



## RESEARCH ARTICLE

### GLOMERULAR FILTRATION RATE AND CYSTATIN C AS MARKERS FOR DIABETIC NEPHROPATHY

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#### ARTICLE INFO

##### Article History:

Received 21<sup>st</sup> March, 2016  
Received in revised form  
18<sup>th</sup> April, 2016  
Accepted 22<sup>nd</sup> May, 2016  
Published online 15<sup>th</sup> June, 2016

##### Key words:

Diabetes,  
Glomerular filtration rate,  
Cystatin C- Diabetic nephropathy,  
Hyperfiltration.

#### ABSTRACT

Diabetes is recognized as a major risk factor for the development of kidney disease. Diabetic subjects with kidney disease may be at high risk for developing other health complications. The early detection of kidney dysfunction in subject with type 1 diabetes is of vital important to treat the progression to diabetic nephropathy. The aim of this study is to assess the level of cystatin C for studying early renal function decline in type 1 diabetic patients. This study included 64 type 1 diabetes mellitus patients (male and female) all the patients were hyperglycaemic with poor glucose control and control group included 30 healthy age matching subjects. Levels of HbA1c, serum fasting blood glucose, lipid profile, uric acid, creatinine and cystatin C were determined. Glomerular filtration rate was detected according to cockcroft-gault (CG) and modification of diet in renal disease (MDRD) formulas. For uric acid level a significant reduction was found in patients with  $GFR_{MDRD} > 90$ . The serum levels of cholesterol showed a significant increase ( $P < 0.05$ ) and a very highly significant increase ( $P < 0.005$ ) in high density lipoprotein in patients with  $GFR_{MDRM} < 90$  ml/min/1.73m<sup>2</sup>, meanwhile the serum level of low density lipoprotein showed a non-significant increase in patients with  $GFR_{MDRM} < 90$  ml/min/1.73m<sup>2</sup>. A highly significant decrease was found in cystatin C and creatinine ( $P < 0.01$ ) and a very highly significant decrease ( $P < 0.001$ ), ( $P < 0.000$ ) in both formulas CG and MDRD  $> 90$ . This study may suggest that age, BMI, gender, glycaemic control and duration of the diabetes may influence the level of cystatin C and the GFR provides a good index for the decline of kidney function.

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Citation: Hana Mohamed Gashlan and Heba Abdullah Alghamdi. 2016. "Glomerular filtration rate and cystatin c as markers for diabetic nephropathy", International Journal of Current Research, 8, (06), 32337-32342.

## INTRODUCTION

Diabetes mellitus developing as a major public health problem in Saudi Arabia in parallel with the worldwide diabetes epidemic, which is having a particular impact upon the Middle East and the third world (El-Hazmi *et al.*, 1995 ; Al-Nozha and Al-Maatouq, 2004). Diabetes causes unique changes in kidney structure, tubular (Brito *et al.*, 1998) and interstitial (Katz *et al.*, 2002) changes are also present. Diabetic nephropathy is considered one of the major microvascular complications of diabetes (Felehgari *et al.*, 2011). Approximately 20–30% of patients with type 1 or type 2 diabetes develop evidence of nephropathy (Pantalone *et al.*, 2015). Diabetic nephropathy develops in about 40% of patients with diabetes, even when high glucose levels are maintained for long periods of time. The serum level of cystatin C has been used as an endogenous marker of renal function that is

accurate even at the low concentrations found when Glomerular filtration rate (GFR) is normal or high (Westhuyzen, 2006). Cystatin C is unique among cystatins as it seems to be produced by all human nucleated cells (Čabarkapa, 2015). It is freely filtered by the renal glomerulus and primarily catabolized in the renal tubules, if kidney functions and glomerular filtration rate decline, the blood levels of cystatin C rise (Newman, 2002). This observation obviously implied an insignificant peritubular uptake of cystatin C. In human, cystatin C is normally degraded by proximal tubular cells after its passage through the glomerular membrane (Jacobsson *et al.*, 1995). Several studies stated that cystatin C does not appear to be affected by age, gender, diet, lean body mass, body fat content, inflammatory processes, liver functions or malignancies (Randers *et al.*, 1998 and Schuck *et al.*, 2004). Glomerular hyperfiltration occurs in the large majority of young Type 1 diabetes and is considered a characteristic functional abnormality in insulin-dependent diabetic patients. It is also a proposed mechanism for kidney injury in several clinical circumstances (Palatini, 2012). The aim of this study was to evaluate the significance of glomerular filtration rate and serum

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cystatin C as a marker for early detection of renal impairment in Saudi patients with type 1 diabetes mellitus.

