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**Assessment of plasma and urine β 2-Microglobulin,
Microalbumin as biomarker for early detection of renal
impairment and correlate with Lipid Profile in Sudanese
with type 2 Diabetes**

*A Dissertation Submitted in full fulfillment for the Requirements of the degree of PhD in
clinical chemistry.*

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Abstract

This study is case control study was conducted in Jaber Aboalaz Diabetes Center in Khartoum state during the period from March 2013 to September 2015.

The study aimed to compare of the mean of plasma levels of total cholesterol, triacylglycerides, HDL, LDL, Creatinine and urine of Creatinine, Microalbumin and Beta2 Microglobulin and Microalbumin/creatinine ratio and serum level of Beta2 Microglobulin and Body Mass Index (BMI) and blood HbA_{1c} of 300 Sudanese patients with type 2 Diabetes mellitus (155 males and 145 females) as a test group and 150 Sudanese healthy subjects (81 males and 69 females) as control group, all the participants in this study were matched in term of age.

The plasma levels of triacylglycerides and total cholesterol, HDL, LDL, Creatinine and urine Creatinine were measured using Biosystem BTS- 305 spectrophotometer and commercial kits from Biosystem Company and blood HbA_{1c} was measured using Nycocard® READER II in Accurate medical Lab, and urine of Microalbumin, Beta2 Microglobulin and serum level of Beta2 Microglobulin were measured using Biosystem A25 automated system in Alryan medical Center Lab, Microalbumin/creatinine ratio and Body Mass Index(BMI) were calculated.

The results were analyzed statically using SPSS and it was found that the mean of plasma total cholesterol, triacylglycerides, HDL, LDL, Creatinine and urine of Creatinine, Microalbumin and Beta2 Microglobulin and Microalbumin/creatinine ratio and serum level of Beta2 Microglobulin and BMI and blood HbA_{1c} were significant increased in diabetic patient compared with control group ($P \leq 0.050$).

The results were found that a significant elevation of the plasma levels of triacylglycerides, HDL, Creatinine and urine Microalbumin and blood HbA_{1c} and

Microalbumin/creatinine ratio was observed in type2 uncontrolled patients compared with controlled patients ($P = \leq 0.05$).

The results were found that a significant increase in the plasma levels of total cholesterol, HDLc, LDLc, Creatinine and urine Microalbumin and BMI and Microalbumin/creatinine ratio in Male types 2 diabetic patients compared with Female types 2 diabetic patients ($P = \leq 0.05$)

In present study, statistically insignificant correlation was found between serum level of beta2 microglobulin(B2M) and blood HbA_{1c} and plasma levels of total cholesterol, triacylglycerides and HDLc, and weak significant positive correlations between serum level of beta2 microglobulin(B2M) and urine creatinine and plasma level of LDLc, also was found a highly significant positive correlation between serum level of beta2 microglobulin(B2M) and urine microalbumin, plasma level of creatinine and microalbumin/creatinine ratio in type2 diabetic patients.

In this study insignificant correlations between body mass index and urine microalbumin and Strong significant positive correlations were observed between body mass index and plasma levels of Cholesterol and LDLc, and moderate significant positive correlations between body mass index and plasma level of triacylglycerides, and week significant positive correlations between body mass index and serum level of beta2 microglobulin(B2M) and serum level of creatinine in type2 diabetic patients.

From the results of this study it is concluded that:

Plasma levels of total Cholesterol, triacylglycerides, HDL, LDL, Creatinine and urine of Creatinine, Beta2 Microglobulin, Microalbumin and serum level of Beta2 Microglobulin and Microalbumin/creatinine ratio, BMI and blood HbA_{1c} were increased in type2 Diabetic patients compared with non diabetic subjects, this lead to increase the risk of cardiovascular disease, atherosclerosis and nephropathy complications.

مستخلص الدراسة

اجريت هذه الدراسة في مركز جابر ابو العز للسكري في ولايه الخرطوم في الفتره من مارس 2013 الي سبتمبر 2015م. حيث تم تقييم مستويات الدهون في بلازما الدم (الكلوستيرول ، ثلاثي الغليسريد، البروتين الدهني عالي الكثافه و البروتين الدهني منخفض الكثافه)، وكرياتنين المصل ، كرياتنين البول ، الميكروالبيومين في البول، علاقته الميكروالبيومين بالكرياتنين، الميكروغلوبولين في مصل الدم ، الميكروغلوبولين في البول، مؤشر كتلة الجسم والهيموغلوبين المجلز في الدم (هيموغلوبين A_{1c}) عند 300 من المرضى السودانيين المصابين بداء السكري من النوع الثاني (155 من الرجال و 145 من النساء) مع 150 من المتطوعين الاصحاء الغير مصابين بمرض السكري (81 من الرجال و 69 من النساء) كمجموعة ضابطه، وكان هنالك تطابق في العمر بين المرضى والمجموعه الضابطه.

