relatively narrow range. Glucoregulatory hormones include insulin, glucagon,

amylin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), epinephrine, cortisol, and growth hormone. Of these, insulin and amylin are derived from the β-cells, glucagon from the α-cells of the pancreas, and GLP-1 and GIP from the L-cells of the intestine.

In the bi-hormonal model of glucose homeostasis, insulin is the key regulatory hormone of glucose disappearance, and glucagon is a major regulator of glucose appearance. After reaching a post-meal peak, blood glucose slowly decreases during the next several hours, eventually returning to fasting levels. In the immediate post-feeding state, glucose removal into skeletal muscle and adipose tissue is driven mainly by insulin. At the same time, endogenous glucose production is suppressed by 1) the direct action of insulin on the liver, and 2) the paracrine effect or direct communication within the pancreas between the α- and β-cells, which results in glucagon suppression (Wallum et al., 1992).

Type 2 diabetes is a disorder characterized by lack of insulin action and/or secretion that induces hepatic glucose output by inhibiting glycogen synthesis and stimulating glycogenolysis and gluconeogenesis then increased rates of hepatic glucose production result in the development of overt hyperglycemia, especially fasting hyperglycemia (Defronzo et al., 1992 and Michael et al., 2000).

In such conditions, lipolysis in adipose tissue is promoted leading to elevated circulating levels of free fatty acids. Ketones are produced, and are found in large quantities in ketosis, the liver converts fat into fatty acids and ketone bodies which can be used by the body for energy (Botion and Green, 1999). In addition, excess fatty acids in serum of diabetics are converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins (Jaworski et al., 2007). Several studies showed that cholesterol, triglycerides and LDL-C are elevated in diabetic patients (Barrett-Connor et al., 1982,). In contrast, other studies documented that HDL-C was decreased (Yassin et al., 2011).

2.4.2 Risk factors of type 2 diabetes

The most common risk factors for type 2 diabetes comprise obesity, poor diet, sedentary lifestyle, increased age and family history; diabetes tends to run in families (Fujita, 2009 and Pijl et al., 2009). Not everyone with type 2 diabetes has symptoms, particularly in the early stages of the disease. In fact, 5.7 million of the 23.6 million people with diabetes are unaware that they even have the disease. Of those, 90 to 95% are those with type 2 diabetes (Centers for Disease Control and Prevention, CDC, 2008).

2.4.3 Complications of type 2 diabetes mellitus

Complications of type 2 diabetes include acute and chronic complications. The acute complications comprise diabetic ketoacidosis, hyperosmolar hyperglycemic non ketotic coma, lactic acidosis and hypoglycemia (Bardin and wayne, 1994 and Harris et al., 1995). The chronic complications include cardiovascular disease, peripheral vascular disease, cerebrovascular disease, diabetic retinopathy, diabetic nephropathy and diabetic neuropathy (Savage, 1996; Dyck et al., 2002; Bate et al., 2003; Katalin, 2003 and The National Eye Institute, 2006).

2.5 The Kidneys

2.5.1 Location and structure

The kidneys lie against the dorsal body wall beneath the parietal peritoneum in superior lumbar region where they receive some protection from the lower part of the rib cage. Each kidney has a medial indentation (the hilus) in which there is two renal arteries, renal vein, and ureter. A fibrous renal capsule encloses each kidney. The kidney has three regions, outer granulated layer called renal cortex, renal medulla that consists of cone shaped tissue masses called medullary pyramids, and renal pelvis which is a central space or cavity that is continuous with the ureter (Figure 2.1) (Marieb, 2003).

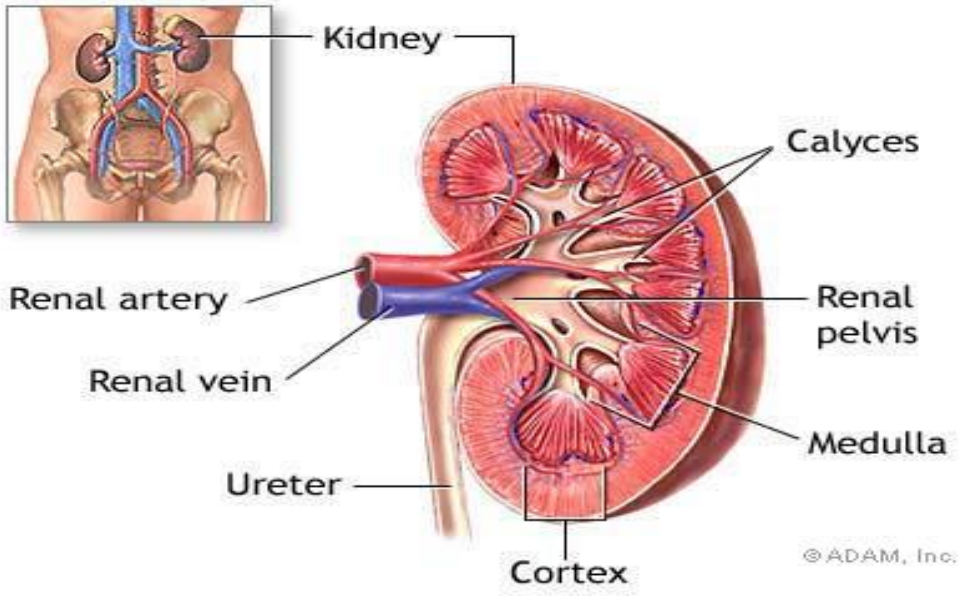


Figure 2.1 Location and structure of the kidney

Each kidney contains approximately one million tiny structures called nephrons (Figure 2.2). Nephrons are responsible for the processes of filtration, reabsorption, and secretion that go on in the kidney to form the urine product. The nephron consists of two main structures, a glomerulus, which is a knot of capillaries, and a renal tubule. The closed end of the renal tubule is enlarged and cup-shaped and completely surrounds the glomerulus. This portion of the renal tubule is called Bowman's capsule. In order from Bowman's capsule they are the proximal convoluted tubule, loop of Henle, and the distal convoluted tubule. Urine from many nephrons is collected in the collecting ducts, which deliver the final urine product into the calyces and pelvis of the kidney (Thibodeau and Patton, 1999).

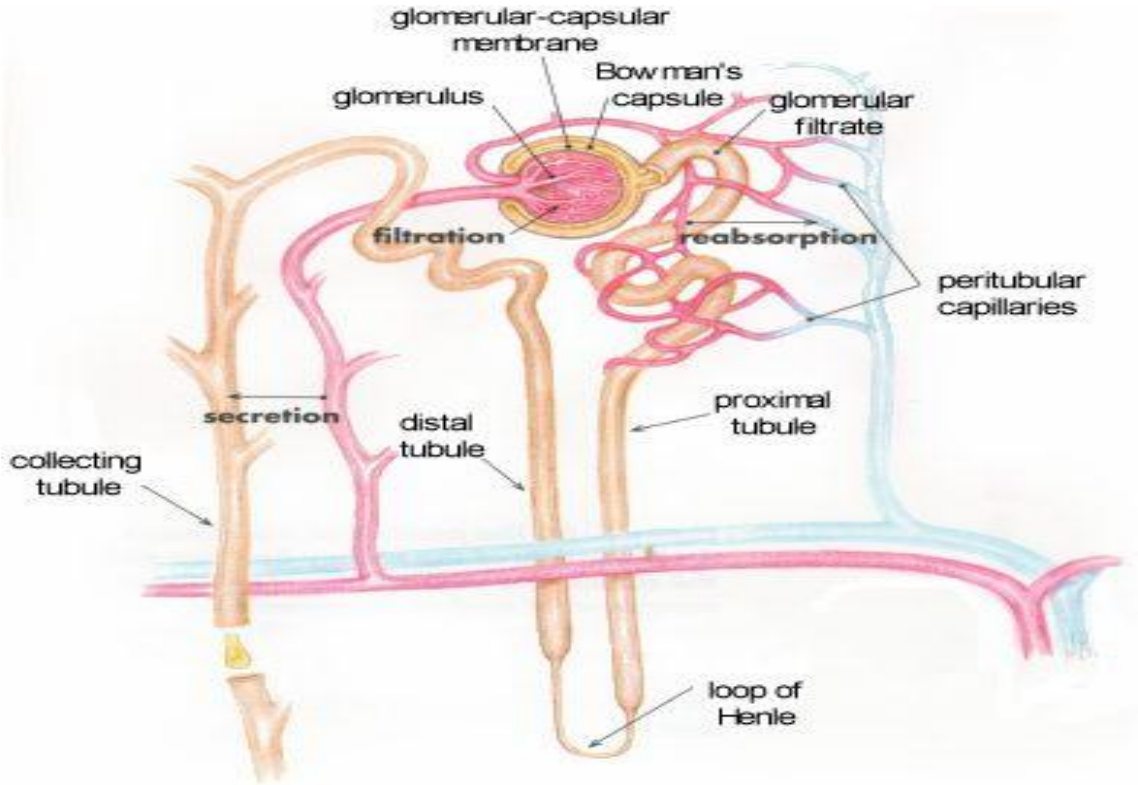


Figure 2.2 Structure of the nephron

Every nephron is associated with two capillary beds: The glomerulus and the peritubular capillary bed. The glomerulus is both fed and drained by arterioles. The afferent arteriole is the feeder vessel, and the efferent arteriole receives blood that has passed through the glomerulus. The efferent arteriole then breaks up to form the peritubular capillary bed, which closely clings to the whole length of the tubule. The peritubular capillaries then drain into an interlobular vein that leaves the cortex (Marieb, 2003).

2.5.2 Role of the kidneys

The kidneys perform two main functions: eliminate soluble waste products of metabolism and preserve the internal environment of the cells (maintain water balance, pH, ionic equilibrium, and fluid osmotic pressure). In addition, the kidney does perform other functions such as, synthesis of erythropoietin and 1-hydroxylation of 25-hydroxy-vitamin D_3 (Kaplan and Szabo,1983).

2.5.3 Glomerular filtration barrier

The glomerulus is the filtering unit of the mammalian kidney; it is a complex knot of capillaries and filtration takes place across the capillary wall into Bowman.s space. About 180 liters of water and small molecules are freely filtered in large quantities through glomerular daily in a 70 kg adult. Glomerular perm selectivity involves two adjacent molecular filters: the glomerular basement membrane (GBM) and the slit diaphragm (Nosadini et al., 2002). The GBM in humans is a complex three layered structure comprised of endothelial cells with fenestrations, the dense fibrillar GBM itself, and podocytes, the outer visceral epithelial cells (Figure 2.3). Between the interdigitating foot processes of the podocytes arises the slit diaphragm, a zipper-like complex. The basement membrane layer, attached to endothelial and visceral epithelial cells, has a thickness of 250-350 nm (Yang, 1994). The GBM is made up of unusual highly restricted isoforms of laminin and type IV collagen together with various proteoglycans, including heparan sulphate, grinand perlecan (Christiansen et al. 1985 and American Diabetes Association, 2002). The latter impart a highly negative electrostatic charge on the GBM, and much of the older literature on glomerular permeability focused on the charge characteristics of the GBM as a means of resisting the passage of albumin molecules, which are also negatively charged (chan et al., 1975). Podocyte stabilize glomerular architecture by counteracting distensions of the GBM (Steffes et al., 2001) and maintain a large filtration surface through the slit diaphragms (Cohen et al., 1997).

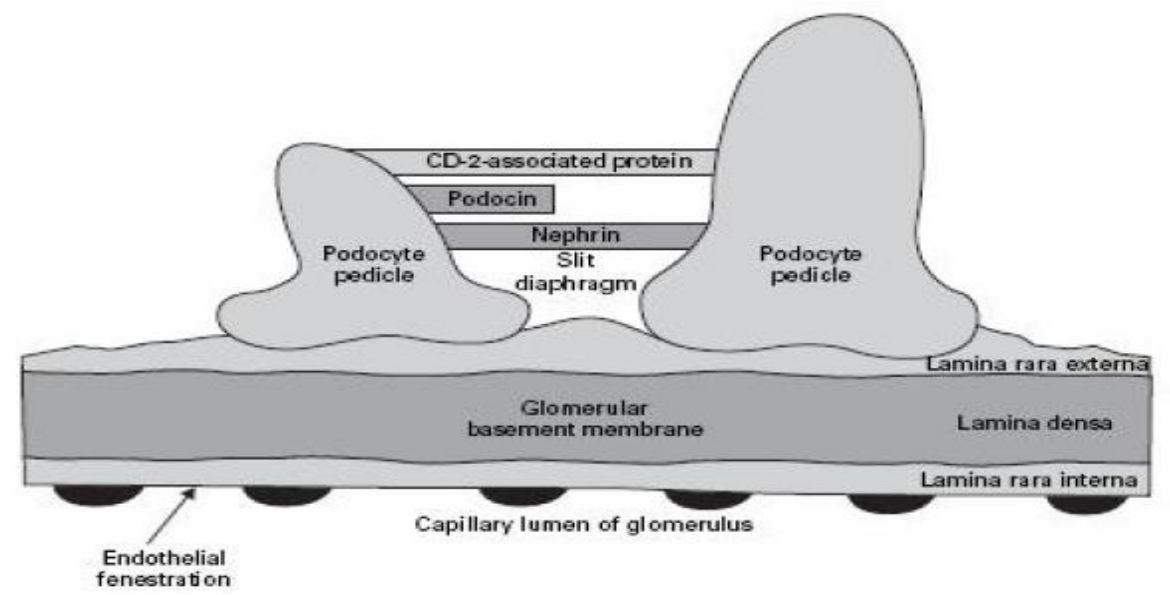


Figure 2.3 the barrier to proteinuria. Schematic drawing of the visual glomerular epithelial cells (podocytes) lining the outer aspect of the GBM. Foot processes are connected by the slit diaphragm with nephrin, podocin, and other proteins (Drumond et al., 1994).

2.6 Formation of urine

Urine formation starts with the ultrafiltration of blood in the kidney glomerulus. Plasma flows through the pores in the endothelium, while blood cells are retained in the capillary lumen. The GBM is thought to act as a prefilter, which prevents proteins from passing through the capillary wall. Finally, the filtrate is guided through a slit between two podocyte cell protrusions to the Bowman.s space and further to the tubular system of nephron, where it is concentrated to become the final urine (Vesa, 2004).