## MATERIALS AND METHODS

### Subjects

This study included 64 patients with T1DM (male and female) attending outpatient's clinics of King Abdulaziz University Hospital in Jeddah, Saudi Arabia with age range between (13-48 years) and duration of diabetes from (1 year to 19 years). The 64 patients were subdivided into two groups according to estimated glomerular filtration rate: One included diabetic patients with GFR more than 90 ml/min. The other included diabetic patients with GFR less than 90 ml/min. The control group included thirty healthy age matching subjects not suffering from any disease. All the patients gave written, informed consent and the study was approved by the Ethics and Research Committee.

### Samples Collection

Five milliliters of venous blood were withdrawn from both normal and diabetic subjects after an overnight fasting. Two milliliters were put into a tube containing EDTA as anticoagulant while the other 3ml were put into a plain tube and left to clot then centrifuged at 3000 rpm for 10 minutes to separate serum and stored at -20 C° until assayed.

### Biochemical Analysis

Whole blood of glycosylated haemoglobin HbA1C level was measured according to the method described by (Calisti and Tognetti S, 2005), uric acid was measured according to (Kalckar, 1947). Determination of serum levels of creatinine employed a modification of the kinetic Jaffe reaction reported by Larsen (1972). Serum Cystatin C was determined according to the method described by Grubb *et al.* (1985).

### Calculation of glomerular filtration rate

The most widely used equations to calculate the estimated GFR (eGFR) in adults are Cockcroft-Gault (CG) 1976 and the Modification of Diet in Renal Disease (MDRD) (Levey *et al.*, 1999), both equations use serum creatinine levels to determine the eGFR.

$$\begin{aligned} \text{eGFR}_{\text{CG}}, \text{ mL/min} &= \frac{[140 - \text{age}] \times \text{body weight}(\text{kg})}{72 \times \text{serum creatinine}(\text{mg/dl})} \\ &\times (0.85 \text{ if female}) \end{aligned}$$

$$\text{eGFR}_{\text{MDRD}}, \text{ mL/min/1.73m}^2 = 186.3 \times \text{Creatinine, mg/dL}^{-1.154} \times \text{age}^{-0.203} \times (0.742, \text{ if female})$$

### Statistical Analysis

Statistical analysis was performed using Social Package for Social Science (SPSS) software package for data analysis version 21. Data was represented as mean  $\pm$  standard deviations of the mean. Comparison between two groups was performed by using student (t) test. Pearson's correlation test was used for

correlating variables. Probability  $P < 0.05$  was considered statistically significant.

## RESULTS

Serum levels of LDL and HDL displayed a highly significant increase in T<sub>1</sub>DM group compared to control. Meanwhile serum levels of cholesterol and TGL showed a non-significant increase as compared to control. BMI showed non-significant changes (Table 1). From Table 2, HbA1c and serum glucose levels showed a very highly significant increase, while a very highly significant decrease was found in the serum levels of uric acid and creatinine in T<sub>1</sub>DM compared to control group. Cystatin C level showed on difference between the two groups. The serum levels of cholesterol, LDL and HDL showed a non-significant increase in patients with  $\text{GFR}_{\text{CG}} > 90$  ml/min, while a non-significant decrease was shown in the serum levels of TGL in patients with  $\text{GFR}_{\text{CG}} > 90$  ml/min compared to  $\text{GFR}_{\text{CG}} \leq 90$  ml/min (Table 3). The serum level of LDL showed a non-significant increase in patients with  $\text{GFR}_{\text{MDRM}} 90$  ml/min/1.73m<sup>2</sup> compared to patients with  $\text{GFR}_{\text{MDRD}} \leq 90$  ml/min/1.73m<sup>2</sup>. The serum levels of cholesterol and HDL showed a significant increase in patients with  $\text{GFR}_{\text{MDRM}} 90$  ml/min/1.73m<sup>2</sup> as compared to patients with  $\text{GFR}_{\text{MDRD}} \leq 90$  ml/min/1.73m<sup>2</sup> (Table 3). The serum levels of TGL showed a non-significant decrease in patients with  $\text{GFR}_{\text{MDRM}} 90$  ml/min/1.73m<sup>2</sup>. The body mass index showed non-significant increase in patients with  $\text{GFR}_{\text{CG}} > 90$  ml/min compared to  $\text{GFR}_{\text{CG}} \leq 90$  ml/min. At the same time, BMI showed a very highly significant decrease in patients with  $\text{GFR}_{\text{MDRM}} 90$  ml/min/1.73m<sup>2</sup> as compared to patients with  $\text{GFR}_{\text{MDRD}} \leq 90$  ml/min/1.73m<sup>2</sup>.