لقد تم استخدام جهاز قياس الطيف الضوئي والمحاليل من شركة الانظمه الحيويه الالمانيه شبه اتوماتيكي BTS- 305 لقياس مستويات الدهون في بلازما الدم (الكلوستيرول ، ثلاثي الغليسريد، البروتين الدهني عالي الكثافه و البروتين الدهني منخفض الكثافه)، كرياتنين المصل و كرياتنين البول وتم استخدام جهاز نايوكارد ريدر II لقياس مستوي الهيموغلوبين المجلز في الدم (هيموغلوبين A_{1c}) في مختبر اكيورت الطبي.

كما تم استخدام جهاز قياس الطيف الضوئي من شركة الانظمه الحيويه الالمانيه اتوماتيكي A25 لقياس مستويات الميكروالبيومين في البول، الميكروغلوبولين في مصل الدم ، الميكروغلوبولين في البول في مختبر الريان المركزي. اما علاقته الميكروالبيومين بالكرياتنين ومؤشر كتلة الجسم تم حسابهما رياضيا.

اظهرت الدراسه ارتفاع ذو دلالة احصائيه في كل المستويات الوسطيه للدهون في بلازما الدم (الكلوستيرول ، ثلاثي الغليسريد، البروتين الدهني عالي الكثافه و البروتين الدهني منخفض الكثافه)، و كرياتنين المصل، كرياتنين البول، الميكروالبيومين في البول، علاقته الميكروالبيومين بالكرياتنين، الميكروغلوبولين في مصل الدم ، الميكروغلوبولين في البول، مؤشر كتلة الجسم والهيموغلوبين المجلز في الدم (هيموغلوبين A_{1c}) في مرضي السكري من النوع الثاني عند مقارنتهم بالاصحاء، القيمه الاحتماليه (اقل من 0.05)

كما اظهرت الدراسه ارتفاع ذو دلالة احصائيه في كل المستويات الوسطيه لبلازما الدم للدهون ثلاثية الغليسريد، البروتين الدهني عالي الكثافه، كرياتنين، الميكروالبيومين في البول، علاقته الميكروالبيومين بالكرياتنين والهيموغلوبين المجلز في الدم (هيموغلوبين A_{1c}) في مرضي السكري المنضبطين عند مقارنتهم بمرضي السكري من النوع الثاني الغير منضبطين، القيمه الاحتماليه (اقل من 0.05).

كما اظهرت الدراره ارتفاع ذو دلالة احصائية في كل المستويات الوسطيه لبلازما الدم للكلوستيرول ، البروتين الدهني عالي الكثافه، البروتين الدهني منخفض الكثافه، وكرياتنين، الميكر والبيومين في البول، علاقه الميكر والبيومين بالكرياتنين ومؤشر كتلة الجسم في مرضي السكري الذكور من النوع الثاني عند مقارنتهم بمرضي السكري الاناث من النوع الثاني، القيمه الاحتماليه (اقل من 0.05).

لم يكن هنالك ارتباط ذو دلالة احصائية معنويه بين الميكر وغلوبيولين في مصل الدم ووبلازما الدم للكلوستيرول، الدهون ثلاثيه الغلسريد، البروتين الدهني عالي الكثافه والهيموقلوبين المجلکز في الدم (هيموقلوبين A_{1c})، كما انه يوجد ارتباط موجب ضعيف ذو دلالة احصائية معنويه بين الميكر وغلوبيولين في مصل الدم وكرياتنين البول والبروتين الدهني منخفض الكثافه في بلازما الدم ويوجد ايضا ارتباط موجب قوي ذو دلالة احصائية معنويه بين الميكر وغلوبيولين في مصل الدم والميكر والبيومين في البول، علاقه الميكر والبيومين بالكرياتنين، كرياتنين في بلازما الدم في مرضي السكري من النوع الثاني.