2.7 Diabetic nephropathy

2.7.1 Definition

By convention, diabetic nephropathy (DN) is defined as the appearance of persistent clinical albuminuria in an individual with diabetes for more than 5 years and concomitant retinopathy, in the absence of urinary tract infection, other renal diseases and heart failure (Stephen et al., 2006).

2.7.2 Development of diabetic nephropathy

Diabetic nephropathy is a multi-stage condition that takes several years to become clinically overt. There are usually changes in renal function such as glomerular hyperfiltration, increased renal blood flow and hypertrophy of the kidney. Most of these changes can be reversed at an early stage by good glycemic control, but they persist in many patients and may be important in the later development of clinical nephropathy (Stephen et al., 2006).

Normal human urine contains only very small quantities of albumin, <30 mg of albumin being excreted by healthy adults in 24 hours. The dysfunction of the glomerular filter, leading to extensive leakage of plasma proteins. Diffuse alteration of podocyte foot processes is observed in various forms of glomerulonephritis (Masao, 2004). The development of DN is characterized by glomerular hyperfiltration and increased albumin excretion rate (Sho-ichi, 2002). Accurate quantification of the amount of albumin lost in the urine has important clinical connotation: excretion of amounts in excess of 300 mg in 24 h is termed macroalbuminuria and excretion of lesser amounts of albumin, between 30 and 300 mg in 24 h, is termed microalbuminuria (Peter, 2004).

Once overt nephropathy occurs, the GFR gradually falls over a period of several years at a rate that is highly variable from individual to individual about 2-20 ml/min/year (American Diabetes Association, ADA, 2002). End stage renal disease (ESRD) develops in 50% of type 1 diabetic individuals with overt nephropathy within 10 years and in >75% by 20 years. A higher proportion of individuals with type 2 diabetes are found to have microalbuminuria and overt nephropathy shortly after the diagnosis of their diabetes, because diabetes is actually present for many years before the diagnosis is made. Twenty to forty percentages of type 2 diabetic patients with microalbuminuria progress to overt nephropathy, but by 20 years after onset of overt nephropathy, only ~20% will have progressed to ESRD (ADA, 2002).

The natural history of DN in type 1 patients leads to death 6 years after the start of persistent proteinuria (Mark, 2005). The overall sequence is similar

in type 2 patients, but uncertainties may exist because of inaccurate dating of the onset of diabetes. The decline is more variable in type 2 patients and the progression rate to ESRD may be as low as 20% over 20 years. Improvements in the management of nephropathy have extended the time course from persistent proteinuria to renal failure (ADA, 2002).

2.7.3 Macroalbuminuria

Is defined as the range in between urinary excretion of albumin ≥ 300 mg/24 hours (Chan et al., 2001). Arterial hypertension usually occurs with persistent proteinuria, and over time the protein loss may increase to cause nephritic syndrome with hypoalbuminaemia and peripheral edema, lipid disturbances and atherosclerotic complications are prominent in this phase.

2.7.3.1 Mechanisms of proteinuria in diabetic nephropathy

It is accepted that diabetic proteinuria is caused by changes in hemodynamic pressure gradient across the GBM and factors intrinsic to the filtration barrier, including the pore size and extent of anion charges (Figure 2.4) (Brenner et al., 1978).

Angiotensin II exerts complex hemodynamic and nonhemodynamic actions that may contribute to DN, including induction of systemic vasoconstriction, increased glomerular arteriolar resistance, increase in glomerular capillary pressure, increased glomerular capillary permeability, reduction in the filtration surface area, stimulation of extracellular matrix proteins, and stimulation of renal proliferation and fibrogenic chemokines (Wolf et al., 1997, Amann et al., 2003 and Cao Z et al., 2000).

One of the most prominent ultrastructural abnormalities in DN is podocyte injury and loss (Steffes et al., 2001). Visceral epithelial cell injury and reduction in the number of podocytes per glomerulus leave fewer podocytes to cover the surface area (Vestra et al., 2003). Increased synthesis of collagen by podocytes could lead to GBM thickening, or excessive amounts

of secreted vascular endothelial growth factor could enhance permeability of the barrier to macromolecules.

Nephrin is a 1,241-residue transmembrane cytoskeleton protein gene product localized to the filtration slit area between foot processes of the podocytes, and is essential for the formation of the zipper-like slit diaphragm structure (Huber et al., 2004). Reduced nephrin mRNA and protein expression may be associated with podocyte ultrastructural abnormalities, providing one potential mechanism for proteinuria in DN (Pettersson-Fernholm et al., 2003, and Benigni et al., 2004).

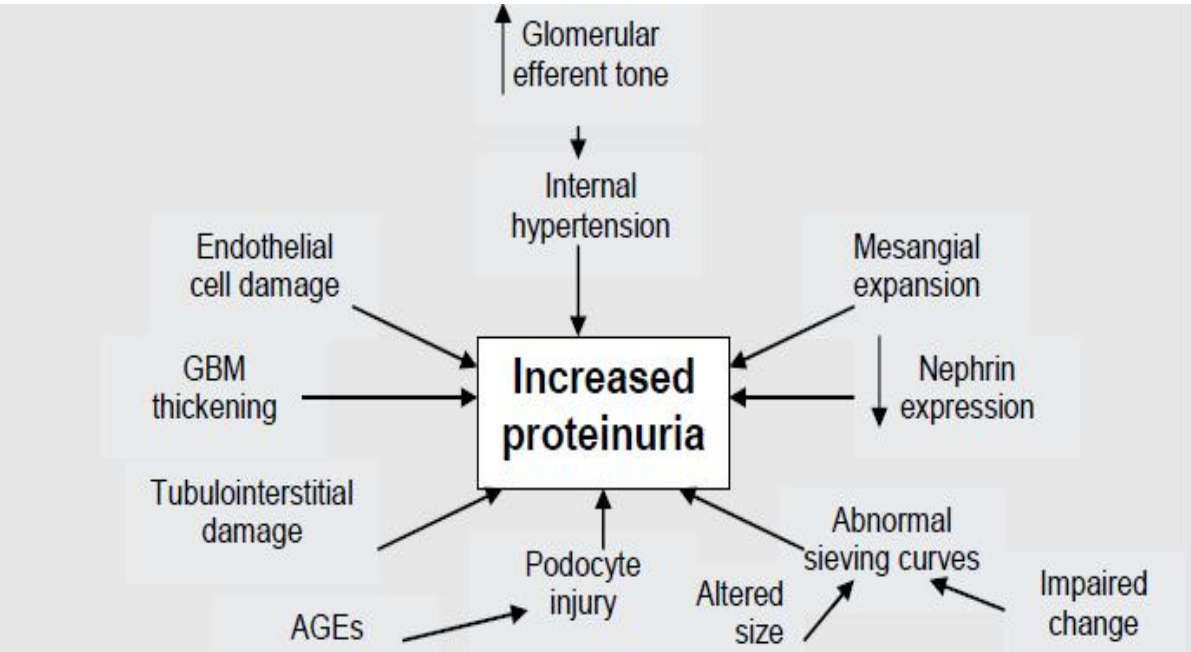


Figure 2.4 mechanisms of proteinuria in DN

AGEs: advanced glycation end-product

2.7.4 Microalbuminuria

Microalbuminuria is defined as the range in between urinary excretion of albumin 30 to 300 mg/24 hours (Keane et al., 1999). It can also be defined in terms of the urinary albumin to creatinine ratio. A ratio greater than 30 mg/g in a random spot collection is considered abnormal.

Screening for microalbuminuria should be performed after a semiquantitative dipstick test in type 2 diabetic urine sample for protein. If the dipstick test is negative, the presence of microalbuminuria should be measured. Microalbuminuria rarely occurs with short duration of type 1 diabetes; therefore, screening should begin after 5 years of the disease duration (ADA, 2002). Because of the difficulty in precise dating of the onset of type 2 diabetes, such screening should begin at the time of diagnosis.

2.8 Homocysteine

2.8.1 Definition and structure

Homocysteine is an amino acid with the formula HSCH2CH2CH(NH2)CO2H. It is a homologue of the amino acid cysteine, differing by an additional methylene (-CH2-) group. Homocysteine exists at neutral pH values as a zwitterion: Betatine form of (S)-Homocysteine and (R)-Homocysteine.

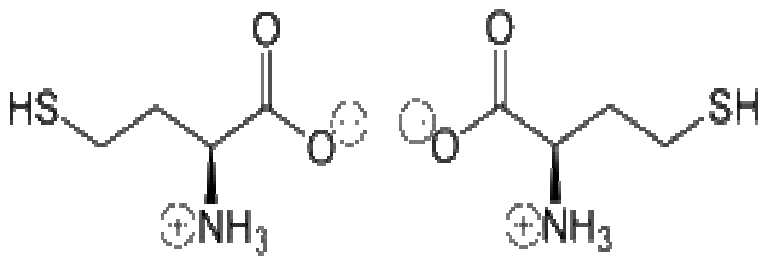


Figure 2.5 Structure of homocysteine. Betatine form of (S)-Homocysteine (left) and (R)-Homocysteine (right). Sited from Nelson et al (2000).

2.8.2 Biosynthesis of Homocysteine

Homocysteine is not obtained from the diet (Selhub,1999). Instead, it is biosynthesized from methionine via a multi-step process. First, methionine receives an adenosine group from ATP, a reaction catalyzed by S-adenosyl-methionine synthetase, to give S-adenosyl methionine (SAM). SAM then transfers the methyl group to an acceptor molecule, (i.e., norepinephrine as an acceptor during epinephrine synthesis, DNA methyltransferase as an intermediate acceptor in the process of DNA methylation). The adenosine is then hydrolyzed to yield L-homocysteine. L-Homocysteine has two primary fates: conversion via tetrahydrofolate (THF) back into L-methionine or conversion to L-cysteine (Champe et al., 2008).

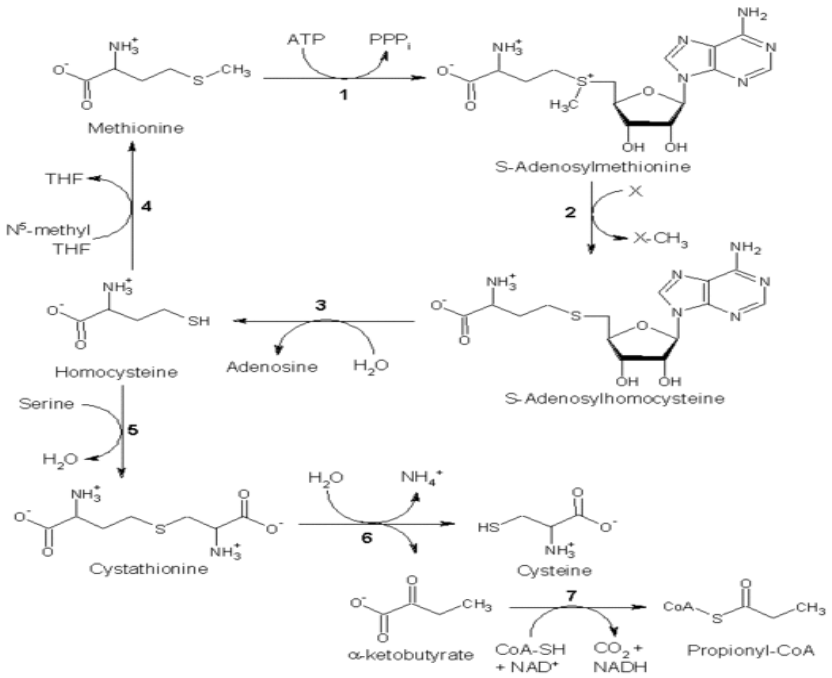


Figure 2.6 Biosynthesis of homocysteine. Sited from Champe et al. (2008).

2.8.3 Homocysteine species

Several homocysteine species have been identified in human plasma including albumin-(protein)-bound, free circulating disulfides and sulfhydryl forms (Maron & Loscalzo, 2007). However, total homocysteine plasma

concentrations are commonly reported in the literature as current analytical methodology involves the reduction of homocysteine disulfide bonds, quantifying all forms as free total homocysteine (Perla-Kajan et al., 2007 and Maron and Loscalzo, 2009).

2.8.4 Metabolism of homocysteine

The metabolism of homocysteine can be divided into three distinct pathways (Figure 2.7): (1) the remethylation of homocysteine to methionine by the vitamin B12 dependent methionine synthase; (2) the transsulfuration pathway, converting homocysteine to cystathionine and then cysteine via vitamin B6 dependent cystathionine β-synthase (CBS) enzyme; (3) in the liver and kidneys, homocysteine can be remethylated back to methionine by betaine-homocysteine methyltransferase (Maron and Loscalzo, 2009).

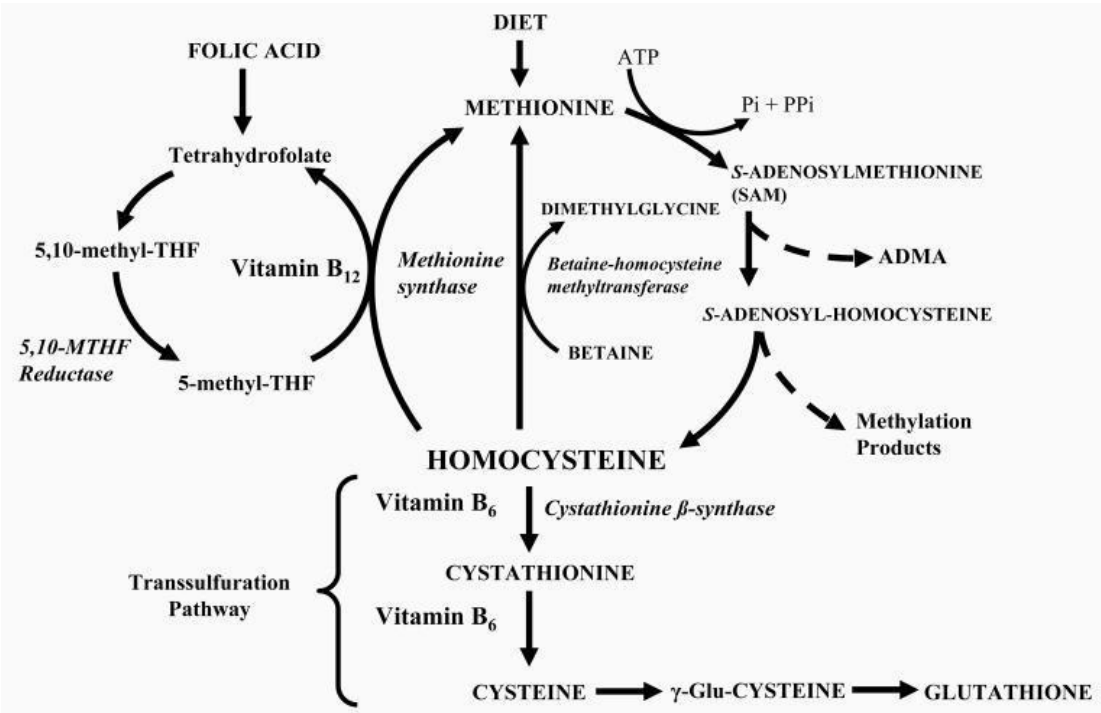


Figure 2.7 Homocysteine metabolic cycle. Sited from Maron and Loscalzo, (2009).

