Original Article

Assessment of Plasma Cystatin C among Sudanese Patients with Type II Diabetes Mellitus

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ABSTRACT

Background: Cystatin C is mainly used as a biomarker of kidney function. It is freely filtered by glomerulus, and does not return to the blood stream or secreted by renal tubules. It has been suggested to be closer to the "ideal" endogenous marker.

Objectives: To assess the plasma levels of cystatin c, creatinine clearance, blood HbA1C% and body mass index among Sudanese with type 2 Diabetes.

Materials and methods: This is a case-control study conducted in diabetic centers in Khartoum state, Sudan, from March 2010 to November 2013. A total of 300 Sudanese patients with type2 diabetes (49% males and 51% females) as a test group, and 150 healthy subjects (48%males and 52%females) as a control group were enrolled in this study. Both groups were matched for gender and age. The plasma levels of Cystatin C, creatinine and blood HbA1c were measured using Nephelometry technique. Creatinine clearance was calculated for each participant. SPSS was used for analysis of data.

Results: The means of the plasma levels of Cystatin C, HbA1c% and the body mass index (BMI) were significantly raised in the diabetic group compared to the control group ($p<0.05$).There is significant moderate negative correlation between Cystatin C and creatinine clearance ($r=0.69$, $p=0.015$) in the diabetic group, and there is a significant strong positive correlation between the plasma levels of Cystatin C and HbA1c%($r=o.78$, $p=0.044$).

Conclusion: The present data indicates that among Sudanese patients with Type 2 Diabetes Mellitus, plasma levels of Cystatin C are significantly raised and has a significant strong positive correlation with glycated haemoglobin % and a significant moderate negative correlation with creatinine clearance.

Key words: Type 2 Diabetes, Cystatin C, Glycated Heamoglobin, Sudan.

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels, which result from defects in insulin secretion, action, or both[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 micro vascular 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]. 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. From an economic perspective, the total annual cost of diabetes in 1997 was estimated to be 98 billion dollars in the United States[3]. Diabetes is the third leading cause of death in the United States after heart disease and cancer[4]. Diabetes, the most common non-communicable disease in Sudan, is having an increasing impact on rates of morbidity and mortality. The spread of

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sedentary lifestyles and adoption of western dietary habits that is high in refined carbohydrates and fat-are driving an increase in the number of people with obesity-related type 2 diabetes. Knowledge of the diabetes epidemic in Sudan is limited, and the most recent data come from a small-scale study indicated a prevalence of 3.4% but recent research estimates about four millions, around 95% of whom have type 2 diabetes mellitus[5].
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. 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[6]. Because of its small size and basic pH (9.0), Cystatin C is freely filtered by renal glomeruli. Cystatin C does 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[7].

MATERIALS AND METHODS:

This is an analytical, case-control and hospital- based study, that conducted in Jabir Abualizz and Almolazmeen diabetic centers in Khartoum State, Sudan, during the period from March 2010 to November 2013.A total of 300 Sudanese patients with type 2 diabetes mellitus were enrolled in this study as a test group, in contrast to 150 healthy volunteers as a control group. Both groups were matched for age and gender. Patients with type1 diabetes, gestational diabetes, renal insufficiency, cancer, Alzheimer's disease, multiple sclerosis and hereditary Cystatin C amyloid angiopathy were excluded from this study.
A venous blood sample (4mls) was collected from each participant by standard procedures and divided into two containers, 2mls in EDTA container for HbA1c %(whole blood) and 2mls in heparin containers to ,which was centrifuged at 300rpm for 3mintutes to get plasma that kept at -20C^{o} until used. The serum level of Cystatin C was measured using N latex Cystatin C (NCYSC) SIEMENS Nephelometry, and HbA1c for each sample was measured using Cobas system (SIEMENS Nephelometry). Plasma creatinine was measured using Jaffe reaction and then creatinine clearance for each participant was calculated using the Cockroft-Gault formula:
Creatinine clearance (GFR estimation) =

$$\frac{(140 - \text{age}) \times \text{weight (kg)} \times 1.23\ (0.85\text{for female})}{\text{Serum creatinine (mol/l)}}$$

The body mass index for each participant was calculated by measuring the height and the weight and using the following formula (BMI=Wt/heigt^2). The Statistical Package for Social Science (SPSS version11.5) computer software was used for data analysis, independent t-test was used for comparison and the significant level was set at $p \leq 0.05$.