The serum levels of glucose and uric acid showed non-significant decrease in  $\text{GFR}_{\text{CG}}$  group with  $> 90$  ml/min compared to the  $\text{GFR}_{\text{CG}} \leq 90$  ml/min group, by contrast the blood level of HbA1c showed a non-significant increase in  $\text{GFR}_{\text{CG}}$  group with  $> 90$  ml/min compared to the  $\text{GFR}_{\text{CG}} \leq 90$  ml/min group (Table 4). The blood level of HbA1c and the serum level of glucose showed a non-significant decrease in  $\text{GFR}_{\text{MDRD}}$  group with  $> 90$  ml/min/1.73m<sup>2</sup> compared to the  $\text{GFR}_{\text{MDRD}} \leq 90$  ml/min/1.73m<sup>2</sup> group, while the serum level of uric acid showed a significant decrease in patient with  $\text{GFR}_{\text{MDRD}} > 90$  ml/min/1.73m<sup>2</sup> compared to  $\text{GFR}_{\text{MDRD}} \leq 90$  ml/min/1.73m<sup>2</sup> group (Table 4). The serum levels of cystatin C and creatinine showed highly significant decrease in patients with  $\text{GFR}_{\text{CG}} > 90$  ml/min compared to  $\text{GFR}_{\text{CG}} \leq 90$  ml/min as well as in patients with  $\text{GFR}_{\text{MDRM}} 90$  ml/min/1.73m<sup>2</sup> compared to patients with  $\text{GFR}_{\text{MDRD}} \leq 90$  ml/min/1.73m<sup>2</sup>.

## DISCUSSION

Diabetic nephropathy considered one of the main microvascular complications of diabetes and from the most common single cause of renal disease (Domingueti *et al.*, 2015). Mechanism connecting hyperglycemia with complications of long-term diabetes includes formation of glycated proteins and an increase in oxidative stress (Nadhini and Anuradha, 2003). Table 1 showed a very highly significant increase of glucose and HbA1c ( $P < 0.000$ ) levels while a very highly significant decrease in the serum levels of uric acid ( $P < 0.000$ ) in all the diabetic patients compared to control group.

**Table 1. Serum levels of Cholesterol, TGL, LDL, HDL and BMI in control and type1 diabetic patients**

Parameters Groups	Cholesterol mg/dl	TGL mg/dl	LDL mg/dl	HDL mg/dl	BMI Kg/m <sup>2</sup>
Control Range n= 19	136-197	53-69	77.2-125.2	35-59	21.3-33.5
Mean ± SD	176.5 ± 20.7	60 ± 7.9	104.1 ± 15.1	45.9 ± 7.45	26.8± 4.39
T <sub>1</sub> DM Range n= 64	104-267	54-84	65.4-175.2	16-90	14.8- 43.8
Mean ± SD	185.7 ± 31.3	69±8.9	115.8 ± 25.9	52.2 ± 14.8	25.5± 6.64
P Value <	NS	NS	0.01	0.01	NS

SD: Standard deviations  
P Value 0.01: Significant.  
NS: Non-significant

**Table 2. Blood level of HbA1c and serum levels of Glucose, uric acid Cystatin C and Creatinine in control and type 1 diabetic patients**

Parameters Groups	HbA1c%	Glucose mg/dl	Uric Acid mg/dl	Cystatin C mg/L	Creatinine mg/dl
Control Range n= 19	4.3-5.8	79-106	2.9-6.2	0.54-0.8	0.6-1.3
Mean ± SD	4.75 ± 0.37	94.4 ± 8.09	4.57 ± 1.07	0.69±0.07	0.94±0.19
T <sub>1</sub> DM Range n= 64	5.5-14.7	72-492	1.9-6.4	0.43-1.46	0.4-1.6
Mean ± SD	9.75 ± 2.11	222.1 ± 95.5	3.45 ± 1.03	0.65±0.14	0.81±0.21
P Value	0.000	0.000	0.000	NS	0.05