2.8.5 Homocysteine values

The American Heart Association released an advisory statement classifying total homocysteine plasma concentrations as follows: 5-15 µmol/l homocysteine as normal, 16-30 µmol/l homocysteine as moderate, 31-100 µmol/l homocysteine as intermediately elevated and total homocysteine levels above 100 µmol/l as severely elevated concentrations (Malinow et al., 1999).

2.8.6 Hyperhomocycteinemia

Hyperhomocysteinemia, is defined as total homocyteine concentrations elevated above 15 µmol/l. There are several indications that whole body homocysteine metabolism is altered in renal insufficiency (van Guldener, 2005). Hyperhomocysteinemia has been implicated in patients with diabetic nephropathy (Arnadottir et al., 2001; Sandhu et al., 2004; Li et al., 2007 and Cho et al., 2010). Hyperhomocysteinemia has also been associated with the pathogenesis of cardiovascular disease (Al-obaidi et al., 2001; Lubos et al., 2007 and coldea et al., 2011). Deficiencies of the vitamins folic acid (B9), pyridoxine (B6), or B12 (cyanocobalamin) can lead to hyperhomocysteinemia (Brosnan, 2004 and Abraham et al., 2010). Hyperhomocysteinemia also occur in the rare hereditary disease homocystinuria and in the methylenetetrahydrofolate reductase polymorphism genetic traits (Qi et al., 2003).

2.9 Homocysteine studies in diabetic nephropathy

Chico et al. (1998) measured fasting homocysteine concentrations in the plasma of 165 diabetic patients (75 with type 1 diabetes, 90 with Type 2 diabetes) and 56 non-diabetic control subjects. Other measurements included the prevalence of diabetic complications, glycaemic control, lipid and lipoprotein levels, vitamin status and renal function tests. Patients with type 2

diabetes had higher homocysteine levels than control subjects, whereas type 1 diabetic patients did not (9.2±4.5 vs 7.7±2 μmol/l, p<0.01; and 7.0±3 vs 7.4±2 μmol/l, p>0.05). Albumin excretion rate was the parameter with the strongest independent association with homocysteine. Patients with both types of diabetes and nephropathy had higher plasma homocysteine concentrations than those without nephropathy. Increases of homocysteine in plasma were related to increases in the severity of the nephropathy. Fasting hyperhomocysteinaemia was considered as the mean of the plasma homocysteine for all control subjects (7.5 ± 2.1 μmol/l) + 2 SD (cut-off =11.7 μmol/l). Nephropathy was present in 80% of diabetic patients with fasting hyperhomocysteinaemia.

The impacts of insulin resistance and renal function on plasma total homocysteine (tHcy) levels in 75 patients with type 2 diabetes with a wide range of nephropathy were assessed (Emoto et al., 2001). Fifty four healthy subjects were used as controls. Plasma tHcy levels and their log-transformed values (log tHcy) were significantly higher in all diabetic patients than in controls (tHcy, 12.0±0.7 vs. 8.7±0.3 μmol/l, P<0.0001; log tHcy, 1.040±0.021 vs. 0.920±0.016 mmol/l, P<0.0001). Plasma tHcy levels in diabetic patients were significantly increased according to degree of nephropathy (P<0.0001). On simple regression analyses, log tHcy correlated with insulin sensitivity indexes (r=–0.319, P=0.005) as well as creatinine clearance (r=0.634, P<0.0001) in all diabetic patients. Multiple regression analyses showed that insulin sensitivity indexes (β=–0.245) as well as creatinine clearance were independent contributors to log tHcy in all diabetic patients (R^2=0.750, P<0.0001). For the 59 diabetic patients with creatinine clearance>60 ml/min, insulin sensitivity indexes were also shown to be a significant contributor to log tHcy (β=–0.438, R^2=0.561, P<0.001).

Ximei et al. (2002) investigated the relationship between homocysteine and incipient diabetic nephropathy in 67 type 2 diabetic patients. Forty seven persons were used as controls. The plasma homocysteine level was higher in type 2 diabetic patients than that in controls (15.27±6.04 μmol/L vs 12.10±1.86 μmol/L, P<0.01), and the plasma homocysteine level was higher

in incipient diabetic nephropathy patients than that in non nephropathy patients (17.86±8.04 μmol/L vs 13.83±4.00 μmol/L, P<0.01). Diabetic patients with hyperhomocysteinmia had a higher diabetic nephropathy incidence rate (52.0% vs 26.2% P<0.05) compared with no hyperhomocysteinmia patients, and the former had higher plasma thrombomodulin level than the latter (P<0.05). Through Logistic regression analysis, it was concluded that homocysteine was related to the occurrence of incipient diabetic nephropathy. In addition, Looker et al., (2003) reported that incidence of nephropathy was associated with homocysteine concentrations: Incident rate ratio (IRR)=1.42 (95% CI, 1.09–1.84, p=0.01).

A prospective study on plasma homocysteine and endogenous insulin in 50 Indian type 2 diabetic patients with nephropathy was carried out (Sandhu et al., 2004). The results were compared with 25 diabetics without nephropathy, and 25 age and sex matched healthy controls. The mean values of plasma homocysteine were significantly elevated in diabetic nephropathy (21.3±7.2 mmol/L) and diabetics without nephropathy (19.4±7.1) when compared to healthy control (11.5±2.3). The insulin levels and BMI were significantly higher in diabetics as compared to controls. There was no correlation between homocysteine and insulin, homocysteine and BMI, and homocysteine with the degree of renal failure.

Li et al. (2006) explored the relationship between tHcy in plasma and the occurrence and clinical phasing of diabetic nephropathy in 76 Type 2 diabetic nephropathy patients. Fifty one healthy individuals were used as controls. The tHcy levels in diabetic patients was 11.85±7.79 μmol/L, significantly higher than that of the controls of 7.59±1.23μmol/L (P=0.001). While the level of tHcy in diabetic patients without complications was 8.66±2.66 μmol/L. The level of tHcy in diabetic patients at early stage was 13.48±3.36 μmol/L. The tHcy level in clinical diabetic patients was 25.50±12.41 μmol/L. The differences between each groups were significant (P=0.01). The authors concluded that tHcy can be used as an indicator in monitoring the occurrence and development of the diabetic nephropathy.

The relationship between glycated haemoglobin (HbA1c), hypertension and microalbuminuria onset in 200 type 2 diabetic patients was established. Also, the metabolic action of homocysteine on LDL fatty acids and on renal function was ascertained (Kassab et al., 2008). Creatinine and microalbuminuria significantly increased in type 2 diabetes when compared with controls. Microalbuminuria was significantly correlated with HbA1c and with the presence of high blood pressure. Homocysteinaemia significantly correlated with creatinine clearance in diabetes. Linoleic acid (C18:2ω6) did not differ between groups. C18:2ω6/C18:3ω3 ratio was three times higher in diabetics than in controls. Total saturated fatty acids, homocysteine, H2O2 and LDL-thiobarbituric reactive substances significantly increased in microalbuminuric when compared with normoalbuminuric diabetes. Total polyunsaturated fatty acids, arachidonic acid (C20:4ω6), LDL-cholesterol, apo B and creatinine clearance significantly decreased in microalbuminuric when compared with normoalbuminuric diabetes.

Cho et al. (2010) determined whether hyperhomocysteinemia can be a risk factor for the development of microalbuminuria in 887 type 2 diabetic patients who did not have microalbuminuria at baseline, 76 developed microalbuminuria during follow-up (36.0±11.7 months; range, 18-76 months). The control group consisted of 152 age- and sex-matched subjects who did not develop microalbuminuria. Baseline plasma homocysteine concentrations and mean HbA1C levels during follow-up were significantly higher in patients who developed microalbuminuria than in those who remained normoalbuminuric. Multivariate logistic regression analysis showed that baseline plasma homocysteine level and mean HbA1C were independent predictors of microalbuminuria in type 2 diabetes. The results support the concept that hyperhomocysteinemia has an etiologic role in the pathogenesis of diabetic nephropathy. In addition, Krajnc et al. (2011) observed significant difference in serum homocystiene between patients on haemodialysis and patients with type 2 diabetes.

The mutation in C677T which considered the Methylenetetrahydrofolate reductase (MTHFR) gene predisposed type 2 diabetes patients to the development of diabetic nephropathy. (Sharaf et al., 2012). MTHFR is a regulatory enzyme of homocysteine metabolism The T allele of this mutation presumably acting by elevating homocysteine levels and seems to be associated with a faster progression of nephropathy to end-stage renal failure. that

Chapter 3

Materials and Methods

3.1 Study design

This study is a case control design.

3.2 Study population

The study population comprised type 2 diabetic patients aged 40-65 years from hospitals and diabetic clinics in Gaza. Controls were non diabetic healthy individuals.

3.3 Sampling and sample size

Type 2 diabetic patients, previously diagnosed according to the WHO diagnostic criteria for diabetes (World Health Organization, 2006), were selected as cases from Al Shifa hospital, UNRWA and Al Rimal diabetic clinics in Gaza. These are the main diabetic centers which are representative to Gaza City. Healthy non diabetic individuals from Gaza City were served as controls. Cases and controls were matched with age and gender. The sample size was 120 diabetic patients distributed as follows:

* Group I: 40 normoalbuminurics (20 males and 20 females).

* Group II: 40 microalbuminurics (20 males and 20 females).

* Group III: 40 macroalbuminurics (20 males and 20 females).

The control group included 40 non diabetic healthy individuals (20 males and 20 females).

3.4 Selection criteria

3.4.1 Inclusion criteria

* Cases: Type 2 diabetic patients aged 40-65 years.

* Controls: Non diabetic healthy individuals aged 40-65 years.

3.4.2 Exclusion criteria (for cases and controls)

* Cases and controls aged <40 and >65 years.

* Blood pressure ≥140/90 mmHg

* Urinary tract infection

* Pregnant women and women under hormonal therapy.

* Patients with history of other diseases.

3.5 Ethical Consideration

The necessary approval to conduct the study was obtained from the local ethical committee in the Gaza Strip.

3.6 Data collection

3.6.1 Questionnaire interview

A meeting interview was used for filling in a questionnaire which designated for matching the study need (Annex 1). All interviews were conducted face to face by the researcher himself. The questionnaire was based on diabetic clinics questions, and on that used in similar studies with some modifications (Al Rimal Medical Center, 2006, Altawil, 2009 and Abu sunyma, 2012). During the study the interviewer explained to the participants any of the confused questions that were not clear to them. Most questions were the yes/no questions, which offer a dichotomous Choice (Backestrom and Hursh-Cesar, 1981). The validity of the questionnaire was tested by six specialists in the fields of epidemiology, public health, endocrinology and nutrition. The questionnaire was piloted with 10 patients. The questionnaire includes

questions on the personal profile of the study population (age, gender and education), socioeconomic data (employment and family history of diabetes), diet, clinical data (duration of diabetic nephropathy and the most common self-reported complications of diabetes) and food intake.

3.6.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg/height in meter square. Patients and controls were asked to remove heavy clothes and shoes before measurement of weight and height. Medical balance (Seca model 762, Germany) was used for weight measurement. People with BMI=18.5–24.9 was considered to have normal weight, people with BMI=25.0–29.9 was classified overweight, people with BMI≥30.0 was considered obese (WHO, 2000).

3.6.3 Urine analysis

Random urine samples were collected from both diabetic patients and non diabetic healthy controls for urine analysis. The urine samples were centrifuged at room temperature at 4000 rpm/10 minutes to precipitate all the debris. About 0.5 ml of urine was transferred to the autoanalyzer (Konelab 60 Chemistry Autoanalyzer, Finland) for the detection of microalbuminuria, and urine protein. Then, patients were classified accordingly to three groups as follows:

* Group I: 40 normoalbuminurics (urinary albumin <30 mg/g)
* Group II: 40 microalbuminurics (urinary albumin 30-300 mg/g)
* Group III: 40 macroalbuminurics (urinary albumin >300 mg/g)

Urine samples were also collected from 40 non diabetic healthy controls (control group) and analyzed for microalbuminurea and urine protein.

3.6.4 Blood collection and biochemical analysis

Twelve hours fasting overnight venous blood samples were collected from 120 type 2 diabetic patients and 40 healthy non diabetic controls. Blood samples (6 ml each) were drawn by the researcher himself into plastic tubes

from each control and diabetic patient. About 2 ml blood was placed into EDTA tube to analyze HbA1c for controls and cases. The remainder quantity of blood (4 ml) was left for a while without anticoagulant to allow blood to clot. Then, serum samples were obtained by centrifugation at room temperature at 4000 rpm/10 minutes. Serum homocysteine, glucose, urea, creatinine, cholesterol, triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol were determined. In addition, the whole blood HbA1c was measured.

3.7 Biochemical analysis

3.7.1 Determination of urine microalbuminuria

Microalbuminuria was determined by Immunoturbidometry-Latex method (Harmoinen et al., 1985) using BioSystems kit, Spain.

Principle

Albumin in the urine sample causes agglutination of the latex particles coated with anti-human albumin. The increase of the particles agglutination is proportional to the albumin concentration and can be measured immunoturbidometrically.