لم يكن هنالك ارتباط ذو دلالة احصائية معنويه بين مؤشر كتلة الجسم والميكر والبيومين في البول وكرياتنين البول كما انه يوجد ارتباط موجب قوي ذو دلالة احصائية معنويه بين مؤشر كتلة الجسم وبلازما الدم للكلوستيرول والبروتين الدهني منخفض الكثافه، كما انه يوجد ارتباط موجب متوسط ذو دلالة احصائية معنويه بين مؤشر كتلة الجسم و الدهون ثلاثيه الغلسريد في بلازما الدم، كما انه يوجد ارتباط موجب ضعيف ذو دلالة احصائية معنويه بين مؤشر كتلة الجسم و بلازما الدم للميكر وغلوبيولين و الكرياتنين في مرضي السكري من النوع الثاني.

وعليه تلخص نتائج هذه الدراره : ان هنالك ارتفاع في مستويات الدهون في بلازما الدم (الكلوستيرول ، ثلاثي الغلسريد، البروتين الدهني عالي الكثافه و البروتين الدهني منخفض الكثافه)، كرياتنين المصل ، كرياتنين في البول، الميكر والبيومين في البول، علاقه الميكر والبيومين بالكرياتنين، الميكر وغلوبيولين في مصل الدم ، الميكر وغلوبيولين في البول، مؤشر كتلة الجسم والهيموقلوبين المجلکز في الدم (هيموقلوبين A_{1c}) عند المرضي السودانيين المصابين بداء السكري من النوع الثاني ممايؤدي الي زياده خطر الاصابه بامراض القلب والشرابين وتصلب الشرايين واعتلال الكليه.

Introduction

1. Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both; Diabetes causes about 5% of all deaths globally each year. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke^(1,2)).

Diabetic patients with accompanied (but often unnoticed) dyslipidemia are soft targets of cardiovascular deaths (CVD). Patients with type 2 diabetes often exhibit an atherogenic lipid profile, which greatly increases their risk of CVD compared with people without diabetes. An early intervention to normalize circulating lipids has been shown to reduce cardiovascular complications and mortality^(3,4).

Glycated hemoglobin (HbA_{1c}) is a routinely used marker for long-term glycemic control. In accordance with its function as an indicator for the mean blood glucose level, HbA_{1c} predicts the risk for the development of diabetic complications in diabetes patients.² Apart from classical risk factors like dyslipidemia, elevated HbA_{1c} has now been regarded as an independent risk factor for CVD in subjects with or without diabetes. Estimated risk of CVD has shown to be increased by 18% for each 1% increase in absolute HbA_{1c} value in diabetic population.⁵ Positive relationship between HbA_{1c} and CVD has been demonstrated in non-diabetic cases even within normal range of HbA_{1c}^(6,7).

The current criteria for diagnosis of diabetes rely on the etiology of diseases. Four forms of diabetes have been classified. These four forms are type 1, type 2, gestational diabetes, and other specific causes of diabetes.⁸ Aglycated hemoglobin, is an indicator of long-term glycemic control. In adults, hemoglobin is a mixture of three forms: Hb A₁, Hb A₂, and Hb F, with HbA₁ predominating. Hemoglobin A₁ consists of three subforms : Hb A_{1a}, HbA_{1b}, and HbA_{1c}, with HbA_{1c} predominating. The term glycated hemoglobin describes a chemically stable conjugate of any of the forms of hemoglobin with glucose. Glycated forms of hemoglobin are formed slowly, nonenzymatically, and irreversibly at a rate that is proportional to the concentration of glucose in the blood^(8, 9).

Diabetes mellitus incidence varies from country to country. It is estimated to be about 15% in the Caribbean⁽¹⁰⁾. Many of these patients have hypertension which accelerates the vascular complications associated with diabetes, including those of the retinal, renal cardiac and peripheral vascular systems.¹¹ One of the more chronic complications is diabetic nephropathy which can lead to end stage renal disease (ESRD). Early detection of nephropathy is important as intense glycaemic control⁽¹²⁾, and treatment with Angiotensin Converting Enzyme (ACE) inhibitors⁽¹³⁾, may delay its onset and progression.

Diabetic nephropathy is a serious microvascular complication of diabetes. Diabetic nephropathy is the most common cause of end stage renal disease and constitutes approximately 40% of all patients needing renal replacement therapy⁽¹⁴⁾. Type 2 diabetes is the largest and fastest growing disease which is in need of renal replacement therapy. The risk factors for nephropathy are older age, male sex, non-Caucasian race, and poor blood pressure, glycemic, and lipid control⁽¹⁵⁾.