SD: Standard deviations  
P Value 0.05: Significant.  
P Value 0.005: very highly significant.  
NS: Non-significant

**Table 3. Comparison between the serum levels of Cholesterol, TGL, LDL, HDL and BMI in type 1 diabetic patients with GFR<sub>CG</sub> and GFR<sub>MDRD</sub>**

	GFR <sub>CG</sub> ≤90 ml/min	GFR <sub>CG</sub> >90 ml/min	P Value	GFR <sub>MDRD</sub> ≤90 ml/min/1.73m <sup>2</sup>	GFR <sub>MDRD</sub> >90 ml/min/1.73m <sup>2</sup>	P Value
Number	18	46		13	51	
Cholesterol mg/dl	178.8±28.2	187±32.1	NS	173.3±30.4	190.6±30.7	0.05
LDL mg/dl	112.9±27.1	116.5±25.9	NS	109.7±24.2	118.2±26.5	NS
TGL mg/dl	70±10.3	65±9.36	NS	75±8.56	68±8.45	NS
HDL mg/dl	50.9±13.5	52.6±15.3	NS	44.3±12.2	55.3±14.7	0.005
BMI	22.9±6.65	26.1±6.55	NS	30.4±6.87	23.5±5.51	0.000

SD: Standard deviations  
P Value 0.05: Significant.  
P Value 0.005: very highly significant.  
NS: Non-significant

**Table 4. Comparison between the levels of glucose, HbA1c, uric acid, creatinine and cystatin c in T<sub>1</sub>DM patients with GFR<sub>CG</sub> and GFR<sub>MDRD</sub>**

	GFR <sub>CG</sub> ≤90 ml/min	GFR <sub>CG</sub> >90 ml/min	P Value	GFR <sub>MDRD</sub> ≤90 ml/min/1.73m <sup>2</sup>	GFR <sub>MDRD</sub> >90 ml/min/1.73m <sup>2</sup>	P Value
Number	18	46		13	51	
Glucose mg/dl	250.2±105.3	215.0±2.6	NS	251.9±102.9	210.5±90.9	NS
HbA1C%	9.44±2.24	9.83±2.09	NS	10.2±2.18	9.59±2.09	NS
Uric Acid mg/dl	3.53±0.95	3.43±1.06	NS	3.85±1.07	3.29±0.99	0.05
Creatinine mg/dl	0.99±0.28	0.77±0.16	0.01	1.03±0.21	0.73±0.13	0.000
Cystatin C mg/l	0.73±0.17	0.63±0.12	0.01	0.74±0.16	0.61±0.11	0.001

SD: Standard deviations  
P Value 0.01: Significant.  
P Value 0.05: Significant.  
P Value 0.001: very highly significant.  
P Value 0.005: very highly significant.  
NS: Non-significant

A highly positive correlation was found between HbA1c and glucose level ( $r=0.514$ ). Serum glucose levels are the main determinant of HbA1c levels. In high serum glucose concentration glucose can be combined into proteins non-enzymatically by an unregulated glycation reaction. Hussein *et al.* (2007) reported that HbA1c levels provides an index of integrated glucose concentration over the life span of the red blood cell normally about 100-120 days. Uric acid a weak organic acid plays an important role in renal disease. It is excreted mostly in the kidney and has been historically linked to acute kidney damage (Pasalic *et al.*, 2012). The results of the current study of serum levels of Cholesterol, TGL, LDL and HDL in all the diabetic patients and control were represented in table 2. Guy *et al.* (2009) stated that youth with type 1 diabetes have abnormal lipid levels and atherogenic changes in lipoprotein composition, even after relatively short disease duration. James and Pometta (1990) stated that under normal circumstances, triglyceride-rich particles are rapidly hydrolyzed by lipoprotein lipase. The enzyme is induced in adipose tissue by insulin, and thus, intensive insulin therapy is typically associated with a marked fall of triglyceride-rich particles, which may explain the results obtained from the current study.

