

Plasma Cyclophilin-A as a Novel Biomarker in Chronic Nephropathy

Mohy Eldin Abd EL-Fattah* and Taghrid Bahig Elabasiri

Department of Science, Cairo University, Giza, Egypt

*Corresponding author: Mohy Eldin Abd EL-Fattah, Department of Science, Cairo University, Giza, Egypt; E-mail: mohy_yassen@yahoo.com

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Abstract

Background: Type 2 diabetes mellitus (DM) is the most common cause of end-stage renal disease. Albuminuria is the foremost commonly utilized marker to anticipate onset of diabetic nephropathy (DN) without sufficient affectability and specificity to identify early DN.

Aim: This study aimed to evaluate plasma Cyclophilin A (CypA) as a new biomarker for early DN.

Methods: This cross-sectional study included 125 Egyptian subjects attending the outpatients Clinic of the Department of Internal Medicine, 10th of Ramadan City Health Insurance Hospital and divided into: control group, patient with diabetic mellitus, patients with Diabetic nephropathy and patient with diabetic nephropathy and other complications. Patients were subjected to measurement of plasma α -klotho, FBS, HbA1C, serum Creatinine, serum urea, serum uric acid, K, Na, serum phosphorus, Albumin: Creatinine Ratio, GFR, Chol, TG, LDL HDL, AST, ALT, T.BIL, D.BIL ALB, TP, GLB and A/G ratio.

Results: Results showed that plasma α -klotho was significantly correlated with haemoglobin A1C, potassium, GFR, Albumin, TP and GLB. Meanwhile, plasma α -klotho was negatively correlated with duration of DM, CR, Urea, UR.A, Na, phosphorus, ACR, Chol, TG, LDL, AST, ALT, T.BIL, and D.BIL. However, there were no significant correlations between plasma α -klotho and FBS, HDL and A/G ratio. At cut-off level ≥ 84.14 , cyclophilin A had 91% sensitivity and 62% specificity for diagnosing diabetic nephropathy.

Conclusion: CypA can be used as an early marker for DN as we found early significant high levels of urinary CypA in diabetic patients with stage 2 DN even before the appearance of albuminuria.

Keywords: Plasma Cyclophilin-A; Albuminuria; Haemoglobin

numerous novel drugs for DM, there are no particular healing medicines however for DN.

Reasons for destitute result incorporate insufficient markers and the complicated components of DN [2]. Now, severity of this disease is decided agreeing to the levels of albuminuria. Albuminuria is the foremost commonly utilized marker to foresee onset and movement of DN clinically. In any case, this conventional marker for DN needs both affectability and specificity to identify early organizes of DN [3].

However, some DN patients with ESRD do not present with significant albuminuria [4-6]. There is lack of association between Glomerular Filtration Rate (GFR) and albuminuria suggests that an alternative to this albuminuria-based staging system is needed. Some studies have noted the existence of pathological change before micro albuminuria. Therefore, even if micro albuminuria can be regarded as the earliest manifestation of DN, it is possible that a new biomarker for DN exists. Recently, different markers of DN were reviewed including fibroblast growth factor 23, tubular markers inflammatory markers (interleukin 6 [IL-6], IL-8, monocyte chemo attractant protein 1, and interferon γ -inducible protein) urinary 8-hydroxy-2'-deoxyguanosine, serum cystatin C, and so on. Among these, genetic susceptibility almost always leads to irreversible DN, and detection of the clinical markers mostly occurs too late to diagnose and monitor the progression of DN. As such, it is crucial to find an earlier and reliable marker for DN. Earlier diagnosis and intervention may provide an opportunity to stop the permanent damage caused by DN. Cyclophilin A (CypA) is an 18-kDa protein with ubiquitous characteristics. It is mostly distributed in the cytoplasm and facilitates protein folding and protein trafficking. It also acts as a cellular receptor for Cyclosporine A (CsA). The expression of CypA is relatively high in the kidney, where Proximal Tubular Epithelial Cells (PTECs) are reported to contain considerably more CypA than other kidney tissues. With respect to kidney diseases, the majority of research has been on the cellular relationship between CypA and CsA, which is used as an immunosuppressant, and leaves behind its secreted form. This secreted CypA (sCypA) was reported to be correlated with cardiovascular disease (CVD), asthma, Rheumatoid Arthritis (RA), and lung and liver injury. sCypA has been suggested to be a potential biomarker and mediator in CVD.

