SUDAN UNIVERSTY OF SCIENCE AND TECHNOLOGY

COLLEGE OF GRADUATE STUDIES

Comparative Assessment Of plasma Cystatin C, β2 Microgloulin and Creatinine as Markers of Renal Function in Sudanese patients with Type2 Diabetes

مقارنه تقويميه لبلازما السيستاتين سي ، البيتا2وقلوبيولين الدقيق وا لكرياتينين كدلائل لوظايف الكلي لدي المرضي السودانين المصابين بمرض السكري من النوع الثاني

A Thesis Submitted in Fulfillment for PhD Degree in Clinical Chemistry

By

NOON BABIKER MOHAMMED AHMED:

BSc in Medical Laboratory Sciences Clinical Chemistry, Ahlia University (1999)

MSc in Clinical Chemistry Al-Zaiem Al-Azhari University (2004).

Supervisor

Dr. BADER ELDIEN HASSAN ELABID:

MB BS, University of Khartoum, MD Clinical Pathology, Associate Professor of Chemical Pathology, University of Science and Technology.

Co supervisor

Dr. Samia Mahdi Ahmed:

Assistant Professor, Department of Medical Laboratories,College of Applied Medical Sciences, Taibah University, Almadeena Almonawara, Saudi Arabia.

April 2014

Dedication

This Research is lovingly dedicated to my respective mother who has been my constant source of inspiration. I dedicate this research also to my husband and my kids, my sisters, all my friend, my teacher and my student. They have given me the drive and discipline to tackle any task with enthusiasm and determination. Without their love and support this project would not have been made possible.

Noon

Acknowledgments

First of all my thanks to ALLAH.I wish to express my thanks and gratitude to my supervisor Dr. BADER ELDIEN HASSAN ELABID, associate Professor of Chemical Pathology University of Science and Technology, Sudan, for his close supervision, assistance and continuous support during this work. Without his help, this work could not have been accomplished.

My gratitude is also extended to my Co supervisor Dr. Samia Mahadi Ahmed: Assistant Professor at Taibah University, Saudi Arabia . I am also indebted to everyone who taught me and to all those who stood behind me and gave me kind, personal, close and distant support.

My sincere thanks are due to the staff of Jabir Abu Elaaz and Almolazmeen Diabetic centers.

I feel indebted to all participant who donated samples for the current study.

Finally, I would like to thank and express my deepest gratitude to Dr: Magdi Obaied, my husband, for his assistance in clinical examination of the participants.

Abstract

Background: Cystatin C and B2-macroglobulin, are mainly used as biomarkers of kidney function. Because of their small size, Cystatin C andB2-microglobulin are freely filtered by the glomeruli, and dose not return to the blood stream or secreted by renal tubules. They have been suggested to be closer to the "ideal" endogenous marker, and emerging as a biomarker superior to serum creatinine for estimating the glomerular filtration rate and predicting the risk of death and cardiovascular events. Diabetic nephropathy cause gradual renal impairment, that may end with end stage renal failure. In glomerular kidney disease, the glomeruli can't filter cystatin C and B2-microglobulin out of the blood, so levels increase in blood and decrease in urine.

Objectives: To assess the plasma levels of cystatin C, B2-microglobulin, creatinine, creatinine clearance, blood HbA_{1C}% and body mass index among Sudanese patients with type 2 diabetes in comparison with healthy controls.

Materials and methods: This is a case-control study conducted in Jabir Abulizz and Almolazmeen diabetic centers in Khartoum state, Sudan, during the period from November 2010 to March 2014. A total of 300 Sudanese patients with type2 diabetes mellitus (49%males and 51% females)as a test group and 150 healthy subjects(48%males and 52%female) as a control group

were enrolled in this study. The test group and the control group were matched in term of gender and age. The plasma levels of cystatin c, B2-microglobulin, creatinine and blood HbA_{1c}% were measured using Nephelometry technique. creatinine clearance was calculated and the body mass index was determined for each participant. SPSS was used for analysis of data. Student's "t" test was used for comparison between the test group and the control group and Pearson's correlation was used to assess correlations between variables.

Results: In the current study the majority of diabetic patients were found to be obese (71%), with long standing diabetes (65%) and had uncontrolled diabetes (69%). The means of the plasma levels of cystatin C, B2-microglobulin, creatinine , blood HbA_{1c}% and body mass index were significantly raised in the diabetic group when compared to the control group ($p<0.05$).The study showed a significant positive correlation between the plasma levels of cystatin C and creatinine , and a significant moderate positive correlation between the plasma levels of cystatin c and HbA_{1c} % of the diabetic group. In the present study there was significant moderate positive correlations between HbA_{1c}% and plasma levels of both; cystatin C and B2-microglobulin, whereas creatinine clearance had significant inverse moderate correlations with both; cystatin C and B_2-microglobulin.

Conclusion: From the results of this study it is concluded that: plasmacystatin c, B2-microglobulin,creatinine,HbA_{1c}% and body mass index are raised in Sudanese with type 2diabetes. Plasma cystatin C and B2-microglobulin have significant inverse correlations with the glomerular filtration rate. Both cystatin C and B2-microglobulin plasma levels have significant positive moderate correlations with HbA_{1c}%.Tthe plasma levels of cystatin C and B2-microglobulin could be used as markers of renal function and glycemic control in patients with type 2 diabetes.

مستخلص الدراسة

خلفية: يستخدم السيتاتين سي وبيتا 2 ماكروقلوبيولين كمؤشرات بيولوجية لوظائف الكلية. ونسبة لأحجامها الصغيرة فإن السيتاتين سي وبيتا 2 ماكروقلوبيولين يتم رشحها بحرية بواسطة الكبيبات الكلوية، ولا تعود إلى مجرى الدم أو يتم إفرازها بواسطة الكببات الكلوية. لذا يعتقد أنها الأقرب إلى المؤشرات ذات المنشأ الداخلي المثالية، وتظهر كمؤشرات بيولوجية ذات فائدة لتقييم معدل الرشح الكبيبي وتوقع مخاطر الوفاة ومشكلات القلب والأوعية الدموية والاضطرابات الكلوية بسبب السكري والتي تسبب خلل تدريجي في الكلية قد يؤدي في النهاية إلى حدوث فشل كلوي. في حالة مرض الكلية الكيبيبي لا تتمكن الكبيبات من رشح السيتاتين سي وبيتا 2 ماكروقلوبيولين من الدم البول، بالتالي فإن مستوياتها تزيد في الدم وتقل في البول.

الأهداف: تقييم معدلات خروج السيتاتين سي وبيتا 2 ماكروقلوبيولين والكرياتينين في بلازما الدم بالإضافة إلى إخراج الكرياتين والنسبة المئوية الهيموجبوبين المجلكز في الدم ومؤشر كتلة الجسم للمرضى السودانيين المصابين بمرض السكر من النوع الثاني مقارنة بمجموعة ضابطة من الأصحاء.

المواد وطرق البحث: هذه الدراسة أجريت في مركزي جابر أبو العز والملازمين للسكري بولاية الخرطوم بالسودان في الفترة ما بين مارس 2010 إلى نوفمبر 2014م. شملت عينة الدراسة 300 من المرضى السودانيين المصابين بمرض السكر من النوع الثاني (49% منهم ذكور، 51% إناث) كمجموعة تجريبية و150 أصحاء (48% ذكور، 52% إناث) كمجموعة ضابطة. المجموعة التجريبية والمجموعة الضابطة كانت متطابقة من حيث النوع والعمر. تم قياس مستويات السيتاتين سي وبيتا 2 ماكروقلوبيولين والكرياتينين في الدم والنسبة المئوية الهيموجلوبين المجلكز باستخدام تقنية نفيلوميتري. معدل خروج الكريتانين ومؤشر كتلة الجسم تم حسابها لكل المشاركين في الدراسة. تم استخدام برنامج الحزم الإحصائية للعلوم الاجتماعية لتحليل البيانات. لمقارنة النتائج بين المجموعة

التجريبية والضابطة حيث تم استخدام اختبار (ت) واستخدم معامل ارتباط بيرسون لمعرفة العلاقات الارتباطية بين المتغيرات.

النتائج: في هذه الدراسة كان غالبية مرضى السكري (71%) يعانون من السمنة، والغالبية أيضاً تعاني من طول فترة المرض (65%)، ونسبة الذي ليس لديهم سيطرة على مرض السكري (69%). كانت متوسطات مستويات البلازما من السيتاتين سي وبيتا 2 ماكروقلوبيولين والكرياتينين والنسبة المئوية لهيموجلوبين أ 1 سي ومؤشر كتلة الجسم مرتفعة بدرجة ذات دلالة إحصائية في مجموعة مرضى السكري مقارنة بمجموعة الأصحاء (القيمة الاحتمالية كانت أقل من 0.05). أظهرت الدراسة وجود علاقة ارتباط متوسط وموجب ذات دلالة إحصائية بين مستويات البلازما من السيتاتين سي والكرياتينين وعلاقة ارتباط متوسطة موجبة ذات دلالة إحصائية بين مستويات البلازما من السيتاتين والنسبة المئوية الهيموجلوبين المجلكز لدى مجموعة مرضى السكري. بينما كانت هنالك علاقة ارتباطة متوسطة ذات دلالة إحصائية موجبة بين النسبة المئوية للهيموجلوبين المجلكز و السيتاتين وبيتا 2 ماكروقلوبيولين، كانت العلاقة الارتباطية عكسية ذات دلالة إحصائية بين معدل إخراج الكرياتينين و السيتاتين سي وبيتا 2 ماكروقلوبيولين.

الخلاصة: من خلال النتائج تخلص الدراسة إلى أن مستويات البلازما من السيتاتين سي وبيتا 2 ماكروقلوبيولين والكرياتينين والنسبة المئوية الهيموجلوبين المجلكز ومؤشر كتلة الجسم كانت مرتفعة لدى المرضى السودانيين المصابين بمرض السكري من النوع الثاني. هنالك علاقة عكسية ذات دلالة إحصائية موجبة بين السيتاتين سي وبيتا 2 ماكروقلوبيولين مع معدل الرشح الكيبيبي. توجد علاقة ارتباطية موجبة بين مستويات البلازما للسيتاتين سي وبيتا 2 ماكروقلوبيولين والنسبة المئوية للهيموجلوبين المجلكز. بناءً عليه يمكن قياس مستويات السيتاتين سي وبيتا 2 ماكروقلوبيولين في البلازما كمؤشرات لتقييم كفاءة عمل الكلي وكذلك كمقياس للتحكم في مستوى سكر الدم لدى مرضى السكري من النوع الثاني.

List of contents

Chapter Three

3. Materials And Methods

List of tables

List of figures

List of Abbreviations

ADA: American Diabetes Association

AIDS: Acquired Immune Deficiency Syndrome

BMI: body mass index

BUN: Blood Urea Nitrogen

CG: Cockcroft-Gault formula.

CKD: Chronic Kidney Disease

CRCL: creatinine clearance

CVA: cerebrovascular accident

CVD: cerebrovascular disease

DIDMOAD: diabetes mellitus, optic atrophy, and deafness

DKA: Diabetic ketoacidosis

DM: Diabetes Mellitus

ESRD: End Stage Renal Disease

GDM: Gestational diabetes mellitus

GFR: glomerular filtration rate

HbA1C: Glyclated haemoglobin

HHNKC: hyperosmolar hyperglycemic nonketotic coma

HHS: Hyperglycemic Hyperosmolar State

HIV: human immunodeficiency virus

HNS: hyperosmolar nonketotic state

HONKC: hyperosmotic non-ketotic coma

IDDM: Insulin Dependent Diabetes Mellitus

LDL: low-density lipoprotein

MDRD: Modification of Diet in Renal Disease

MODY: Maturity- onset diabetes of youth

MRDM: malnutrition-related diabetes mellitus

NHANES: National Health and Nutrition Examination Survey

NICE: National Institute for Health and Clinical Excellence

NIDDM: Non Insulin Dependent Diabetes Mellitus

PSL: prenidsolone

ROS: reactive oxygen species

SPSS: Statistical Package for Social Sciences

TTAB: Teteradecyl trimethyl ammonium bromide

UK: United Kingdom

WHO: World Health Organization

Chapter One

Introduction

Chapter one

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels, which result from defects in insulin secretion, or action, or both. Diabetes mellitus, commonly referred to as diabetes, was first identified as a disease associated with “sweet urine”, and excessive muscle loss in the ancient world. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine. Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas. Insulin lowers the blood glucose level. When the blood glucose elevates (for example, after eating food), insulin is released from the pancreas to normalize the glucose level. In patient with diabetes, the absence or insufficient production of insulin causes hyperglycemia. Diabetes is a chronic medical condition, meaning that although it can be controlled, it lasts lifetime.[(1)]

Over time, Diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage are the result of damage to small vessels, referred to as microvascular disease. Diabetes is also an important factor in accelerating hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel disease [(2)]. This is referred to as macrovascular disease. Diabetes affects approximately 17 million (about 8% of the population) in

the United States. In addition, an estimated additional 12 million people in the United States have diabetes and don't even know it[2]. From an economic perspective, the total annual cost of diabetes in 1997 was estimated to be 98 billion dollars in the United States. The capita cost of resulting from diabetes in 1997 amounted to $10.071. While healthcare cost of $2.699. During this same year, 13.9million days of hospital stay were attributed to diabetes, While 30.3 million physician office visits were diabetes related.

Remember, these numbers reflect only population in the United States. Diabetes is the third leading cause of death in the United States after heart disease and cancer [2].

Diabetes mellitus is one of the leading causes of morbidity and mortality in the United States because of its role in the development of optic, renal, neuropathic, and cardiovascular disease.

Diabetes in Sudan

Until recently diabetes had been considered rare in African countries, probably

due to the lack of awareness of diabetes by doctors and compounded by the lack of diagnostic facilities, but in the urban population of Sudan, chronic non-communicable diseases such as diabetes mellitus and hypertension are now emerging as important health problems.

Type 1 diabetes is rarer in African countries than type 2 and for this reason type 2 diabetes will be the subject of this review. By the year2025, the number of people with diabetes is expected to increase from 135million (today) to 300 million.5 In Sudan and other African countries the growing wave of urbanization has 324 Practaltered many people's lifestyles. These lifestyle changes include the increased use of motorized vehicles, little or no time devoted to regular exercise, too many sedentary hours watching TV and large amounts of sugar, refined cereals and fat consumed instead of the healthy traditional foods. For many people, obesity is highly desirable as it is seen as a sign of strength and affluence in men and beauty and attractiveness in women. Physical underactivity and obesity resulting from these factors are known predisposing factors for diabetes.

Consequently, diabetes is now one of the major health problems in Sudan resulting in 10% of all hospital admissions and mortality(3). The actual number of people with diabetes in Sudan is not known. A small population-based study in 1993 of a sample of 1284 adult men, showed a prevalence of 3.4% of type 2 diabetes(4).

The influence of environmental factors in the cause diabetes needs to be mentioned. One small study questioned the role of malaria falciparum (13 million cases in 1995) as a possible cause of diabetes.9 Large scale studies in Sudan confirmed its absence; malnutrition-related diabetes mellitus (MRDM) was

reported to be common in the Indian subcontinent but this is relatively rare among Africans. Generally, studies of hospital admissions in Africa are rare due to the poor or unsatisfactory quality of patient records. In Sudan, diabetes is the commonest cause of hospital admissions due to a non-communicable disease. One study showed that people with diabetes constituted 7% of all hospital admissions, a value higher than that reported for other African countries[5].

Type 2 diabetes is associated with an overall age-adjusted mortality that is about twice that of the non-diabetic population and the life expectancy is reduced by 5–10 years. Currently, diabetes in Sudan (and most of Africa) is believed to have one of the highest mortality rates for a non-infectious disease. One study indicated that 10%of adult patient deaths in hospitals were caused by diabetes. This figure may be underestimated as patients who died at home or were unable to reach hospital due to lack of transportation or economic constraints were not included. Acute complications, especially DKA, are the commonest cause of diabetes-related mortality in Sudan.

There is a delay in recognition of early signs of DKA (and therefore treatment) due to doctors being unaware or due to self-treatment of the precipitating factors such as malaria. Other major causes of death include septicaemia (secondary to septic foot) and end stage renal disease[6].

Cystatin C is a 122-amino acid, 13-KDa protein that is a member of the family of cysteine proteinase inhibitors. It is the product of a gene expressed in all nucleated cells and is produced at a constant rate. The imbalance between cystatin c and cysteine proteinases, is associated with inflammation, renal failure, cancer, Alzheimer's disease, multiple sclerosis and hereditary cystatin c amyloid angiopathy[8]. Because of its small size and basic pH (9.0), cystatin c is freely filtered by the glomeruli[9].

Beta2-microglobulin is a protein found on the surface of many cells. Testing of beta2-microglobulin is done primarily when evaluating a person for certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and multiple myeloma or kidney disease.

Beta2-microglobulin is plentiful on the surface of white blood cells. Increased production or destruction of these cells causes Beta2-microglobulin levels in the blood to increase. This increase is seen in people with cancers involving white blood cells, but it is particularly meaningful in people newly diagnosed with multiple myeloma[9]. When kidney disease is suspected, comparing blood and urine levels helps identify where the kidney is damaged. Beta2-microglobulin normally is filtered out of the blood by the kidney's glomeruli, only to be partially reabsorbed back into the blood when it reaches the kidney's tubules. In glomerular kidney disease, the glomeruli can't filter it

out of the blood, so levels increase in the blood and decrease in the urine. In tubular kidney disease, the tubules can't reabsorb it back into the blood, so urine levels rise and blood levels fall. After a kidney transplant, increased blood levels may be an early sign of rejection [10].

Increased urinary levels of B_2-microlobulin are found in people with kidney damage caused by high exposure to the heavy metals cadmium and mercury. Periodic testing of workers exposed to these metals helps to detect beginning of kidney damage.

1.1 Rationale:

Determination of glomerular filtration rate (GFR) by clearance methods is time consuming and labor intensive. Due to the inaccuracies associated with these methods, the measurement of endogenous blood substances to estimate GFR in the common practice. Properties of an ideal endogenous blood substance to estimate GFR should include release in to the blood stream at constant rate, free filtration by the glomerulus, no reabsorption or secretion by renal tubules, and exclusive elimination via the kidneys.

In the last 40 years, plasma creatinine has become the most commonly used endogenous marker of renal function. Its rate of appearance in the blood stream is related to muscle mass, so that interaindividual concentrations are affected by age and gender.

Calculation of creatinine clearance (CRCL) by determining its concentration in timed urine collections and simultaneously in blood correlates with exogenous gold standard methods better than creatinine . However collection of timed urine is cumbersome and prone to error in outpatient setting. Measurement of creatinine clearance using time (24-hour) urine collection does not improved the estimation of GfR over that provided by prediction equations.

Cystatin C is a 122-amino acid, 13-KDa protein that is a member of the family of cysteine proteinase inhibitors. It is the product of a "housekeeping" gene expressed in all nucleated cells and is produced at a constant rate Because of its small size and basic pH (9.0), cystatin c is freely filtered by glomulus. Cystatin C dose not return to the blood stream and is not secreted by renal tubules, it has been suggested to be closer to the "ideal" endogenous marker.

1.2 Objectives:

1. General objective:

To compare and to assess the serum levels of cystatin c and creatinine as markers of renal function in Sudanese patients with type 2 diabetes.

2. Specific objectives:

i- To measure the serum levels of cystatin c and creatinine in Sudanese patients with type 2diabetes mellitus.

ii- To correlate between the serum levels of cystatin c, creatinine and the glommerlar filtration rate (GFR).

iii-To asses the relationship between the plasma levels of cystatin c, Beta2microglobulin and creatinine versus:

i- Glyclated haemoglobin (HbA1C).

ii- Body Mass Index (B M I).

iii- Duration of diabetes.

Chapter Two

Literature Rev

Chapter Two

2. Literature Review

2.1 Diabetes Mellitus

Diabetes mellitus is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose that caused by relative or absolute deficiency of insulin, which enables cells to absorb glucose in order to turn it into energy. In diabetes, the body either fails to properly respond to its own insulin, does not make enough insulin, or both. This causes glucose to accumulate in the blood, often leading to various complications [11; 12]. Many types of diabetes are recognized: The principal three are: Type 1: Results from the body's failure to produce insulin. It is estimated that 5%-10% of Americans who are diagnosed with diabetes have type 1 diabetes. Almost all persons with type 1 diabetes must take insulin injections. Type 2: Results from Insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with relative insulin deficiency. Most people who are diagnosed with diabetes have type 2 diabetes. Many people who to develop type 2 diabetes spend many years in a state of Pre-diabetes: Termed "America's largest healthcare epidemic," pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. In 2009 there are 57 million Americans who have pre-diabetes [13].

All forms of diabetes have been treatable since insulin became medically available in 1921, but there is no cure for the common types except a pancreas transplant (gestational diabetes usually resolves after delivery). Diabetes and its treatments can cause many complications. Acute complications including hypoglycemia, diabetic ketoacidosis, or non ketotic hyperosmolar coma may occur if the disease is not adequately controlled. Serious long-term complications include cardiovascular disease, chronic renal failure, retinal damage that can lead to blindness, several types of nerve damage, and micro vascular damage that may cause erectile dysfunction and poor wound healing. Poor healing of wounds, particularly of the feet, can lead to gangrene, possibly requiring amputation [14].

2.1.1 Epidemiology of Diabetes Mellitus

In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an

environmental (dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented [15]. For at least 20 years, diabetes rates in North America have been increasing substantially. In 2008 there were about 24 million people with diabetes in the United States alone, from those 5.7 million people remain undiagnosed. Other 57 million people are estimated to have pre-diabetes. The Centers for Disease Control has termed the change an epidemic. The National Diabetes Information Clearing-house estimates that diabetes costs $132 billion in the United States alone every year. About 5%–10% of diabetes cases in North America are type 1, with the rest being type 2. The fraction of type 1 in other parts of the world differs; this is probably due to both differences in the rate of type 1 and differences in the rate of other types, most prominently type 2. Most of this difference is not currently understood. The American Diabetes Association cite the 2003 assessment of the National Center for Chronic Disease Prevention and Health Promotion (Centers for Disease Control and Prevention) that 1 in 3 Americans born after 2000 will develop diabetes in their lifetime. According to the American Diabetes Association, approximately 18.3% (8.6 million) of Americans age 60 and older have diabetes. Diabetes mellitus prevalence increases with age, and the numbers of older persons with diabetes are expected to grow as the elderly population increases in number. The

National Health and Nutrition Examination Survey (NHANES III) demonstrated that, in the population over 65 years old, 18% to 20% have diabetes, with 40% having either diabetes or its precursor form of impaired glucose tolerance. [16; 17]. Indigenous populations in first world countries have a higher prevalence and increasing incidence of diabetes than their corresponding non-indigenous populations. In Australia the age-standardized prevalence of self-reported diabetes in Indigenous Australians is almost 4 times that of non-indigenous Australians. Preventative community health programs such as Sugar Man (diabetes education) are showing some success in tackling this problem [18].

In Africa which is a large continent, but the health care systems of African countries face similar challenges in the delivery of health care. Resources are limited and systems are strained. Diabetes mellitus is no longer rare in Africa. Meta-analytic estimates and recent investigations based on the step-wise approach for monitoring the risk factors of non-communicable diseases indicate a prevalence of between 1% and 20%[19].

The prevalence of DM in African communities is increasing due to an ageing population and lifestyle changes associated with rapid urbanization and westernization. Traditional rural communities still have very low prevalence, at most 1-2%, except in some specific high-risk groups, whereas up to 13% or more adults in urban communities have DM. Type 2

diabetes is the predominant form (70-90%), the rest being represented by typical type 1 patients and patients with atypical presentations. Due to the high urban growth rate, unhealthy dietary changes, reduction in physical activity and increasing obesity it is estimated that the prevalence of diabetes is going to triple within the next 25 years[19]. In addition, long-term complications occur early in the course of diabetes and affect a high proportion of patients, and that could be partly explained by uncontrolled hypertension, poor metabolic control and possible ethnic predisposition. The combination of the rising prevalence of diabetes and the high rate of long-term complications in Africans will lead to a drastic increase of the burden of diabetes on health care systems of African countries. The design and implementation of an appropriate strategy for early diagnosis and treatment, and population-based primary prevention of diabetes in these high-risk populations is therefore a public health priority [20].

The disease burden is very high. Recent literature regarding healthcare for diabetes arises from a limited number of countries in Africa. Where assessments of health care have been made it is clear that the cover and quality of services are well below any reasonable minimum [21]. Unknown diabetes in Africa is in the order of 60% to 80% in cases diagnosed in Cameroon, Ghana and Tanzania [22]. The rate of limb amputations varies from 1.4% to 6.7% of diabetic foot cases. Annual mortality linked to

diabetes worldwide is estimated at more than one million [23]. In some countries of the Region, the mortality rate is higher than 40 per 10 000 inhabitants. Diabetes is particularly common in Egypt, with a prevalence of 4.3% [24].

The rising prevalence of diabetes, its increasing morbidity and mortality, its disproportionate effect on disadvantaged individuals, communities and nations, and its high human and economic costs clearly establish diabetes as a significant global public health problem [25; 26].

The prevalence and incidence rates of DM in Sudan, as in many other low-income countries, are increasing to epidemic proportions, leading to the emergence of a public health problem of major socio-economic impact. In the northern states the crude prevalence in 1992 reached 3.4% in those less than 25 years of age [27; 28]. It was found to be 5.5% in the Northern State and 8% in Khartoum State. The prevalence was particularly high (10.8%) in a certain community in the Northern State [29]. Type I DM is not rare in Sudan, the prevalence being approximated to 0.1 % among children 7-14 years of age [30].

Diabetes mellitus in Sudan is associated with poor glycaemic control, a high prevalence of complications, a low quality of life, and particularly with morbidity [29]. Patients with a median duration of diabetes of 9 years showed a high prevalence of micro- and macro vascular complications [31].

2.1.2 Classification of Diabetes Mellitus

In 1979, the national diabetes data group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into two broad categories, Type (1) Insulin- Dependent Diabetes Mellitus (IDDM); and Type (2), Non Insulin Dependent Diabetes Mellitus (NIDDM). Established in 1995, the International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, working under the sponsorship of the ADA, was given the task of updating the 1979 classification system. The proposed changes included eliminating the older terms of IDDM and NIDDM. The categories of type 1 and type 2 were retained, with adoption of Arabic numerals instead of Roman numerals [32].