Reagents

Reagent	Component	Concentration
Reagent 1	Borate buffer sodium azide pH	0.1 mol/L 0.95 g/L 10.0
Reagent 2	Suspension of latex particles coated with anti-human albumin antibodies Sodium azide	0.95 g/L
Albumin	Human albumin	47mg/L

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	200
Serum volume (µl)	2
Calibrator 1 (mg/L)	0.0
Calibrator 2 Alb (mg/L)	47
Incubation time (s)	130
Wavelength (nm)	540
Calibrator type	Linear
Measurement Type	End point

3.7.2 Determination of urine protein

Urine protein was determined by Pyrogallol Red method (Watanabe et al., 1986) using BioSystems kit, Spain.

Principle

Protein in the urine sample reacts with pyrogallol red and molybdate in acid medium forming a colored complex which can be measured photometrically.

Reagents

Reagent	Component	Concentration
Reagent 1	Pyrogallol red Sodium molybdate Succinate pH Detergent	60 µmol/L 40 µmol/L 50 µmol/L 2.3
Urine Protein Standard	Bovine albumin	1081 mg/L

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

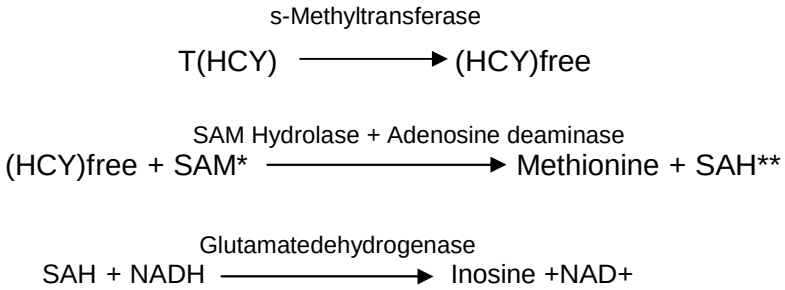
Parameter	Value
Reagent volume (µl)	170
Serum volume (µl)	3
Calibrator 1 (mg/L)	0.0
Calibrator 2 Alb (mg/L)	1081
Incubation time (s)	600
Wavelength (nm)	600
Calibrator type	Linear
Measurement Type	End point

3.7.3 Determination of serum homocysteine

Serum homocysteine was determined by enzymatic colorimetric method for the quantitative determination of homocysteine (Refsum, 2002), using Globe diagnostics kit, Italy.

Principle

The Globe Diagnostics Enzymatic Test for the quantitative homocysteine determination (HCY) is based on a series of enzymatic reactions causing a decrease in absorbance value due to NADH oxidation to NAD+. HCY concentration in the sample is directly proportional to the quantity of NADH converted to NAD+ (ΔA 340nm). The enzymatic reactions are the following:



* SAM = S-Adenosyl-methionine

** SAH = S-Adenosyl-homocysteine

Reagents

Reagent	Concentration
Reagent A:	
S-adenosylmethionine	0.1 mmol/l
NADH*	0.2 mmol/l
TCEP	0.5 mmol/l
2-oxoglutarate	5.0 mmol/l
Reagent B:	
Glutamate dehydrogenase	10 KU/l
SAH hydrolase	3.0 KU/l
Adenosyne deaminase	5.0 KU/l
HCY** methyltransferase	5.0 KU/l

*Nicotinamide Adenine Dinucleotide (NADH)

**Homocysteine (HCY)

Analytical procedure

About 0.5 ml of serum was transferred to the Mindray BS-300 chemistry auto analyzer to perform the test according to these parameters:

Parameter	Value
Reagent 1 (µl) Reagent 2 (µl)	180 47
Serum (µl)	9
Incubation period (s)	25 cycles (5 minutes)
Reaction type	Fixed time
Wavelength (nm)	340
Reaction	Descending

Calculation of results

The ΔA (A2 - A1) calculated for blank and each calibrator against its concentration (concentrations are reported on the calibrator vial label).Results was found by comparing the sample ΔA against the plotted curve. A curve fitting system software was suggested to achieve more precise results.

Reference value

National Institute of Standards and Technology (NIST) standardized study shows 15 µmol/l as the cut-off value for normal level of homocysteine for adults.

Standarization	NIST
Adult normal cut-off	≤ 15 µmol/l

3.7.4 Determination of serum glucose

Serum glucose was determined by glucose oxidase (GOD)/glucose peroxidase (POD) method (Trinder,1969) using Labkit Kit, Spain.

Principle

Determination of glucose after enzymatic oxidation by glucose oxidase. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase.

$$\text{Glucose} + O_2 \xrightarrow{GO} \text{Gluconic acid} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{POD} \text{Quinoneimine} + 4\ H_2O$$

Reagents

Reagent	**Concentration**
Phosphate buffer (pH 7.5) Phenol 4-Aminoantipyrine Glucose oxidase (GOD) Peroxidase (POD)	250 mmol/l 5 mmol/l 0.5 mmol/l ≥ 15 ku/l ≥ 1 ku/l
Standard	100 mg/dl

Assay procedure

Wavelength: 500 nm

Optical path: 1 cm

Temperature: 37 °C

Measurement: Against reagent blank.

- Ten µl of standard (sample or control) was added to 1 ml of the reagent and mixed well.
- The mixture was incubated for 10 min at 37 ºC.
- The absorbance was measured within 60 min.

Calculation

$$\text{Glucose [mg / dl]} = \frac{\Delta A \text{ sample} \times \text{concentration of standard}}{\Delta A \text{ standard}}$$

Reference value (fasting glucose)

(Palestinian Clinical Laboratory Tests Guide, PCLTG, 2005)

Child	60 – 100 mg/dl
Adult	70 – 110 mg/dl

3.7.5 Determination of Glycohemoglobin (HbA1c) in whole blood

HbA1c was determined by the colorimetric determination of glycohemoglobin in whole blood (Trivelli et al., 1971) using Stanbio Kit, Texas-USA.

Principle

A preparation of hemolyzed whole blood is mixed with a weakly binding cation exchange resin. The non-glycosylated hemoglobin HbA0 binds to the resin, leaving HbA1 free to be removed by means of a resin separator in the supernate. The percent of HbA1 is determined by measuring the absorbance values at 415 nm of the HbA1 fraction and of the total hemoglobin fraction, calculating the ratio of absorbances (R), and comparing this ratio to that of a glycohemoglobin standard carried through the same procedure. Results are express as HbA, but can be converted or derived as HbA1c by using a conversion factor or when using HbA1c value for the standard.

Reagents

Glycohemoglobin lon Exchange Resin. Each tube contains 3.0 mL cation exchange resin 8 mg/dL. pH 6.9
Glycohemoglobin Lysing Reagent Contains potassium cyanide 10 mmol/L and surfactants.
Glycohemoglobin Standard (Lyophilized) (1 vial) Prepared from packed human erythrocytes.

Procedure

Hemolysate Preparation

1. Pipette 0.5 mL (500 µl) Lysing reagent into tubes labeled Standard (S), Unknown (U) and Control (C).
2. Pipette 0.1mL (100 µl) of each well-mixed blood sample into appropriately labeled tube and mix.
3. Allow to stand for 5 minutes at room temperature (15-30°C) to complete hemolysis.

Glycohemoglobin separation and assay

1. Label resin tubes Standard (S), Unknown (U) and Control (C).
2. Pipette 0.1 mL (100 µl) of the prepared hemolysate into appropriately labeled resin tube.
3. Position a resin separator in the tube so rubber sleeve is approximately 1-2 cm above liquid level.
4. Mix tubes on a hematology rocker for 5 minutes. Alternatively tubes may be mixed by hand if held above the resin.
5. At the end of the 5 minute mixing, push resin separator into tube until resin is firmly packed in bottom of the 13mm tube.
6. Pour each supernate directly into separate cuvettes for absorbance measurements.
7. Read absorbance (Agly) of Standard, Unknown and Control vs. water at 415 nm within 60 minutes.

Total hemoglobin assay

1. Pipette 5.0 mL deionized water into tubes labeled Standard (S), Unknown (U) and Control (C).
2. Pipette 20 µl of hemolysate into appropriately labeled tube. Mix well and transfer to cuvette for absorbance reading.
3. Read absorbance (Atot) of Standard, Unknown and Control vs. water at 415 nm within 60 minutes.

Calculation

For each Standard and Unknown calculate the ratio (R) of the glycohemoglobin absorbance to the hemoglobin absorbance as follows:

$$(R) = Agly / Atot$$

$$\text{Hemoglobin (\%)} = \frac{\text{(R) Unknown x Hemoglobin Standard (\%)}}{\text{(R) Standard}}$$

Results may also be reported as HbA1c when compared to the reference A1c method, the Stanbio method showed a 98% correlation with an equation of:

$$Y \text{ (A1c value)} = 0.838 \times \text{(Stanbio value)} - 0.732$$

The value obtained by the Stanbio method may be converted to Calculated A1c value by use of this formula. For a direct calculated A1c value, the value of the standard may be changed to 7.6% in lieu of the 10.0% and the results will be A1c values.

3.7.6 Determination of serum urea

Principle

Serum urea was determined by using "Urease-GLDH": enzymatic UV test, according to Thomas method (Thomas, 1998) using DiaSys reagent kits.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + 2HCO$$

$$\text{2-Oxoglutarate} + NH_4 + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

Reagents

Concentrations are those in the final test mixture.

Reagent	**Concentration**
R1: TRIS	120 mmol/l
2- Oxoglutarate	7 mmol/l
ADP	0.6 mmol/l
Urease	≥ 0.6 ku/l
GLDH	≥ 1 ku/l
R2: NADH	0.25 mmol/l
Standard	50 mg/dl

Assay procedure

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 340 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against distilled water.

- Ten µl of standard (sample or control) was added to 1 ml of working reagent and mixed well.
- The mixture was incubated for 30 sec then absorbance (A1) was recorded.
- After exactly further 60 sec the absorbance (A2) was measured.

Calculation

ΔA = (A1 –A2) sample or standard

$$\text{Urea (mg/dl)} = \frac{\Delta A \text{ sample X concentration of standard}}{\Delta A \text{ standard}}$$

Reference value

(PCLTG, 2005)

Child	5 - 30 mg/dl
Adult	13 - 43 mg/dl

3.7.7 Determination of serum creatinine

Serum creatinine was determined by using kinetic test without deproteinization according to Newman and Price method (Newman and Price, 1999) using DiaSys reagent kits.

Principle

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid $\longrightarrow$ creatinine picrate complex

Reagents

Concentrations are those in the final test mixture.

Reagent	Concentration
R1: Sodium hydroxide (pH approx. 13)	0.16 mol/l
R2: Picric acid (pH approx. 1.2)	4.0 mmol/l
Standard	2.0 mg/dl

Assay procedure

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 490 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against distilled water.

- Fifty µl of standard (sample or control) was added to 1 ml of working reagent add and mixed well.
- The Mixture was incubated for 60 sec then absorbance (A1) was recorded.
- After exactly further 120 sec the absorbance (A2) was measured.

Calculation

ΔA = (A1 – A2) sample or standard

$$\text{Creatinine (mg/dl)} = \frac{\Delta A \text{ sample X concentration of standard}}{\Delta A \text{ standard}}$$

Reference value (in serum) (PCLTG, 2005).

Infant	0.2 – 0.4 mg/dl
Child	0.3 - 0.7 mg/dl
Adolescent	0.5 - 1.0 mg/dl
Adult: Male Female	0.6 - 1.2 mg/dl 0.5 -1.1 mg/dl

3.7.8 Determination of serum cholesterol

Serum cholesterol was determined by cholesterol oxidase (COD)/POD method (Meiatlini et al., 1978) using BioSystems kit, Spain.

Principle

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured photometrically.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + 1/2\ O_2 + H_2O \xrightarrow{\text{COD}} \text{Cholestenone} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\ H_2O$$

Reagents

Concentrations are those in the final test mixture.

Reagent	Components	Concentration
Reagent 1	Pipes	35 mmol/L
	Sodium cholate	0.5 mmol/L
	Phenol	28 mmol/L
	Cholesterol esterase	> 0. 2 U/mL
	COD	> 0.1 U/mL
	POD	> 0.8 U/mL
	4-ammoantipyrine	0.5 mmol/L
	pH	7.0

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2
Calibrator 1(mg/dl)	0.0
Calibrator 2 CALS(mg/dl)	236
Incubation time (s)	240
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	End point

3.7.9 Determination of serum triglycerides

Serum triglyceride was determined by Glycerol phosphate oxidase/peroxidase method (Bucolo and David, 1973) using BioSystems kit, Spain.

Principle

Triglycerides in the sample onginates, by means of the coupled reactions described below colored complex that can be measured photometrically

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyaceton phosphate} + H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine} + \text{4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCl + 4H_2O$$

Reagents

Reagent	Components	Concentration
Reagent 1	Pipes	45 mmol/L
	Magnesium chloride	5 mmol/L
	4-chlorophenol	6 mmol/L
	Lipase	> 100 U/mL
	Glycerol kinase	> 1.5 U/mL
	Glycerol-3-phosphate oxidase	> 4 U/mL
	Peroxidase	> 0.8 U/mL
	A-aminoantipyrine	0.75 mmol/L
	ATP	0.9 mmol/L
	pH	7.0

Procedure

About 0.5 ml of serum was transferred to the Konelab 60 Chemistry Autoanalyzer, to perform the test according to these parameters:

Parameter	Value
Reagent volume (µl)	140
Serum volume (µl)	2
Calibrator 1(mg/dl)	0.0
Calibrator 2 CALS(mg/dl)	157
Incubation time (s)	240
Wavelength (nm)	510
Calibrator type	Linear
Measurement Type	End point

3.7.10 Determination of serum high density lipoprotein cholesterol (HDL-C)

HDL-C was determined by precipitating method (Grove, 1979) using Labkit kit, Spain.

Principle

The VLDL and LDL-C from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant is containing high density lipoproteins (HDL-C) and used for the determination of it.

Reagents

Reagent	Concentration
Monoreagent contain: Magnesium chloride Phosphotungstic acid	2 mmol/l 14 mmol/l

Procedure

1. Pipette into a centrifuge tube 25 µl of HDL-C reagent and 250 µl serum. Mix well. Allow to stand for 10 minutes at room temperature.