In addition, sCypA is associated with inflammatory or infectious diseases such as RA, asthma, and periodontitis. Interestingly, sCypA was also detected in diabetic patients'

Introduction

Type 2 diabetes mellitus (DM) is the most common single cause of End-Stage Renal Disease (ESRD) [1]. ESRD in nearly half of patients is due to diabetic nephropathy (DN), and these cases have the most exceedingly bad result compared to patients with other causes of ESRD. In spite of the fact that there are

plasma and was shown to be secreted by monocytes in response to hyperglycaemia, indicating that sCypA could be a potential secretory marker in type 2 DM. Furthermore, a relatively high expression level of CypA in normal kidneys has led to speculation that sCypA may be associated with solid organ damage. As a product directly produced by kidney, urine could be best measure for renal injury detection. So this study aimed to evaluate Plasma Cyclophilin A as biomarkers in chronic diabetic nephropathy.

Aim

The study aimed to evaluate Plasma Cyclophilin A as biomarkers in chronic diabetic nephropathy.

Patients and Methods

Study design

Cross sectional study, aiming to evaluate Plasma Cyclophilin A as biomarkers in chronic diabetic nephropathy.

Study setting

The study was carried out at Clinic of the Department of Internal Medicine, 10th of Ramadan city Health Insurance Hospital.

Target population

Diabetic patients attending the Out Patients Clinic of the Department of Internal Medicine, 10th of Ramadan city Health Insurance Hospital. This study included 125 Participants who were divided into:-

Control group: 20 healthy subjects whose age ranged between 30-50 years old were taken as control group.

Study group: including 105 patients divided into.

Group 1 : 20 patient with diabetic mellitus whose age ranged between 30-50 years old

Group 2 : 65 patients Diabetic nephropathy whose age ranged between 30-50 years old

Group 3: Diabetic nephropathy and other complications whose age ranged between 30-50 years old.

Inclusion criteria

- Patients were free from infectious disease.
- Patients were free from inflammatory disease.
- Patients were free from liver disease.
- Patients were free from malignancy.
- All were non-smokers.

Exclusion criteria

- Patients who took drugs for hypertension.
- Patients who took drugs for DM.
- Patients who took drugs for hyperlipidemia.
- Patients who took drugs for CVD.
- Patients who took drugs for hyperuricemia.

- Patients who took drugs for gout.

All patients were subjected to the following:

a) Collection of demographic data as required in the attached sheet including age, occupation, anthropometric measurements of height, weight, waist circumference, and history of disease.

b) Collection of morning urine samples in vacutaniner cup and also collection of 10 venous blood samples from the overnight fasted 5 ml blood were collected on plane tubes and other 5 ml blood were collected on EDTA tubes by vacutaniner system under complete aseptic conditions and HbA1C first done and the samples centrifuged for 10 min at 2,500 g within 30 min separated serum and plasma were stored at 20°C the measurement of plasma cyclophyline A concentration, serum fasting glucose, serum creatinine, serum urea n, serum uric acid , serum potassium k, serum sodium Na serum phosphorus, Albumin: Creatinine Ratio, GFR concentration, serum cholesterol and serum triglycide . AST, ALT, T.BIL, D.BIL ALB, TP, GLB and A/G ratio.

The collected data was revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science (SPSS 23). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. The following tests were used:

Mean, Standard deviation (\pm SD) and range for parametric numerical data, while Median and Interquartile range (IQR) for non-parametric numerical data. Frequency and percentage of non- numerical data.

ANOVA test of significance was used when comparing between means of more than two groups.

Post-hoc test after ANOVA for significance between each two groups.

Chi-Square test was used to examine the relationship between two qualitative variables.

Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.

Correlation analysis (using Pearson's method) to assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables.

Results

A total of 125 subjects were enrolled in this study; their mean age was 55.8610.4 years (range, 24–82 years), and there were 71 men and 54 women. Age, BMI, Duration of D.M, F.B.G, C.P.A, HBA1C, S. Creatinine, S. urea, UR.A, Na, ACR, GFR, Cholesterol, Triglycerides, HDL, LDL, AST, ALT, ALB, T.BIL, and D.BIL were significantly higher in diabetic patients than non-diabetic control. Meanwhile, K, Ph, T.P, AG ratio and CPA were

Significantly lower in diabetic patients than in non-diabetic controls. Other parameters did not differ significantly between the diabetes group and non-diabetic controls.

Age, BMI, Duration of D.M, F.B.G, C.P.A, HBA1C, S. Creatinine, S. urea, UR.A, Na, ACR, GFR, Cholesterol, Triglycerides, HDL, LDL, AST, ALT, ALB, T.BIL, D.BIL, K, Ph, T.P, AG ratio and CPA were significantly different between four groups. Cyclophilin A was significantly correlated with duration of DM, CR, Urea, UR.A, Na, phosphorus, ACR, Chol, TG, LDL, AST, ALT, T.BIL, D.BIL. Meanwhile, Cyclophilin A was negatively correlated with HA1C, K, GFR, HDL, ALB, TP, GLB and A/G ratio. However, there were no significant correlations between Cyclophilin A and FBS, HA1C and A/G ratio.