Therefore, the ADA/Word Health Organization (WHO) guidelines recommend these categories which are, Type 1diabetes, Type 2 diabetes, other specific types of diabetes and Gestational diabetes mellitus (GDM) [18].

(i) Type 1 Diabetes Mellitus

Is characterized by inappropriate hyperglycemia primarily result of pancreatic islets β- cell destruction and a tendency to ketoacidosis. Type 2 diabetes, in contrast, includes hyperglycemia cases that result from insulin resistance with an insulin secretory defect. An intermediate stage, in which the fasting glucose increased above- normal limits but not to the level of diabetes, has been named impaired fasting glucose. The

term impaired glucose tolerance to indicate glucose tolerance values above normal but below diabetes levels was retained. Also, the term gestational diabetes mellitus was retained for women who develop glucose intolerance during pregnancy[19].

Type 1 diabetes mellitus is a result of cellular- mediated autoimmune destruction of β cells of the pancreas, causing an absolute deficiency of insulin secretion. Upper limit 110 mg/dl on the fasting plasma glucose is designated as the upper limit of normal blood glucose. Type1 constitutes only 10-20% of all diabetes and commonly occur in childhood and adolescence. This disease is usually initiated by an environmental factor or infection (usually a virus) in individuals with a genetic predisposition and causes the immune destruction of the β cells of the pancreas, and therefore, a decreased production of insulin. Characteristic of type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency. This diabetic type is genetically related. One or more of the following markers are found in 85-90% of individuals with fasting hyperglycemia: islet cell autoantibodies, insulin autoantibodies, glutamic acid decarboxylase, autoantibodies, and tyrosine phosphate 1A-2 and 1A-2B autoantibodies [32].

Sign and symptoms include polydipsia (excessive thirst), polyphagia (increased food intake), polyuria (excessive urine production), rapid weight loss, hyperventilation, mental confusion, and possible loss, of consciousness (due to increase

glucose to brain).complications include micro vascular problems such as nephropathy, neuropathy, and retinopathy. Increased heart disease is also found in patients with diabetes. Idiopathic type 1 diabetes is a form of type 1 diabetes that's has no known etiology, is strongly inherited, and dose not have β cell autoimmunity. Individuals with this form of diabetes have episodic requirements for insulin replacement [13].

(ii) Type 2 diabetes Mellitus:

Type 2 characterized by hyperglycemia as result of individual resistance to insulin with insulin secretory defect. This resistance results in a relative, not an absolute, insulin deficiency. Type 2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abdominal region. This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, with patients at increased risk with an in age, obesity, and lack of physical exercise. Characteristics usually include adult onset of the disease and milder symptoms than in type 1, with ketoacidosis seldom occurring. How ever, these patients are more likely to go into a hyperosmolar coma and are at an increased risk of developing macro vascular and micro vascular complications [32].

There are many factors which can potentially give rise to or exacerbate type 2 diabetes. These include obesity, hypertension, elevated cholesterol (combined hyperlipidemia), and with the

condition often termed metabolic syndrome (it is also known as Syndrome X, Reavan's syndrome) [33]. Other causes include acromegaly, Cushing's syndrome, thyrotoxicosis, pheochromocytoma, chronic pancreatitis, cancer and drugs. Additional factors found to increase the risk of type 2 diabetes include aging, high-fat diets and a less active lifestyle [34].

Subclinical Cushing's syndrome (cortisol excess) may be associated with DM type 2 [35]. The percentage of subclinical Cushing's syndrome in the diabetic population is about 9% [36]. Diabetic patients with a pituitary micro adenoma can improve insulin sensitivity by removal of these micro adenomas [37].

Hypogonadism is often associated with cortisol excess, and testosterone deficiency is also associated with diabetes mellitus type 2 [38], even if the exact mechanism by which testosterone improve insulin sensitivity is still not known [39].

(iii) Other specific types of diabetes (secondary Diabetes)

Other types associated with certain conditions (secondary), including genetic defect of β- cell function or insulin action, pancreatic disease, diseases of endocrine origin, drug or chemical induced insulin receptor abnormalities, and certain genetic syndromes. The characteristic and prognosis of this form of diabetes depends on the primary disorder. Maturity- onset diabetes of youth (MODY) is a rare form of diabetes that is inherited in an autosomal dominant fashion [40].

(iv) Gestational diabetes mellitus

Is “any degree of glucose intolerance with onset or first recognition during pregnancy”, causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal postpartum. However, this disease is associated with increased perinatal complications and an increased risk for development of diabetes and an increased risk for respiratory distress syndrome, hypocalcemia, and hyperbilirubinemia. Fetal insulin secretion is stimulated in the neonate of a mother with diabetes. However, when the infant is born and the umbilical cord is severed, the infant's oversupply of glucose is abruptly terminated, causing sever hypoglycemia [32].

2.1.3 Pathophysiology of Diabetes Mellitus

In both type1and type 2diabetes, the individual is hyperglycemic, which can be sever. Glucosuria can also occur after the renal tubular transporter system for glucose becomes saturated. This happens when the glucose concentration of plasma exceeds roughly 180mg/dl in an individual with normal renal function and urine output. As hepatic glucose overproduction continues, the plasma glucose concentration reaches a plateau around 300-500mg/dl (17-28mmoL/L). Provided renal output is maintained, glucose excretion will match the overproduction, causing the plateau. The individual with type1 diabetes has a higher tendency to produce ketones, patient with type 2diabetes seldom generate ketones, but instead have a greater tendency to develop hyperosmolar nonketotic states. The difference in glucagons and

insulin concentrations in these two groups appear to be responsible for the generation of ketones through increased β-oxidation. In type 1, there is an absence of insulin with an excess of glucagons. This permits gluconeogenesis and lipolysis to occur. In type 2, insulin is present as is (at times) hyperinsulinemia; therefore, glucagons is attenuated. Fatty acid oxidation is inhibited in type2. This causes fatty acid to be incorporated into triglycerides for release as very- low density lipoproteins [31].

The laboratory findings of a patient with diabetes with ketoacidosis tend to reflect dehydration, electrolyte disturbances, and acidosis. Acetoacetate, β- hydroxybutyrate, and acetone are produced form the oxidation of fatty acids. The two former ketone bodies contribute to the acidosis. Lactate, fatty acid, and other organic acid can also contribute to a lesser degree. Bicarbonate and total carbon dioxide are usually decreased due to kussmaul- kien respiration (deep respiration).this a compensatory mechanism to blow off carbon dioxide and remove hydrogen ions in the process. The anion gap in this acidosis can exceed 16 mmol/L. serum osmolality is high as a result of hyperglycemia; sodium concentrations tent to be lower due in part to losses(polyuria) and in part to a shift of water from cells because of the hyperglycemia. The sodium value should not be falsely underestimated because of hypertriglyceridemia. Grossly elevated triglycerides will displace plasma volume and give the

appearance of decreased electrolytes when flame photometry or pre diluted, ion-specific electrodes are used for sodium determinations. Hyperkalemia is almost always present as a result of the displacement of potassium from cells in acidosis. This somewhat misleading because the patient's total body potassium is usually decreased [33].

More typical of untreated patient with type 2 diabetes is a nonketotic hyperosmolar state. The individual presenting with this syndrome has an overproduction of glucose; however, there appears to be an imbalance between production and elimination in urine. Often, this state is precipitated by heart disease, stroke, or pancreatitis. Glucose concentrations exceed 300-500 mg/dL (17-28mmol/L) and sever dehydration is present. The sever dehydration contributes to the inability to excrete glucose in the urine. Mortality is high with this condition. Ketones are not observed because the severe hyperosmolar state inhibits the ability of glucagons to stimulate lipolysis. The laboratory findings of nonketotic hyperosmolar coma include plasma glucose values exceeding 1000 mg/dL(55 mmol/L), normal or elevated plasma sodium or potassium, slightly decreased bicarbonate, elevated blood urea nitrogen (BUN) and creatinine, and an elevated osmolality. The gross elevation in glucose and osmolality, the elevation in BUN, and the absence of ketones distinguish this condition from diabetic ketoacidosis. Other form of impaired glucose metabolism that does not meet the criteria

for diabetes mellitus includes impaired fasting glucose and impaired glucose tolerance [32].

2.1.4 Genetics of Diabetes Mellitus

Both type 1 and type 2 diabetes are at least partly inherited. Type 1 diabetes appears to be triggered by some (mainly viral) infections, with some evidence pointing at Coxsackie B4 virus. There is a genetic element in individual susceptibility to some of these triggers which has been traced to particular HLA genotypes (i.e., the genetic "self" identifiers relied upon by the immune system). However, even in those who have inherited the susceptibility, type 1 diabetes mellitus seems to require an environmental trigger. There is a stronger inheritance pattern for type 2 diabetes. Those with first-degree relatives with type 2 have a much higher risk of developing type 2, increasing with the number of those relatives. Concordance among monozygotic twins is close to 100%, and about 25% of those with the disease have a family history of diabetes. Genes significantly associated with developing type 2 diabetes, include TCF7L2, PPARG, FTO, KCNJ11, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1, and HHEX. KCNJ11 (potassium inwardly rectifying channel, subfamily J, member 11), encodes the islet ATP-sensitive potassium channel Kir6.2, and TCF7L2 (transcription factor 7–like 2) regulates proglucagon gene expression and thus the production of glucagon-like peptide-1. Moreover, obesity

(which is an independent risk factor for type 2 diabetes) is strongly inherited [41].

Various hereditary conditions may feature diabetes, for example myotonic dystrophy and Friedreich's ataxia. Wolfram's syndrome is an autosomal recessive neurodegenerative disorder that first becomes evident in childhood. It consists of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness, hence the acronym DIDMOAD. Gene expression promoted by a diet of fat and glucose as well as high levels of inflammation related cytokines found in the obese results in cells that "produce fewer and smaller mitochondria than is normal," and are thus prone to insulin resistance[42].

2.1.5 Diagnosis of Diabetes Mellitus

The diagnosis of type1 diabetes and many cases of type 2, is usually prompted by recent-onset symptoms of excessive urination (polyuria) and excessive thirst (polydipsia), and often accompanied by weight loss. These symptoms typically worsen over days to weeks; about a quarter of people with new type 1 diabetes have developed some degree of diabetic ketoacidosis (Ketoacidosis is a type of metabolic acidosis which is caused by high concentrations of ketone bodies, formed by the breakdown of fatty acids and the deamination of amino acids), by the time the diabetes is recognized. The diagnosis of other types of diabetes is usually made in other ways. These include ordinary health screening; detection of hyperglycemia during other

medical investigations; and secondary symptoms such as vision changes or unexplainable fatigue. Diabetes is often detected when a person suffers a problem that is frequently caused by diabetes, such as a heart attack, stroke, neuropathy, poor wound healing or a foot ulcer, certain eye problems, certain fungal infections, or delivering a baby with macrosomia or hypoglycemia. Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the followings, fasting plasma glucose level at or above 126 mg/dL(7.0 mmol/L), plasma glucose at or above 200 mg/dL (11.1 mmol/L) two hours after a 75 g oral glucose load as in a glucose tolerance test. Symptoms of hyperglycemia and casual plasma glucose at or above 200 mg/dL(11.1 mmol/L). A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above-listed methods on a different day. Most physicians prefer to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/L) is considered diagnostic for diabetes mellitus. Patients with fasting glucose levels from 100 to 125 mg/dL (6.1 and 7.0 mmol/L) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140

mg/dL or 7.8 mmol/L, but not over 200, two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two pre-diabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus as well as cardiovascular disease. [43; 44; 45]. While not used for diagnosis, an elevated level of glucose irreversibly bound to hemoglobin (termed glycated hemoglobin or HbA1c) of 6.0% or higher (the 2003 revised U.S. standard) is considered abnormal by most labs; HbA1c is primarily used as a treatment-tracking test reflecting average blood glucose levels over the preceding 90 days (approximately) which is the average lifetime of red blood cells which contain hemoglobin in most patients. However, some physicians may order this test at the time of diagnosis to track changes over time. The current recommended goal for HbA1c in patients with diabetes is 6.5% [46; 47].

2.1.6 Monitoring of blood glucose

Self-monitoring of blood glucose may not improve outcomes in some cases, that is among "reasonably well controlled non-insulin treated patients with type 2 diabetes". Nevertheless, it is very strongly recommended for patients in whom it can assist in maintaining proper glycemic control, and is well worth the cost (sometimes considerable) if it does. It is the only source of current information on the glycemic state of the body, as changes are rapid and frequent, depending on food, exercise, and medication (dosage and timing with respect to both diet and

exercise) and secondarily, on time of day, stress (mental and physical) and infection [48].

The National Institute for Health and Clinical Excellence (NICE), UK released updated diabetes recommendations on 30 May 2008. They indicate that self-monitoring of blood glucose levels for people with newly diagnosed type 2 diabetes should be part of a structured self-management education plan. However, a recent study found that a treatment strategy of intensively lowering blood sugar levels (below 6%) in patients with additional cardiovascular disease risk factors poses more harm than benefit, and so there appear to be limits to benefit of intensive blood glucose control in some patients [49].

2.1.7Glycated hemoglobin (HbA1C)

Also named as glcosylated hemoglobin, is formed by a post-translational, non-enzymatic, substrate-concentration dependent irreversible process of combination of aldehyde group of glucose and other hexoses with the amino-terminal valine of the alpha-chain of hemoglobin and the rate of combination is directly proportional to the plasma glucose concentration. Because the average red blood cell lives approximately 120 days, the glycated hemoglobin level at any one time reflect the average blood glucose level over the previous 2-3 months [50]. Glycated hemoglobin is recommended for both, checking blood sugar control in people who might be pre-diabetic and monitoring blood sugar control in patients with more elevated levels, termed

diabetes mellitus. There are a significant proportion of people who are unaware of their elevated HbA1c level before they have blood lab work. The American Diabetes Association guidelines are similar to others in advising that the glcosylated hemoglobin test be performed at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals, also it added the A1c ≥ 6.5% as another criteria for the diagnosis of diabetes [15; 51]. Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals. Two individuals with the same average blood sugar can have A1C values that differ by as much as 3 percentage points. In general, the reference range (that found in healthy persons), is about 4%–5.9%. Higher levels of HbA1c are found in people with persistently elevated blood sugar, as in diabetes mellitus. While diabetic patient treatment goals vary, many include a target range of HbA1c values. A diabetic person with good glucose control has a HbA1c level that is close to or within the reference range [52; 53].

In autoimmune hemolytic anemia, concentrations of hemoglobin A1 (HbA1) is undetectable. Administration of prenidsolone (PSL) will allow the HbA1 to be detected. The alternative fructosamine test may be used in these circumstances and it

similarly reflects an average of blood glucose levels over the preceding 2 to 3 weeks [54].

2.1.8 Complications of Diabetes mellitus

Patient education, understanding, and participation are vital since the complications of diabetes are far less common and less severe in people who have well-controlled blood sugar levels. Wider health problems accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. According to one study, women with high blood pressure (hypertension) were three times more likely to develop type 2 diabetes as compared with women with optimal BP after adjusting for various factors such as age, ethnicity, smoking, alcohol intake, body mass index (BMI), exercise, family history of diabetes[52].

In diabetes mellitus, severe hyperglycemia may result from absolute or relative insulin deficiency. In some patients, the condition may culminate in diabetic ketoacidosis or hyperglycemic hyperosmolar nonketotic coma. Profound hypoglycemia may result from a relative excess of insulin. Symptoms associated with acute hyperglycemia generally develop more slowly (over hours or days) than do symptoms associated with an acute fall in the level of blood glucose (over minutes) [45].

(i) Hypoglycemia

Hypoglycemia is the medical term for a state produced by a lower than normal level of blood glucose level below approximately 60 mg/dL. Hypoglycemia can produce a variety of symptoms and effects but the principal problems arise from an inadequate supply of glucose as fuel to the brain, resulting in impairment of function (neuroglycopenia), other symptoms associated with adrenergic symptoms (apprehension, tremors, sweating, or palpitations). Usually, the symptoms of low blood glucose are mild, related to catecholamine release, and easily treated by the patient. Effects can range from vaguely "feeling bad" to seizures, unconsciousness, and (rarely) permanent brain damage or death. The most common forms of moderate and severe hypoglycemia occur as a complication of treatment of diabetes mellitus with insulin or oral medications. Hypoglycemia is less common in non-diabetic persons, but can occur at any age, from many causes. Among the causes are excessive insulin produced in the body, inborn errors of carbohydrate, fat, amino acid or organic acid metabolism, medications and poisons, alcohol, hormone deficiencies, certain tumors, prolonged starvation, and alterations of metabolism associated with infection or failures of various organ systems [55; 56].

(ii) Diabetic ketoacidosis (DKA)

It is a potentially life-threatening complication in patients with diabetes mellitus. It happens predominantly in those with type 1

diabetes, but it can occur in those with type 2 diabetes under certain circumstances. DKA was first described in 1886; until the introduction of insulin therapy in the 1920s it was almost universally fatal. It still carries a significant mortality of up to 5%[57; 58]..

DKA may be the first symptom of previously undiagnosed diabetes, but it may also occur in known diabetics due to a variety of causes, such as intercurrent illness or poor compliance with insulin therapy. Vomiting, dehydration, deep gasping breathing, confusion and occasionally coma are typical symptoms. DKA is diagnosed with blood and urine tests; it is distinguished from other, rarer forms of ketoacidosis by the presence of high blood sugar levels. Treatment involves intravenous fluids to correct dehydration, insulin to suppress the production of ketone bodies, treatment for any underlying causes such as infections, and close observation to prevent and identify complications[59; 60].

(iii) Nonketotic hyperosmolar coma

Nonketotic hyperosmolar coma (nonketotic hyperglycemia) is a type of diabetic coma associated with a high mortality seen in diabetes mellitus type 2. The preferred term used by the American Diabetes Association is hyperosmolar nonketotic state (HNS). Other commonly used names are hyperosmolar hyperglycemic nonketotic coma (HHNKC) or hyperosmotic non-ketotic coma (HONKC). It is also called Hyperglycemic

Hyperosmolar State (HHS), as some patients may have some ketonuria and it does not necessarily cause coma. Nonketotic coma is usually precipitated by an infection, myocardial infarction, stroke or another acute illness [61; 62]. A relative insulin deficiency leads to a serum glucose that is usually higher than 33 mmol/l (600 mg/dl) and a resulting serum osmolarity that is greater than 350 mOsm. This leads to polyuria (an osmotic diuresis), which, in turn, leads to volume depletion and hemoconcentration that causes a further increase in blood glucose level. Ketosis is absent because the presence of some insulin inhibits lipolysis, unlike diabetic ketoacidosis. The increasing hemoconcentration and volume depletion may result in hyperviscosity, disordered mental functioning, and neurologic sign and ultimately, if untreated, will lead to death. Treatment of HHS begins with reestablishing tissue perfusion using intravenous fluids. People with HHS can be dehydrated by 8 to 12. Attempts to correct this usually take place over 24 hrs with initial rates of normal saline often in the range of 1 l/hr for the first few hours [63].

(b) Chronic complications of diabetes

(i) Atherosclerosis

It is commonly referred to as a hardening or furring of the arteries by the formation of multiple plaques within the arteries, also defined as condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol [64].

Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized by free radicals, particularly reactive oxygen species (ROS). When oxidized LDL comes in contact with an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized LDL. The LDL molecule is globular shaped with a hollow core to carry cholesterol throughout the body. Cholesterol can move in the bloodstream only by being transported by lipoproteins. The body's immune system responds to the damage to the artery wall caused by oxidized LDL by sending specialized white blood cells (macrophages and T-lymphocytes) to absorb the oxidized-LDL forming specialized foam cells. Unfortunately, these white blood cells are not able to process the oxidized-LDL, and ultimately grow then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle[65]. Eventually the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery, reduces the blood flow and increases blood pressure. These complications of advanced atherosclerosis are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures, causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery in approximately 5 minutes. Since atherosclerosis is a body-wide

process, similar events occur also in the arteries to the brain, intestines, kidneys, legs. Numerous studies have shown that patients with diabetes mellitus have accelerated atherosclerotic vascular disease, and major advances in understanding it pathogenesis have been made as mention above[66].

(ii) Stroke

A stroke (sometimes called a cerebrovascular accident (CVA)is the rapidly developing loss of brain function(s) due to disturbance in the blood supply to the brain, caused by a blocked or burst blood vessel. This can be due to ischemia (lack of glucose and oxygen supply) caused by thrombosis or embolism or due to a hemorrhage. As a result, the affected area of the brain is unable to function, leading to inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or inability to see one side of the visual field [67; 68]. The traditional definition of stroke, devised by the World Health Organization, is a "neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours". This definition was supposed to reflect the reversibility of tissue damage and was devised for the purpose, with the time frame of 24 hours being chosen arbitrarily. The 24-hour limit divides stroke from transient ischemic attack, which is a related syndrome of stroke symptoms that resolve completely within 24 hours [68; 69]. With the availability of treatments that, when given early, can reduce stroke severity, many now prefer

alternative concepts, such as brain attack and acute ischemic cerebrovascular syndrome (modeled after heart attack and acute coronary syndrome respectively), that reflect the urgency of stroke symptoms and the need to act swiftly [70].

A stroke is occasionally treated with thrombolysis (clot buster), but usually with supportive care (speech and language therapy, physiotherapy and occupational therapy) in a "stroke unit" and secondary prevention with antiplatelet drugs (aspirin and often dipyridamole), blood pressure control, statins, and in selected patients with carotid endarterectomy and anticoagulation. Stroke symptoms typically start suddenly, over seconds to minutes and depend on the area of the brain affected, loss of consciousness, headache, and vomiting usually occurs more often in hemorrhagic stroke than in thrombosis because of the increased intracranial pressure from the leaking blood compressing on the brain [68].

(iii) Eye disease (Diabetic Retinopathy)

Diabetic retinopathy is retinopathy (damage to the retina) caused by complications of diabetes mellitus, which can eventually lead to blindness. It is an ocular manifestation of systemic disease which affects up to 80% of all patients who have had diabetes for 10 years or more. Despite these intimidating statistics, research indicates that at least 90% of these new cases could be reduced if there was proper and vigilant treatment and monitoring of the eyes [11; 71].

Diabetes mellitus is a major cause of blindness in the United States and is the leading cause of new blindness in working-aged Americans. Diabetic retinopathy alone accounts for at least 12% of new cases of blindness each year in the United States. People with diabetes are 25 times more at risk for blindness than the general population. Over a lifetime, 70% of people with insulin-dependent diabetes mellitus (IDDM) will develop proliferative diabetic retinopathy, and 40% will develop macular edema. Both complications, if untreated, frequently lead to serious visual loss and disability. Diabetic retinopathy is often asymptomatic in its most treatable stages. Unfortunately, only about half of persons with diabetes receive adequate eye care. So that early detection of diabetic retinopathy is very critical [72; 73].

Retinopathy was evident in approximately 43%, dipstick proteinuria in 22% and neuropathy in 37%. Cardiovascular disease was reported in 28%. Peripheral vascular disease was reported in 10% and cerebrovascular accidents in 5.5%. As expected, patients with complications were significantly older, had longer disease duration and had higher serum cholesterol and triglyceride concentrations. The glycaemic control was only acceptable (1-IBA1c <7.5%) in 12.5% of the patients. The reasons for poor metabolic control are not difficult to understand. Drugs are extremely costly relative to income[74].

(iv) Other cardiovascular complications

Other cardiovascular complication include, Heart Failure: Heart failure is a chronic condition in which the heart cannot pump blood properly—it does not mean that the heart suddenly stops working. Heart failure develops over a period of years, and symptoms can get worse over time. People with diabetes have at least twice the risk of heart failure as other people. One type of heart failure is congestive heart failure, in which fluid builds up inside body tissues. If the buildup is in the lungs, breathing becomes difficult [16].

(v) Neuropathy and foot problems

Persons with diabetes who develop neuropathy may have no symptoms or may experience pain, sensory loss, weakness and autonomic dysfunction. Neuropathy may result in significant morbidity and may contribute to other major complications, such as lower extremity amputation. Diabetes is reaching epidemic proportions and with it carries the increased risk of complications. Disease of the foot is among one of the most feared complications of diabetes [75; 76]. The term 'Diabetic Foot' consists of a mix of pathologies including diabetic neuropathy, peripheral vascular disease, Charcot's neuroarthropathy, foot ulceration, osteomyelitis and the potentially preventable endpoint, limb amputation. The lifetime risk of a person with diabetes developing foot ulceration is reported to be as high as 25% [11; 77]. It is estimated that more than a million people with diabetes require limb amputation each

year, suggesting that one major amputation is performed worldwide every 30 s. Amputation is associated with significant morbidity and mortality, besides having immense social, psychological and financial consequences [78; 79].

(vi) Kidney disease (Diabetic Nephropathy)

Diabetic nephropathy represents a distinct clinical syndrome characterized by albuminuria, hypertension, and progressive renal insufficiency. Diabetic nephropathy can lead to end-stage renal disease (ESRD), a serious condition in which a patient's survival depends on either dialysis or kidney transplantation. Among persons who have had insulin-dependent diabetes mellitus (IDDM) for 20 years, the incidence of ESRD approaches 40%. [80; 81]. Renal disease is a common and often severe complication of diabetes.45 Approximately 35% of patients with type 1 diabetes of 18 years' duration will have signs of diabetic renal involvement. Up to 35% of new patients beginning dialysis therapy have type 2 diabetes. End-stage renal disease (ESRD) appears to be especially common among Hispanics, blacks, and Native Americans with diabetes. For patients with diabetes who are on renal dialysis, mortality rates probably exceed 20% per year. When diabetes is present, CVD is the leading cause of death among patients with ESRD [82; 83]

Diabetic nephropathy (nephropatiadiabetica), also known as Kimmelstiel-Wilson syndrome, or nodular diabetic glomerulosclerosis [84] and intercapillary glomerulonephritis, is a

progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. It is characterized by nephrotic syndrome and diffuse glomerulosclerosis. It is due to longstanding diabetes mellitus, and is a prime indication for dialysis in many Western countries.