RESULTS:

This study was conducted on 300 Sudanese patients with type2 diabetes mellitus (49% males and 51% females) as a test group and 150 healthy (non- diabetic) subjects (48% males and 52% female) as a control group. The test group and the control group were matched in term of gender and age. The mean age of the test group was 58.40±7.20 year and that of the control group was 56.8±10.0 year, (p=0.094).
Table (1) shows means of the plasma levels of Cystatin C, creatinine clearance, HbA1c% and body mass index of the test group when compared with the control group.

Figure (1) shows the correlation between the plasma Cystatin C and creatinine clearance. Figure (2) shows the correlations between the plasma levels of Cystatin C and HbA1c%, whereas Figure (3) shows the correlation between Cystatin C and body mass index.

DISCUSSION:

Cystatin C has low molecular weight (approximately 13.3 kilo Dalton), and it is measured from blood stream by the glomerular filtration in the kidneys. If kidney function and glomerular filtration rate decline,

Table (1): Comparison of the serum levels of Cystatin C, creatinine clearance, HbA1c and body mass index of the diabetic group (test group) and the control group.

Variables	Test group (n=300)	Control group (n=150)	(P)
Cystatin C mg/l	(1.35±1.06) (0.74- 3.87)	(0.84±0.18) (0.39- 1.28)	0.026*
Creatinine clearance ml/min	(80.66±33.17) (71.91, 120.00)	(93.37±30.61) (90.90, 138.89)	0.015*
HbA1C %	8.51±1.74 (3.75-11.32)	3.22±4.31 (2.35-5.11)	0.0157*
BMI kg/m^2	(25.69±3.77) (18.62,34.68)	(23.43±4.31) (18.43, 35.42)	0.043*

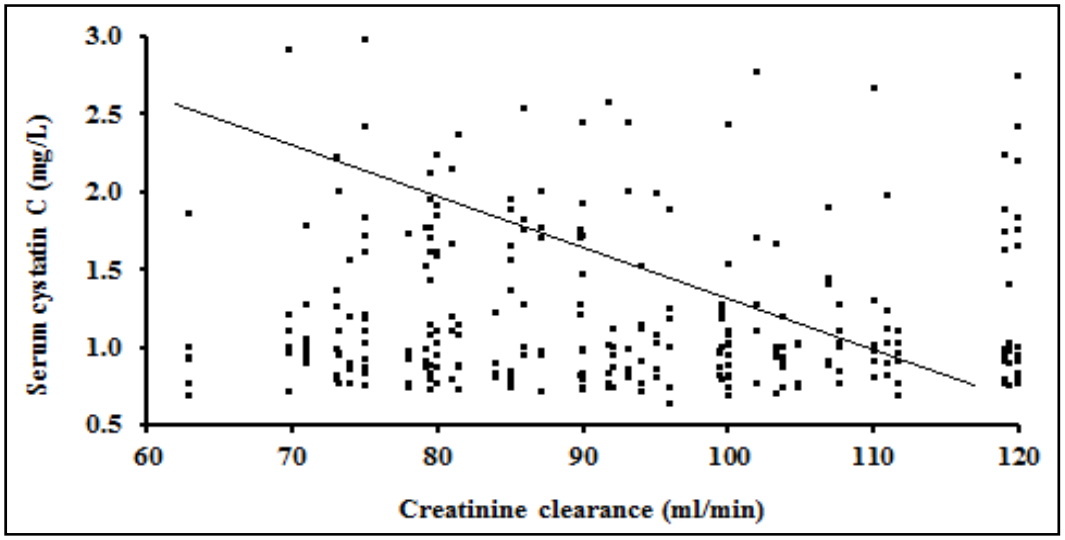

Figure (1): The relationship between creatinine clearance and serum cystatin C (r=-0.69; P=0.015)