2. Centrifuge at 4000 rpm for 10 minutes. Collect the supernatant and test HDL-C.

3. Pipette into centrifuge tube 1 ml cholesterol reagent and 10 µl of the supernatant.

Mix well. Allow to stand for 10 minutes at room temperature.

4. Set the Unicam spectrophotometer, United Kingdom, at 505 nm and adjust it to zero with blank reagent. Read the Absorbance (A) of the test, and standard against reagent blank.

Calculation:

$$\text{HDL Concentration} = \frac{\text{(A) Test} \times \text{(C) Standard}}{\text{(A) Standard}}$$

3.7.11 Determination of serum low density lipoproteins cholesterol (LDL-C)

LDL-C can be calculated using the empirical relationship of (Friedewald et al., 1972)

Principle

The ultracentrifugal measurement of LDL-C is time consuming and expensive and requires special equipment. For this reason, LDL-C is most commonly estimated from quantitative measurements of total and HDL-C and plasma triglycerides (TG) using the empirical relationship of Friedewald.

The Equation

$$\text{LDL-C} = \text{Total Cholesterol} - \text{HDL-C} - \text{TG}/5$$

3.8 Statistical analysis

Data were computer analyzed using SPSS/ PC (Statistical Package for the Social Science Inc. Chicago, Illinois USA, version 18.0) statistical package.

- Simple distribution of the study variables and the cross tabulation were applied.
- Chi-square (χ^2) was used to identify the significance of the relations, associations, and interactions among various variables. Yates's continuity correction test, $\chi^2_{(corrected)}$, was used when not more than 20% of the cells had an expected frequency of less than five and when the expected numbers were small.
- Analysis of variance (ANOVA) was applied.
- The independent sample t-test procedure was used.
- Pearson's correlation test was applied.
- The results in all the above mentioned procedures were accepted as statistical significant when the p-value was less than 5% ($p<0.05$).
- Range as minimum and maximum values were used.
- Microsoft Excel program version 11.0 was used for correlation graph plotting.

Chapter 4

Results

The present study is a case control design comprised 120 type 2 diabetic patients classified into 3 groups, each group contains 20 males and 20 females as follows: group I included 40 normoalbuminuric patients (urinary albumin <30 mg/g), group II included 40 microalbuminuric patients (urinary albumin=30-300 mg/g) and group III included 40 macroalbuminuric patients (urinary albumin >300 mg/g). Control group also contained 40 healthy individuals (20 males and 20 females).

4.1 Personal profile of the study population

Table 4.1 summarizes personal profile of the study population. The mean ages of control group, groups I, II and III were 56.9±6.2, 57.7±7.1, 57.8±5.4 and 58.5±6.3 years. The ANOVA test showed no significant difference between mean ages of control and patients groups (F=0.263, P=0.852). Analysis of the educational status of the study population indicated that diabetic nephropathy is more prevalent among less educated individuals ($\chi^2_{(corrected)}$=21.756, P=0.041).

Table 4.1 Personal profile of the study population

Personal character	Control group (n=40)	Diabetic nephropathy			test		P-value
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)			
	No. (%)	No. (%)	No. (%)	No. (%)			
Gender							
Male	20 (50.0)	20 (50.0)	20 (50.0)	20 (50.0)	χ^2	0.075	1.000
Female	20 (50.0)	20 (50.0)	20 (50.0)	20 (50.0)			
Mean age± SD *(min-max)*	56.9±6.2 *(44-65)*	57.7±7.1 *(40-65)*	57.8±5.4 *(49-65)*	58.5±6.3 *(42-65)*	F	0.263	0.852
Education							
University	8 (20.0)	0 (0.0)	4 (10.0)	0 (0.0)			
Secondary school	14 (35.0)	12 (30.0)	6 (15.0)	6 (15.0)			
Preparatory school	6 (15.0)	10 (25.0)	8 (20.0)	16 (40.0)	χ^2	21.756	0.041*
Primary school	7 (17.5)	12 (30.0)	12 (30.0)	10 (25.0)			
Illiterate	5 (12.5)	6 (15.0)	10 (25.0)	8 (20.0)			

*P-value of $\chi^2_{(corrected)}$ test. P>0.05: not significant, P<0.05: significant.

4.2 Socioeconomic data of the study population

Socioeconomic data of the study population is provided in Table 4.2. The employed controls and patient groups I, II and III were 23 (57.5%), 11 (27.5%), 10 (25.0%) and 8 (20.0 %), compared to their unemployed counterparts of 17 (42.5%), 29 (72.5%), 30 (75.0%) and 32 (80.0%). The difference among control and patients groups was significant (χ^2=15.726, P=0.001) with more frequent diabetic nephropathy among unemployed individuals. Regarding family history, significant difference was also recorded between various groups with diabetic nephropathy more prevalent among individuals with family history of diabetes (χ^2=18.912, P=0.000).

Table 4.2 Socioeconomic data of the study population

Character	Control group (n=40)	Diabetic nephropathy			test		P-value
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)			
	No. (%)	No. (%)	No. (%)	No. (%)			
Employment							
Yes	23 (57.5)	11 (27.5)	10 (25.0)	8 (20.0)	χ^2	15.726	0.001
No	17 (42.5)	29 (72.5)	30 (75.0)	32 (80.0)			
Family history of diabetes							
Yes	10 (25.0)	19 (47.5)	28 (70.0)	25 (62.5)	χ^2	18.912	0.000
No	30 (75.0)	21 (52.5)	12 (30.0)	15 (37.5)			

P<0.05: significant.

4.3 Diet among the study population

Table 4.3 shows diet among the study population. Although the percentages of controls and groups I, II and III who were on diet (7.5%, 25.0%, 10.0% and 25.0% respectively), were lower than those who were not (92.5%, 75.0%, 90.0% and 75.0% respectively), the difference between two groups was not significant ($\chi^2_{(corrected)}$=5.480, P=0.140).

Table 4.3 Diet among the study population

Character	Control group (n=40)	Diabetic nephropathy			test		P-value*
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)			
	No. (%)	No. (%)	No. (%)	No. (%)			
Diet							
Yes	3 (7.5)	10 (25.0)	4 (10.0)	10 (25.0)	χ^2	5.480	0.140
No	37 (92.5)	30 (75.0)	36 (90.0)	30 (75.0)			

*P-value of $\chi^2_{(corrected)}$ test. P>0.05: not significant.

4.4 Self-reported complications among the study population

The main self-reported complications among the study population are illustrated in Table 4.4. In general, the prevalence of self-reported complications is significantly increased with the development of diabetic nephropathy. (Retinopathy: χ^2=22.828, P=0.000; CVD: $\chi^2_{(corrected)}$=8.067, P=0.045; Neuropathy: $\chi^2_{(corrected)}$=20.104, P=0.000).

Table 4.4 the main self-reported complications among the study population

Complication	Control group (n=40)	Diabetic nephropathy			χ^2	P-value
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)		
	No. (%)	No. (%)	No. (%)	No. (%)		
Retinopathy						
Yes	6 (15.0)	22 (55.0)	20 (50.0)	26 (65.0)	22.828	0.000
No	34 (85.0)	18 (45.0)	20 (50.0)	14 (35.0)		
CVD**						
Yes	2 (5.0)	8 (20.0)	12 (70.0)	12 (62.5)	8.067	0.045*
No	38 (95.0)	32 (52.5)	28 (30.0)	28 (37.5)		
Neuropathy						
Yes	4 (10.0)	22 (55.0)	16 (40.0)	22 (55.0)	20.104	0.000*
No	36(90.0)	18 (45.0)	24 (60.0)	18 (45.0)		

*P-value of $\chi^2_{(corrected)}$ test. P<0.05: significant. **CVD: Cardiovascular disease.

4.5 Distribution of diabetic patients by diabetes duration

Table 4.5 presents distribution of diabetic patients by diabetes duration. Patients with diabetes since less than 5 years were 30 (25.0%), whereas those with diabetic duration of 5-10 years were 34 (28.3%). The rest of patients 56 (46.7%) had diabetes for more than 10 years.

Table 4.5 distribution of diabetic patients (n=120) by diabetes duration

Duration of diabetes (Year)	No.	%
< 5	30	25.0
5-10	34	28.3
>10	56	46.7

4.6 Self-reported complications in relation to duration of diabetes

The main self-reported complications in relation to duration of diabetes are illustrated in Table 4.6. In general, the longer duration of diabetes mellitus, the higher the percentage of retinopathy, cardiovascular disease and neuropathy among patients. This positive relationship was statically significant (χ^2=7.957, P=0.019; $\chi^2_{(corrected)}$=7.016, P=0.030 and χ^2=6.432, P=0.040, respectively).

Table 4.6 the main self-reported complications and their relation to duration of diabetes

Complication	Duration of diabetes (Year)			χ^2	*P-Value* *
	< 5 (n=30) **n (%)**	**5-10** (n=34) **n (%)**	**> 10** (n=56) **n (%)**		
Retinopathy					
Yes	13 (43.3)	14 (41.1)	38 (67.8)	7.957	0.019
No	17 (56.7)	20 (58.9)	18 (32.2)		
CVD**					
Yes	4 (13.3)	6 (17.6)	22 (39.2)	7.016	0.030*
No	26 (86.7)	28 (82.4)	34 (60.8)		
Neuropathy					
Yes	8 (26.6)	18 (52.9)	30 (53.5)	6.432	0.040
No	22 (73.4)	16 (47.1)	26 (46.5)		

* P- value for chi – Square Test, CVD**: Cardiovascular diseases

4.7 Meat and fish intake among study population.

The numbers of patients in groups I, II and III who eat meat daily 4 (10.0%), 2 (5.0%) and 2 (5.0%) were higher than controls 1 (2.5%). The difference between groups was significant ($\chi^2_{(corrected)}$ =19.387, P=0.022). However, the difference between control and diabetic groups in term of eating fish was not significant ($\chi^2_{(corrected)}$ =11.765, P=0.067).

Table 4.7 food intake of the study population

Food intake	Control group (n=40)	Group I Normo-albuminuria (n=40)	Diabetic nephropathy Group II Micro-albuminuria (n=40)	Diabetic nephropathy Group III Macro-albuminuria (n=40)	χ^2	P-value*
	No. (%)	No. (%)	No. (%)	No. (%)		
Meat						
Daily	1 (2.5)	4 (10.0)	2 (5.0)	2 (5.0)		
Twice/week	8 (20.0)	14 (35.0)	8 (20.0)	10 (25.0)	19.387	0.022
Once/week	24 (60.0)	22 (55.0)	30 (75.0)	28 (70.0)		
None	7 (17.5)	0 (0.0)	0 (0.0)	0 (0.0)		
Fish						
Daily	0 (0.0)	0 (0.0)	0(0.0)	0 (0.0)		
Twice/week	9 (22.5)	22 (55.0)	16 (40.0)	14 (35.0)	11.765	0.067
Once/week	29 (72.5)	14 (35.0)	24 (60.0)	24 (60.0)		
None	2 (5.0)	4 (10.0)	0 (0.0)	2 (5.0)		

*P-value of $\chi^2_{(corrected)}$ test. P<0.05: significant**.** P>0.05: not significant**.**

4.8 Anthropometric measurements of the study population

Table 4.8 shows anthropometric measurements of the study population. The means of BMI for controls, groups I, II and III were 25.6±3.7, 31.3±7.0, 29.1±3.5 and 28.7±5.0 kg/m2, respectively. The ANOVA test showed significant difference in BMI among different groups (F=7. 351, P=0.000).

Table 4.8 anthropometric measurements of the study population

Anthropometric measurement	Control group (n=40)	Diabetic nephropathy			F	P-value
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)		
	mean±SD	mean±SD	mean±SD	mean±SD		
Weight (kg)* *(min-max)*	75.8±13.8 *(55-100)*	87.2±21.2 *(47-150)*	82.7±9.5 *(70-106)*	82.1±18.0 *(55-145)*	2.697	0.050
Height (m) ** *(min-max)*	1.71±0.11 *(1.52-1.92)*	1.66±0.10 *(1.50-1.85)*	1.69±0.06 *(1.50-1.78)*	1.69±0.09 *(1.58-1.88)*	1.179	0.322
BMI*** *(min-max)*	25.6±3.7 *(18.5-34.7)*	31.3±7.0 *(18.8-49.0)*	29.1±3.5 *(23.7-36.7)*	28.7±5.0 *(19.0-41.0)*	7.351	0.000

*Kg: kilogram, ** m: meter. ***BMI: Body mass index (Kg/m^2): People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight, people with BMI≥30.0 were considered obese (WHO, 2000). All values are expressed as mean±SD. P>0.05: not significant, P<0.05: significant

4.9 Urinary albumin concentration of the study population

Urinary albumin concentration of the study population is presented in Table 4.9. There is a marked increase in the mean albumin concentration with the development of diabetic nephropathy (22.3±5.2, 146.7±80.7 and 348±37.6 mg/g in groups I, II and III, respectively) compared to control group (15.9±4.8 mg/g). The change among different groups was statically significant (F=220.2, P=0.000).

Table 4.9 urinary albumin concentrations of the study population.

Urine parameter (mg/g)	Control group (n=40)	Diabetic nephropathy			F	P-value
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)		
	mean±SD	mean±SD	mean±SD	mean±SD		
Albumin *(min-max)*	15.9±4.8 *(6-21)*	22.3±5.2 *(11-29)*	146.7±80.7 *(34-268)*	348±37.6 *(304-417)*	220.2	0.000

P<0.05: significant.

4.10 Serum homocysteine level of the study population

Table 4.10 demonstrates the mean levels of serum homocysteine of the study population. Serum homocysteine was progressively increased showing mean levels of 13.4±3.7, 17.1±4.8, 20.3±5.9 and 22.9±5.5 µmol/l in controls, groups I, II and III, respectively. The ANOVA test showed significant difference in the means level of homocysteine among different groups (F=21.563 and P=0.000).

Table 4.10 serum homocysteine levels of the study population.

Parameter (µmol/l)	Control group (n=40)	Diabetic nephropathy Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)	F	P-value
	mean±SD	mean±SD	mean±SD	mean±SD		
Homocysteine	13.4±3.7	17.1±4.8	20.3±5.9	22.9±5.5	21.563	0.000
(min-max)	*(7-20)*	*(11-27)*	*(12-38)*	*(16-33)*		

P>0.05: not significant, P<0.05: significant.