The syndrome was discovered by British physician Clifford Wilson (1906–1997) and German-born American physician Paul Kimmelstiel (1900–1970) and was published for the first time in 1936. [85]

The syndrome can be seen in patients with chronic diabetes (usually less than 15 years after onset) after about 5 years in type 1 diabetes. Clinical nephropathy secondary to glomerular disease usually manifests 15–25 years after diagnosis of diabetes and affects 25-35% of patients under the age of 30 years. It is the leading cause of premature death in young diabetic patients.(between 50 and 70 years old). The disease is progressive and may cause death two or three years after the initial lesions, and is more frequent in men. Diabetic nephropathy is the most common cause of chronic kidney failure and end-stage kidney disease in the United States. People with both type 1 and type 2 diabetes are at risk. The risk is higher if blood-glucose levels are poorly controlled. Furthermore, once nephropathy develops, the greatest rate of progression is seen in patients with poor control of their blood pressure. Also people with high

cholesterol level in their blood have much more risk than others[84].

The earliest detectable change in the course of diabetic nephropathy is a thickening in the glomerulus. At this stage, the kidney may leak more serum albumin (plasma protein) than normal in the urine (albuminuria), and this can be detected by sensitive medical tests for albumin. This stage is called "microalbuminuria". As diabetic nephropathy progresses, increasing numbers of glomeruli are destroyed by progressive nodular glomerulosclerosis. Consequently, urine albumin increases to the point that it may be detected by ordinary urinalysis techniques. At this stage, a kidney biopsy generally clearly shows diabetic nephropathy [85].

The Armani-Ebstein change (or Armani-Ebstein cells) consists of deposits of glycogen in the tubular epithelial cells (pars straight of proximal convoluted tubule and loop of Henle). Because most diabetics are treated before this stage, it is very rare to see it at the present time. It appears in decompensated diabetics with glycemia higher than 500 mg/dL and in the presence of severe glycosuria; it is a reversible alteration without functional manifestations. The interstitium shows unspecific chronic changes [84].

Kidney failure provoked by glomerulosclerosis leads to fluid filtration deficits and other disorders of kidney function. There is an increase in blood pressure (hypertension) and fluid retention

in the body plus a reduced plasma oncotic pressure causes edema. Other complications may be arteriosclerosis of the renal artery and proteinuria [85].

Throughout its early course, diabetic nephropathy has no symptoms. They develop in late stages and may be a result of excretion of high amounts of protein in the urine or due to renal failure [85]:

- The first laboratory abnormality is a positive microalbuminuria test. Most often, the diagnosis is suspected when a routine urinalysis of a person with diabetes shows too much protein in the urine (proteinuria). The urinalysis may also show glucose in the urine, especially if blood glucose is poorly controlled. Serum creatinine and BUN may increase as kidney damage progresses.
- A kidney biopsy confirms the diagnosis, although it is not always necessary if the case is straightforward, with a documented progression of proteinuria over time and presence of diabetic retinopathy on examination of the retina of the eyes.

2.2 The kidney:

The kidneys are paired, bean-shaped organs located retroperitoneally on either side of the spinal column. Macroscopically, a fibrous capsules of connective tissue .encloses each kidney. When dissected longitudinally, two regions can be clearly discerned—an outer region called the cortex and an inner region called the medulla. The pelvis can also be seen. It is a basin-like cavity at the upper end of the ureter into which newly formed urine passes. The bilateral ureters are thick-walled canals, connecting the kidneys to the urinary bladder. Urine is temporarily stored in the bladder until voided from the body by way of the urethra. Kidney, functional units of the kidney that can only be .seen microscopically. Each kidney contains approximately million nephrons [(86).]

The nepheron is composed of [(87)]:

(i) The glomerulus—a capillary tuft surrounded by the expanded end of a renal tubule known as Bowman's capsule. Each glomerulus is supplied by an afferent arteriole carrying the blood in and an efferent arteriole carrying the blood out. The efferent arteriole branches into peritubular capillaries that supply the tubule.

(ii) The proximal convoluted tubule—located in the cortex.

(iii) The long loop of Henle—composed of the thin descending limb, which spans the medulla, and the ascending limb, which is

located in both the medulla and the cortex, composed of a region that is thin and then thick.

(iv) The distal convoluted tubule—located in the cortex.

(v)The collecting duct—formed by two or more distal convoluted tubules as they pass back down through the cortex and the medulla to collect the urine that drains from each nephron. Collecting ducts eventually merge and empty their contents into the renal pelvis. The following section describes how each part of the nephron normally functions[87].

2.2.1 Renal Functions:

The kidneys perform a wide range of functions for the body, most of which are essential for life. Some renal functions have obvious logical and necessary connections to each other. Others seem to be totally independent. Most involve matching renal excretion of substances out of the body to inputs into the body (ie, providing a balance between input and output) [(88)].

A popular view considers the kidney to be an organ primarily responsible for the removal of metabolic waste products from the body. Although this is certainly one function of the kidney, there are other functions that are arguably more important. These functions include[(89)]:

(i) : Regulation of Water and Electrolyte Balance:

The balance concept states that our bodies are in balance for any substance when the inputs and outputs of that substance are matched. Any difference between input and output leads to an increase or decrease in the amount of a substance within the body. Our input of water and electrolytes is enormously variable and is only sometimes driven in response to body needs. Although we drink water when thirsty, we drink much more because it is a component of beverages that we consume for reasons other than hydration. We also consume food to provide energy, but food often contains large amounts of water. The kidneys respond by varying the output of water in the urine, thereby maintaining balance for water (ie, constant total body

water content). Minerals like sodium, potassium, magnesium, and so on are components of foods and generally present far in excess of body needs. As with water, the kidneys excrete minerals at a highly variable rate that, in the aggregate, matches input. One of the amazing feats of the kidneys is their ability to regulate each of these minerals independently (ie, we can be on a high-sodium, low-potassium diet or low-sodium, high-potassium diet, and the kidneys will adjust excretion of each of these substances appropriately) [90].

One point to emphasize, and one that is commonly misunderstood, is that when we have an unusually high or low level of a substance in our body relative to normal, this does not imply that we are perpetually out of balance. To raise the level of a substance in the body, we must be transiently in positive balance. However, once that level reaches a constant value with input and output again equal, we are back in balance. Consider the case of urea, a substance the liver synthesizes continuously. In normal conditions, the kidneys excrete urea at the rate it is synthesized in the body. We are normally in balance for urea. If damage to the kidneys occurs, excretion is transiently decreased, and urea accumulates in the body. The higher levels of urea in the blood restore renal urea excretion to its former value despite the damaged kidneys, and we are back in balance even though levels in the body remain high. The same applies to more complex substances, such as acids and bases. When we develop a

metabolic acidosis, acid input transiently exceeds acid output. This leads to an accumulation of acids, which, in turn, stimulates renal excretion of acid. Soon excretion matches input (we are back in balance), but there is an elevated amount of acid in the body[89].

(ii) Excretion of Metabolic Waste products:

Our bodies continuously form end products of metabolic processes. In most cases, those end products serve no function and are harmful at high concentrations. Some of these waste products include urea (from protein), uric acid (from nucleic acids), creatinine (from muscle creatine), the end products of hemoglobin breakdown (which give urine much of its color), and the metabolites of various hormones, among many others[87].

(iii)Excretion of Bioactive Substances (Hormones and Many Foreign Substances, Specifically Drugs) That Affect Body Function:

Physicians have to be mindful of how fast the kidneys excrete drugs in order to prescribe a dose that achieves the appropriate body levels. Hormones in the blood are removed in many ways, mostly in the liver, but a number of hormones are removed in parallel by renal processes [88].

(iv)Regulation of Arterial Blood Pressure:

Although many people appreciate at least vaguely that the kidneys excrete waste substances like urea (hence the name urine) and salts, few realize the kidneys' crucial role in

controlling blood pressure. Blood pressure ultimately depends on blood volume, and the kidneys' maintenance of sodium and water balance achieves regulation of blood volume. Thus, through volume control, the kidneys participate in blood pressure control. They also participate in regulation of blood pressure via the generation of vasoactive substances that regulate smooth muscle in the peripheral vasculature [90].

(v)Regulation of Red Blood Cell Production:

Erythropoietin is a peptide hormone that is involved in the control of erythrocyte (red blood cell) production by the bone marrow. Its major source is the kidneys, although the liver also secretes small amounts. The renal cells that secrete it are a particular group of cells in the interstitium. The stimulus for its secretion is a reduction in the partial pressure of oxygen in the kidneys, as occurs, for example, in anemia, arterial hypoxia, and inadequate renal blood flow. Erythropoietin stimulates the bone marrow to increase its production of erythrocytes. Renal disease may result in diminished erythropoietin secretion, and the ensuing decrease in bone marrow activity is one important causal factor of the anemia of chronic renal disease [86].

(vi)Regulation of Vitamin D Production:

When we think of vitamin D, we often think of sunlight or additives to milk. In vivo vitamin D synthesis involves a series of biochemical transformations, the last of which occurs in the kidneys. The active form of vitamin D (1,25-dihydroxyvitamin

D3), is actually made in the kidneys, and its rate of synthesis is regulated by hormones that control calcium and phosphate balance [87].

(vii)Gluconeogenesis:

Our central nervous system is an obligate user of blood glucose regardless of whether we have just eaten sugary doughnuts or gone without food for a week. Whenever the intake of carbohydrate is stopped for much more than half a day, our body begins to synthesize new glucose (the process of gluconeogenesis) from noncarbohydrate sources (amino acids from protein and glycerol from triglycerides). Most gluconeogenesis occurs in the liver, but a substantial fraction occurs in the kidneys, particularly during a prolonged fast [88].

2.2.2 Assesment of Renal Functions:

2.2.2.1 Assessment of Glomerular Function

The glomerular filtrate is an ulterafiltrate of plasma and has the same composition as plasma without most of the protiens. Plasmais filtered by the glomeruli at a rate of approximately 140 ml/minute. Anormal glomerular filtration rate (GFR) will depend on there being normal renal blood flow and pressure. GFR is directly related to body size, and consequently is higher in men than women. It is also affected by age declining in the elderly. If the [GFR] falls due to of the renal blood supply or as a result of destruction of nephrons by renal disease, there is retention of the waste products of metabolism in the blood. In chronic disease

anew steady state is reached with a constant elevation in the serum concentration of substances such as urea and creatinine. As the renal disease progresses, urea and creatinine concentration may increase slowly over many months [86].

(i)Clearance:

The problem is how to measure the GFR. It helps to consider firstly the concept of clearance. Consider any plasma constituent which is also present in urine the amount excreted can be calculated by measuring the urinary concentration (*U*, say mmoles per litre) and multiplying that by the volume of urine collected in a given time (*V*, say litres in a 24h period). Thus the amount excreted in urine is *U* x *V*. the volume of plasma which would have contained that amount can be worked out by dividing the amount excreted (*U x V*) by the plasma concentration of the substance (*P*), volume of plasma = $\frac{U \text{ x } V}{P}$ [91]

This is the volume of plasma which would have to be completely cleared of the substance in the time specified to give the amount seen in urine. This is the clearance for that substance [90].

The maximum rate that the plasma can be cleared of any substance is equal to the GFR. This could be calculated from the clearance of some plasma constituent which is freely filtered at the glomerulus, and is neither reabsorbed nor secreted in the tubule .Cr-EDTA or inulin (a plant carbohydrate) can be infused and used to measure GFR, but in practice creatinine, which is already present in blood as normal product of muscle metabolism, comes close to fulfilling the above requirements [89].

-Creatinine clearance

An estimate of GFR can be calculated from the creatinine content of a 24h urine collection and the plasma concentration within this period. The clearance of creatinine from plasma is directly related to the GFR provided that [91]:

- The urine volume is collected accurately
- There are no ketones or heavy proteinuria present to interfere with the creatinine determination.

The GFR is calculated as follows: $GFR = \frac{U \times V}{P}$

U = Urine concentration of creatinine

P = serum or plasma concentration of creatinine

V = urine flow in ml/min

A common mistake is to consider Vas urine volume, which it is not. It is the urine volume collected in 24 hours [90].

-Serum creatinine and urea

The concentrations of creatinine and urea in serum samples are used as convenient, but insensitive, measures of glomerular function.

A normal clearance creatinine does not necessarily mean all is well the reference intervals will vary with age and body size.

Serum urea concentration is less useful as a measure of glomerular function. Dietary protein intake affects serum urea concentration. Gastrointestinal bleeding will case serum urea to be elevated, and this does not indicate that glomerular

filtration is compromised. Urea is reabsorbed in the tubules. This reabsorption increases at low urine flow rates [91].

Most laboratories will measure serum creatinine and urea. The ratio of the tow is of value in the investigation of renal disorders [89].

(ii)Proteinuria

The glomerular basement membrane does not usually allow passage of albumin and large proteins. A small amount of a mount of albumin, usually less than 25 mg/24h is found in urine. When larger amount of albumin in excess of 250 mg/24h are detected, significant damage to the glomerular membrane has occurred. Quantitiv urine protein measurements should always be made on complete 24-hour urine collections. Albumin excretion in the range 25-300 mg/24h is termed microalbuminuria[90].

2.2.2.2 Renal tubular function

The glomeruli provide an efficient filtration mechanism for ridding the body of waste products and toxic substances. To ensure that important constitutes such as water, sodium, glucose and amino acids are not lost from the body. tubular reabsorption must be equally efficient . There are no easily performed test which measure the tubular function in a quantitive manner [87].

-Tubular dysfunction

Some disorders of tubular function are inhibited. Renal tubular damage is often secondary to other conditions such as exposure to heavy metals or nephron-toxic drugs, or amyloidosis [91].

-Investigation of tubular function

(i)Osmolality measurements in plasma and urine

Of all tubular functions, the one most frequently affected by disease is the ability to concentrate the urine. If the tubules and collecting ducts are working efficiently and if AVP is present, able to be reabsorb water. Just how will can be assessed by measuring urine concentration. This is conveniently done by determining the osmolality, and then comparing this to the plasma. In normal individuals on an average fluid intake the urine: plasma osmolality ratio is usually between 1.0 and 3.0. In other words, the urine is more concentrated than the plasma. When the urine: plasma osmolality ratio is 1.0 or less, the renal tubules are not reabsorbing water [90].

-The water deprivation test

It may be necessary to deprive a patient of water in an effort to find the cause of excessive for urea. The water deprivation test involves complete fluid deprivation during a 24-hour period, with measurement of the osmolality of all the urine specimens past during the second 12 hours of the test. And osmolality of greater than 700 mmol/kg should be attended and the urine: plasma osmolality ratio should be 2.0 are above. In polyuria of

diabetes insipidus, where the hormone AVP is lacking, the ratio will remain between 0.2 and 0.7 even after fluid restriction. In the polyuria associated with so-called compulsive water drinkers, the ratio may be normal without fluid restriction but increases after fluid restriction. In some hospitals this form of concentration test involves fluid restriction overnight (8pm-10 am(and measuring the osmolality of urine voided in the morning[88].

In practice, the water deprivation test is extremely unpleasant for the patient. It is potentially dangerous if there is severe inability to retain water. The test must be terminated if weight loss exceeds 3 Kg or if more than 3 litres of urine is passed.

Administration of AVP as the synthetic analogue DDAVP will result in increased urinary concentration (osmolality greater than 700 mmol/Kg) if the failure to concentrate is due o diabetes incipidus. No response will be obtained if the AVP receptors cannot respond to the hormone. This called nephrogenic diabetes insipidus[86].

(ii)The acid load test

The acid load test is occasionally used for the diagnosis of renal tubular acidosis, conditions in which metabolic acidosis arises from diminished tubular secretion of hydrogen ions. Ammonium chloride is administrated orally in gelatin capsules. Urine samples are collected for the following 8 hours. With normal renal function, the pH of the at least one sample should be less

than 5.3 if necessary in adifficult diagnosis, the excretion rates of titratable acid and ammonium iron, and serum by carbonate concentration, are all measure. This test should not be performed on patients how are already acidotic or how liver disease renal tubular acidosis having may be characterized as follows [90]:

1. *Type I.* there is defective hydrogen iron scretion in the distal tubul which may be inhereted or aquired.
2. *Type II.* the capacity to reabsorbe the bicarbonate in the proxymal tubul is reduced.
3. *Type IV.*by carbonate reabsorption by the renal tubule is impared as the consequence of aldosteron difficency, aldosteron defects, or drugs which block aldosteron action.

There is no sepreate type three renal tubular acidosis.

(iii)Specific proteinuria

beta-2 microglobulin and alpha-1 microglobulin are small proteins which are filterd at the glomeruli an dusually reabsorbed by the tubular cells. and incrase concentration of this protins in urine is a sensitive indicator of renal tubular cell damage[86].

(iv)Glycosuria

The presence of glucose in urine when blood glucose is normal usually reflects the inability of the tubules to reabsorb glucose because of a specific tubular lesion but here, the renal threshold(the capacity for the tubules to reabsorb the substance in question) has been reached. this called renal glycosuria and is a

benign condition. glycosuria can also present in association with other disorder of tubular function the fanconi syndrome[90].

(v)Aminoaciduria

Normally, amino acids in the glomerular filterate are reabsorbed in the proxymal tubules. they may be present in urine in excessive amount because the plasma concentration exceeds the renal threshold, or because there is specific failure of normal tubular reabsorpive mechanisms, such as in the inherited metabolic disorder, cystinuria, or more commonly because of acquired renal tubular damage[90].

2.3 Cystatin C:

The cystatin superfamily encompasses proteins that contain multiple cystatin-like sequences. Some of the members are active cysteine protease inhibitors, while others have lost or perhaps never acquired this inhibitory activity[92]. There are three inhibitory families in the superfamily, including the type 1 cystatins (stefins), type 2 cystatins and the kininogens. The type 2 cystatin proteins are a class of cysteine proteinase inhibitors found in a variety of human fluids and secretions, where they appear to provide protective functions. The cystatin locus on the short arm of chromosome 20 contains the majority of the type 2 cystatin genes and pseudogenes[93].

The CST3 gene is located in the cystatin locus and comprises 3 exons (coding regions, as opposed to introns, non-coding regions within a gene), spanning 4.3 kilo-base pairs. It encodes the most

abundant extracellular inhibitor of cysteine proteases. It is found in high concentrations in biological fluids and is expressed in virtually all organs of the body (CST3 is a housekeeping gene). The highest levels are found in semen, followed by breastmilk, tears and saliva. The hydrophobic leader sequence indicates that the protein is normally secreted. There are three polymorphisms in the promoter region of the gene, resulting in two common variants. Several single nucleotide polymorphisms have been associated with altered cystatin C levels[94].

Cystatin C is a non-glycosylated, basic protein (isoelectric point at pH 9.3). The crystal structure of cystatine C is characterized by a short alpha helix and a long alpha helix running across a large antiparallel, five-stranded beta sheet. Like other type 2 cystatines, it has two disulfide bonds. Around 50% of the molecules carry a hydroxylatedproline. Cystatine C forms dimers (molecule pairs) by exchanging subdomains; in the paired state, each half is made up of the long alpha helix and one beta strand of one partner, and four beta strands of the other partner[95].

Cystatin C was first described as 'gamma-trace' in 1961 as a trace protein together with other ones (such as beta-trace) in the cerebrospinal fluid and in the urine of patients with renal failure[96]. Grubb and Löfberg first reported its amino acid sequence[96]. They noticed it was increased in patients with advanced renal failure[97]. It was first proposed as a measure of glomerular filtration rate by Grubb and coworkers in 1985[98; 99].

2.3.1Cystatin C as a GFR Marker

Diabetic nephropathy is the single most frequent cause of ESRD and is predominantly due to type 2 diabetes. Although measurements of albumin excretion rate are useful in clinical practice, there is a 40% day to day variability in albumin excretion rate[100].67 Numerous studies have investigated the use of cystatin C in the diabetic population[101; 102; 103; 104]. Hoek et al. showed that not only was cystatin C a better indicator of GFR than creatinine, it was the parameter which had the best correlation with changes in GFR over two years, making it a useful analyte for follow up of patients with diabetes[102]. A four year follow-up study in Pima Indians demonstrated close correlation between longitudinal trends in Iothalamate clearance and the trends in renal function estimated from serum cystatin C[105].72 In contrast, the trends for commonly used creatinine-based estimates of GFR, such as serum creatinine, the CG formula, and the MDRD formula compared poorly with trends in Iothalamate clearance. The annual percentage change in GFR based on Iothalamate clearance and cystatin C were also found to be similar, again emphasising the accuracy of cystatin C in monitoring small changes of GFR[105].

The clinical reliability of cystatin C as a measure of GFR has also been documented in type 1 diabetes[106; 107]. In their recent study, Pucci *et al.* [104] reported that levels of cystatin C could discriminate degrees of renal impairment in a population of type

1 and 2 diabetic patients[104]. Cystatin C was found to correlate more strongly with Iohexol clearance than creatinine[104].

Cystatin C was also found to be able to predict progression to pre-diabetes stages in normoglycaemic subjects. The Western New York Health study found that elevated baseline cystatin C levels was associated with a three fold risk of progression from normoglycaemia to pre-diabetes over a six year period[108].

A substantial body of evidence has developed over the past several years which supports the use of Cystatin C as an alternative and more sensitive endogenous marker for the estimation of GFR than serum creatinine and serum creatinine based GFR estimations[105; 106].

Cystatin C is a small 13 –kDa protein that is a member of the cysteine proteinase inhibitor family that is produced at a constant rate by all nucleated cells. Due to its small size it is freely filtered by the glomerulus, and is not secreted but is fully reabsorbed and broken down by the renal tubules. This means the primary determinate of blood Cystatin C levels is the rate at which it is filtered at the glomerulus making it an excellent GFR marker[107].

A recent meta-analysis demonstrated that serum Cystatin C is a better marker for GFR than serum creatinine[108].

Unlike creatinine , Cystatin C serum levels are virtually unaffected by age (>1 yr), muscle mass, gender and race. A number of very simple formulas have been introduced which can

be used to obtain an estimated GFR using Cystatin C[109]. Multiple studies have found Cystatin C to be more sensitive to actual changes in GFR in the early stages of CKD than creatinine based GFR estimates[110]. (A significant advantage of Cystatin C based formulas, unlike creatinine based equations, is that Cystatin C based estimated GFR formulas are not biased according to GFR(13-14) and there is no GFR blind area with Cystatin C.)[11; 112; 113].

2.3.2Cystatin C and the MDRD

The creatinine based MDRD underestimates GFR in healthy subjects and shows decreased accuracy in older patients (with decreased muscle mass) and patients with body mass indexes (BMI) <21 and >30. While MDRD and serum creatinine show good diagnostic accuracy in severe renal failure (GFR <15 mL/min per 1.73 m2) creatinine based measurements show a lack of sensitivity in stage 2 and stage 3 renal disease when early intervention may improve outcomes. Cystatin C based estimates of GFR have been reported to be a more sensitive maker of decline in GFR especially in the earliest stages of CKD[114].

2.3.3 Cystatin C for Early Detection of CKD in Diabetes

Estimation of the prevalence of earlier stages of chronic kidney disease (CKD) in the US population and ascertainment of trends over time is central to disease management and prevention planning, particularly given the increased prevalence of obesity and diabetes [115]. To prevent this increase, screening for CKD

and early intervention are necessary. In diabetic patients, the early detection of diabetic nephropathy has focused on the measurement of urinary albumin excretion rate. The elevated urinary albumin excretion rate within microalbuminuric level (30-299 mg/24 hr or a spot urine albumin-to-creatinine ratio of 30-299 mg/g) allows the detection of patients with an increased risk for the development of overt diabetic nephropathy with persistent macroalbuminuria. Moreover, impaired renal function may be present even in patients with normal urinary albumin excretion rate[116]. Gold standard procedures for glomerular filtration rate (GFR) measurement, based on the clearance of 51Cr-EDTA or iohexol, are impractical in clinical settings and for larger research studies[116].

Cystatin C, a cysteine protease inhibitor, is freely filtered by the renal glomeruli, metabolized by the proximal tubule and identified as a promising marker of renal failure [117]. Cystatin C is produced at a constant rate by nucleated cells and released into bloodstream with a half-life of 2 hr. Its concentration is almost totally dependent on GFR. Other studies have demonstrated that serum cystatin C is an early renal marker in diabetic patients [118], but not all studies have done so [119].

Multiple reports indicate that Cystatin C is a reliable marker of GFR in patients with mild to moderate impairment of kidney function (stages 2-3 of CKD) [120]. This high degree of sensitivity has been demonstrated in both Type 1 and Type 2 Diabetes[121].

In addition, several studies indicate that although clinical proteinuria was associated with both MDRD and Cystatin C estimates of GFR only Cystatin C was associated with microalbuminuria. This finding supports the enhanced sensitivity of Cystatin C based formulas for the early detection of kidney damage. Elevated serum Cystatin C levels have also recently been identified as a significant prognostic indicator for the development of cardiovascular disease in people with diabetes[122].

2.3.4 Beta2-microglobulin:

Beta2-microglobulin is a protein found on the surface of many cells. Testing is done primarily when evaluating a person for certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and multiple myeloma or kidney disease[123].

Beta2-microglobulin is plentiful on the surface of white blood cells. Increased production or destruction of these cells causes Beta2-microglobulin levels in the blood to increase. This increase is seen in people with cancers involving white blood cells, but it is particularly meaningful in people newly diagnosed with multiple myeloma. Multiple myeloma is a malignancy (cancer) of a certain kind of white blood cell, called a plasma cell. At the time of diagnosis, the Beta2-microglobulin levels

reflect how advanced the disease is and the likely prognosis for that person[124].

When kidney disease is suspected, comparing blood and urine levels helps identify where the kidney is damaged. Beta2-microglobulin normally is filtered out of the blood by the kidney's glomeruli (a round mass of capillary loops leading to each kidney tubule), only to be partially reabsorbed back into the blood when it reaches the kidney's tubules. In glomerular kidney disease, the glomeruli can't filter it out of the blood, so levels increase in the blood and decrease in the urine. In tubular kidney disease, the tubules can't reabsorb it back into the blood, so urine levels rise and blood levels fall. After a kidney transplant, increased blood levels may be an early sign of rejection[123].
Increased urinary levels are found in people with kidney damage caused by high exposure to the heavy metals cadmium and mercury. Periodic testing of workers exposed to these metals helps to detect beginning kidney damage[123].
Beta2-microglobulin levels also rise during infection with some viruses, including cytomegalovirus and human immunodeficiency virus (HIV). Studies show that as HIV disease advances, beta2-microglobulin levels rise[124].