Figure 1 show that at cut off level ≥ 84.14 , cyclophilin A had 91% sensitivity and 62% specificity for diagnosing diabetic nephropathy.

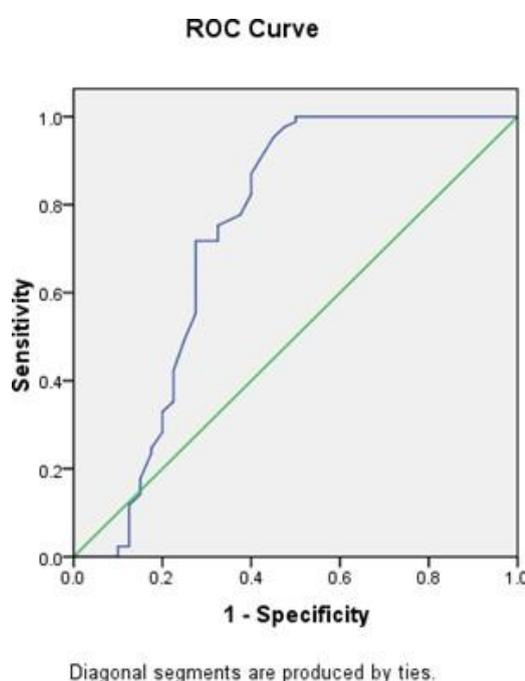


Figure 1: ROC curve of C.P.A for diabetic nephropathy.

Discussion

Diabetic Nephropathy (DN) is one of the most common micro vascular complications of diabetes and it is considered as a leading cause of end-stage renal disease since there are no specific treatments for it till now. Therefore earlier diagnosis and intervention may provide an opportunity to stop the permanent renal damage caused by DN.

In our study, we tried to find out the possibility of using the plasma cyclophilin A (CypA) as a new marker for diagnosis of diabetic nephropathy as early as possible. Our study showed that there was a statistically significant difference in the level of plasma CypA between the three main groups ($p < 0.01$) being higher in G3 and G2 than the control (G1).

The CypA in GIII (diabetics with albumin uric DN) (6.01 ± 1.61 ng/ml) was statistically significant higher than in

GII (diabetics without albuminuria) (1.69 ± 0.87 ng/ml, $t=12.93$, $p < 0.001$) and in GI (control) (0.55 ± 0.14 ng/ml, $t=18.55$, $p < 0.0001$). In GII the CypA was statistically significant higher than in GI ($t=7.04$, $p < 0.01$). We also found that the level of plasma CypA was statistically significant higher in group 4 (105.5 ± 5.26 ng/ml) than in group 3 (84.14 ± 7 ng/ml, $p < 0.001$).

Study which was the first study to use CypA in early detection of DN. It was conducted on 120 subjects; 20 healthy control group and 20 diabetic patients in each stage of DN (5 stages). Samples were collected to determine the expression of CypA. They also treated mesangial (MES-13) and tubular (HK-2) cells with glucose or free radicals to observe the expression of secreted CypA in Western blot analysis. They found that the levels of CypA were higher in groups of DN than in normal one. There was a highly statistical significant difference ($p < 0.001$) in levels of CypA between all groups except between normal and stage 1 DN groups where there was no significant difference in level of CypA between them. The lowest levels of CypA was found in control and stage 1 DN, wherein the CypA increased gradually with progression of DN till it reached the highest levels in stage 5 DN (ESRD).

In addition, due to that either GFR-based or albuminuria-based classifications of DN correlated significantly with urinary CypA. When comparing different stages of DN or Chronic Kidney Disease (CKD), there was only a trend of higher CypA in higher CKD stages, but truly statistically significant difference existed among the different DN stages. This finding supports the notion that CypA is better correlated using the albuminuria-based classification, which is the better and earlier detection method for monitoring DN in clinical practice. This will enable us to detect stage 2 DN early, so intensive blood sugar monitoring, timely diet restriction and exercise education would be useful to avoid further silent deterioration of DN.

Conclusion

CypA was higher in diabetics with macro albumin uric DN than those with micro albumin uric DN who in turn had higher levels of urinary CypA than diabetics with normoalbuminuric DN. CypA had a positive correlation with serum Creatinine, urinary albumin Creatinine ratio and duration of diabetes, while it had a negative correlation with estimated glomerular filtration rate. CypA can be used as an early marker for DN as we found early significant high levels of urinary CypA in diabetic patients with stage 2 DN even before the appearance of albuminuria.

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