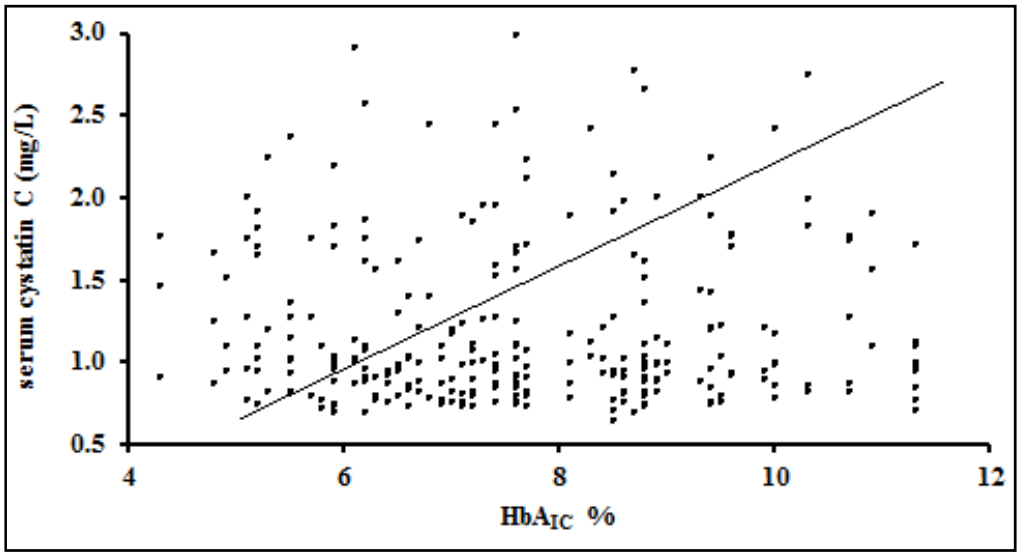

Figure (2): The relationship between HbAIC % and serum cystatin C (r=0.78; P=0.044)

the blood levels of Cystatin C raise. Plasma levels of Cystatin C are a more precise test of kidney function than plasma creatinine[8,9]. In the present study the results show that the mean of the plasma levels of Cystatin C, creatinine clearance, blood HbA1c% and body mass index were raised. Figure (1) 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 of Cystatin C. Creatinine clearance in the diabetic group was significantly reduced compared to the healthy controls 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. Figure (2) which show strong correlation between HbA1c % plasma and cystatin C level. This means uncontrolled diabetes with abnormal raised glycated heamoglobin 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.

Two large studies[10,11] with consistent results found that Cystatin C is influenced by many variables (age, sex, body mass index, smoking, hypertension, coronary heart disease, C-reactive protein level) other than renal function alone, even after adjustment for kidney function. Both studies excluded patients with moderate and severe renal failure, and in both Cystatin C were highly correlated with age. In conclusion, although multiple factors in addition to renal function may influence cystatin C. The current study provides convincing evidence that Cystatin C may be more useful for detecting early renal impairment in type 2 diabetic patients than are creatinine and commonly employed creatinine-derived formulas[10]. These results are remarkable in light of data suggesting that Cystatin C is a useful indicator of the association of mild kidney dysfunction with increased risk for cardiovascular events, peripheral arterial disease, heart failure, and death[11]. Furthermore, recent studies suggest that very early renal failure may be considered the early marker of the underlying progressive kidney damage associated with diabetes.

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