Serum and plasma beta2 microglobulin values have emerged as markers for the activation of the cellular immune system, as well

as a tumor marker in certain hematologic malignancies. Urine beta2 microglobulin values indicate renal filtration disorders. Measurement of values in both serum and urine can help distinguish a problem of cellular activation from a renal disorder[124].

Low serum levels of beta2 microglobulin essentially indicate decreased disease activity in conditions for which beta2 microglobulin is used as a prognostic marker (multiple myeloma, lymphoma, leukemia) or the absence of such a disease process. However, low beta2 microglobulin levels are never used to rule out a particular disease (eg, lymphoma) in the absence of other more definitive tests[126; 127].

Increased serum beta2 microglobulin levels reflect increased activity of the disease process in question and can be an exquisitely sensitive marker for this purpose in many hematologic disorders. The absolute value is less important than the historical values, except in certain situations such as multiple myeloma, in which a value of less than 4 µg/mL was found to correlate with increased survival [127].

Increased urine beta2 microglobulin levels reflect tubular disorders of the kidney. In such cases, serum beta2 microglobulin levels are usually normal, since the dysfunction is in tubular

reabsorption. Increased CSF beta2 microglobulin levels are seen in certain conditions such as multiple sclerosis, AIDS dementia complex, and meningeal spread of hematologic tumors [128].

Chapter Three

Materials and Methods

CHAPTER THREE

3. MATERIALS and METHODS

3.1 Study design:

This is a quantitative, descriptive, analytic, cross- sectional and hospital-based study.

3.2 Study area and period:

This study was conducted in Khartoum state, capital and central of Sudan country. Patients enrolled in this study came to the refer clinics of different diabetic centers and hospitals in Khartoum state clinic of Jabir AbuElizz, Almolazmeen Diabetes Centres which are specialized centres for diabetes mellitus. The study was conducted during the period from March 2010 to November 2013.

3.3 Study population and sample size:

Population of this study included a test group of 300 diabetic patients who attended Jabir Abulizz and Amolazmeen diabetes centres for routine follow up and a control group of 150 healthy volunteers non- diabetics. Both, the test group and the control group were matched for age and gender.

3.4 Ethical consideration:

Firstly the permission of this study was obtained from the local authorities in the area of the study and from the medical directors of Jabir AbuElizz andAlmolazmeen Diabetes Centres, The objective of the study were explained to all participating in this study. An informed consent was obtained from each participant in the study.

3.5 Inclusion criteria:

The test group included patients with type 2 DM, whereas the control group included apparently healthy volunteers (non-diabetics).

3.6 Exclusion criteria:

Patient with type 1 diabetes, gestational diabetes and secondary diabetes were excluded from this study.

3.7 Data collection and clinical examination:

Clinical data for every participant was collected using a questionnaire. Whereas clinical assessment for both the test group and control group was done by clinicians in the above mentioned centers.

3.8 Blood sample collection:

The blood samples were collected from the patients group and the control group, blood samples “5ml” were collected from fasting subjects of the study group after fulfillments of questionnaire as

well as control group, using disposable syringe and sprit for sterilization the area of collection. Collected blood was drawn in two containers with heparin and EDTA. Blood in all containers was gently mixed with anticoagulant to obtain plasma and whole blood consecutively. Heparin container was centrifuged at 10000 rpm at -4 0_C using cold centrifuge. Hemolyzed and lipamic samples were rejected and excluded from the study. Whole blood was use for testing of glycocylated hemoglobin, pl. asma samples were preserved at -20 C prior to processing, plasma from fluoride oxalate for testing fasting glucose, while heparinized plasma for testing creatinine level, Cystatin C level andBeta2microglobulin .

3.9 Biochemical measurements:

3.9.1 Measurement of plasma Cystatin C:

The Diazyme Cystatin C assay method Roche Hitachi 917 chemistry analyzer.

Principle:

Diazyme Cystatin C assay is based on a latex enhanced immunoturbidimetric assay. Cystatin C in the sample binds to anti-Cystatin C antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of Cystatin C in the sample. The instrument calculates the Cystatin C

concentration of a patient specimen by interpolation of the obtained signal on a 6-point calibration curve.

Reagent composition, reagent preparation, procedure and calculation: See appendix (1).

3.9.2 Measurement of Plasma creatinine :

Serum levels of creatinine were measured using creatinine Jaffe Gen.2(a Roche Hitachi 917cobas chemistry analyzer.

. Principle:

This kinetic colorimetric assay was based on Jaffe method. In alkaline solution, creatinine formed a yellow-orange complex with picrate. The rate of dye formation was proportional to the creatinine concentration in the specimen.

Createnine + picric acid -----→ yellow-orange complex

.Reagent composition, reagent preparation, procedure and calculation:

See appendix (2).

3.9.3 Determination of creatinine clearance:

createnine clearance for each participant was calculated using the Cockroft-Gault formula:

Createnine clearance=

(140-age)×weight(kg)× 1.23 (0.85for female)

Plasma creatinine (mol/L)

3.9.4 Measurement of plasma β2-Microglobulin:

Plasma levels of β2-Microglobulin was measured by N Latex β2-Microglobulin (a Roche Hitachi 917 cobas chemistry analyzer.

Principle:

Polystyrene particles coated with specific antibodies to human β2-Microglobulin are aggregated when mixed with sample containing β2-Microglobulin. These aggregates scattered a beam of light passed through the sample. The intensity of the scattered light was proportional to the concentration of the relevant protein in sample. The result was evaluated by comparison with a standard of known concentration.

.Reagent composition, reagent preparation, procedure and calculation: See appendix (3).

3.9.5 Measurement of blood Glycated hemoglobin:

Principle:

This method uses TTAB (Teteradecyl trimethyl ammonium bromide) as the hemolysing reagent to eliminate interference from leukocytes (TTAB dose not lyse leukocyte). Sample pretreatment to remove labile HbA1c is not necessary.

All hemoglobin variants which are glycated at β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are measured by this assay.

Sample:

A venous blood sample (2mls) was collected from each participant by standard procedures, 2mls in EDTA container for HbA1c(whole blood).

.Reagent composition, reagent preparation, procedure and calculation: See appendix (4).

3.10 Body mass index (BMI):

The body mass index for each participant was calculated by measuring the height and the weight and using the following formula:

$$BMI = \frac{\text{Body weight (kg)}}{\text{Height (meters)}^2}$$

3.11Quality control:

The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

3.12 Statistical analysis:

The data was recorded and analyzed using Statistical Package for Social Sciences (SPSS-version 17) on programmed computer.

The mean and standard deviation of variables were calculated for both the test group and the control group and the P value for comparison was obtained .P value $\leq$ 0.05 was considered significant.

Pearson's Correlation and linear regression were used to assess the relationship between different variables.

Chapter Four

Results

Chapter Four

4. Results

This study was a case-control study conducted in Jabir Abulizz and Almolazmeen diabetic centers in Khartoum state, Sudan, during the period from March 2010 to November 2013.The study aimed to assess the plasma levels of cystatin c, β2-microglobulin, creatinine , creatinine clearance, blood HbA_{1C} % and body mass index of 300 Sudanese patients with type2 diabetes mellitus (49%males and 51% females)as a test group, and 150 healthy volunteers non diabetic (48%males and 52%females) as a control group. The test group and the control group were matched in term of gender and age.

Table (4.1) shows baseline characteristics of the test group and the controls. This table shows insignificant difference (P=0.712) between the mean of age of the test group and the control group. Also shows a significant difference (P=0.031) between the mean of the body mass index (BMI) of the test group and the control group.

Table (4.2) shows a significant difference (P=0.026) between the means of plasma levels of cystatin c of the test group and the control group. Also shows a significant difference (P= 0.038) between the mean of plasma levels of creatinine of the test group and the control group. Also shows a significant difference (P= 0.015) between the mean of creatinine clearance of the test group and the control group. Also shows a significant difference (P= 0.035) between the mean of plasma levels of β2 microglobulin of the test group and the control group.

Table (4.3) shows a significant difference(p=0.039) between the means of blood HbA_{1c} % of the controlled test group and the uncontrolled test group. Also shows a significant difference (P= 0.015) between the mean of plasma

levels of cystatin c of the controlled test group and the uncontrolled test group. Also shows a significant difference (P= 0.021) between the mean of creatinine of the controlled test group and the uncontrolled test group. Also shows a significant difference (P= 0.001) between the mean of creatinine clearance of the controlled test group and the uncontrolled test group. Also shows a significant difference (P= 0.042) between the mean of plasma levels of β2 microglobulin controlled test group and the uncontrolled test group.

Figure (4.1) shows a significant week positive correlation between the plasma levels of cystatinc, and the duration of diabetes in the diabetic group (r = 0.27, P = 0.045).

Figure (4.2) shows a significant weak positive correlation between the plasma levels of creatinine , and the duration of diabetes in the diabetic group (r = 0.31, P = 0.22).

Figure (4.3) shows a significant weak positive correlation between the plasma levels of β2microglobulin, and the duration of diabetes in the diabetic group (r = - 0.36, P = 0.04).

Figure (4.4) shows a significant moderate negative correlation between the plasma levels of cystatin c and creatinine clearance (r=-0.49,p=0.015) in the diabetic group.

Figure (4.5) shows a significant week negative correlation between the plasma levels of creatinine and creatinine clearance (r=-0.33,p=0.045) in the diabetic group.

Figure (4.6) shows a significant moderate negative correlation between the plasma levels of β2 microglobulin and creatinine clearance (r=-0.46,p=0.023) in the diabetic group.

Figure (4.7) showsa significant week positive correlation between the plasma levels of cystatin c and the body mass index (r=0.28,p=0.024) of the diabetic group.

Figure (4.8) shows a significant weak positive correlation between the plasma levels of creatinine and the body mass index (r=0.27,p=0.038) of the diabetic group.

Figure (4.9) shows a significant weak positive correlation between the plasma levels of β2 microglobulin and the body mass index(r=0.29,p=0.031) of the diabetic group.

Figure (4.10) shows a significant moderate positive correlation between the plasma levels of cystatin c and blood HbA_{1c} %(r=o.48,p=0.024).

Figure (4.11) shows a significant weak positive correlation between the blood plasma of creatinine and blood HbA_{1c} % among the diabetics(r=o.39,p=0.029).

Figure (4.12) shows a significant moderate positive correlation between the plasma levels of β2 microglobulin and blood HbA_{1c} %(r=o.45,p=0.013).

Table (4.1): Baseline characteristics of the test group and the control group

Variable	Test group (n=300)	Control group (n=150)	P-value
Age (years)	59.40±7.20 (45.0-82.0)	58.00±17.63 (33.0-85.0)	$0.712^{n.s}$
Weight (kg)	73.42±11.34 (39.0-94.0)	68.16±15.69 (41.5-114.7)	0.002^{**}
Height (cm)	169.84±10.68 (154-188)	170.78±7.82 (157-189)	$0.061^{n.s}$
BMI (kg/m^2)	27.69±3.77 (18.62-34.68)	23.22±4.31 (18.43-35.42)	0.031^{*}

Table shows the mean±standard deviation, range between brackets and level of significance (P-value). t-test was used for comparison between the test group and control group.

P-value ≤0.05 is considered significant

Table (4.2): Comparison of the mean of the serum levels of cystatin C, creatinine, β2 microglobulin and creatinine clearance.

Variables	Test group (n=300)	Control group (n=150)	P-value
Plasma Cystatin C (mg/L)	1.35±1.06 (0.74-3.87)	0.84±0.18 (0. 39-1.28)	0.026*
Plasma Creatinine (mg/dL)	1.23±0.89 (0.67-9.20)	0.87±0.19 (0.49-1.29)	0.038*
Creatinine clearance(ml/min)	80.66±33.17 (71.91-125.00)	83.37±30.61 (90.90-138.89)	0.015*
Plasma β_2 microglobulin (mg/ml)	2.38±1.22 (0.82-4.00)	1.31±0.48 (1.12, 2.00)	0.035*

Table shows the mean±standard deviation, range between brackets and level of significance (P-value).t-test was used for comparison between the test group and control group.

P-value ≤0.05 is considered significant.

Table (4.3): Comparison of the mean of HbA_{1C}, cystatin C, creatinine, creatinine clearance and β_2 microglobulin of controlled and uncontrolled patients

Variables	Controlled (n=158)	Uncontrolled (n=142)	P-value
Blood HbA_{1C} (g/dl)	5.96±1.12 (5.1-6.0)	8.52±2.74 (6.1-9.9)	0.039*
Plasma Cystatin C (mg/L)	1.09±0.23 (0.74-1.45)	2.86±1.06 (1.47-3.87)	0.015*
Plasma Creatinine (mg/dL)	2.13±0.97 (0.67-4.25)	6.20±1.76 (4.5-9.2)	0.021*
Creatinine clearance (ml/min)	78.92±15.44 (71.91-95.31)	105.32±17.46 (96.0-125.00)	0.001**
Plasma β_2 microglobulin (mg/ml)	1.04±0.87 (0.82-1.12)	3.58±1.07 (1.13-4.00)	0.042*

Table shows the mean±standard deviation, range between brackets and level of significance (P-value).t-test was used for comparison between the test group and control group.

P-value ≤0.05 is considered significant.

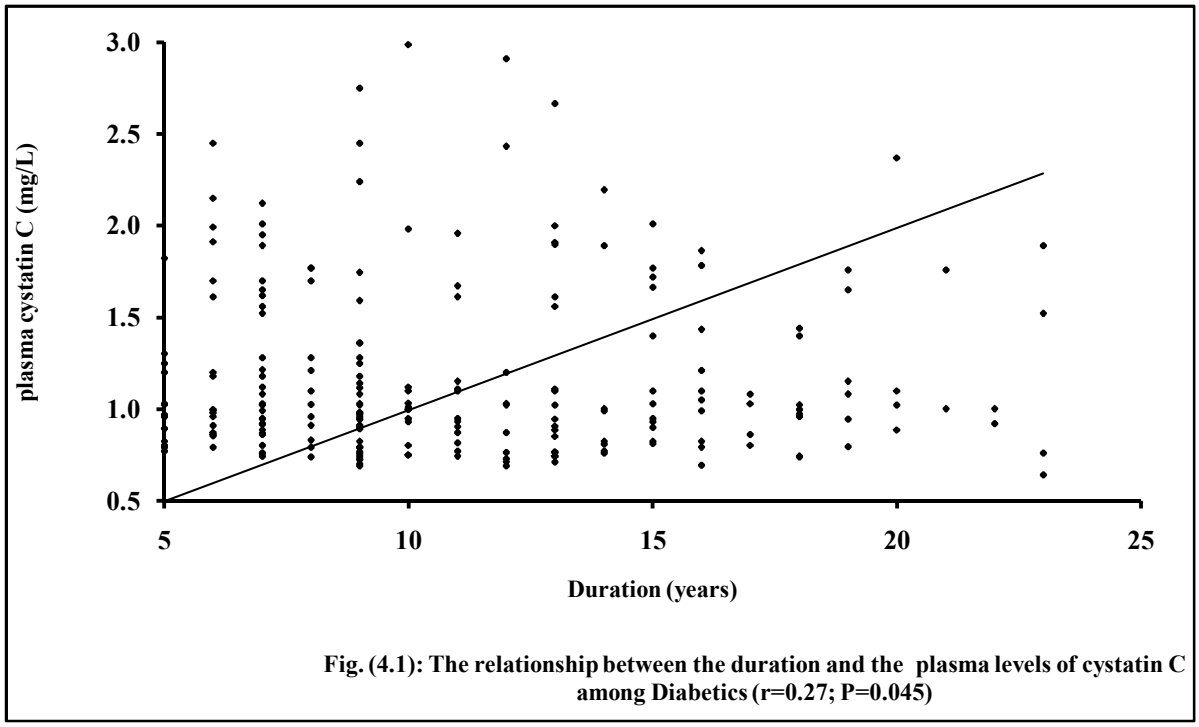


Fig. (4.1): The relationship between the duration and the plasma levels of cystatin C among Diabetics (r=0.27; P=0.045)

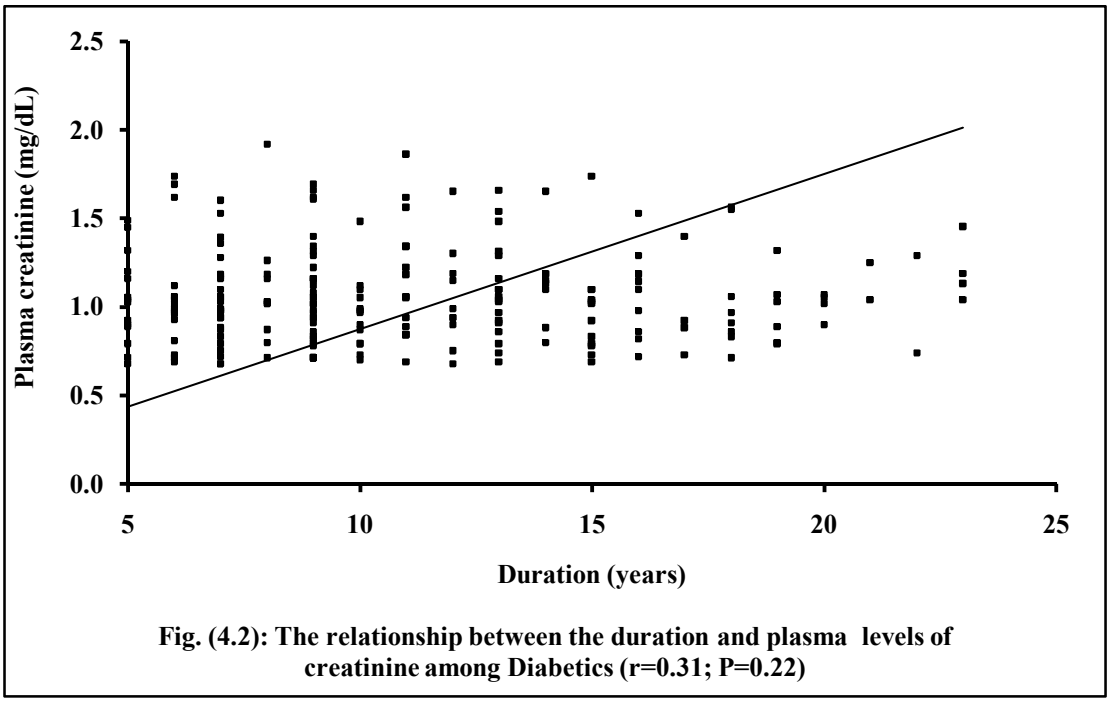


Fig. (4.2): The relationship between the duration and plasma levels of creatinine among Diabetics (r=0.31; P=0.22)

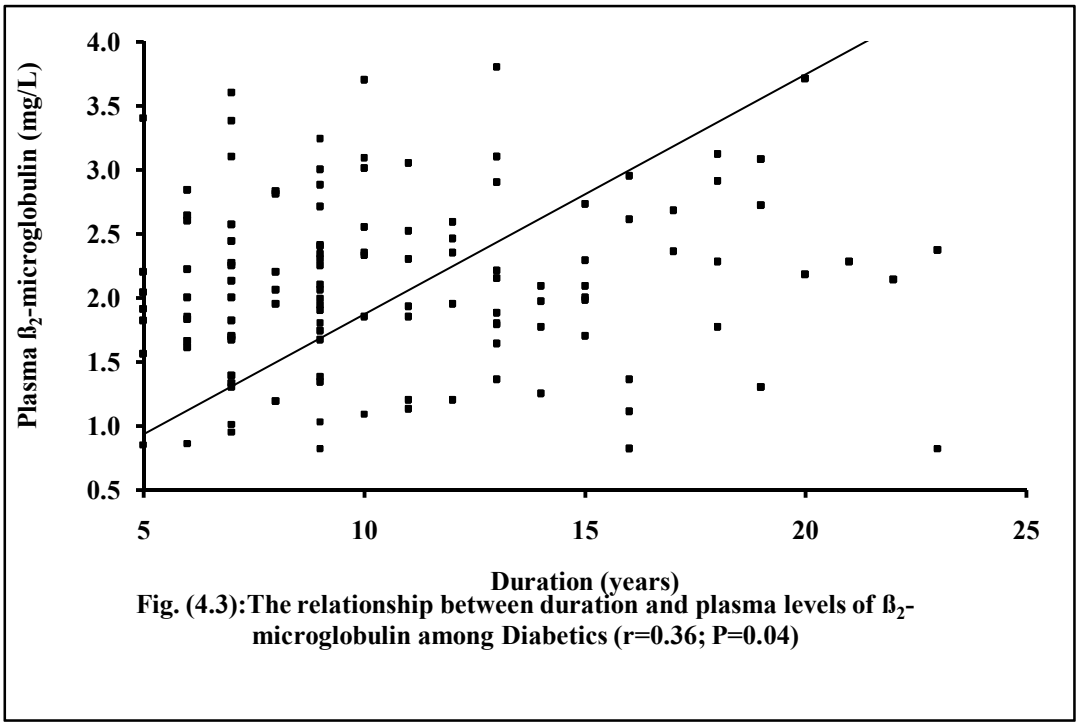


Fig. (4.3):The relationship between duration and plasma levels of ß$_2$-microglobulin among Diabetics (r=0.36; P=0.04)

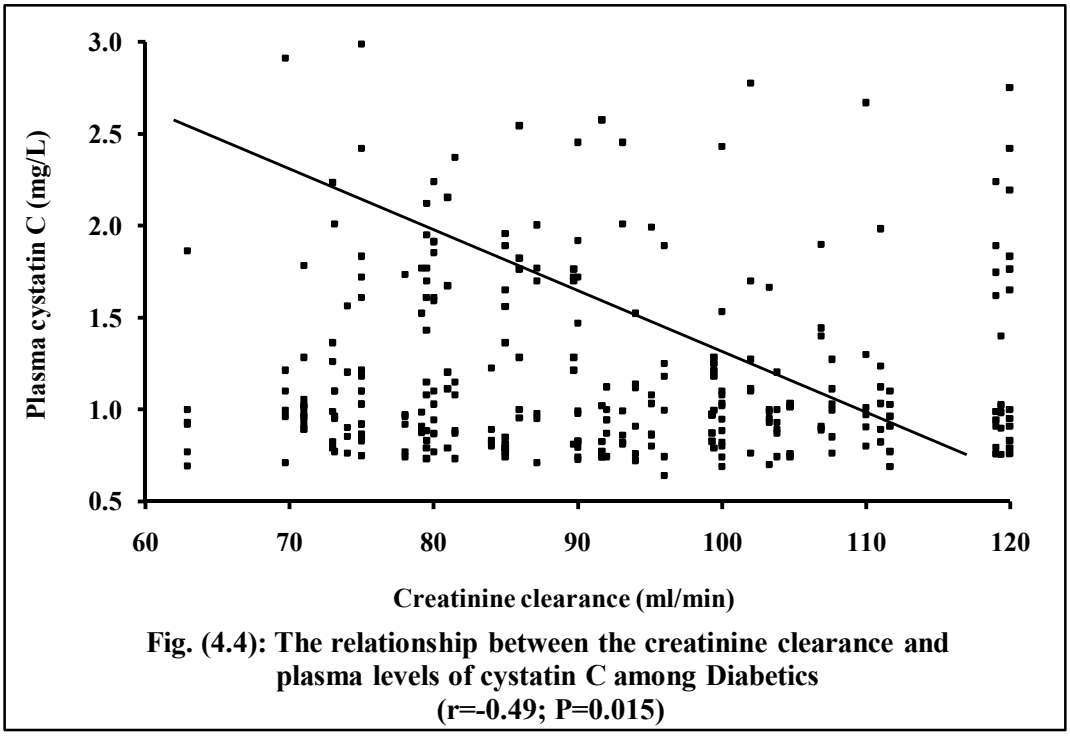


Fig. (4.4): The relationship between the creatinine clearance and plasma levels of cystatin C among Diabetics (r=-0.49; P=0.015)

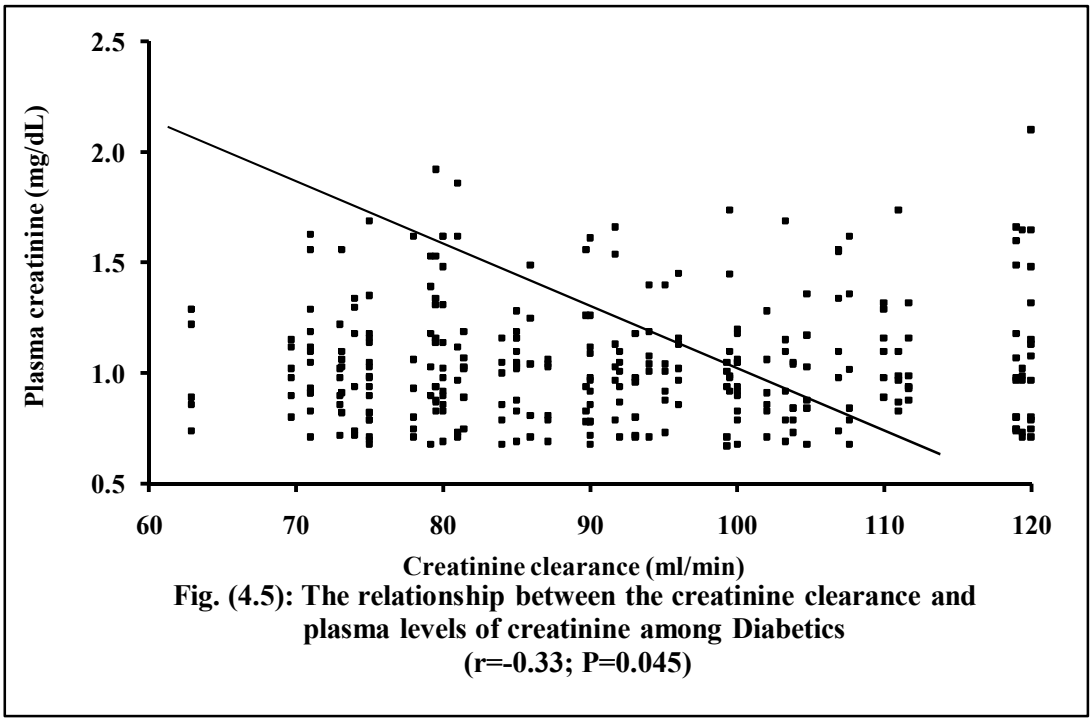


Fig. (4.5): The relationship between the creatinine clearance and plasma levels of creatinine among Diabetics (r=-0.33; P=0.045)