4.11 Serum glucose and blood HbA1c levels of the study population

As indicated in Table 4.11, the mean levels of serum glucose in groups I, II and III are gradually increased with the development of diabetic nephropathy (225.6±51.1, 251.3±104.3 and 288.7±176.2 mg/dl, respectively) compared to control group (109.7±14.8 mg/dl). Such change in glucose level among different groups was statically significant (F=21.530, P=0.000). In parallel, diabetic patients in groups I, II and III showed an increasing level of HbA1c (7.6±1.1, 8.6±1.3, 8.8±1.4, respectively) whereas HbA1c in control group was 4.7±0.6, with significant difference among various groups (F=88.666, P=0.000).

Table 4.11 Serum glucose and blood HbA1c levels of the study population

Parameter	Control group (n=40)	Diabetic nephropathy			F	P-value
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)		
	mean±SD	mean±SD	mean±SD	mean±SD		
Glucose (mg/dl) *(min-max)*	109.7±14.8 *(70-148)*	225.6±51.1 *(146-348)*	251.3±104.3 *(100-461)*	288.7±176.2 *(72-754)*	21.530	0.000
HbA1c (%) *(min-max)*	4.7±0.6 *(3.8-6.0)*	7.6±1.1 *(6.0-9.0)*	8.6±1.3 *(7.0-12.0)*	8.8±1.4 *(7.0-11.0)*	88.666	0.000

P<0.05: significant. Reference ranges of glucose and HbA1c are 70-115 mg/dl and 4%-6%, respectively.

4.12 Serum urea and creatinine concentrations of the study population

Table 4.12 present serum urea and creatinine concentrations of the study population. There was a progressive increase in urea concentration with the development of diabetic nephropathy in groups I, II and III (48.7±38.1, 54.6±40.0 and 72.1±62.6 mg/dl, respectively) with respect to control group (26.0±7.5 mg/dl). This increase among different groups was statically significant (F=7.888, P=0.000). Similarly, creatinine concentration was significantly increased recording values of 0.72±0.16, 0.89±0.62, 1.23±1.25 and 1.68±1.87 mg/dl in controls, group I, II and III, respectively (F=4.347, P=0.006).

Table 4.12 serum urea and creatinine concentrations of the study population

Parameter (mg/dl)	Control group (n=40)	Diabetic nephropathy			F	P-value
		Group I Normo-albuminuria (n=40)	Group II Micro-albuminuria (n=40)	Group III Macro-albuminuria (n=40)		
	mean±SD	mean±SD	mean±SD	mean±SD		
Urea *(min-max)*	26.0±7.5 *(15-50)*	48.7±38.1 *(18-203)*	54.6±40.0 *(25-189)*	72.1±62.6 *(18-210)*	7.888	0.000
Creatinine *(min-max)*	0.72±0.16 *(0.45-1.10)*	0.89±0.62 *(0.42-3.38)*	1.23±1.25 *(0.48-6.38)*	1.68±1.87 *(0.6-7.4)*	4.347	0.006

P<0.05: significant.

4.13 Serum lipid profile of the study population

Table 4.13 demonstrates serum lipid profile including cholesterol, triglycerides, HDL-C and LDL-C of the study population. There were significant increases in the means level of cholesterol, triglycerides and LDL-C showing mean values of 182.2±31.2, 290.9±141.2, 227.9±55.4 and 253.5±157.2 mg/dl for cholesterol, 133.6 ±69.2, 248±146.8, 256.8±148.3 and 269.8±160.2 mg/dl for triglycerides and 113.8±27.9, 196.1±123.3, 137.1±45.5 and 159.8±136.9 mg/dl for LDL-C in controls, groups I, II and III, respectively (F=6.329, P=0.001 for cholesterol, F=8.570, P=0.000 for triglycerides and F=4.576, P=0.005 for LDL-C). In contrast, the mean level of HDL-C showed significant decrease with values of 46.3±7.1, 42.9±4.8, 38.0±10.7, and 37.7±10.0 mg/dl in controls, groups I, II and III, respectively (F=7.234, P=0.000).

Table 4.13 Serum cholesterol, triglycerides, high density lipoprotein (HDL-C) and low density lipoprotein cholesterol (LDL-C) of the study population

Lipid Profile (mg/dl)	**Control group (n=40)**	**Group I Normo-albuminuria (n=40)**	**Diabetic nephropathy**		**F**	**P-value**
			Group II Micro-albuminuria (n=40)	**Group III Macro-albuminuria (n=40)**		
	mean±SD	**mean±SD**	**mean±SD**	**mean±SD**		
Cholesterol *(min-max)*	182.2±31.2 *(117-267)*	290.9±141.2 *(126-574)*	227.9±55.4 *(161-412)*	253.5±157.2 *(108-787)*	6.329	0.001
Triglycerides *(min-max)*	133.6±69.2 *(47-280)*	248±146.8 *(78-662)*	256.8±148.3 *(76-595)*	269.8±160.2 *(71-659)*	8.570	0.000
HDL-C *(min-max)*	46.3±7.1 *(32-61)*	42.9±4.8 *(35-50)*	38.0±10.7 *(13-59)*	37.7±10.0 *(15-56)*	7.234	0.000
LDL-C *(min-max)*	113.8±27.9 *(54-159)*	196.1±123.3 *(52.8-477)*	137.1±45.5 *(78-271)*	159.8±136.9 *(29-601)*	4.576	0.005

P<0.05: significant.

4.14 Serum homocysteine level in relation to the studied parameter

4.14.1 Serum homocysteine level in relation to personal and socioeconomic characters of the study population

The relationship of serum homocysteine with personal and socioeconomic characters of the study population is provided in Table 4.14. Results showed that the lower the educational level, the higher the level of homocysteine. Using ANOVA test, this negative relationship was found to be significant (F=2.727, P=0.035). The t-test showed that homocysteine level in unemployed individuals was significantly higher than that in employed ones (19.2±6.1 vs.16.1±5.2µmol, t=2.297, P=0.026). In addition, individuals with family history of diabetes had higher homocysteine levels than those without (t=2.595, P=0.011).

Table 4.14 Serum homocysteine level in relation to the personal and socioeconomic characters of the study population

Character	Homocysteine level (µmol/l) Mean±SD	Statistical test		P-value
Education				
University	14.8±4.6			
Secondary school	18.9±4.2			
Preparatory school	19.1±6.9	**F**	2.727	0.035
Primary school	19.3±6.5			
Illiterate	19.7±5.9			
Employment				
Yes	*16.1±5.2*	**t**	2.297	0.026
No	*19.2±6.1*			
Family history				
Yes	19.4±5.0	**t**	2.595	0.011
No	16.5±6.3			

P<0.05: significant.

4.14.2 Serum homocysteine level in relation to meat and fish intake of the study population

Table 4.15 gives serum homocysteine level in relation to meat and fish intake of the study population. The highest level of homocysteine (21.6±6.4 µmol/l) was found among individuals who ate meat daily whereas homocysteine level was the highest (20.4±6.42) among individual who did not eat fish compared to other periods of such food intake (F=2.876, P=0.048 and F=6.619, P=0.002, respectively).

Table 4.15 Serum homocysteine level in relation to meat and fish intake of the study population

Food intake	Homocysteine level (µmol/l) Mean±SD	F	P-value
Meat			
Daily	21.6±6.4	2.876	0.048
Once/week	18.8±5.3		
Twice/week	16.5±5.3		
Fish			
Once/week	16.1±5.53	6.619	0.002
Twice/week	14.2±3.35		
None	20.4±6.42		

P<0.05: significant.

4.14.3 Serum homocysteine level in relation to BMI of the study population

The relationship between serum homocysteine level and BMI of the study population is presented in Table 4.16. Homocysteine level showed no significant correlation with body mass index (r=0.144, P= 0.146).

Table 4.16 Serum homocysteine level in relation to body mass index (BMI) of the study population

Parameter (Kg/m^2)	Homocysteine (μmol/l)	
	Pearson correlation (r)	P-value
BMI	0.144	0.146

P>0.05: not significant.

4.14.4 Serum homocysteine level in relation to urinary albumin of the study population

Table 4.17 and Figure 4.1 illistraute serum homocysteine level in relation to urinary albumin of the study population. The Pearson correlation test showed positive significant correlation between serum homocysteine and urinary albumin (r=0.564 and P=0.000).

Table 4.17 Serum homocysteine level in relation to urinary albumin of the study population

Parameter (mg/g)	Homocysteine (μmol/l)	
	Pearson correlation (r)	P-value
Urinary albumin	0.564	0.000

P<0.05: significant.

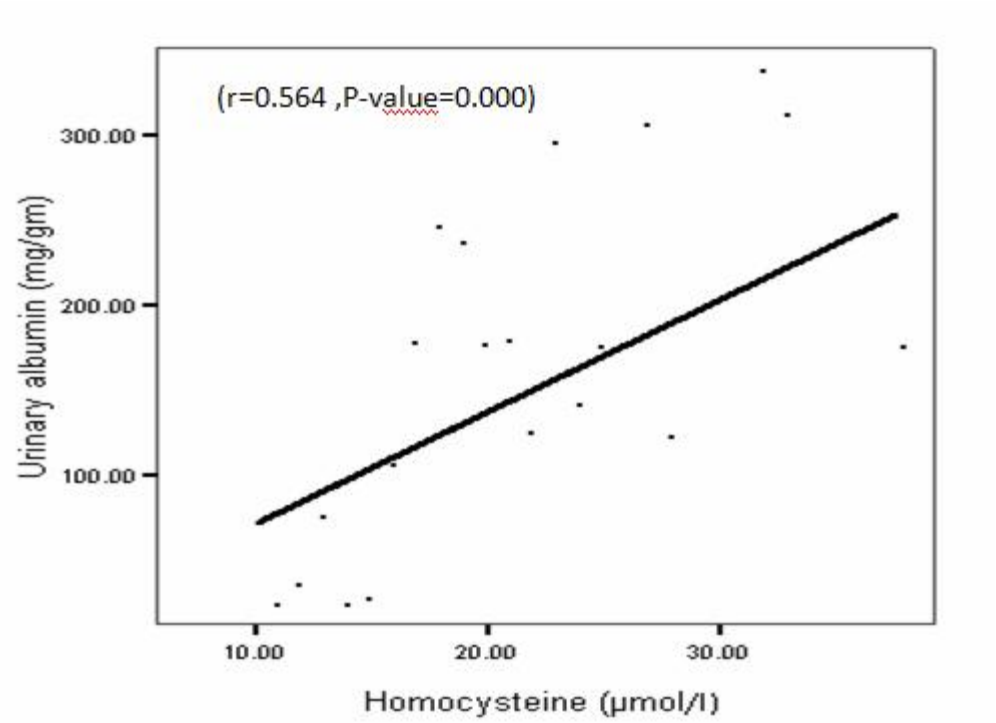


Figure 4.1 Correlation between homocysteine levels with urinary albumin of the study population

4.14.5 Serum homocysteine level in relation to serum glucose and blood HbA1c of the study population

Serum homocysteine level in relation to serum glucose and blood HbA1c of the study population is presented in Table 4.18 and figures 4.2 and 4.3. There are positive significant correlations between homocysteine level with glucose and HbA1c levels (r=0.465, P=0.000 and r=0.517, P=0.000, respictively).

Table 4.18 Serum homocysteine level in relation to serum glucose and blood HbA1c of the study population

Parameter	Homocysteine (µmol/l)	
	Pearson correlation (r)	P-value
Glucose (mg/dl)	0.465	0.000
HbA1c (%)	0.517	0.000

P<0.05: significant.

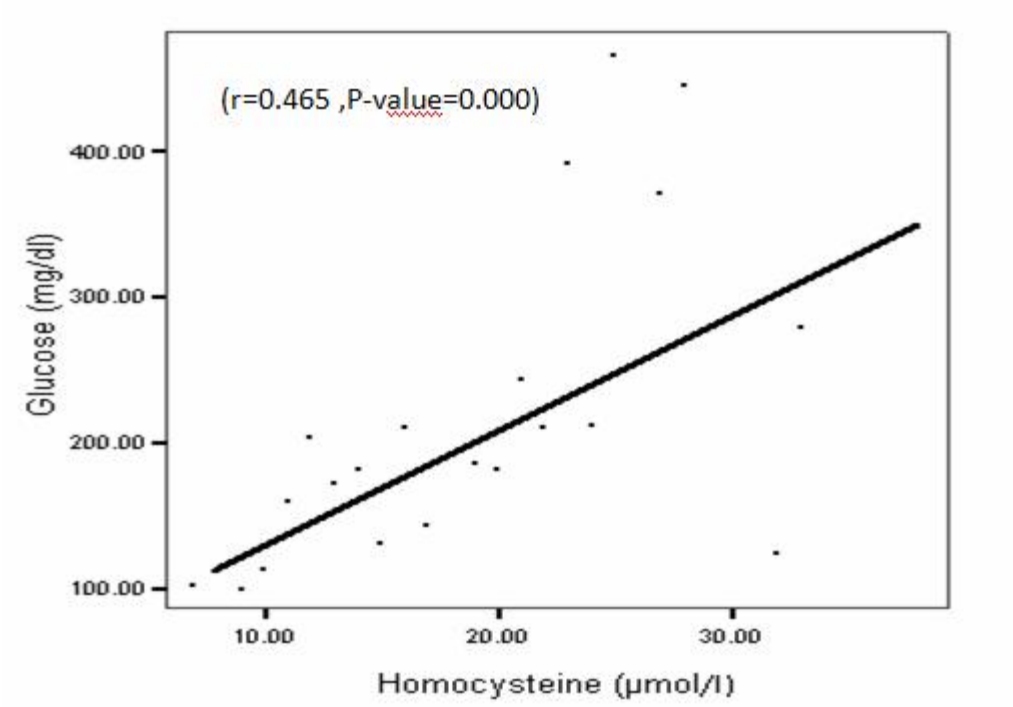


Figure 4.2 Correlation between homocysteine levels with glucose of the study population