The first sign of diabetic nephropathy is usually albuminuria and the first symptom peripheral oedema. The grade of decline in GFR in the natural history of diabetic

nephropathy has been found to be highly variable (2-20 ml/min/year) with a mean of 12 ml/min/year⁽¹⁵⁾.

Diabetic nephropathy is characterised by changes in the glomerulus filtration rate, expansion of extracellular matrix in the mesangial part, i.e. in the central part of the glomerulus, glomerular capillary crowding and overt renal occlusion leading to kidney failure.¹⁶ The clinical hallmark of diabetic nephropathy is persistent proteinuria greater than 500 mg/24 hours. This is equivalent to urinary albumin excretion rate of 300 mg/day (or over 200 µg/min) in a person with diabetes, and without any other renal disease⁽¹⁷⁾.

The current study aims to assess the relationship of glycaemic control and duration of diabetes mellitus to lipid profile and renal function in Sudanese with type 2 diabetic patients by determination the insulin, serum albumin, Glycohemoglobin (HbA_{1c}) and insulin resistance, serum creatinine, microalbumin, β₂-Microglobulin(B2M).

Renal function will be assessed by measuring s. creatinine, creatinine clearance, microalbuminuria and β₂-Microglobulin.

1.1 Rationale:

Diabetes mellitus is the medical condition that can be life-threatening if not treated properly. Type 2 Diabetes in Sudan is a common disease with increasing incidence. The majority are people above 45 years old. . More than two hundred million people worldwide have diabetes. It is clear that hyperglycemia is associated with a wide variety of pathological changes in both diabetic and nondiabetic patients. In both diabetic and nondiabetic critically ill patients, hyperglycemia is associated with impaired immunity that results in increased susceptibility to infection. The prevalence of cardiovascular complication is also abnormally elevated in Sudanese patients with diabetes type 2 due to poor Diabetes control in addition to risk factors for atherosclerosis related to alteration in lipid and lipoprotein profile. The microvascular pathologies in the retina, renal glomerulus, and nervous system, which occur as chronic complications of diabetes in many patients, may produce blindness, renal disease, and neuropathy, respectively. Hyperglycemia of diabetes is associated with cardiovascular disorders, stroke, and limb amputation. Controlling hyperglycemia reduces the risk of such consequences and improves patient outcome. Glycosylated Haemoglobin could give a clue for the glycemic condition of the individual for the past few months. That gives good indicator to uncontrolled or controlled diabetic patients. Many studies reported abnormal results for serum level of creatinine and creatinine clearance in association with type 2 diabetes in different countries. This study aims to show are there any significant differences in serum level of creatinine in Sudanese patients with type 2 diabetes mellitus ? .

This study aims to answer the question do uncontrolled Diabetic patients have an increase risk of atherosclerosis more than controlled Diabetic patients?

1.2 Study objectives:

1.2.1 General Objective:

To assess the plasma and urine levels of β 2-Microglobulin and urine Microalbumin as biomarker for early detection of renal impairment and correlate with Plasma Lipid Profile in Sudanese patients with type2 Diabetes in comparison with apparently healthy volunteers as controls.

1.2.2. Specific Objective:

1- To measure blood Glycosylated Haemoglobin (HbA_{1c}) % and plasma levels of triglycerides, total cholesterol, LDL, HDL and creatinine and serum level of β 2-Microglobulin and urine for microalbuminuria, creatinine and β 2-Microglobulin in patients and control groups.

2- To assess the relationship between serum level of β 2-Microglobulin to blood Glycosylated Haemoglobin(HbA_{1c}) and plasma levels of total cholesterol, triglycerides,LDL,HDL and creatinine and urine for creatinine, microalbuminuria and β 2-Microglobulin and microalbuminuria/ creatinine ratio and body mass index in type 2 diabetic patients.

3- To assess the relationship of the body mass index to serum level of β 2-Microglobulin to blood Glycosylated Haemoglobin (HbA_{1c}) and plasma levels of triglycerides, total cholesterol, LDL,HDL and creatinine and urine microalbuminuria, creatinine and β 2-Microglobulin and microalbuminuria/ creatinine ratio in type 2 diabetic patients.