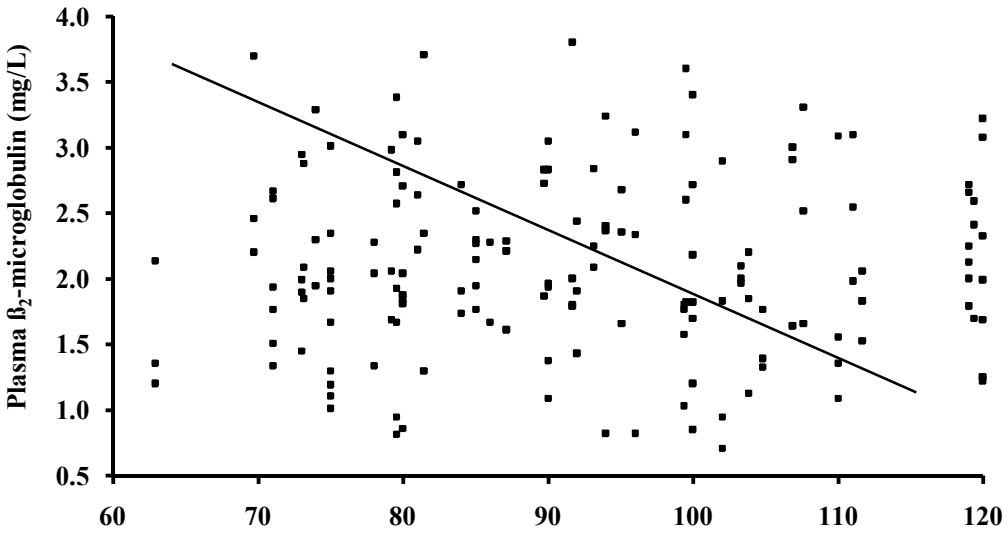


Fig. (4.6): The relationship between the creatinine clearance and plasma levels of ß$_2$-microglobulin among Diabetics (r=-0.46; P=0.023)

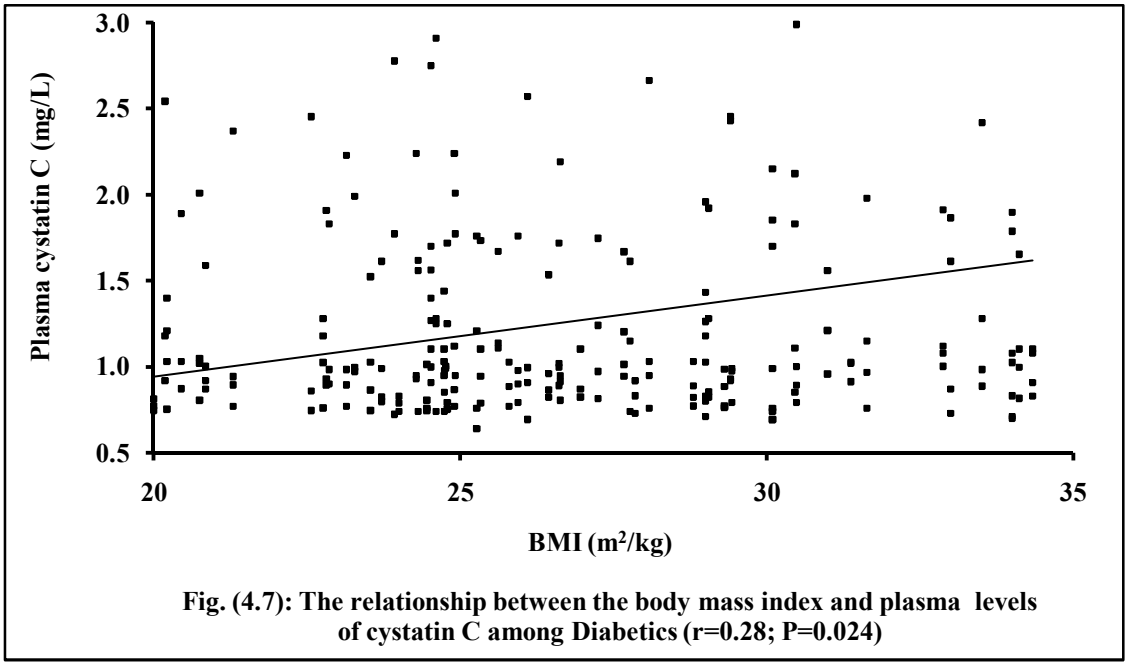


Fig. (4.7): The relationship between the body mass index and plasma levels of cystatin C among Diabetics (r=0.28; P=0.024)

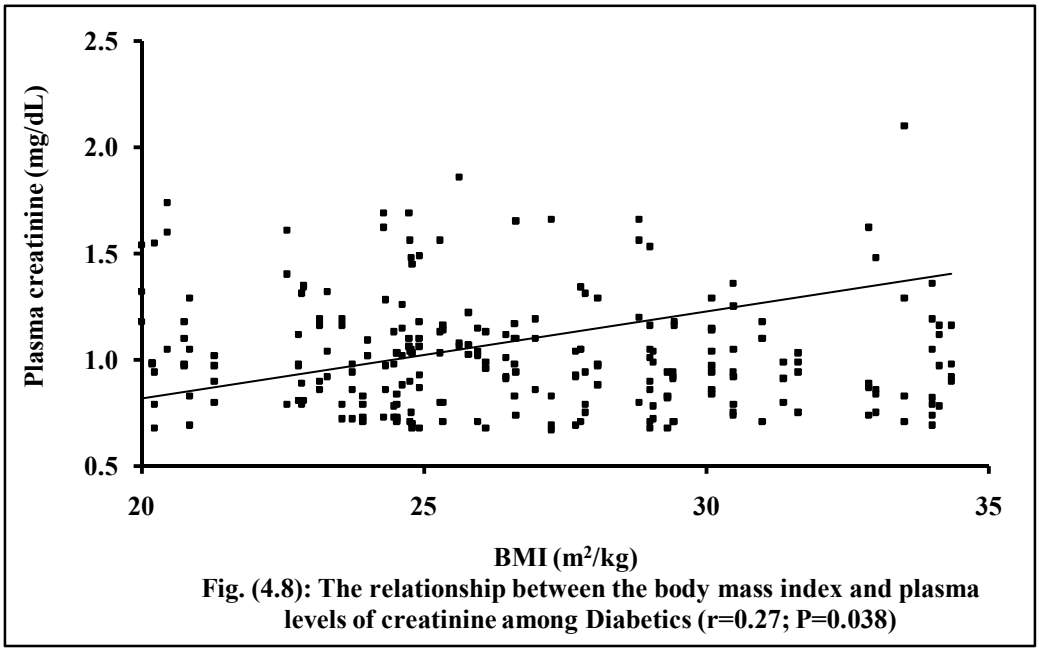


Fig. (4.8): The relationship between the body mass index and plasma levels of creatinine among Diabetics (r=0.27; P=0.038)

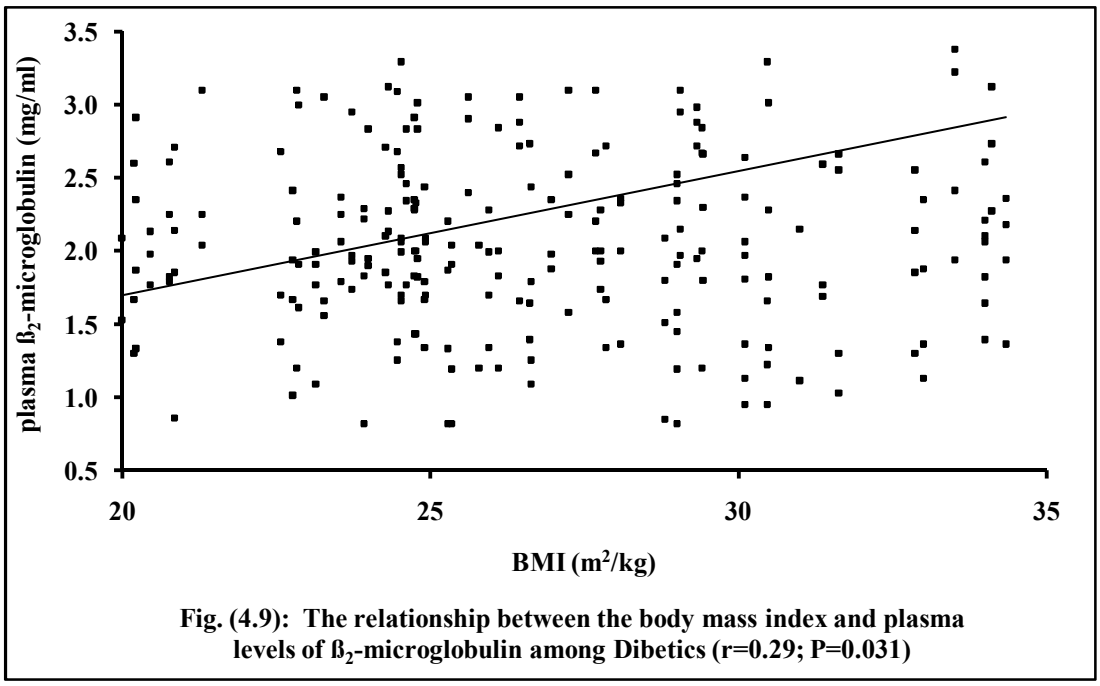


Fig. (4.9): The relationship between the body mass index and plasma levels of ß$_2$-microglobulin among Dibetics (r=0.29; P=0.031)

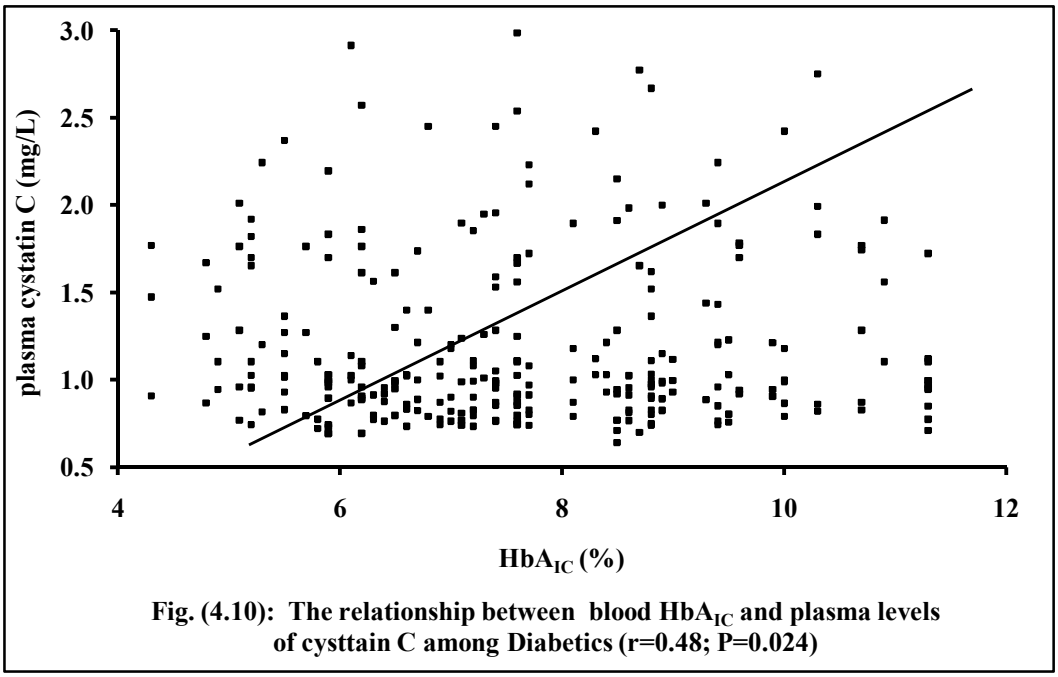


Fig. (4.10): The relationship between blood HbA_{IC} and plasma levels of cysttain C among Diabetics (r=0.48; P=0.024)

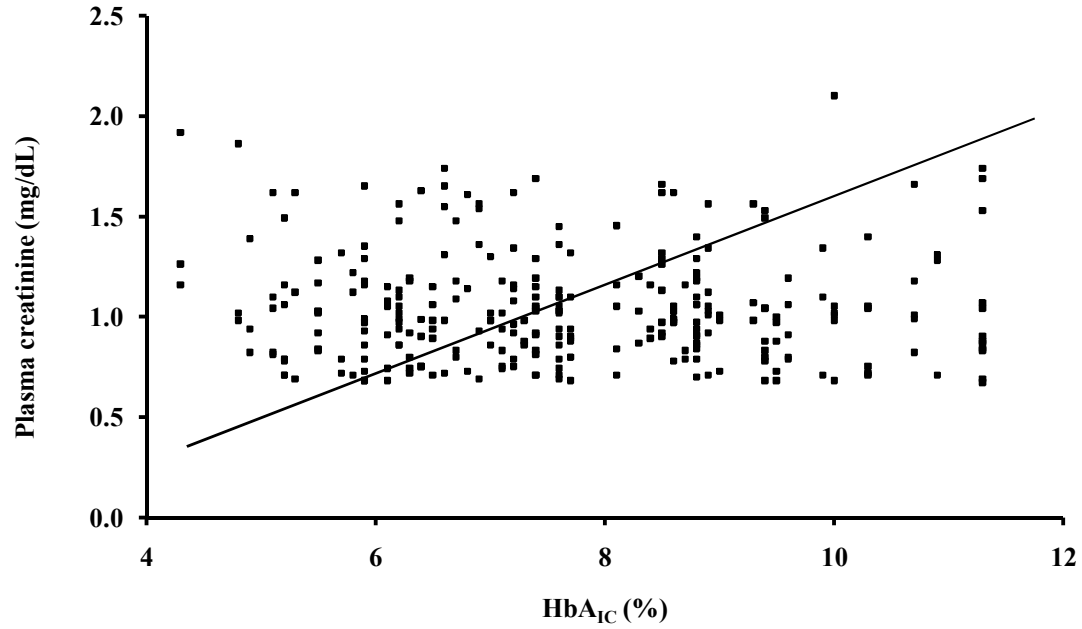


Fig. (4.11): The relationship between the blood HbA_{IC} and plasma levels of creatinine among Diabetics (r=0.39; P=0.029)

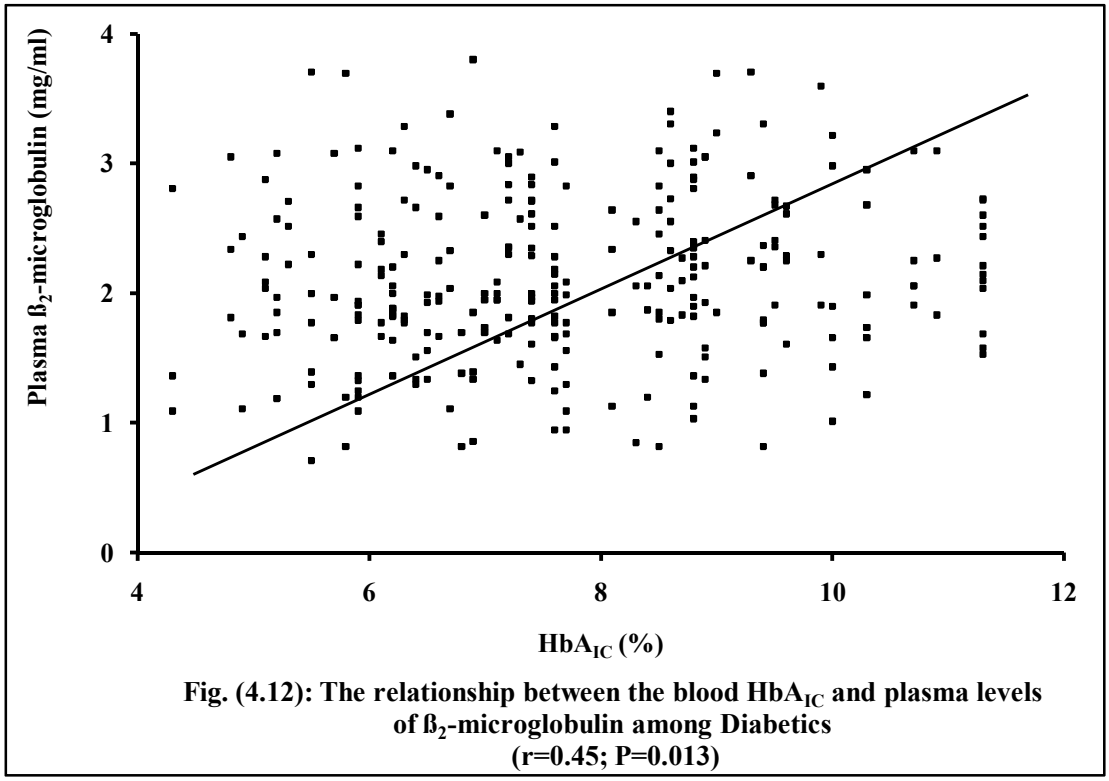


Fig. (4.12): The relationship between the blood HbA_{IC} and plasma levels of $ß_2$-microglobulin among Diabetics (r=0.45; P=0.013)

Chapter Five

Discussion

Chapter Five

5. Discussion

The current study aimed to assess the plasma levels of cystatin C, B2-microglobulin, creatinine , creatinine clearance, blood HbA_{1C}% and body mass index among Sudanese patients with type 2 diabetes in comparison with healthy controls.

Cystatic C and B2microglobulin have low molecular weight, and there execration affected by the glomerular filtration in the kidneys. If kidney function and glomerular filtration rate decline, the blood levels of Cystatin C and B2microglobulin raise. Plasma levels of Cystatin C are a more precise test of kidney function than plasma creatinine[108]. In the present study the results show that the mean of the plasma levels of Cystatin C, creatinine B2-microglobulin, clearance, blood HbA1c% and body mass index were significantly raised.

The clinical reliability of cystatin C as a measure of GFR has also been documented in type 1 diabetes[106; 107]. In their recent study, Pucci *et al.* [104] reported that the levels of cystatin C could discriminate degrees of renal impairment in a population of type 1 and 2 diabetic patients. Cystatin C was found to correlate more strongly with Iohexol clearance than createnine[104].

Cystatin C was also found to be able to predict progression to pre-diabetes stages in normoglycaemic subjects. The Western New

York Health study found that elevated baseline cystatin C levels was associated with a three fold risk of progression from normoglycaemia to pre-diabetes over a six year period[108].

The current study shows a moderate negative correlation between creatinine clearance and plasma Cystatin C levels, that means reduction of creatinine clearance, a marker of renal function, is associated with raised levels plasma Cystatin C. Creatinine clearance in the diabetic group was significantly reduced compared to the healthy group and this could be due to the long standing effect of diabetes on the kidneys because the majority of the diabetic patients enrolled in this study have diabetes for more than 10 years. The study also shows a strong positive correlation between HbA1c % plasma and cystatin C level. This means uncontrolled diabetes with abnormal raised glycatedheamoglobin is associated with high Cystatin C plasma levels.

In this study cystatin c levels have significant weak negative correlation with body mass index in the diabetic group. Numerous studies have investigated the use of cystatin C in the diabetic population for assessment of renal function[101; 102; 103; 104]. Hoek et al. [102] showed that not only was cystatin C a better indicator of GFR than creatinine, it was the parameter which had the best correlation with changes in GFR over two years, making it a useful analyte for follow up for patients with diabetes[102]. A four year follow-up study in Pima Indians demonstrated close correlation

between longitudinal trends in Iothalamate clearance and the trends in renal function estimated from serum cystatin C[105].

In the present study the results showed that the mean of the plasma levels of Beta2-microglobulin, were significantly raised in the diabetic group when compared to the control group. Increased level of B2-microglobulin may be an early indication of diabetic nephropathy and reflect glomerular kidney disease that cause elevation of plasma levels of B2microglobulin. This result agrees with the results of the study reported a significant high levels of B2-microglobulin in patients with type 2 diabetes[118]. In our study the majority of patients (71%) were obese and 69% were found to have uncontrolled diabetes, with HbA1c% ≥ 6.5%. In the current study, the glomerular filtration rate assessed by creatinine clearance was significantly reduced, this could be due to the impact of long standing of diabetes mellitus on the kidneys, that cause progressive diabetic nephropthy. The current study showed moderate negative correlation between creatinine clearance and plasma Beta2-microglobulin levels, that means reduction of creatinine clearance is associated with raised serum levels of Beta2-microglobulin. In the present study HbA1 % has a significant positive moderate correlation with the plasma levels of B2-microglobulin, this means uncontrolled diabetes with abnormal raised glyclated heamoglobin is associated with high serum Beta2-microglobulin levels.

Two large studies[120; 121; 122] with consistent results found that cystatin and Beta2-microglobulin is influenced by many variables (age, sex, body mass index, smoking, hypertension, coronary heart disease and C-reactive protein level) rather than renal function alone. Both studies excluded patients with moderate and severe renal failure, and in both Beta2-microglobulins were highly correlated with age. In conclusion, although multiple factors in addition to renal function may influence Beta2-microglobulin, our study provides convincing evidence that Beta2-microglobulin is significant raised and positively correlated with HbA1c and inversely correlted with creatinine clearance in patients with diabetes mellitus, so, it can be used as a marker of renal damage in diabetic patients10. These results are remarkable in light of data suggesting that Beta2-microglobulin is a useful indicator of the association of mild kidney dysfunction with increased risk for cardiovascular events, peripheral arterial disease, heart failure, and death.

Chapter Six

Conclusion and Recommendations

Chapter Six

6. Conclusion and Recommendations

6.1 Conclusion

From the results of this study, it is concluded that in Sudanese patients with Type 2 diabetes:

1. The means of the plasma levels of Cystatin C, B_2-microglobulin, creatinine, blood HbA_{1c}% and body mass index are significantly raised.
2. Creatinine clearance is significantly reduced.
3. There is a moderate inverse correlation between creatinine clearance and plasma Cystatin C levels.
4. There is a moderate positive correlation between blood HbA_{1c} % and plasma cystatin C levels.
5. The plasma level of Cystatin c have a significant weak positive correlation with the body mass index.
6. There is a moderate negative correlation between creatinine clearance and plasma level of Beta2-microglobulin levels.
7. The plasma levels of B2-microglobulin has a significant moderate positive correlation with blood HbA_{1c}%.
8. The plasma levels of B2-microglobulin have a significant weak positive correlation with the body mass index.

6.2 Recommendations

From the results of this study, it is recommended that:

1. The plasma level of cystatin c and B2-microglobulin should be measured regularly in addition to the traditional markers (plasma ceratinine and creatinine clearance) to assess renal function in patients with type 2 diabetes.
2. Patients with diabetes should achieve good glycemic control to delay deterioration of renal function, especially in those who are observed with long standing diabetes.
3. Obese diabetics should be advised to reduce their weights which may improve glycemic control.

Further studies should be done to assess the plasma levels of cystatin C and B2-microglobulin in diabetic patients with complications such as ischemic heart disease, hypertension and peripheral neuropathy.

References

References

1. Abraira C, Henderson WG, Colwell JA. Response to intensive therapy steps and to glipizide dose in combination with insulin in type 2 diabetes. Diabetes Care. 1998; 21 (4) :574-9.
2. Abraira C, Colwell J, Nutgall F. Cardiovascular events and correlates in the Diabetes mellitus .. Arch Intern Med . 1997; 157 (2): 181-8.
3. Sudan Annual Statistical Report. Ministry of Health, Sudan. 2012.
4. McLarty DG, Athaida IA, Swai AB. Diabetesdetection in Tanzania. Pract. Diab Dig 1990,7: 65–67.
5. Gill G. Good bye 'IDDM' – hello 'Type 1'.Diabetes Int. 2000; 10: 34.
6. Murray CJ. Lopez AD. Harvard School of PublicHealth on Behalf of the World Health Organization and the World Bank. The Global Burden of Disease. Harvard University Press: London, 1996.
7. Ahmed AM, Ahmed NH. Hospitalization patterns of diabetic patients in Sudan. Diabetes Int.2000; 10: 18–19.
8. James G.D, Sealey J.E, Alderman M, Ljungman S, Mueller F.B.A longitudinal study of urinary creatinine and creatinine clearance in normal subjects . Race, sex, and age differences. Am. J. Hypertens. 1988; 1:124- 31.

9. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Executive Summary. New York, NY , National kidney foundation 2002.
10. Abrahamson M, Olafsson I, Palsdottir A . Structure and expression of the human cystatin c gen. Biochem. J .1990; 268: 287-94.
11. Rother K. Diabetes treatment—bridging the divide. *The New England Journal of Medicine*, 2007; 356 (15): 1499–501
12. Tierney, L., McPhee, S., and Papadakis, M. *Current medical Diagnosis & Treatment. International edition*. New York: Lange Medical Books/McGraw-Hill. 2002, pp. 1203–15.
13. Handelsman, Y. A Doctor's Diagnosis: Prediabetes." *Power of Prevention,* 2009; Vol 1, Issue 2.
14. Mailloux, L. Up To Date Dialysis in diabetic nephropathy". Up To Date. 2007; in http://patients.uptodate.com/, accessed March 21, 2014.
15. Wild S. Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004; 27 (5): 1047–53.
16. American Diabetes Association. "Total Prevalence of Diabetes & Pre-diabetes" 2005. http://www.diabetes.org/, accessed March 21, 2014.
17. Harris MI, Flegal KM, Cowie CC. "Prevalence of diabetes, impaired fasting glucose, and impaired glucose

tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988-1994". Diabetes Care, 1998; 21 (4): 518–24.

18. Australian Institute for Health and Welfare. "Diabetes, an overview". http://www.aihw.gov.au/. accessed March 21, 2014.

19. Whiting, D.R., Hayes L , and Unwin N.C. Diabetes in Africa. Challenges to health care for diabetes in Africa. *J Cardiovasc Risk,* 2003; 10(2): p. 103-10.

20. Sobngwi, E., Onkamo P, Vaananen S, Karvonen M, Tuomileho J. Diabetes in Africans. Part 1: epidemiology and clinical specificities. *Diabetes Metab,* 2001; 27(6): p. 628-34.

21. Lawrence, J., Contreras, R., Chen, W., Sacks, A., "Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005". *Diabetes Care* 20008; 31(5): 899–904.