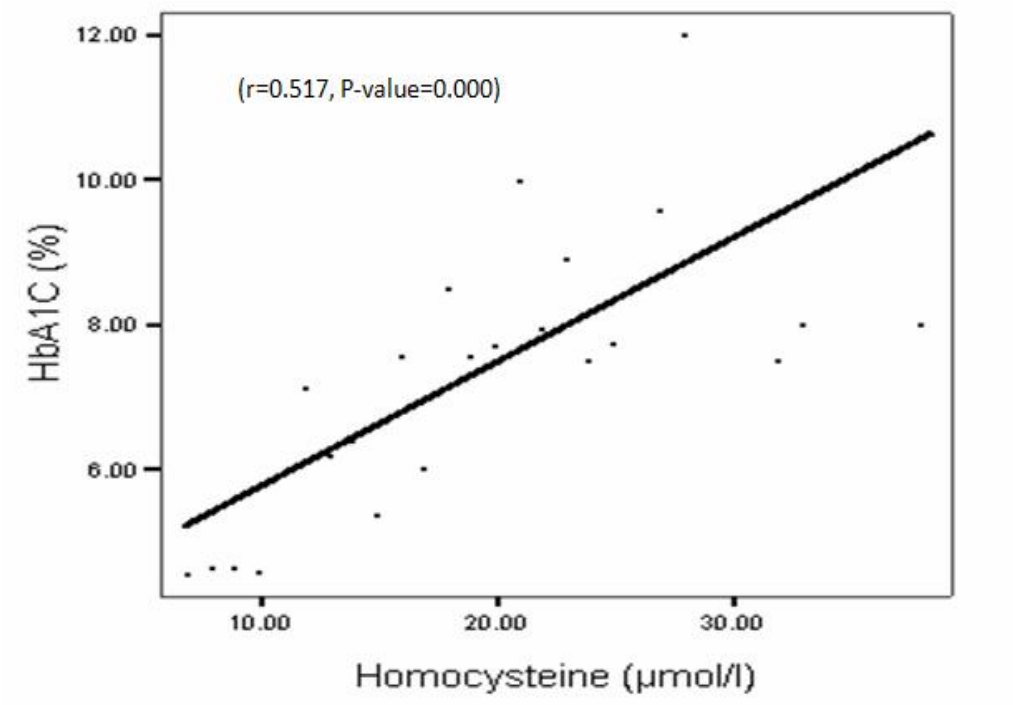


Figure 4.3 Correlation between homocysteine levels with HbA1c of the study population

4.14.6 Serum homocysteine level in relation to serum urea and creatinine concentrations of the study population

Table 4.19 and Figures 4.4 and 4.5 illustrate the correlation between serum homocysteine levels with serum urea and creatinine concentrations of the study population. The Pearson correlation test showed positive significant corrolations between homocysteine level with urea and creatinine concentrations (r=0.654, P=0.000 and r=0.561, P=0.000, respectively).

Table 4.19 Serum homocystiene in relation to serum urea and creatinine concentrations of the study population

Parameter	Homocystiene (µmol/l)	
	Pearson correlation (r)	P-value
Urea (mg/dl)	0.654	0.000
Creatinine (mg/dl)	0.561	0.000

P<0.05: significant.

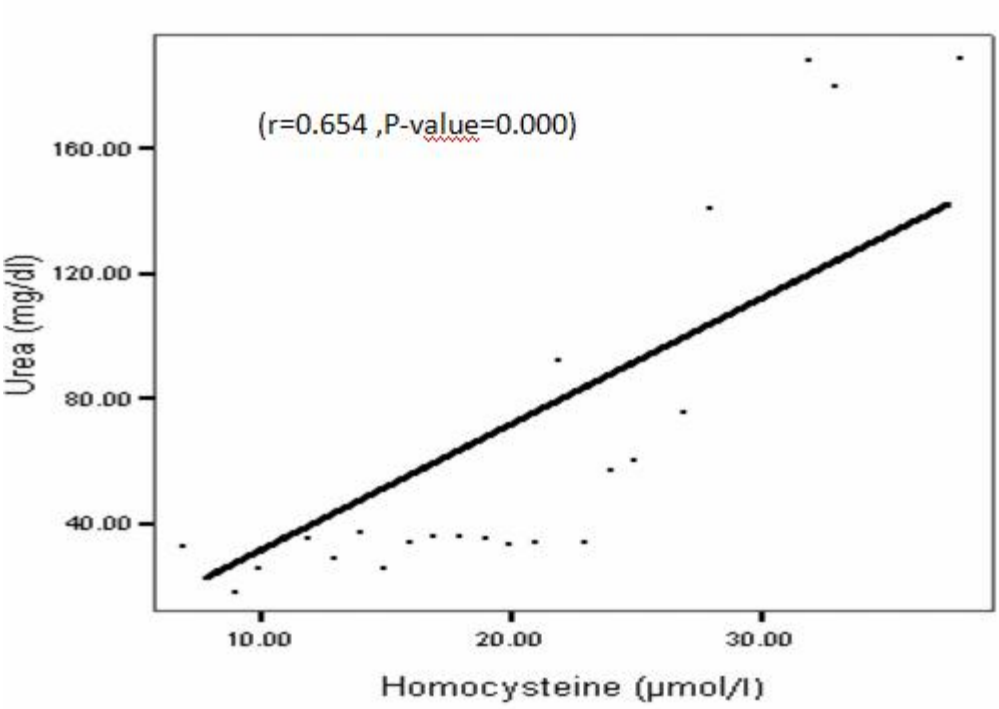


Figure 4.4 Correlation between homocysteine levels with urea of the study population

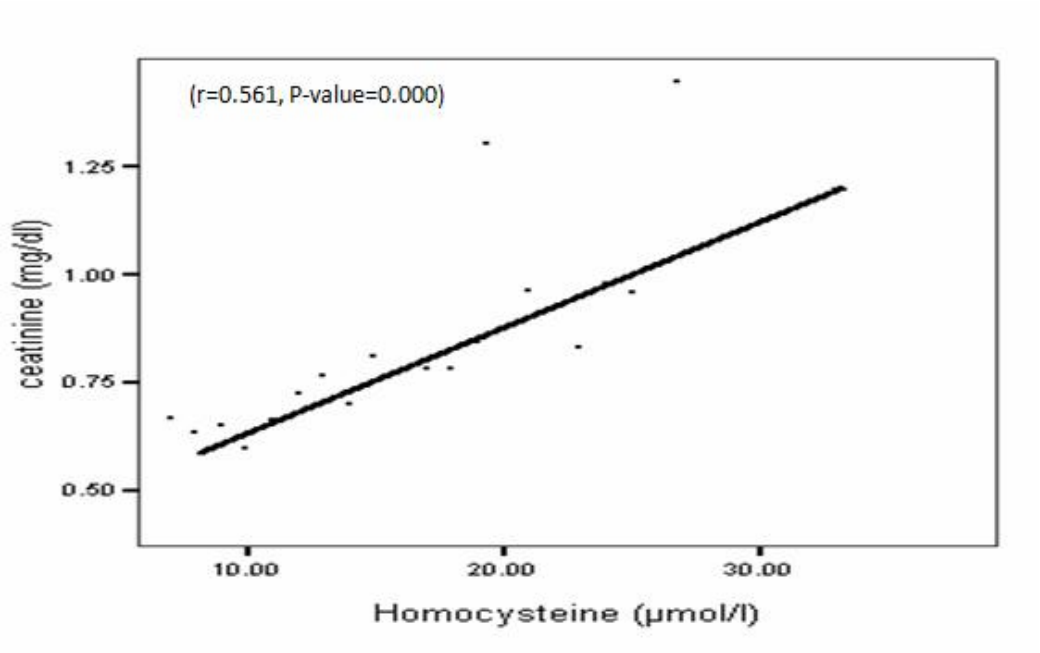


Figure 4.5 Correlation between homocysteine levels with creatinine of the study population

4.14.7 Serum homocysteine level in relation to serum lipid profile of the study population

Table 4.20 summarizes serum homocysteine level in relation to serum lipid profile including cholesterol, triglyceride, HDL-C and LDL-C of the study population. Pearson correlation test revealed positive significant correlation between homocystiene and triglyceride (Figure 4.6, r=0.320, P=0.001). On the other hand, there was a negative significant correlation between homocystiene and HDL-C levels (Figure 4.7, r=-0.517, P=0.000).

Table 4.20 Serum homocystiene in relation to serum lipid profile of the study population (n=160)

Lipid Profile (mg/dl)	Homocystiene (μmol/l)	
	Pearson correlation (r)	P-value
Cholesterol	0.147	0.138
Triglycerides	0.320	0.001
HDL-C*	-0.517	0.000
LDL-C**	0.114	0.252

*HDL-C: High density lipoprotein cholesterol. **LDL-C: Low density lipoprotein cholesterol, P>0.05: not significant, P<0.05: significant.

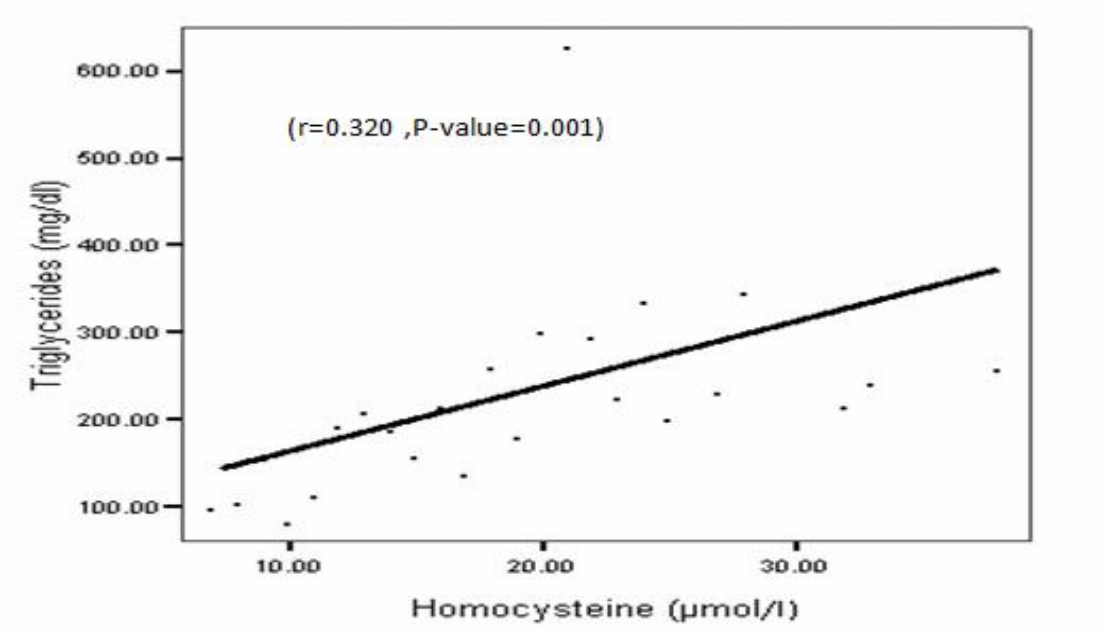


Figure 4.6 Correlation between homocysteine level with triglycerides of the study population

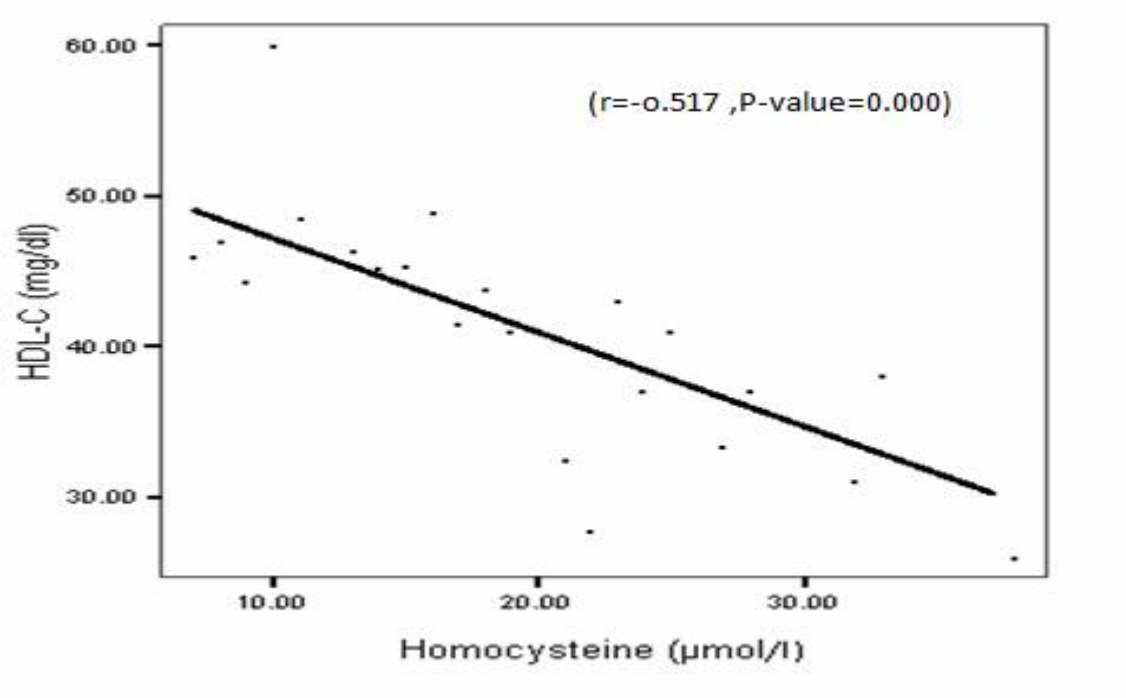


Figure 4.7 Correlation between homocysteine level with HDL-C of the study population.

Chapter 5

Discussion

Over the past three decades, the number of people with diabetes mellitus has more than doubled globally (expected to reach 366 million in the year 2030, Wild et al., 2004), making it one of the most important public health challenges to all nations. In Palestine, the mortality rates of diabetes constituted 5.9 per 100,000 population in the year 2009 (Ministry of Health 2009), and this figure raised to 8.5 per 100,000 population in the year 2010 (Ministry of Health 2010). Despite that, there are under-diagnosis and under-reporting of the disease in the Gaza strip. Biochemical tests of the disease were restricted to monitoring blood glucose level when the patient visits the clinic. Although few studies have been recently assessed some early markers of diabetic nephropathy (Altibi, 2007, Shubair, 2008 and Abu Mustafa, 2011), no perverse study investigated homocysteine status in diabetic nephropathy patients with type 2 diabetes in the Gaza Strip. Therefore, the present study is the first to demonstrate homocysteine levels in different stages of diabetic nephropathy among type 2 diabetic patients in the Gaza Strip.