In this study, the participants were divided into two groups according to their estimated glomerular filtration rate.  $GFR \leq 90$  and  $> 90$  which calculated by cockroftgult formula (CG) and modification of diet in renal disease (MDRD) formulas. Table 3 were adjusted for the comparison between the levels of glucose, HbA1c, uric acid, creatinine and cystatin C in T1DM patients according to  $GFR_{CG}$  and  $GFR_{MDRD}$ . The mean values of creatinine and cystatin C in patients with  $GFR \leq 90$  calculated with both formulas (CG and MDRD) was the same. Also there were no differences in the results of creatinine and serum cystatin C in patients with  $GFR > 90$  in both formulas. The results of this study indicated that all the patients with  $GFR > 90$  or  $GFR \leq 90$  were hyperglycaemic with poor glucose control and a significant decrease was found in the level of uric acid ( $P < 0.05$ ) in patients with  $GFR_{MDRD} > 90$  ml/min/1.73m<sup>2</sup>. In concomitant with this study, Yokoyama *et al.* (2009) found that high baseline GFR and high HbA1c to be a significant predictors in subjects with diabetes.

Mogensen and Andersen (1975) declared that in type 1 diabetes the most important determinant of hyperfiltration is uncontrolled hyperglycaemic. Perkins *et al.* (2007) indicated high HbA1c as a risk of early renal function decline. Regarding to low level of serum uric acid Goliket *et al.* (1993) found that hypouricemia could be a marker for the onset of the diabetic kidney disease. Bo *et al.* (2001) stated that hypouricemia would be associated with poor disease control, hyperfiltration or a late onset nephropathy. Suryawanshi *et al.*, (2006) observed that in diabetic patients, high levels of total cholesterol appear due to increased cholesterol synthesis and the triglyceride levels changes according to the glycemic control. The increase may be due to over production of VLDL-TGL. The result of this study may not show typical dyslipidemia profile but it showed a disturbance in lipid profile as a result of diabetes. The results of this study suggested that cystatin C level may not be used to estimate renal function unless one accounts for other factors such age, BMI, gender, glycaemic control and duration of the

diabetes that may influence its level. It can be concluded that longer diabetes duration, poorer glycaemic control and BMI may associated with development of kidney function decline. Moreover, the glomerular filtration rate may provide the best index of kidney function. For this study cystatin C may consider as an alternative for serum creatinine but not a sensitive indicator. It may due to that the diabetic patients in this study despite they were hyperglycaemic with poor glucose control but they did not reach the stage of renal damage. Also, to take into account the glomerular hyperfiltration which may be strong predictive of the development of renal impairment parallel to cystatin C. In this study, serum creatinine and serum cystatin C concentration showed a normal level in  $GFR_{CG}$  and  $GFR_{MDRD}$ . In cohort study, Mussap *et al.* (2002) found that the correlation of cystatin C and creatinine was lower in patients with a normal GFR ( $>80$  ml/min), as a consequence of different pathophysiological factors that may affect the serum concentration. In addition, the GFR calculated with CG and MDRD formulas revealed that most of diabetic patients have a  $GFR > 90$  which consider in other studies normal. Glomerular filtration rate is determined by renal plasma flow, the transmembrane hydraulic pressure gradients and the ultrafiltration coefficient. Renal plasma flow is dependent on changes between afferent and efferent renal arteriolar resistance; the transmembrane hydraulic pressure gradients is the difference between the mean pressure in the glomerular capillary and the proximal tubule; and the ultrafiltration coefficient is consistent with the filtration surface area and its permeability. In patients with diabetes, all of these factors are altered (Steffes *et al.*, 1992 and Bak *et al.*, 2000).

Disturbance of glomerular hemodynamic is an early event in type 1 diabetes mellitus, and glomerular hyperfiltration has been supported as a determinant of progression to clinical nephropathy. Hyperfiltration is a prerequisite for nephropathy (Rudberg *et al.*, 1992). Yip *et al.* (1996) confirmed that the rate of renal function decline was faster in those with hyperfiltration than those without. From this study, the GFR calculated by CG and MDRD formulas was higher than 140 ml/min in about 25% of the diabetic population while, about 7.8% of the diabetic patients registered GFR between (120-140 ml/min). Previous studies indicated that the threshold for hyperfiltration has ranged from 125 to 140 ml.min-1.1.73 m-2 (Dahlquist *et al.*, 2001, Amin *et al.*, 2005 and Magee *et al.*, 2009). The state of hyperfiltration reflect hyperglycaemic and can be reversed with intensive insulin therapy. In conclusion for this study the level of cystatin C did not show any marked difference than serum creatinine level. It may consider as an alternative for serum creatinine but not a sensitive marker to detect the early changes in renal function. The diabetic patients in this study despite they were hyperglycemic with poor control of glucose level but they maybe did not reach yet the stage of renal damage. This study recommend to take into account the glomerular hyperfiltration which may be a strong predictive of the development of renal impairment.

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