22. International Diabetes Federation, Diabetes Mellitus., 2nd Edition, Brussels. 2003.

23. WHO, Preventing chronic diseases: A vital investment, Geneva. World Health Organization, 2005; www.who.int, accessed March 2014.

24. WHO, The burden of mortality attributable to diabetes, Geneva. World Health Organization, 2004, www.who.int, accessed March 2014.

25. King, H., R.E. Aubert, and W.H. Hennan, Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care, 1998; 21(9): p. 1414-31.

26. Vinicor, F., Is diabetes a public-health disorder? *Diabetes Care,* 1994; 17 Suppl 1: p. 22-7.

27. Beran, D. and Yudk J.S. in Diabetes care in sub-Saharan Africa. *Lancet,* 2006; 368(9548): p. 1689-95.

28. United Nations Population Division. Department of economic and social affairs Word urbanization prospects. Khartoum. 1999.

29. Elbagir, MN., A population-based study of the prevalence of diabetes and impaired glucose tolerance in adults in northern Sudan. *Diabetes Care,* 1996; 19(10): p. 1126-8.

30. Elamin, A. Childhood Type I (Insulin Dependent) Diabetes Mellitus in the Sudan. *Acta University Upsaliensis 348 (Thesis).* Uppsala University. 1992.

31. Eltom, M. and C. Thompson, Management of diabetes in Sudan. Diabetes Foum, *Norwegian Diabetes Association,* 2006; 2(2): p. 32-4.

32. Michael L. P., Edward P. F., Larry E. S. Clinical chemistry principles, procedures and correlations, 10th ed. Lippincott Williams, London 2010: 314,320.

33. Hu FB. 'Sedentary lifestyle and risk of obesity and type 2 diabetes'. Lipids 2003; 38(2): 103-108 .

34. Iwasaki Y, Takayasu S, Nishiyama M, et al. 'Is the metabolic syndrome an intracellular Cushing state? Effects of multiple humoral factors on the transcriptional activity of the hepatic glucocorticoid-activating enzyme (11beta-hydroxysteroid dehydrogenase type 1) gene'. Molecular and Cellular Endocrinology, 2008; 285(1-2): 10-18.

35. Chiodini I, Torlontano M, Scillitani A, Arosio M, Bacci S, Di Lembo S, Epaminonda P, Augello G, Enrini R, Ambrosi B, Adda G, Trischitta V Association of subclinical hypercortisolism with type 2 diabetes mellitus: a case-control study in hospitalized patients. Eur J Endocrinol 2005; 153:837–844.

36. Taniguchi, J. Ceriello, PA. Tuncer N. Oxidative stress and diabetes associated complications. Endo Prac 2008; 12; 60-62.

37. Saad F, Gooren L. 'The role of testosterone in the metabolic syndrome: a review'. The Journal of Steroid Biochemistry and Molecular Biology 2003; 114(1-2): 40-53.

38. Farrell JB, Deshmukh A, Baghaie AA. 'Low testosterone and the association with type 2 diabetes'. The Diabetes Educator, 2008; 34(5): 799-806.

39. Sakagashira S, Sanke T, Hanabusa T, et al. 'Missense mutation of amylin gene (S20G) in Japanese NIDDM patients'. Diabetes 1996; 45(9): 1279-1281.

40. Malchoff C. D. Diagnosis and classification of diabetes mellitus. Conn Med 1991: 55(11): 625.

41. Walley AJ, Blakemore AI, Froguel P. "Genetics of obesity and the prediction of risk for health". Hum. Mol. Genet. 2006; 15(Spec No 2): R124–30.

42. Barrett TG. "Mitochondrial diabetes and other inherited diabetes syndromes". Best Pract. Res. Clin. Endocrinol. Metab. 2001; 15(3): 325–43.

43. World Health Organization. Department of Non-communicable Diseases Surveillance. 'Definition, diagnosis and classification of diabetes mellitus and its complications: 1999; http://www.who.int/, accessed January 19th 2013.

44. Saydah SH, Loria CM, Eberhardt MS, et al. Subclinical states of glucose intolerance and risk of death in the U.S. Diabetes Care. 2001; 25:447–53.

45. Sniderman, AD; Bhopal R, Prabhakaran D, Sarrafzadegan N, Tchernof A. "Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis". International journal of epidemiology, 2007; 36(1): 220–5.

46. Genuth S. "Insights from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study on the use of intensive glycemic treatment to reduce the risk of complications of type 1 diabetes". Endocr Pract 2006; 12(Suppl 1): 34–41.

47. Lee, A., S. Mirrett, L. B. Reller, and M. P. Weinstein. Detection of bloodstream infections in adults: how many blood cultures are needed? J. Clin. Microbiol. 2007; 45:3546-3548

48. Gerstein H, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, Hanefeld M, Hoogwerf B, Laakso M, Mohan V, Shaw J, Zinman B, Holman R. "Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial". Lancet, 2006; 368(9541): 1096–105.

49. Bolen S et al. Systematic Review: Comparative Effectiveness and Safety of Oral Medications for Type 2 Diabetes Mellitus. Ann Intern Med 2007;147:6.

50. Michael L. P., Edward P. F., Larry E. S. Clinical chemistry principles, procedures and correlations, 5th ed. Lippincott Williams and Wilkins, London 2005: 270,271.

51. American Diabetes Association. "Standards of medical care in diabetes—2007; Diabetes Care 30 (Suppl 1): S4–S41.

52. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. "Translating the A1C assay into estimated average glucose values.". Diabetes Care 31 2008; (8): 1473–8.

53. Ruchi M, and William C. S. Hemoglobin A1c Test. 2007.

54. Bays H, Blond L, Rosenson R. Adiposopathy: how do diet, exercise and weight loss drug therapies improve

metabolic disease in overweight patients? Expert Rev Cardiovasc Ther. 2006;4:871–95.

55. Philip E, Jefferson, L, Cherrington A, Goodman, H. Handbook of Physiology; Section 7, The Endocrine System. New York: Oxford University Press. 2001; 1057-1092.

56. Wilkin TJ.Testing the Accelerator Hypothesis: Body Size, {beta}-Cell Function, and Age at Onset of Type 1 (Autoimmune) Diabetes: Response to Dabelea et al. Diabetes Care, 2006; 29(6): 1462–1463.

57. Kitabchi AE, Umpierrez GE, Murphy MB, Kreisberg RA. "Hyperglycemic crises in adult patients with diabetes: a consensus statement from the American Diabetes Association". Diabetes Care, 2006; 29(12): 2739–48.

58. Eledrisi MS, Alshanti MS, Shah MF, Brolosy B, Jaha N. "Overview of the diagnosis and management of diabetic ketoacidosis". *American Journal of Medical Science*, 2006; 331(5): 243–51.

59. Dunger DB, Sperling MA, Acerini CL . "European Society for Paediatric Endocrinology/Lawson Wilkins Pediatric Endocrine Society consensus statement on diabetic ketoacidosis in children and adolescents". *Pediatrics*, 2004; 113(2): e133–40.

60. Kasper, DL, Braunwald E, Fauci, AS, Harrison's Principles of Internal Medicine (16th ed), New York, NY: McGraw-Hill. 2005, p 2152-2180.

61. Cirasino L, Thiella G, Invernizzi R, Silvani A, Ragaini S. Hypersmolar hyperglycemic nonketotic coma in Waldenstrom's macroglobulinemia associated with type II diabetes and complicated by pulmonary tuberculosis. 1992; 83 (4): 122-145.

62. Stoner, GD. "Hyperosmolar hyperglycemic state". American Family Physician, 2005; 71(9): 1723–30. PMID 15887451.

63. Tintinalli, Judith E.; Kelen, Gabor D.; Stapczynski, J. Stephan; American College of Emergency Physicians Neuroradiology. 2007; 49(4):299-305

64. Maton, Anthea; Roshan L. Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, Jill D. Wright. Human Biology and Health. Englewood Cliffs, NJ: Prentice Hall. 1993.

65. Fabricant CG, Fabricant J. "Atherosclerosis induced by infection with Marek's disease herpesvirus in chickens". Am Heart J, 1999; 138(5 Pt 2): S465–8.

66. Colwell J A, Lopes-Virella M, Halushka P V. Pathogenesis of atherosclerosis in diabetes mellitus. Diabetes Care January 1981 vol. 4 no.1 121-133.

67. Sims NR, Muyderman H. "Mitochondria, oxidative metabolism and cell death in stroke". Biochimica et Biophysica Acta, 2009; 1802 (1): 80–91.

68. Donnan GA, Fisher M, Macleod M, Davis SM. "Stroke". Lancet 2008; 371(9624): 1612–23.

69. World Health Organization. Cerebrovascular Disorders (Office Publications). Geneva. 1978.

70. Kidwell CS, Warach S. "Acute ischemic cerebrovascular syndrome: diagnostic criteria". Stroke 2003; 34 (12): 2995–8.

71. International Diabetes Federation. Hypertension in people with Type 2 diabetes: knowledge-based diabetes-specific guidelines. Diabet Med 2006;20:972-987.

72. Aiello LM, Rand LI, Sebestyen JG. The eye and diabetes. In: Marble A, Krall LP, Bradley RS, Christlieb AR, Soeldner JS, eds Joslin's Diabetes Mellitus. 12th edition Philadelphia: Lea and Febriger, 1985.

73. Klein R. Hyperglycemia and microvascular and macrovascular disease in diabetes. Diabetes Care 1995;18:258–268.

74. Zhang, P., et al. Application of economic analysis to diabetes and diabetes care. *Ann Intern Mcd,* 2004; 140(11): p. 972-7.

75. Broadstone VL, Cyrus J, Pfeifer, MA, Greene DA. Diabetic peripheral neuropathy, part I: sensorimotor neuropathy, The diabetes educator. 1987.

76. Cyrus T.J., Larson M.G., Vasan R.S., Cheng S., Rhee E.P., McCabe E. Metabolite profiles and the risk of developing diabetes. Nat Med. Apr 2011; 17(4):448-53.

77. Singh, C. Weiss J, Sumpio B. "Review of prevalence and outcome of vascular disease in patients with diabetes mellitus". *Eur J Vasc Endovasc Surg,* 2006; 31 (2): 143–50.

78. Boulton AJM, Malik RA, Arezzo J, Sosenko JM: Diabetic neuropathy: technical review. Diabetes Care 2000; 27: 1458–1487.

79. Reiber GE, Vileikyte L, Lavery L, Boyko EJ, Boulton AJM: Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. Diabetes Care, 1999; 22:157–162.

80. Lauren C. Brown, Jeffrey A. Johnson, Sumit R. Majumdar, Ross T. Tsuyuki, Finlay A. McAlister. Evidence of suboptimal management of cardiovascular risk in patients with type 2 diabetes mellitus and symptomatic atherosclerosis. CMAJ. 2004; 171(10): 1189–1192.

81. Hofer TP, Zemencuk JK, Hayward RA. When there is too much to do: how practicing physicians prioritize among recommended interventions. J Gen Intern Med. 2004; 19(6):646-53. Erratum in: J Gen Intern Med. 2004 Aug;19(8):903.

82. American Diabetes Association: Standards of medical care in diabetes, Diabetes Care, 2004; 27 (Suppl. 1):S15 –S35.

83. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Executive

Summary . New York, NY , National kidney foundation 2002

84. Berkman, James; Rifkin, Harold. "Unilateral nodular diabetic glomerulosclerosis (Kimmelstiel–Wilson): Report of a case". Metabolism 1973; 22(5): 715–722.

85. Zelmanovitz, T., Grechman, F., Balthazar, A. P. Diabetic nephropathy. Diabetol metab synd. 2009; 21:1 – 10.

86. Theodore A. Kotchen. Hypertensive vascular disease, Dan L. Longo, Anthony S. Fauci, Dennis L.Kasper, Braunwald E, Hauser SL, , Jameson JL, et al. Harrison's Principles of Internal Medicine, 18th ed, Volume II, McGraw Hill Company's; 2012; Chapter 247: 2042.

87. Kara L. Lynch, Alan H. B. Wu, Michaeil L. Bishop, Edward P. Fody, Larry E. Schoeff, Renal function. Clinical chemistry techniques, principles, correlations; Wolters kluwer (India) Pvt. Ltd., New Delhi; 2010; 557 – 564.

88. Edmund J. Lamb, Christopher P. Price, Carl A. Burtis, Edward R. Ashwood, David E. Bruns. Creatinine, Urea and Uric acid. Tietz fundamentals of clinical chemistry, 6th edition, Elsevier, New Delhi; 2008; 366- 367.

89. Mitchell H. Rosner, MD, and W. Kline Bolton, MD., Core curriculum in nephrology, renal function testing, American Journal of Kidney Diseases, 2006; 47(1): 174-183.

90. Stevens LA, Levey AS. Clinical implications for estimating equations for GFR. Ann Intern Med 2004; 141; 12:959-961.

91. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31-41.

92. Kolodziejczyk, R.; Michalska, K.; Hernandez-Santoyo, A.; Wahlbom, M.; Grubb, A.; Jaskolski, M. "Crystal structure of human cystatin C stabilized against amyloid formation". FEBS Journal, 2010; 277(7): 1726–1737.

93. Zahran A, El-Husseini A, Shoker A. "Can cystatin C replace creatinine to estimate glomerular filtration rate? A literature review". Am. J. Nephrol. 2007; 27(2): 197–205.

94. Muntner P, Winston J, Uribarri J, Mann D, Fox CS. "Overweight, obesity, and elevated serum cystatin C levels in adults in the United States". Am. J. Med. 2008; 121(4): 341–348.

95. Hwang SJ, Yang Q, Meigs JB, Pearce EN, Fox CS. "A genome-wide association for kidney function and endocrine-related traits in the NHLBI's Framingham Heart Study". BMC Med. Genet. 2007; 8 Suppl 1: S10.

96. Janowski R, Kozak M, Jankowska E et al.. "Human cystatin C, an amyloidogenic protein, dimerizes through three-dimensional domain swapping". Nature Structural & Molecular Biology 2001; 8(4): 316–320.

97. Grubb A, Löfberg H. "Human gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis". Proc. Natl. Acad. Sci. U.S.A. 1982; 79(9): 3024–3027.

98. Löfberg H, Grubb AO. "Quantitation of gamma-trace in human biological fluids: indications for production in the central nervous system". Scand. J. Clin. Lab. Invest. 1979; 39(7): 619–626.

99. Grubb A, Simonsen O, Sturfelt G, Truedsson L, Thysell H. "Serum concentration of cystatin C, factor D and beta 2-microglobulin as a measure of glomerular filtration rate". Acta Med Scand 1985; 218(5): 499–503.

100. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. New Engl J Med. 1984;310:356–60.

101. Mussap M, Dalla Vestra M, Fioretto P, Saller A, Varagnolo M, Nosadini R. Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. Kidney Int. 2002;61:1453–61.

102. Hoek FJ, Kemperman FA, Krediet RT. A comparison between cystatin C, plasma creatinine and the Cockroft and Gault formula for the estimation of glomerular filtration rate. Nephrol Dial Transplant. 2003;18:2024–31.

103. MacIsaac RJ, Tsalamandris C, Thomas MC, Premaratne E, Panagiotopoulos S, Smith TJ, The accuracy of cystatin C

and commonly used creatinine-based methods for detecting moderate and mild chronic kidney disease in diabetes. Diabet Med. 2007;24:443–8.

104. Pucci L, Triscornia S, Lucchesi D, Fotino C, Pellegrini G, Pardini E, et al. Cystatin C and estimates of renal function: searching for a better measure of kidney function in diabetic patients. Clin Chem. 2007;53:480–8.

105. Perkins BA, Nelson RG, Ostrander BE, Blouch KL, Krolewski AS, Myers BD, et al. Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study. J Am Soc Nephrol. 2005;16:1404–12.

106. Buysschaert M, Joudi I, Wallemacq P, Hermans MP. Performance of serum cystatin-C versus serum creatinine in subjects with type 1 diabetes. Diabetes Care. 2003;26:1320.

107. Tan GD, Lewis AV, James TJ, Altmann P, Taylor RP, Levy JC. Clinical usefulness of cystatin C for the estimation of glomerular filtration rate in type 1 diabetes: reproducibility and accuracy compared with standard measures and iohexol clearance. Diabetes Care. 2002;25:2004–9.

108. Donahue RP, Stranges S, Rejman K, Rafalson LB, Dmochowski J, Trevisan M. Elevated cystatin C concentration and progression to pre-diabetes: the Western New York study. Diabetes Care. 2007;30:1724–9.

109. Rigalleau V, Beauvieux MC, Le Moigne F, Lasseur C, Chauveau P, Raffaitin C, Perlemoine C, Barthe N, Combe C, Gin H. Cystatin C improves the diagnosis and stratification of chronic kidney disease, and the estimation of glomerular filtration rate in diabetes. Diabetes Metab. 2008;34:482–489.

110. Rigalleau V, Lasseur C, Raffaitin C, Beauvieux MC, Barthe N, Chauveau P, Combe C, Gin H. Normoalbuminuric renal-insufficient diabetic patients: a lower-risk group. Diabetes Care. 2007;30:2034–2039.

111. Kramer CK, Leitão CB, Pinto LC, Silveiro SP, Gross JL, Canani LH. Clinical and laboratory profile of patients with type 2 diabetes with low glomerular filtration rate and normoalbuminuria. Diabetes Care. 2007;30:1998–2000.

112. Stevens LA, Levey AS. Measurement of kidney function. Med Clin North Am. 2005;89:457–473.

113. Uslu S, Efe B, Alataş O, Kebapçi N, Colak O, Demirüstü C, Yörük A. Serum cystatin C and urinary enzymes as screening markers of renal dysfunction in diabetic patients. J Nephrol. 2005;18:559–567.

114. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem. 2006;52:5–18.

115. Gregg EW, Cheng YJ, Cadwell BL, Imperatore G, Williams DE, Flegal KM, Narayan KM, Williamson DF. Secular trends in cardiovascular disease risk factors according to body mass index in US adults. JAMA. 2005;293:1868–1874.

116. Olivarius Nde F, Andreasen AH, Keiding N, Mogensen CE. Epidemiology of renal involvement in newly-diagnosed middle-aged and elderly diabetic patients. Cross-sectional data from the population-based study "diabetes care in general practice", Denmark. Diabetologia. 1993;36:1007–1016.

117. Comper WD, Osicka TM, Jerums G. High prevalence of immuno-unreactive intact albumin in urine of diabetic patients. Am J Kidney Dis. 2003;41:336–342.

118. Mussap M, Dalla Vestra M, Fioretto P, Saller A, Varagnolo M, Nosadini R, Plebani M. Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. Kidney Int. 2002;61:1453–1461.

119. Oddoze C, Morange S, Portugal H, Berland Y, Dussol B. Cystatin C is not more sensitive than creatinine for detecting early renal impairment in patients with diabetes. Am J Kidney Dis. 2001;38:310–316.

120. Ix JH. Utility of cystatin C measurement--precision or secretion? Nephrol Dial Transplant. 2006;21:3614.

121. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum

creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem. 2006;52:5–18.

122. Swedko PJ, Clark HD, Paramsothy K, Akbari A. Serum creatinine is an inadequate screening test for renal failure in elderly patients. Arch Intern Med. 2003;163:356–60.

123. Huang WC., et al. b2Microglobulin, aa21-119, Recombinant, J Biol Chem. 2010; 285(11):7947-56 2.

124. Morabito A., et al. (2009) Human (B2M, Beta 2 Microglobulin, Hum Immunol. 70(7):492-5.

125. Bethea M, Forman DT. Beta 2-microglobulin: its significance and clinical usefulness. Ann Clin Lab Sci. 1990;20(3):163-8.

126. Evrin PE, Wibell L. The Serum Levels and Urinary Excretion of β2-Microglobulin in Apparently Healthy Subjects. Scandinavian Journal of Clinical & Laboratory Investigation. 1972;29 (1):69-74.

127. Durie BG, Stock-Novack D, Salmon SE, Finley P, Beckord J, Crowley J, et al. Prognostic value of pretreatment serum beta 2 microglobulin in myeloma: a Southwest Oncology Group Study. Blood. 1990;75(4):823-30.

128. Adachi N. Beta-2-microglobulin levels in the cerebrospinal fluid: their value as a disease marker. A review of the recent literature. Eur Neurol. 1991;31(4):181-5.

Appendices

Appendix (1)

خطاب موافقة

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

كلية المختبرات الطبية

قسم الكيمياء السريرية

أقر أنا ..... بالموافقة على مشاركتي في الدراسة بعنوان (تقييم مستويات السيستاتين سي، البيتا 2 ميكروقلوبيولين والكرياتنين في البلازما كؤشرات لوظائف الكلى لدى السودانيين المصابين بالسكري من النوع الثاني) وأخذ عينة دم (5) مل لإجراء الاختبارات المعملية المطلوبة للبحث، بعد شرح أهداف وأغراض الدراسة.

الاسم ..... التوقيع .....

Appendix (2)

SUDAN UNIVERSTY OF SCIENCE AND TECHNOLOGY

COLLEGE OF GRADUATE STUDIES and Scientific Research

Comparative Assessment Of plasma Cystatin C, β2 Microgloulin and Creatinine as Markers of Renal Function in Sudanese patients with Type2 Diabetes

Questionnaire

Name: ……

Age: …….

Sex ……..

Marital status

…….

Educational level ……..

Occupation …….

Weight …….. kg height …… (m) BMI …….

Duration of disease ……

Present history of disease:

- Diabetes……..
- Hypertension……..
- Renal impairment……..
- Gout…….
- Ischemic heart disease…….

- Others…….

Family history of disease:

- Diabetes……..

- Hypertension……..

- Renal impairment…….

- Gout……..

- Ischemic heart disease…….

- Others…….

Investigations:

1. **Random plasma glucose.**
2. **Urine glucose.**
3. **Urine protien.**
4. **Plasma levels of cystatin C.**
5. **Plasma levels of B_2 –microglobulin.**
6. **Blood HbA_{1c} %.**

Appendix (3)

Cystatin C

Manufactured for: IBL America, Inc.
8201 Central Ave, NE, Suite P
Minneapolis, MN 55432, USA
Tel: 763-780-2955 / Fax: 763-780-2988
Email: info@ibl-america.com
Website: www.ibl-america.com

Cystatin C Assay

Configuration

The IBL-America, Inc. Cystatin C Assay reagent is provided in packaging configurations:

Configuration	Catalog No.	Kit size
Universal	IB46123	R1: 1 x 100 mL R2: 1 x 20 mL Cal: IB46123-CAL*
Hitachi 917	IB46123-KH1	R1: 1 x 60 mL R2: 1 x 12 mL Cal: IB46123-CAL*
	IB46123-KH2	R1: 2 x 60 mL R2: 2 x 12 mL Cal: IB46123-CAL*
Beckman CX/LX	IB46123-KB1	R1: 1 x 60 mL R2: 1 x 18 mL Cal: IB46123-CAL*
	IB46123-KB2	R1: 2 x 60 mL R2: 2 x 18 mL Cal: IB46123-CAL*

* Calibrators packaged separately

Intended Use

The IBL-America, Inc. Cystatin C Assay is an in-vitro diagnostic test for the quantitative determination of Cystatin C in serum or plasma by latex enhanced immunoturbidimetric method. The measurement of Cystatin C is used as an aid in the diagnosis and treatment of renal disease. For *in vitro* diagnostic use only.

Clinical Significance

Glomerular Filtration Rate (GFR) is a direct measure of renal function. GFR is measured by using a clearance determined by a biologically inert substance freely filtered through the glomerular membrane and re-entering circulation. Determination of creatinine clearance is the most widely used method for non-invasive estimation of GFR. However, creatinine evaluation is influenced by muscle mass, body surface and food intake; therefore, one must consider age, gender, height, and body composition when evaluating patient samples. Creatinine clearance leads to significant overestimation on GFR in those patients with highly decreased GFR due to tubular secretion. The collection of 24 hour urine is time consuming and creates additional sources of errors.

Cystatin C is a basic proteinase inhibitor with a low molecular mass of 13Kda that is produced at a constant rate in all nucleated cells and appears in human plasma and serum. Cystatin C is freely filtered through the glomerulus, is not secreted by the tubule or eliminated via any extra-renal route, and is almost completely absorbed and metabolized by proximal tubular cells. Therefore, the plasma concentration of Cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), making Cystatin C an excellent indicator of GFR. Cystatin C has advantages over routine clinical measures of renal function. It is more accurate than plasma creatinine and the Cockcroft-Gault estimation of creatinine clearance and is more reliable than the 24-h creatinine clearance. There is a growing body of evidence that suggests that Cystatin C can be used to detect kidney disease at earlier stages than serum creatinine which may help facilitate prevention efforts in the elderly and those with diabetes, hypertension, or cardiovascular disease.[1-6]

Assay Principle

The Cystatin C Assay is based on a latex enhanced immunoturbidimetric assay. Cystatin C in the sample binds to the specific anti-Cystatin C antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of Cystatin C in the sample. The instrument calculates the Cystatin C concentration of a patient specimen by interpolation of the obtained signal of a 6-point calibration curve.

Materials Required but not Provided

An analyzer capable of dispensing two reagents and of measuring absorbance at around 540 nm with temperature control (37°C).

Controls for validating the performance of the Cystatin C reagents are provided separately (IB46123-Con). Saline, which is used for diluting serum samples and used as a zero calibrator, is also not provided.

Reagent Composition

Reagent 1
100 mM Tris-buffer solution, ready to use

Reagent 2
Suspension of anti-human Cystatin C chicken polyclonal antibody coated latex particles (< 0.5%), ready to use

Calibrators
Ready to use liquid calibrators containing Cystatin C

Reagent Preparation

1. The Cystatin C reagents are provided ready to use.
2. Physiological saline is needed to dilute high Cystatin C samples and is also used as a zero calibrator.

Reagent Stability and Storage

The Cystatin C assay reagents, calibrators, and controls should be stored at 2-8°C. **DO NOT FREEZE**. The reagents, calibrators, and controls are stable when stored as instructed until the expiration date on the label. Open reagent bottles are stable for 4 weeks if stored on board the refrigerated compartment of the analyzer. Do not mix reagents of different lots.