5.1 Socio-demographic data of the study population

The present study is a case control design included 4 groups: control group (40 non diabetic healthy individuals), normoalbuminuric group (group I: 40 patients, urinary albumin <30 mg/g), microalbuminuric group (group II: 40 patients, urinary albumin 30-300 mg/g) and macroalbuminuric group (group III: 40 patients, urinary albumin >300 mg/g). The mean age of control group, groups I, II and III were 56.9±6.2, 57.7±7.1, 57.8±5.4 and 58.5±6.3 years. It was reported that type 2 diabetes mellitus usually develops after age 40 years (Rodger, 1991 and Umpierrez et al., 2006). Analysis of the

educational status of the study population showed that the development of diabetic nephropathy is significantly increasing with decreasing educational level among different groups. This finding is in agreement with that obtained by Abu Mustafa (2011) who reported that low educational level is a risk factor of diabetes. In addition, Altawil (2009) concluded that the well educated diabetic patients had a better control of the disease through adoption of healthy diets. The prevalence of diabetic nephropathy was higher among unemployed individuals as well as among individuals with family history of diabetes. This indicates that unemployment and family history are associated with type 2 diabetes. Black (2002) mentioned that among adults aged 45-64 years, 49% of diabetics are unemployed, compared with only 28% of non diabetics. In addition, many studies reported that family history is a risk factor of diabetes (Annis et al., 2005; Harrison et al., 2003; Pijl et al., 2009 and Yassin et al., 2011).

5.2 Diabetes duration, self-reported complications and food intake among the study population

Data presented in this study indicated that quarter of the patients had diabetes since less than 5 years. Such finding does confirm the idea that type 2 diabetes has long asymptomatic pre-clinical phase which frequently goes undetected. At the time of diagnosis, the patient could have one or more diabetes complications i.e. there is a latent phase before diagnosis of type 2 diabetes. During this period of undiagnosed disease, risk factors for diabetic micro- and macrovascular complications are markedly elevated and diabetic complications are developing (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2003 and Waltkins, 2003). With diabetes progression, a significant increase in the frequency of self-reported symptoms among micr- and macralbuminuric patients was observed. The relation between diabetes duration and self-reported complications confirmed this view. However, this point still needs further investigation. The most self-reported symptoms among diabetic patients were neuropathy and retinopathy. The prevalence of such symptoms was positively associated with the progress of the disease i.e. the longer the

duration of diabetes mellitus, the higher the percentage of self-reported complications among patients. Several studies reported similar diabetic complications with increasing rates upon disease progress (Savage, 1996, Dyck et al., 2002, Marshall, 2006 and The National Eye Institute, 2006, Altawil, 2009 and Abu Sunayma, 2012). Concerning food intake the present results showed a significant increase in the number of patients who ate meat in various diabetic groups towards development of diabetic nephropathy compared to control group. This implies that frequent consumption of meat is associated with the development of diabetic nephropathy. Similar result was obtained by National Institutes of Health (2006) and by Odermatt (2011). There is evidence that consumption of cooked meat, in particular, may affect CKD categorization based on estimated GFR (Preiss et al., 2007). It is believed that meat contains high levels of saturated fat which increase blood cholesterol contributed to hypertension and then CKD (Fox and Muntner, 2008). However, there were a lower number of patients who eat fish with the development of diabetic nephropathy. Fish consumption at least in the present study, showed a border line significant association with diabetic nephropathy. This necessitates launching of educational programs to show the importance of food intake in controlling the disease. Waltkins (2003) reported that healthy eating is the cornerstone of diabetic treatment, and control of the diet should always be the first treatment offered to Type 2 diabetic patients before drugs are considered.

5.3 Diabetes and body mass index

Body mass index provides a reliable indicator of body fatness for most people and it is used to screen for weight categories that may lead to health problems (CDC, 2007). Therefore, obesity is commonly defined as a BMI of 30 kg/m2 or higher. This definition distinguishes obesity from being pre-obese or overweight, which is classified as a BMI of 25 kg/m2 but less than 30 kg/m2 (WHO, 2000). In the present study, ANOVA test showed significant increase in BMI among various diabetic groups compared to control group, indicating that diabetes is associated with BMI. The literature

supported the present result in that obesity is a major risk factor for chronic diseases including diabetes (El-Hazmi et al., 1997, Marshall, 2006 and Yassin et al., 2011). It was reported that about 55% of type 2 diabetics were obese (Eberhart et al., 2004).

5.4 Serum homocysteine level of the study population

The results of this study showed a progressive significant increase in the serum homocysteine levels in the three diabetic groups in respect to control group. Such finding is in line with previous studies (Jager et al., 2001; Li et al., 2006; Cho et al., 2010 and Krajnc et al., 2011) and support the hypothesis that homocysteine may play a pathophysiological role in the development of diabetic nephropathy. Friedman et al., (2001) reported that hyperhomocysteinemia is very common in patients with chronic renal insufficiency and is nearly ubiquitous in patients with end-stage renal disease; who have up to a 30 times higher risk of cardiovascular related death than the general population. Two, not mutually exclusive hypotheses for hyperhomocysteinemia in diabetic nephropathy are suggested:1) homocysteine disposal in the kidneys themselves is disturbed and 2) extrarenal homocysteine metabolism is impaired (van Guldener, 2006). The first hypothesis is supported by the observed impairment of kidney function that is manifested in marked elevation in urinary albumin, and serum urea and creatinin concentrations with the development of diabetic nephropathy. In addition, there is a significant positive correlation between urinary albumin and homocysteine. Cho et al. (2010) and Shaikh et al. (2012) showed that hyperhomocysteinemia is associated with a higher incidence of microalbuminuria in patients with type 2 diabetes mellitus.

5.5 Serum glucose and blood HbA1c of the study population

As indicated in the present result, the mean levels of serum glucose in various diabetic groups exhibited significant gradual increase with the development of diabetic nephropathy with respect to control group. In parallel, diabetic patients showed significant increasing levels of HbA1c in different groups compared to

control group. Similar results were obtained by El meleigi et al. (2003), Qi et al. (2007), Nada and Abdul Jalil (2010) and Yassin et al. (2011) who found that type 2 diabetic patients with diabetic nephropathy had higher fasting glucose and HbA1C levels than non-diabetics. In diabetes, prolonged hyperglycemia superdrives nonenzymatic protein glycation, which forms reversible Schiff bases and Amadori compounds. A series of further complex molecular rearrangements then yield irreversible Advanced glycosylated end-products (AGEs). AGEs accumulate in the circulating blood and in various tissues (Furth, 1997). It is reported that the levels of HbA1c in the blood reflect the glucose levels to which the erythrocyte has been exposed during its lifespan (Goldstein, 2004). Therefore, the HbA1c test is attractive as it measures chronic glycaemia, rather than instantaneous blood glucose levels. HbA1c has been used as an objective marker of average glycaemic control for many years, has an accepted place in the monitoring of patients with diabetes, and is relied on for significant management decisions, such as initiation of insulin therapy (d´Emden et al., 2012). In the present study, the Pearson correlation test showed positive significant correlations between homocysteine levels and HbA1c and glucose. Similar result was documented by Drzewoski et al. (2000), Cho et al. (2010), Nada and Abdul jalil (2010). In addition, it was reported that HbA1c is raised in patients with hyperhomocysteinemic diabetic population (Shaikh et al., 2012). The observed positive relationship in this study reflects the change of the glycemic parameters with homocysteine levels and reconfirms the hypothesis that hyperhomocysteinemia may play a pathophysiological role in the development of diabetic nephropathy in type 2 diabetes. However, this point needs further investigation.

5.6 Serum urea and creatinine of the study population

Serum urea and creatinine concentrations showed progression significant increase in different diabetic groups with the development of diabetic nephropathy compared with control group. Such finding is in accord with that obtained by Ozmen et al. (2002), El meleigi et al. (2003) and Abu Mustafa (2011). Urea is formed by the liver as an end product of protein breakdown and is one marker of the kidney function (Debra Manzella, 2008). Increase in serum

urea observed here may be due to impairment in its synthesis as a result of impaired hepatic function and/or due to disturbance in protein metabolism. The significant increases in protein profile detected in the present study do confirm this view. Creatinine is a waste product that is normally filtered from the blood and excreted with the urine. Higher creatinine levels in diabetic patients may be related to disturbance of kidney function (Debra Manzella, 2008). In addition, the observed increases in urea and creatinine may be explained on the basis of impairment in glomerular filtration as a result of impairment in kidney function accompanied diabetic nephropathy. However, it is difficult to determine the onset of such changes and this may lead to controversial results (Varghese et al., 2001, Altibi, 2007 and Yassin et al., 2011). Therefore, it must watch the creatinine levels carefully to determine how much function the kidneys have and this does vary slightly. In the present study, the Pearson correlation test showed positive significant correlations between homocysteine levels and urea and creatinine. Similar result was obtained by Ozmen et al. (2002) and Sharaf et al. (2012). This positive relationship reflects the change of urea and creatinine concentrations with homocysteine levels and suggests an association between homocysteinemia and impaired renal function, as evidenced by increased serum creatinine.

5.7 Serum lipid profile of the study population

In general, there was gradual significant increase in the mean levels of cholesterol, triglycerides and LDL-C whereas the mean level of HDL-C was significantly decreased in diferrent diabetic groups towards the development of diabetic nephropathy. These results are in accordance with that reported by other authors (El meleigi et al., 2003 and Jha et al., 2010). The general increase of serum lipids in diabetic patients may be mainly attributed to increase in the mobilization of free fatty acids from fat depots, since elevation of insulin inhibits the hormone sensitive lipase. Then, excess fatty acids in serum are converted into triglycerides, phospholipids and cholesterol in liver. These three substances with protein may be discharged into blood in the form of lipoproteins (Taskinen, 1992 and Jaworski et al., 2007). When related to homocystiene levels, only triglyceride and HDL-C

showed positive and negative significant correlations respectively. Similar results were documented by Okumura and Aso, (2003), Rudy et al., (2005) and Chauhan et al., (2012). Mutual increment in homocysteine level with increase in triglyceride and decrease in HDL-C could introduce further evidence in the involvement of hyperhomocysteinemia in the pathophysiology of diabetic nephropathy.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

- The mean urinary albumin concentrations were 15.9±4.8, 22.3±5.2, 146.7±80.7 and 348±37.6 mg/g in control group, normoalbuminuric patients (group I), microalbuminuric patients (group II) and macroalbuminuric (group III), respectively.
- The mean age of control group, groups I, II and III were 56.9±6.2, 57.7±7.1, 57.8±5.4 and 58.5±6.3 years.
- Diabetic nephropathy was more prevalent among less educated and unemployment individuals as well as among individuals with family history of diabetes and those who frequently eat meat.
- The main self-reported complications among patients were retinopathy, cardiovascular diseases and neuropathy. The prevalence of such complications was significantly increased with the development of diabetic nephropathy.
- ANOVA test showed significant increase in BMI among various diabetic groups compared to control group.
- There was a progressive significant increase in the serum homocysteine levels in the three diabetic groups in respect to control group.
- The levels of homocysteine were higher in less educated and unemployment individuals, individuals with family history of diabetes, and individuals who frequently eat meat and eat less fish.
- Serum homocysteine levels exhibited positive significant correlation with urinary albumin.
- The mean levels of serum glucose and blood HbA1c in various diabetic groups exhibited significant gradual increase with the development of diabetic nephropathy with respect to control group. The Pearson correlation test

showed positive significant correlations between homocysteine levels and HbA1c and glucose.

- Serum urea and creatinine concentrations showed progression significant increase in different diabetic groups with the development of diabetic nephropathy compared with control group. The Pearson correlation test showed positive significant correlations between homocysteine levels and urea and creatinine.
- There was gradual significant increase in the mean levels of cholesterol, triglycerides and LDL-C whereas the mean level of HDL-C was significantly decreased in diferrent diabetic groups towards the development of diabetic nephropathy. When related to homocystiene levels, only triglyceride and HDL-C showed positive and negative significant correlations respectively.

6.2 Recommendations

- Introducing of serum homocysteine test beside urinary albumin monitoring in Gaza hospitals and clinics is recommended in the assessment of the development of diabetic nephropathy is recommended.

- Launching of health education programs particularly among individuals with family history of diabetes.

- Follow up a healthy diet in terms of reducing meat intake and promoting fish intake.

- Regular visits to optical, cardiovascular and neurological clinics to take early steps to avoid and manage diabetic complications concerning diabetic retinopathy, CV disease and neuropathy.

- Weight control among obese individuals is recommended.

- Evaluation of insulin levels in different stages of diabetic nephropathy and their relations to homocysteine levels is needed.

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Annex 1

Questionnaire

أخي المواطن الكريم /أرجو مساعدتنا في إتمام ههذه الدراسة (بحث ماجستير تحاليل طبية / الجامعة الإسلامية) والتي تختص بمرضى السكري المصابين باعتلال الكلي حيث أن هدفها منع أو تأخير دخول المريض في الفشل الكلوي، وذلك من خلال تعبئة هذا الاستبيان وتبرعك لنا بعينة دم و عينة بول لإجراء بعض الفحوصات و التجارب عليها.

Personal data				
Tel. No.				
Name				
Age				
Gender	□ Male □ Female			
Education	□ University □ Secondary school □ Preparatory school □ Primary school □ illiterate			
Employment	□ Employed □ unemployed			
Family history diabetes	□ Yes □ No			
Diet Do you follow a particular diet	□ Yes □ No			
How often do you eat the following foods	**None**	**Daily**	**Twice weekly**	**Weekly**
Meat				
Fish				
Clinical data *(For patient only)*				
Duration of diabetes				
Complication				
Retinopathy			□ Yes	□ No
Cardiovascular diseases			□ Yes	□ No
Neuropathy			□ Yes	□ No

Agreement: I agree to complete this questionnaire concerning my health statement.

أنا موافق على تعبئة هذا الاستبيان الذي يتعلق بصحتي.

التوقيع: .....

التاريخ: .....

شكرا لكم على حسن تعاونكم

الباحث/ محمود النجار