Specimen Collection and Handling

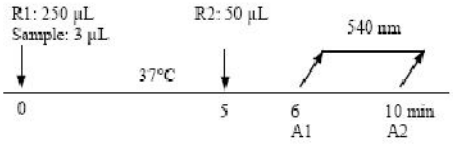
Serum or lithium heparin plasma or EDTA plasma samples can be used for the Cystatin C assay. For serum, collect whole blood by venipuncture and allow clotting. For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. Samples can be stored at 2-8°C, if analyzed within 12 days. Otherwise, samples must be stored at -20° C. It is recommended that frozen samples are thawed at room temperature; samples must be mixed well before analysis.

Precautions

1. The Cystatin C assay reagents, calibrators, and controls should be stored at 2-8°C. DO NOT FREEZE.
2. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
3. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
4. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 888-523-1246.
5. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
6. The reagent contains <0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
7. Do not use the reagents after the expiration date labeled on the outer box.
8. Assay calibration frequency is dependent on instrument used. Additionally, the assay should be recalibrated and controls run with each new lot of reagents.

Test Scheme for Chemistry Analyzer

Cystatin C should be measured according to specific application parameters for specific chemistry analyzers. Below is a general example of the assay test scheme.



Application questions on the Diazyme Cystatin C assay should be addressed to Diazyme technical support. Please call 888-523-1246 or email: info@ibl-america.com

Calibration

Five levels of Cystatin C calibrators (IB46123-Cal) are provided separately and ready to use. For automated analyzers, use saline and the provided calibrator 1-5 for calibration. Assay calibration frequency is dependent on instrument used. Additionally, the assay should be recalibrated and controls run with each new lot of reagents.

Quality Control

We recommend that each laboratory use Cystatin C controls to validate the performance of Cystatin C reagents. A set of normal and abnormal ranges of Cystatin C controls is available from Diazyme Laboratories (Cat. No. IB46123-CON). Each laboratory should follow federal, state, and local guidelines for testing QC material.

Results

Results are printed out in mg/L. Note: Samples with values greater than 8.0 mg/L should be diluted 1:1 with saline and rerun. Multiply results by 2.

Reference Range

The assay reagents were used in a study according to CLSI C28-A3 protocol with serum specimens from apparently healthy adults in the age range of 18-55. The reference range interval was calculated using non-parametric statistics representing the central 95% of the population. Results indicated a reference range of 0.62-1.16 mg/L. In the literature[6] a reference range of 0.5 to 1.03 mg/L is cited. However, it is recommended that each laboratory should establish a range of normal values for the population in their region.

Limitations

1. A sample with a Cystatin C level exceeding the linearity limit of 8.0 mg/L should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.
2. As with any latex turbidimetric immunoassays, Cystatin C assay runs should be followed with appropriate and tho- rough wash steps. Please consult specific instrument manuals for further information.

Performance Characteristics (Hitachi 917)

Precision

The precision of the Cystatin C Assay was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study, three samples containing Cystatin C were tested on Hitachi 917 2 runs per day in duplicates over 20 working days.

Within-Run Precision

	Level 1: 0.9 mg/L	Level 2: 2.5 mg/L	Level 3: 5.4 mg/L
N	80	80	80
Mean	0.91	2.51	5.40
SD	0.03	0.06	0.11
CV%	3.5	2.5	2.0

Within-Laboratory Precision

	Level 1: 0.9 mg/L	Level 2: 2.5 mg/L	Level 3: 5.4 mg/L
N	80	80	80
Mean	0.91	2.51	5.40
SD	0.04	0.08	0.25
CV%	4.6	3.0	4.6

Accuracy

Correlation studies were performed by testing 45 serum samples in comparison with an existing commercial Cystatin C assay method. The linear regression gives a correlation of r^2 value of 0.9922, slope of 0.9999, and y intercept of 0.0715.

Linearity

Eleven levels of the Cystatin C linearity set were prepared by diluting a specimen containing 8.0 mg/L Cystatin C with saline according to Clinical and Laboratory Standards Institute (formerly NCCLS) EP6-Aand tested on Hitachi 917. The Cystatin C assay is linear from 0.2 to 8.0 mg/L.

Limit of Quantitation (LOQ)

The LOQ of the CYSTATIN C Assay was determined on the Hitachi 917 according to CLSI (formerly NCCLS) EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. Specimens with mean measured concentrations ranging from 0.013 to 0.269 were assayed. Based on the EP evaluator-8 fitted model, the LOQ (lowest concentration for which CV is less than a target of 20%) is 0.19 mg/L Cystatin C.

Interference
The following substances do not interfere with this assay at the levels tested (less than 10% bias):

Hemoglobin:	up to 1000 mg/dL
Bilirubin:	up to 40 mg/dL
Bilirubin Conjugated:	up to 40 mg/dL
Triglycerides:	up to 1000 mg/dL
Ascorbic acid:	up to 176 mg/dL
Rheumatoid Factor:	up to 1000 IU/mL

References

1. Filler G, Bökenkamp A, Hofmann W, Le Bricon T, Martinéz-Brú C, Grubb A. Cystatin C as a marker of GFR - history, indications, and future research. Clin Biochem 2005; 38: 1-8.
2. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 2002; 40: 221-226.
3. Grubb A, Björk J, Bondesson P, Lindström V, Sterner G, Nyman U. Cystatin C estimates glomerular filtration rate better than creatinine clearance using the Cockgroft-Gault formula. Scand J Clin Lab Invest 2005; 65: 1-10.
4. Thomassen SA, Johannesen IL, Erlandsen EJ, Abrahamsen J, Randers E. Serum cystatin C as a marker of the renal function in patients with spinal cord injury. Spinal Cord 2002; 40: 524-528.
5. Christensson AG, Grubb AO, Nilsson JA, Norrgren K, Sterner G, Sundkvist G. Serum Cystatin C advantageous compared with serum creatinine in the detection of mild but not severe diabetic nephropathy. J Intern Med 2004; 256: 510-518.
6. Alan H.B. Wu. Tietz Clinical Guide to Laboratory Tests. Fourth Ed. Saunders Elsevier, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. 2006; 328-329

Appendix (4)

Beta 2 Microglobulin

SIEMENS

OQWUG15C0502 (400)

N Latex β_2–Microglobulin

N B2M

See shaded sections - updated information versus edition from April 2008

Intended Use

In vitro diagnostic reagent for the quantitative determination of β_2-microglobulin in human serum, heparinized and EDTA plasma, as well as in urine by means of particle-enhanced immunonephelometry on the BN* Systems.

Summary and Explanation

β_2-microglobulin has a molecular weight of 11,800 and occurs on all nucleated cells as a component of the HLA complex. It is constantly released into the blood in small quantities. β_2-microglobulin is freely filtered in the kidneys where it is reabsorbed and degraded in the renal tubules. Therefore, the serum levels found in healthy individuals remain at consistently low levels, and the urine is found to contain almost no β_2-microglobulin[1]. A rise of the serum concentrations occurs as a result of a higher release of β_2-microglobulin due to increased activity of the immune system, like in infections or rheumatic diseases, cell death, or diminished elimination due to renal damage. The serum concentration of β_2-microglobulin is thus a sensitive marker for the glomerular filtration capacity of the kidneys, particularly in children[1-3]. A rise of the urinary levels of β_2-microglobulin is associated with the presence of tubular damage; thus, the assay of urinary β_2-microglobulin is a suitable means for diagnosis and follow-up assessment of tubulointerstitial renal damage[1-3]. The extremely high serum concentration in dialysis patients can lead to β_2-microglobulin amyloidosis[1].

Furthermore, serum β_2-microglobulin is a good prognostic marker in multiple myeloma[4] as well as for monitoring and therapy assessment of lymphatic neoplasia[1]. Elevated serum levels of β_2-microglobulin can also occur with HIV infection and play a major role in prognosis and therapeutic monitoring[1,2,5].

As the serum concentration of β_2-microglobulin can be elevated in a variety of disease states, its diagnostic application always should follow a clear clinical question and should rule out the presence of other relevant diseases[1]. Elevated concentrations of β_2-microglobulin in serum or plasma are also found in patients with multiple myeloma and chronic lymphatic leukemia[1,2]. Its specificity for situations with increased cell proliferation can be enhanced by determining the β_2-microglobulin/cystatin C ratio[6].

Principle of the Method

Polystyrene particles coated with specific antibodies to human β_2-microglobulin are aggregated when mixed with samples containing human β_2-microglobulin. These aggregates scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.

Reagents

Materials provided

N Latex β_2-Microglobulin, REF *OQWU*

3 x 3 mL N B2M REAGENT, N Latex β_2-Microglobulin Reagent

1 x 2 mL N B2M U STAB, Urine Stabilizer

Composition

N Latex β_2-Microglobulin Reagent consists of a suspension of polystyrene particles coated with mouse monoclonal antibodies to human β_2-microglobulin.

Urine Stabilizer consists of a solution containing detergent.

Preservatives

N Latex β_2-Microglobulin Reagent: Gentamicin 6.25 mg/L, Amphotericin 0.625 mg/L

Warnings and Precautions

1. For *in-vitro*-diagnostic use.
2. Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests found to be in conformance with the In Vitro Diagnostic Directive in the EU or FDA approved tests. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Preparation of the Reagent

N Latex β_2-Microglobulin Reagent is supplied ready-for-use. Shake carefully to mix before the first use.

The Urine Stabilizer is supplied ready-for-use.

Storage and Stability

Stability at +2 to +8 °C:

See expiry date on the label.

Stability once opened:

4 weeks, if stored at +2 to +8 °C securely capped immediately after each use and if contamination (e.g. by microorganisms) is precluded.

Do not freeze.

On-board Stability:

A minimum of five days, at eight hours per day, or comparable period of time.

Note: On-board stability may vary, depending on BN* System used and laboratory conditions. For further details, refer to BN* II and BN ProSpec® System Instruction Manual.

Materials required but not provided

BN* System

N Protein Standard SL, REF OQIM

N/T Protein Control SL/L, REF OQIN

N/T Protein Control SL/M, REF OQIO

N/T Protein Control SL/H, REF OQIP

N Supplementary Reagent L, REF OQTD

N Diluent, REF OUMT

BN* II Evaporation Stoppers (optional), REF OVLE

1 N Sodium hydroxide solution (for alkalizing urine samples)

Additional materials and supplies as described in your BN* System Instruction Manual.

Specimens

Suitable samples are human serum and plasma (EDTA or heparinized), either as fresh as possible (stored for no more than 8 days at +2 to +8 °C) or stored frozen, and also fresh urine samples. Serum and plasma samples can be stored at below -20 °C for up to 2 months, if they are frozen within 24 hours after collection and if repeated freeze-thaw cycles are avoided. Serum samples must be completely coagulated and, after centrifugation, must not contain any particles or traces of fibrin. For β_2-microglobulin determination in urine random and timed urine collections are suitable specimens. The use of urine samples stored frozen is not recommended. Each urine sample must be centrifuged prior to testing, thereafter 1 drop of Urine Stabilizer must be added to 1 mL of the centrifuged urine sample and this sample must then be thoroughly mixed.

Lipemic samples, or frozen samples which became turbid after thawing, must be clarified by centrifugation (10 minutes at approximately 15,000 x g) prior to testing.

For β_2-microglobulin determination urine samples with a pH value < 6.0 must be adjusted to pH 7 – 9 by the addition of 1 N NaOH as soon as possible after receipt.

Urine samples, containing Urine Stabilizer for β_2-microglobulin determination are no longer suitable for the determination of total protein on the BN* Systems.

Procedure

Notes

1. Consult your BN* System Instruction Manual for details regarding operation of the instrument.
2. With a BN* II and BN ProSpec® System reagents and samples stored at +2 to +8 °C can be used immediately.

Assay Protocols for the BN* Systems

The assay protocols, for serum and plasma, and for urine, respectively are given in the BN* System Instruction Manual and software of the instrument.

All steps are performed automatically by the system.

Establishing the Reference Curve

Reference curves are generated by multi-point calibration. Serial dilutions of N Protein Standard SL are prepared automatically by the instrument using N Diluent. The standard dilutions must be used within 4 hours. The reference curve is valid for 4 weeks and can be used beyond this period of time, as long as controls with corresponding method-dependent target values, e. g. N/T Protein Controls SL, M and H for the serum/plasma assay and N/T Protein Controls SL, L and M for the urine assay, are reproduced within their respective confidence interval. If a different lot of reagent is used, a new reference curve must be recorded.

Assay of Specimens

Serum and plasma samples are automatically diluted 1:400 with N Diluent. Urine samples are automatically diluted 1:100 with N Diluent using a separate assay protocol. The diluted samples must be used within 4 hours. If the results obtained are outside the measuring range, the assay can be repeated using a higher or lower (only serum/plasma assay protocol) dilution of the sample. Refer to BN* System Instruction Manual for information on repeat measurements using other dilutions.

Internal Quality Control

Assay N/T Protein Controls SL, M and H, after each establishment of a reference curve, the first opening of a reagent vial as well as with each run of serum or plasma samples. For the determination of β_2-microglobulin in urine N/T Protein Controls SL, L and M should be used accordingly. The controls are assayed and evaluated as for the patient samples. The assigned value and confidence interval are listed in the Table of Assigned Values of the respective control.

If a result of the controls is outside the confidence interval, the determination must be repeated. If the repeated determination confirms the deviation, a new reference curve should be established. Do not release patient results until the cause of deviation has been identified and corrected.

Results

Evaluation is performed automatically in mg/L or in a unit selected by the user on the BN* System.

Limitations of the Procedure

No interference was detected in serum for concentrations of triglycerides up to 20 g/L, of bilirubin up to 0.6 g/L, and of free hemoglobin up to 10 g/L.

Turbidity and particles in the samples may interfere with the determination. Therefore, samples containing particles must be centrifuged prior to testing. Lipemic or turbid samples which cannot be clarified by centrifugation (10 minutes at approximately 15,000 x g) must not be used. β_2-microglobulin is unstable in urine samples with pH < 6.0 and therefore these must be alkalinized to pH 7 - 9 as soon as possible after receipt using 1 N NaOH.

On the BN* II System, after measuring samples with extremely high β_2-microglobulin concentrations, false-high values may be obtained in a β_2-microglobulin determination performed directly afterwards. In suspect cases, a repeat measurement should be performed, particularly if the β_2-microglobulin results obtained are marginally above the upper limit of the reference range.

Patient samples may contain heterophilic antibodies that could react in immunoassays to give a falsely elevated or depressed result[7]. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed.

Siemens Healthcare Diagnostics has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these Instructions for Use.

Results of these tests should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Due to matrix effects, inter-laboratory survey samples and control samples may yield results that differ from those obtained with other methods. It may therefore be necessary to assess these results in relation to method-specific target values.

Reference Intervals

In a study on 183 apparently healthy blood donors from Central Europe, the values obtained with N Latex β_2-Microglobulin were in a range of 1.09 to 2.53 mg/L (2.5th and 97.5th percentiles)[8].

Neonates and infants up to one year show higher values than adults[9]. The β_2-microglobulin concentrations in serum increase with age, probably as a result of declining renal function[1]. A reference interval for β_2-microglobulin in urine has been established on 196 individuals from Central Europe, without renal-tubular diseases and without increased B cell turnover. None of the samples showed a β_2-microglobulin concentration within the measuring range of N Latex β_2-Microglobulin Reagent. Therefore the upper limit of the reference interval for urine is 0.2 mg/L.

Nevertheless, each laboratory should determine its own reference intervals, since values may vary depending on the population studied.

Specific Performance Characteristics

Sensitivity

The analytical sensitivity of the assay is determined by the lower limit of the reference curve and therefore depends on the concentration of the protein in N Protein Standard SL.

Specificity

No cross-reactivity of the applied antibody is known.

Precision

Serum/Plasma assay

The following coefficients of variation (CV) were obtained with N Latex β_2-Microglobulin (n = 40) on a BN* System:

Precision (n = 40)				
Sample	Mean mg/L	Run-to-Run CV (%)	Within-Run CV (%)	Total CV (%)
N/T Protein Control SL/M	1.98	1.9	3.2	3.4
N/T Protein Control SL/H	5.25	1.5	2.1	2.4
Serum pool 1	1.59	2.1	2.0	2.8
Serum pool 2	2.57	1.7	1.1	2.0
Serum pool 3	5.62	4.1	1.9	4.5

The results were evaluated by analysis of variance on the basis of the recommendations in the CLSI Guideline EP5 A[10].

Urine assay

The following coefficients of variation (CV) were obtained with N Latex β_2-Microglobulin (n = 40) on a BN* System:

Precision (n = 40)				
Sample	Mean mg/L	Run-to-Run CV (%)	Within-Run CV (%)	Total CV (%)
N/T Protein Control SL/L	1.02	2.0	2.6	3.1
N/T Protein Control SL/M	1.85	1.6	1.9	2.3
Urine pool 1	2.95	1.9	2.3	2.8
Urine pool 2	0.95	0.7	2.8	2.6

The results were evaluated by analysis of variance on the basis of the recommendations in the CLSI Guideline EP5 A[10].

Method Comparison

Serum/Plasma assay

A comparison of 120 serum samples measured using N Latex β_2-Microglobulin Reagent on a BN* System (y) and in parallel a commercially available β_2-microglobulin method (particle enhanced immunonephelometry) (x) yielded a correlation coefficient of 0.996 with a y-intercept of 0.12 mg/L and a slope of 0.96.

Urine assay

A comparison of 106 urine samples measured using N Latex β_2-Microglobulin Reagent on a BN* System (y) and in parallel a commercially available β_2-microglobulin method (microparticle enzyme immunoassay) (x) yielded a correlation coefficient of 0.986 with a y-intercept of -0.04 mg/L and a slope of 1.03.

Note

The values cited for specific performance characteristics of the assay represent typical values and are not to be regarded as specifications for N Latex β_2-Microglobulin Reagent.

Bibliography

1. Thomas L. β_2-Mikroglobulin. In: Thomas L, ed. Clinical Laboratory Diagnostics. Frankfurt/Main: TH-Books Verlagsgesellschaft,1998:685-8.
2. Bethea M, Forman DT. Beta 2-microglobulin: its significance and clinical usefulness. Ann Clin Lab Sci 1990;20:163-8.
3. Tomlinson PA. Low molecular weight proteins in children with renal disease. Pediatr Nephrol 1992;6:565-71.
4. Kyle RA. Prognostic factors in multiple myeloma. Stem Cells 1995;13 Suppl 2:56-63.
5. Zabay JM, Sempere JM, Benito JM, et al. Serum beta 2-microglobulin and prediction of progression to AIDS in HIV-infected injection drug users. J Acquir Immune Defic Syndr Hum Retrovirol 1995;8:266-72.
6. Norlund L, Fex G, Lanke J, et al. Reference intervals for the glomerular filtration rate and cell-proliferation markers: serum cystatin C and serum beta 2-microglobulin/cystatin C-ratio. Scand J Clin Lab Invest 1997;57:463-70.
7. Kricka LJ. Human anti-animal antibody interference in immunological assays. Clin Chem 1999;45:942-956.
8. Mátrai Z, Németh J, Miklós K, et al. Serum ß2-microglobulin measured by immunonephelometry: expression patterns and reference intervals in healthy adults. Clin Chem Lab Med 2009,47:585-9.
9. Liappis N. Referenzwerte der β_2-Mikroglobulin-Konzentration im Serum von Kindern. Klin Pädiatr 1988;200:67-9.
10. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline. CLSI document EP5-A (ISBN 1-56238- 368-X). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 1999.

* BN is a trademark of Siemens Healthcare Diagnostics.

BN ProSpec is a trademark of Siemens Healthcare Diagnostics.

Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg/Germany
www.siemens.com/diagnostics

CE

Edition August 2010

N Latex β_2–Mikroglobulin

N B2M

Schattierte Abschnitte - Aktualisierte Informationen gegenüber der Ausgabe April 2008

Anwendungsbereich

In-vitro-Diagnostika zur quantitativen Bestimmung von β_2-Mikroglobulin in humanem Serum, Heparin- und EDTA-Plasma sowie Urin mittels partikelverstärkter Immun-Nephelometrie mit den BN* Systemen.

Diagnostische Bedeutung

β_2-Mikroglobulin hat ein Molekulargewicht von 11.800 und kommt auf allen kernhaltigen Zellen als Bestandteil des HLA-Komplexes vor. Es wird in geringer Menge ständig in das Blut abgegeben. Da es in der Niere frei filtriert und tubulär mit nachfolgender Degradation reabsorbiert wird, finden sich im Serum gesunder Personen gleichbleibend geringe Mengen und im Urin nahezu kein β_2-Mikroglobulin[1]. Eine gesteigerte Freisetzung durch erhöhte Aktivität des Immunsystems, z. B. bei Infektionen oder rheumatischen Erkrankungen, Zelltod oder eine verminderte Elimination durch eine Schädigung der Niere im glomerulären Bereich führen zu einem Anstieg der Serumkonzentration. Die Serumkonzentration von β_2-Mikroglobulin ist somit ein empfindlicher Marker für die glomeruläre Filtrationsleistung der Niere, insbesondere bei Kindern[1-3]. Bei Vorliegen eines tubulären Schadens steigt die β_2-Mikroglobulin-Konzentration im Urin an; die β_2-Mikroglobulin-Bestimmung im Urin eignet sich somit zur Diagnostik und Verlaufsbeurteilung tubulointerstitieller Nierenschäden[1-3]. Die stark erhöhte Serumkonzentration bei Dialysepatienten kann zur β_2-Mikroglobulin-Amyloidose führen[1].

Daneben ist β_2-Mikroglobulin im Serum ein guter prognostischer Marker beim multiplen Myelom[4] sowie zur Verlaufs- und Therapiebeurteilung bei lymphoiden Neoplasien[5]. Erhöhtes β_2-Mikroglobulin im Serum kann auch bei HIV-Infektionen vorliegen und spielt eine wichtige Rolle bei der Prognose und Behandlungsüberwachung[1,2,5].

Aufgrund der unterschiedlichen Ursachen der Serum-Konzentrationserhöhungen von β_2-Mikroglobulin erfordert seine diagnostische Anwendung eine klare klinische Fragestellung und den Ausschluss anderer relevanter Erkrankungen[1]. Erhöhte Konzentrationen von β_2-Mikroglobulin im Serum oder Plasma treten außerdem auf bei Patienten mit mutiplem Myelom sowie chronischer lymphatischer Leukämie[1,2]. Seine Spezifität für Zustände mit erhöhter Zellproliferation kann durch die Bestimmung des β_2-Mikroglobulin/Cystatin C-Quotienten gesteigert werden[6].

Prinzip der Methode

Polystyrol-Partikel, die mit spezifischen Antikörpern gegen humanes β_2-Mikroglobulin beladen sind, bilden bei Mischung mit β_2-Mikroglobulin enthaltenden Proben Aggregate, an denen eingestrahltes Licht gestreut wird. Die Intensität des Streulichts ist abhängig von der Konzentration des jeweiligen Proteins in der Probe. Die Auswertung erfolgt durch Vergleich mit einem Standard bekannter Konzentration.

Reagenzien

Inhalt der Handelspackung

N Latex β_2-Mikroglobulin, REF *OQWU*

3 x 3 ml N B2M, REAGENT, N Latex β_2-Mikroglobulin Reagenz

1 x 2 ml N B2M, U STAB, Urinstabilisator

Zusammensetzung

N Latex β_2-Mikroglobulin Reagenz besteht aus einer Suspension von Polystyrol-Partikeln, die mit monoklonalen Antikörpern (Maus) gegen humanes β_2-Mikroglobulin beladen sind.

Urinstabilisator besteht aus einer Detergenzlösung.

Konservierungsmittel

N Latex β_2-Mikroglobulin Reagenz: Gentamicin 6,25 mg/l, Amphotericin 0,625 mg/l

Warnungen und Vorsichtsmaßnahmen

1. Nur zur *in-vitro*-diagnostischen Anwendung.
2. Jede individuelle Blutspende wurde mit negativem Befund auf humane Immundefizienz-Viren (HIV) 1 und 2, Hepatitis B-Viren (HBV) und Hepatitis C-Viren (HCV) getestet. Die eingesetzten Teste entsprachen entweder den Anforderungen der EU Richtlinie über In-vitro-Diagnostika oder waren von der FDA zugelassen. Da kein Test mit völliger Sicherheit die Abwesenheit von Infektionserregern garantieren kann, sollten alle Produkte mit humanen Bestandteilen mit angemessener Sorgfalt behandelt werden.

Vorbereitung des Reagenzes

Das N Latex β_2-Mikroglobulin Reagenz ist gebrauchsfertig. Es ist vor dem ersten Gebrauch behutsam zu durchmischen.

Der Urinstabilisator ist gebrauchsfertig.

Haltbarkeit und Lagerungsbedingungen

Lagerung bei +2 bis +8 °C:

Das Verfallsdatum ist auf dem Etikett angegeben.

Stabilität nach Öffnen:

4 Wochen, sofern unmittelbar nach Gebrauch wieder dicht verschlossen bei +2 bis +8 °C gelagert und eine Kontamination (z.B. durch Mikroorganismen) vermieden wird.

Das Reagenz darf nicht eingefroren werden.

Stabilität auf den BN* Systemen:

Minimal 5 Tage mit jeweils 8 Stunden, oder ein vergleichbarer Zeitraum.

Hinweis: Die „on-board" Stabilität hängt von dem verwendeten BN* System sowie den Laborbedingungen ab. Weiterführende Angaben sind in den Bedienungsanleitungen des BN* II und BN ProSpec® Systems enthalten.

Zusätzlich benötigte Materialien

BN* System

N Protein-Standard SL, REF OQIM

N/T Protein-Kontrolle SL/L, REF OQIN

N/T Protein-Kontrolle SL/M, REF OQIO

N/T Protein-Kontrolle SL/H, REF OQIP

N Zusatzreagenz L, REF OQTD

N-Diluens, REF OUMT

BN* II Evaporation Stoppers (wahlweise), REF OVLE

1 N Natronlauge (zur Alkalisierung von Urinproben)

Verbrauchsmaterial und Ausrüstung wie in den Bedienungsanleitungen der BN* Systeme beschrieben.

Untersuchungsmaterial

Zur Messung sollen möglichst frische (max. 8 Tage bei +2 bis +8 °C aufbewahrte) oder gefroren gelagerte humane Serumproben, Heparin- und EDTA-Plasmaproben, sowie frische Urinproben eingesetzt werden. Werden Serum- und Plasmaproben innerhalb von 24 Stunden nach Entnahme tiefgefroren, so ist eine Lagerung unterhalb von -20 °C bis zu 2 Monaten möglich, wenn wiederholtes Auftauen und Einfrieren vermieden wird. Serumproben müssen vollständig geronnen sein und dürfen nach Zentrifugation keine Partikel oder Spuren von Fibrin enthalten. Für die Bestimmung von β_2-Mikroglobulin im Urin eignen sich Spontan- und Sammelurinproben. Die Messung von gefroren gelagerten Urinproben wird nicht empfohlen. Jede Urinprobe ist vor der Analyse zu zentrifugieren, anschließend ist zu 1 ml der zentrifugierten Urinprobe ein Tropfen des Urinstabilisators zuzugeben und gut zu durchmischen.

Lipämische Proben oder eingefrorene Proben, die nach dem Auftauen trüb sind, müssen vor der Bestimmung durch Zentrifugation (10 min bei ca. 15.000 x g) geklärt werden.

Urinproben mit einem pH-Wert < 6,0 müssen für die β_2-Mikroglobulin-Bestimmung möglichst bald nach Erhalt durch Zugabe von 1 N NaOH auf pH 7 – 9 eingestellt werden.

Urinproben, die zur Bestimmung von β_2-Mikroglobulin mit Urinstabilisator versetzt wurden, können nicht mehr zur Bestimmung des Gesamtproteins an den BN* Systemen eingesetzt werden.

Testdurchführung

Hinweise

1. Einzelheiten zur Bedienung der BN* Systeme sind der entsprechenden Bedienungsanleitung zu entnehmen.
2. Am BN* II und BN ProSpec® System können bei +2 bis +8 °C gelagerte Reagenzien und Proben direkt eingesetzt werden.

Assay-Protokolle an den BN* Systemen

Die Assay-Protokolle jeweils für Serum und Plasma bzw. für Urin sind in der Bedienungsanleitung sowie der Software des jeweiligen Gerätes enthalten. Alle Schritte werden automatisch vom System durchgeführt.

Erstellung der Referenzkurve

Referenzkurven werden über Mehrpunktkalibrierung aufgenommen. Für die Erstellung werden automatisch Verdünnungsreihen des N Protein-Standards SL mit N-Diluens hergestellt. Die Standard-Verdünnungen müssen innerhalb von 4 Stunden verwendet werden. Die Referenzkurve ist 4 Wochen lang gültig. Sie kann über diesen Zeitraum hinaus verwendet werden, solange Kontrollen mit entsprechenden verfahrensabhängigen Sollwerten, wie z. B. die N/T Protein-Kontrollen SL, M und H für die Serum-/Plasmabestimmung und die N/T Protein-Kontrollen SL, L und M für die Urinbestimmung, innerhalb des jeweiligen Vertrauensbereichs wiedergefunden werden. Bei Verwendung einer anderen Reagenzcharge muss eine neue Referenzkurve aufgenommen werden.

Messung der Patientenproben

Serum- und Plasmaproben werden automatisch 1:400 mit N-Diluens verdünnt. Urinproben werden in einem separaten Assay-Protokoll automatisch 1:100 mit N-Diluens verdünnt. Die Verdünnungen müssen innerhalb von 4 Stunden gemessen werden. Bei Messwerten, die außerhalb des Messbereichs liegen, kann die Messung aus einer höheren oder niedrigeren (nur im Serum/Plasma Assay-Protokoll) Probenverdünnung wiederholt werden. Wiederholungsmessungen aus weiteren Probenverdünnungen sind in den Bedienungsanleitungen der BN* Systeme beschrieben.

Appendix (5)

Creatinine

04810716190V10

CREJ2

Creatinine Jaffé Gen.2

cobas®

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Creatinine Jaffé Gen.2			**cobas c** 311	**cobas c** 501
700 tests	Cat. No. **04810716** 190	System-ID 07 6928 2	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm PUC (4 x 3 mL)	Cat. No. **03121313** 122	Code 240		
Precipath PUC (4 x 3 mL)	Cat. No. **03121291** 122	Code 241		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

English

System information

CREJ2: ACN 690 (Rate blanked, compensated, serum and plasma)
CRJ2U: ACN 691 (Rate blanked, urine)
SCRE2: ACN 773 (STAT, compensated, serum and plasma, reaction time: 4)
SCR2U: ACN 774 (STAT, urine, reaction time: 4)

Intended use

In vitro test for the quantitative determination of creatinine in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5]

Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m^2 for three months or more, regardless of cause.
The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted.

Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of glomerular filtration rate (GFR) is given by the creatinine clearance test based on creatinine's concentration in urine and serum or plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum or plasma have been made. Among the various approaches suggested, two have found wide recognition: that of Cockroft and Gault and that based on the results of the MDRD trial. While the first equation was derived from data obtained with the conventional Jaffé method, a newer version of the second is usable for IDMS-traceable creatinine methods. Both are applicable for adults. In children, the Schwartz formula is used.

In addition to the diagnosis and treatment of renal disease, the monitoring of renal dialysis, creatinine measurements are used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase). Numerous methods were described for determining creatinine. Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests.

Test principle[6,7,8]

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses "rate-blanking" to minimize interference by bilirubin. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -26 µmol/L (-0.3 mg/dL).

Creatinine + picric acid $\xrightarrow{\text{Alkaline pH}}$ yellow-orange complex

Reagents - working solutions

R1	Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L; pH ≥ 13.5; preservative; stabilizer
R2/R3	Picric acid: 38 mmol/L; pH 6.5; non reactive buffer

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.
This kit contains components classified as follows according to the European directive 1999/45/EC.


C – Corrosive. R1 contains potassium hydroxide.
R 1: Explosive when dry. R 4: Forms very sensitive, explosive metallic compounds. R 34: Causes burns.
S 24-25: Avoid contact with skin and eyes. S 26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S 35: This material and its container must be disposed of in a safe way.
S 36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S 45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

Reagent handling

Ready for use.

Storage and stability

CREJ2	
Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks
Diluent NaCl 9 %	
Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation[9]

For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-heparin and K_2-EDTA plasma
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

CREJ2

Creatinine Jaffé Gen.2

cobas®

Urine.

Collect urine without using additives. If urine must be collected with a preservative for other analytes, only hydrochloric acid (14 to 47 mmol/L urine, e.g. 5 mL 10 % HCl or 5 mL 30 % HCl per liter urine) or boric acid (81 mmol/L, e.g. 5 g per liter urine) may be used.

Stability in *serum/plasma*:[10]	7 days at 15-25 °C 7 days at 2-8 °C 3 months at (-15)-(-25) °C
Stability in *urine* (without preservative):[10]	2 days at 15-25 °C 6 days at 2-8 °C 6 months at (-15)-(-25) °C
Stability in *urine* (with preservative):[11]	3 days at 15-25 °C 8 days at 2-8 °C 3 weeks at (-15)-(-25) °C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.
General laboratory equipment

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/27-37 - 15-23 (STAT 4/12-19)		
Wavelength (sub/main)	570/505 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mmol/L)		
Reagent pipetting		Diluent (H_2O)	
R1	13 µL	77 µL	
R3	17 µL	30 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	20 µL	80 µL
Increased	10 µL	–	–

Enter the correction value for the non-specific protein reaction as the instrument factor **y = ax + b** for mg/dL or for µmol/L, where **a = 1.0** and **b = -0.3** (mg/dL) or **a = 1.0** and **b = -26** (µmol/L).

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/42-52 - 24-34 (STAT 4/17-27)		
Wavelength (sub/main)	570/505 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mmol/L)		
Reagent pipetting		Diluent (H_2O)	
R1	13 µL	77 µL	
R3	17 µL	30 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	–	–
Decreased	10 µL	20 µL	80 µL
Increased	10 µL	–	–

Enter the correction value for the non-specific protein reaction as the instrument factor **y = ax + b** for mg/dL or for µmol/L, where **a = 1.0** and **b = -0.3** (mg/dL) or **a = 1.0** and **b = -26** (µmol/L).

Application for urine

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/27-37 - 15-23 (STAT 4/12-19)		
Wavelength (sub/main)	570/505 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mmol/L)		
Reagent pipetting		Diluent (H_2O)	
R1	13 µL	77 µL	
R3	17 µL	30 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	6 µL	144 µL
Decreased	10 µL	2 µL	180 µL
Increased	10 µL	10 µL	115 µL

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/42-52 - 24-34 (STAT 4/17-27)		
Wavelength (sub/main)	570/505 nm		
Reaction direction	Increase		
Units	µmol/L (mg/dL, mmol/L)		
Reagent pipetting		Diluent (H_2O)	
R1	13 µL	77 µL	
R3	17 µL	30 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10 µL	6 µL	144 µL
Decreased	10 µL	2 µL	180 µL
Increased	10 µL	10 µL	115 µL

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - and as required following quality control procedures

CREJ2

Creatinine Jaffé Gen.2

cobas®

Traceability: This method has been standardized against ID/MS. For the USA, this method has been standardized against a primary reference material (SRM 914).

Quality control

For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.

Serum/plasma

For quality control use undiluted serum control material as listed above.
Other suitable control material can be used in addition.

Urine

For quality control use Precinorm PUC and Precipath PUC as listed above.
Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: µmol/L x 0.0113 = mg/dL
µmol/L x 0.001 = mmol/L

Limitations – interference[12]

Criterion: Recovery within ± 10 % of initial value at a creatinine concentration of 80 µmol/L (0.90 mg/dL) in serum/plasma and 2500 µmol/L (28.3 mg/dL) in urine.

Serum/plasma

Icterus (*CREJ2*): No significant interference up to an I index of 5 for conjugated bilirubin and 10 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 86 µmol/L (5 mg/dL) and approximate unconjugated bilirubin concentration: 171 µmol/L (10 mg/dL)).

Icterus (*SCRE2*): No significant interference up to an I index of 2 for conjugated bilirubin and 3 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 34 µmol/L (2 mg/dL) and approximate unconjugated bilirubin concentration: 51 µmol/L (3 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic levels using common drug panels.[13,14]

Exception: Cefoxitin causes artificially high creatinine results.

Exception: Cyanokit (Hydroxocobalamin) may cause interference with results.

Values < 15 µmol/L (< 0.17 mg/dL) or negative results are reported in rare cases in children < 3 years and in elderly patients. In such cases use the Creatinine plus test to assay the sample.

Do not use Creatinine Jaffé for the testing of creatinine in hemolyzed samples from neonates, infants or adults with HbF levels ≥ 60 mg/dL for *CREJ2* applications (≥ 30 mg/dL for *SCRE2* applications).[15] In such cases, use the Creatinine plus test (≤ 600 mg/dL HbF) to assay the sample.

Estimation of the Glomerular Filtration Rate (GFR) on the basis of the Schwartz Formula can lead to an overestimation.[16]

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Icterus: No significant interference up to a conjugated bilirubin concentration of 855 µmol/L (50 mg/dL).

Hemolysis: No significant interference up to a hemoglobin concentration of 621 µmol/L (1000 mg/dL).

Glucose < 120 mmol/L (< 2162 mg/dL) and urobilinogen < 676 µmol/L (< 40 mg/dL) do not interfere.

Drugs: No interference was found at therapeutic levels using common drug panels.[14]

Exception: Cyanokit (Hydroxocobalamin) may cause interference with results.

High homogentisic acid concentrations in urine samples lead to false results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Serum/plasma

15-2200 µmol/L (0.17-24.9 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 5.

Urine

375-55000 µmol/L (4.2-622 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3.6 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 3.6.

Lower limits of measurement

Lower detection limit of the test

Serum/plasma

15 µmol/L (0.17 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Urine

375 µmol/L (4.2 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Serum/plasma		
Adults[17]		
Females	44-80 µmol/L	(0.50-0.90 mg/dL)
Males	62-106 µmol/L	(0.70-1.20 mg/dL)
Children[18]		
Neonates (premature)	25-91 µmol/L	(0.29-1.04 mg/dL)
Neonates (full term)	21-75 µmol/L	(0.24-0.85 mg/dL)
2-12 m	15-37 µmol/L	(0.17-0.42 mg/dL)
1-< 3 y	21-36 µmol/L	(0.24-0.41 mg/dL)
3-< 5 y	27-42 µmol/L	(0.31-0.47 mg/dL)
5-< 7 y	28-52 µmol/L	(0.32-0.59 mg/dL)
7-< 9 y	35-53 µmol/L	(0.40-0.60 mg/dL)
9-< 11 y	34-65 µmol/L	(0.39-0.73 mg/dL)
11-< 13 y	46-70 µmol/L	(0.53-0.79 mg/dL)
13-< 15 y	50-77 µmol/L	(0.57-0.87 mg/dL)
Urine		
1st morning urine[17]		
Females	2470-19200 µmol/L	(28-217 mg/dL)
Males	3450-22900 µmol/L	(39-259 mg/dL)

CREJ2

Creatinine Jaffé Gen.2

cobas®

24-hour urine[19]		
Females	7000-14000 µmol/24 h	(740-1570 mg/24 h)
Males	9000-21000 µmol/24 h	(1040-2350 mg/24 h)
Creatinine clearance[19,20]	71-151 mL/min	

Refer to reference 16 for a prospective study on creatinine clearance in children.[21]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. *Serum/plasma:* repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days);
Urine: repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Serum/plasma (CREJ2)

*Repeatability**	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Precinorm U	105 (1.19)	2 (0.03)	2.1
Precipath U	360 (4.07)	4 (0.05)	1.1
Human serum 1	206 (2.33)	3 (0.03)	1.2
Human serum 2	422 (4.77)	5 (0.06)	1.3

*Intermediate precision***	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Precinorm U	101 (1.14)	4 (0.05)	3.5
Precipath U	351 (3.97)	8 (0.09)	2.2
Human serum 3	201 (2.27)	5 (0.06)	2.5
Human serum 4	411 (4.64)	9 (0.10)	2.2

Urine (CRJ2U)

*Repeatability**	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Control Level 1	8083 (91.3)	115 (1.3)	1.4
Control Level 2	15618 (177)	213 (2)	1.4
Human urine 1	19318 (218)	234 (3)	1.2
Human urine 2	7958 (89.9)	130 (1.5)	1.6

*Intermediate precision***	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Control Level 1	8130 (91.9)	164 (1.9)	2.0
Control Level 2	15533 (176)	251 (3)	1.6
Human urine 3	19353 (219)	385 (4)	2.0
Human urine 4	7932 (89.6)	166 (1.9)	2.1

Serum/plasma (SCRE2)

*Repeatability**	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Precinorm U	106 (1.20)	2 (0.02)	2.2
Precipath U	346 (3.91)	5 (0.06)	1.5
Human serum 1	543 (6.14)	6 (0.07)	1.1
Human serum 2	69 (0.78)	2 (0.02)	3.1

*Intermediate precision***	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Precinorm U	100 (1.13)	4 (0.05)	4.0
Precipath U	334 (3.77)	10 (0.11)	3.0
Human serum 3	522 (5.90)	12 (0.14)	2.4
Human serum 4	64 (0.72)	3 (0.03)	5.0

Urine (SCR2U)

*Repeatability**	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Control Level 1	6287 (71.0)	82 (0.9)	1.2
Control Level 2	15252 (172)	182 (2)	1.2
Human urine 1	24174 (273)	212 (2)	0.9
Human urine 2	2146 (24.2)	48 (0.5)	2.2

*Intermediate precision***	*Mean µmol/L (mg/dL)*	*SD µmol/L (mg/dL)*	*CV %*
Control Level 1	6943 (78.5)	114 (1.3)	1.6
Control Level 2	15394 (174)	229 (3)	1.5
Human urine 3	24230 (274)	354 (4)	1.5
Human urine 4	2184 (24.7)	54 (0.6)	2.5

* repeatability = within-run precision
** intermediate precision = total precision / between run precision / between day precision

Method comparison

Creatinine values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined on Roche/Hitachi 917/MODULAR P analyzers (x), using the corresponding Roche/Hitachi reagent.

Serum/plasma (CREJ2)
Sample size (n) = 273

Passing/Bablok[22]	Linear regression
y = 1.000x - 0.65 µmol/L	y = 1.002x - 0.98 µmol/L
τ = 0.973	r = 0.999

The sample concentrations were between 38 and 2178 µmol/L (0.43 and 24.6 mg/dL).

Urine (CRJ2U)
Sample size (n) = 223

Passing/Bablok[22]	Linear regression
y = 0.999x + 20.66 µmol/L	y = 0.999x + 41.55 µmol/L
τ = 0.969	r = 0.999

The sample concentrations were between 934 and 50228 µmol/L (10.6 and 568 mg/dL).

Serum/plasma (SCRE2)
Sample size (n) = 224

Passing/Bablok[22]	Linear regression
y = 1.000x - 14.36 µmol/L	y = 0.996x - 12.17 µmol/L
τ = 0.964	r = 0.999

The sample concentrations were between 66 and 1775 µmol/L (0.75 and 20.1 mg/dL).

Urine (SCR2U)
Sample size (n) = 223

Passing/Bablok[22]	Linear regression
y = 0.999x + 67.83 µmol/L	y = 0.998x + 112.72 µmol/L
τ = 0.973	r = 0.999

The sample concentrations were between 931 and 48729 µmol/L (10.5 and 551 mg/dL).

References

1. Thomas C, Thomas L. Labordiagnostik von Erkrankungen der Nieren und ableitenden Harnwege. In: Thomas L, ed. Labor und Diagnose. 6th ed. Frankfurt/Main: TH-Books 2005;520-85.
2. Lamb E, Newman DJ, Price CP. Kidney function tests. In: Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. St. Louis, MO: Elsevier Saunders 2006;797-835.
3. http://www.kidney.org/
4. http://www.nkdep.nih.gov/
5. Lamb EJ, Tomson CRV, Roderick PJ. Estimating kidney function in adults using formulae. Ann Clin Biochem 2005;42:321-45.

Appendix (6) Glycated HbA_{1c}

04528123190V7

A1C-2

Tina-quant ® Hemoglobin A1c Gen.2

cobas®

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
Tina-quant ® Hemoglobin A1c Gen.2 150 tests	Cat. No. **04528123** 190	System-ID 07 6850 2	•	•
C.f.a.s. HbA1c (3 x 2 mL)	Cat. No. **04528417** 190	Code 674		
HbA1c Control N (4 x 0.5 mL)	Cat. No. **20764833** 322	Code 357		
HbA1c Control P (4 x 0.5 mL)	Cat. No. **20764841** 322	Code 358		
Hemolyzing Reagent Gen.2 (51 mL)*	Cat. No. **04528182** 190	System-ID 07 6873 1		
HbA1c Hemolyzing Reagent for Tina-quant ® HbA1c (1000 mL)	Cat. No. **11488457** 122	For Hemolysate Application only		

* The value encoded in the instrument settings is 45 mL to account for the dead volume of the bottles.

English

System information

Whole Blood Application - Standardized according to IFCC transferable to DCCT/NGSP

HB-W2:	ACN 870	Hemoglobin (Hb)
A1-W2:	ACN 880	Hemoglobin A1c (HbA1c)
RWI2:	ACN 890	% Ratio
A1CD2:	ACN 952	Hemolyzing reagent

Hemolysate Application - Standardized according to IFCC transferable to DCCT/NGSP

HB-H2:	ACN 840	Hemoglobin (Hb)
A1-H2:	ACN 850	Hemoglobin A1c (HbA1c)
RHI2:	ACN 860	% Ratio
A1CD2:	ACN 952	Hemolyzing reagent

Intended use

In vitro test for the quantitative determination of percent hemoglobin A1c [HbA1c (%)] in whole blood or in hemolysate on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6,7,8,9]

Hemoglobin (Hb) consists of four protein subunits, each containing a heme moiety, and is the red-pigmented protein located in the erythrocytes. Its main function is to transport oxygen and carbon dioxide in blood. Each Hb molecule is able to bind four oxygen molecules. Hb consists of a variety of subfractions and derivatives. Among this heterogeneous group of hemoglobins HbA1c is one of the glycated hemoglobins, a subfraction formed by the attachment of various sugars to the Hb molecule. HbA1c is formed in two steps by the nonenzymatic reaction of glucose with the N-terminal amino group of the β-chain of normal adult Hb (HbA). The first step is reversible and yields labile HbA1c. This is rearranged to form stable HbA1c in a second reaction step.

In the erythrocytes, the relative amount of HbA converted to stable HbA1c increases with the average concentration of glucose in the blood. The conversion to stable HbA1c is limited by the erythrocyte's life span of approximately 100 to 120 days. As a result, HbA1c reflects the average blood glucose level during the preceding 2 to 3 months. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus. Glucose levels closer to the time of the assay have a greater influence on the HbA1c level.[1]

The approximate relationship between HbA1c and mean blood glucose values during the preceding 2 to 3 months was analyzed in several studies. A recent study obtained the following correlation:

IFCC standardization (recalculated acc. to ref. 8)

- Mean plasma glucose [mmol/L] = 1.73 x HbA1c (%) + 0.20 or
- Mean plasma glucose [mg/dL] = 31.2 x HbA1c (%) + 3.51

Standardization acc. to DCCT/NGSP[8]

- Mean plasma glucose [mmol/L] = 1.98 x HbA1c (%) - 4.29 or
- Mean plasma glucose [mg/dL] = 35.6 x HbA1c (%) - 77.3

The risk of diabetic complications, such as diabetic nephropathy and retinopathy, increases with poor metabolic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the development of diabetic complications in diabetes patients.[3,5]

For routine clinical use, testing every 3 to 4 months is generally sufficient. In certain clinical situations, such as gestational diabetes, or after a major change in therapy, it may be useful to measure HbA1c in 2 to 4 week intervals.[7]

Test principle[10,11,12]

This method uses TTAB* as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary. All hemoglobin variants which are glycated at the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are measured by this assay. Consequently, the metabolic state of patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, HbAE) can be determined using this assay.[13,14]

*TTAB = Tetradecyltrimethylammonium bromide

Hemoglobin A1c

The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

- Sample and addition of R1 (Antibody reagent): Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, complex formation does not take place.
- Addition of R2 (Polyhapten reagent) and start of reaction: The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be measured turbidimetrically.

Hemoglobin

Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the preincubation phase (sample + R1) of the above immunological reaction. A separate Hb reagent is consequently not necessary.

Ratio definition

The final result is expressed as percent HbA1c and is calculated from the HbA1c/Hb ratio as follows:

Protocol 1 (acc. to IFCC):
HbA1c (%) = (HbA1c/Hb) x 100

Protocol 2 (acc. to DCCT/NGSP):
HbA1c (%) = (HbA1c/Hb) x 87.6 + 2.27

The IFCC and NGSP directed the manufacturers not to change their current HbA1c report out values until further decisions by the ADA/EASD/IDF working group on the HbA1c assay.[15] This means that most countries should continue to report the established DCCT/NGSP values. If you are uncertain of the situation in your country, please contact your local authorities to ensure which approach is appropriate for your laboratory.

Reagents – working solutions

R1 Antibody reagent
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c antibody (bovine serum) ≥ 0.5 mg/mL; stabilizers; preservatives (liquid)

R2 Polyhapten reagent
MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c polyhapten: ≥ 8 µg/mL; stabilizers; preservatives (liquid)

A1C-2

cobas®

Tina-quant ® Hemoglobin A1c Gen.2

cobas c 501 **test definition HbA1c (A1-W2)**

Assay type	2 Point End
Reaction time / Assay points	10 / 34-70
Wavelength (sub/main)	660/340 nm
Reaction direction	Increase
Unit	g/dL

Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	5 µL	2 µL	180 µL
Decreased	5 µL	2 µL	180 µL
Increased	5 µL	2 µL	180 µL

Ratio definition for HbA1c (%) calculation

Protocol 1 (acc. to IFCC):

Abbreviated ratio name	**RWI2 (890)**
Equation	(A1-W2/HB-W2) x 100
Unit	%

Protocol 2 (acc. to DCCT/NGSP):

Abbreviated ratio name	**RWD2**
Equation	(A1-W2/HB-W2) x 87.6 + 2.27
Unit	%

Protocol 1 is already implemented in the application (ACN 890). Percent HbA1c according to Protocol 2 (DCCT/NGSP) must be manually calculated according to the above equation. If requested the formula (ACN 890) can be modified by using the Administrator Level/EDIT Button.
The ratio for HbA1c (%) will be automatically calculated after result output of both tests.
It is recommended to report HbA1c values to one decimal place, which can be entered in the editable field "expected values".

Hemolysate Application for Hb (HB-H2) and HbA1c (A1-H2)

cobas c 311 **test definition Hb (HB-H2)**

Assay type	1 Point
Reaction time / Assay points	10 / 23
Wavelength (sub/main)	660/376 nm
Reaction direction	Increase
Unit	g/dL

Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	–	–
Decreased	5 µL	–	–
Increased	5 µL	–	–

cobas c 311 **test definition HbA1c (A1-H2)**

Assay type	2 Point End
Reaction time / Assay points	10 / 23-57
Wavelength (sub/main)	660/340 nm
Reaction direction	Increase
Unit	g/dL

Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	–	–
Decreased	5 µL	–	–
Increased	5 µL	–	–

cobas c 501 **test definition Hb (HB-H2)**

Assay type	1 Point
Reaction time / Assay points	10 / 34
Wavelength (sub/main)	660/376 nm
Reaction direction	Increase
Unit	g/dL

Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	–	–
Decreased	5 µL	–	–
Increased	5 µL	–	–

cobas c 501 **test definition HbA1c (A1-H2)**

Assay type	2 Point End
Reaction time / Assay points	10 / 34-70
Wavelength (sub/main)	660/340 nm
Reaction direction	Increase
Unit	g/dL

Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R3	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent*
Normal	5 µL	–	–
Decreased	5 µL	–	–
Increased	5 µL	–	–

Ratio definition for HbA1c (%) calculation

Protocol 1 (acc. to IFCC):

Abbreviated ratio name	**RHI2 (860)**
Equation	(A1-H2/HB-H2) x 100
Unit	%

Protocol 2 (acc. to DCCT/NGSP):

Abbreviated ratio name	**RHD2**
Equation	(A1-H2/HB-H2) x 87.6 + 2.27
Unit	%

Protocol 1 is already implemented in the application (ACN 860). Percent HbA1c according to Protocol 2 (DCCT/NGSP) must be manually calculated according to the above equation. If requested the formula (ACN 860) can be modified by using the Administrator Level/EDIT Button.
The ratio for HbA1c (%) will be automatically calculated after result output of both tests.