Association between serum cystatin C levels and cardiovascular disease in type 2 diabetic patients

La cystatine C au cours des complications cardiovasculaires du diabète de type 2

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Abstract. Serum cystatin C concentration was recently reported as a marker of cardiovascular disease (CVD). In the present study, we evaluated the association between the increase of serum cystatin C levels and the risk of CVD in type 2 diabetes. 42 patients with type 2 diabetes were included in the present study; 27 of them have CVD. The control group consisted of 30 healthy adults. Cystatin C, creatinine, microalbuminuria and CRP were measured on Cobas 6000TM. Cystatin C level was significantly higher in patients with CVD. A significant difference in serum cystatin C was found in patients with and without albuminuria. No difference in serum cystatin C levels was found according to number of affected vessels. A cystatin C level above 1.10 mg/L was associated with increase of risk of CVD with significant difference (OR = 42.52; IC 95% 1.455 to 1242.827 and p = 0.029). Our results suggested that the increase of serum cystatin C concentrations is a potential marker for CVD in diabetes.

Key words: cystatin C, type 2 diabetes, cardiovascular disease, microalbuminuria

Résumé. La cystatine C a été décrite comme un marqueur du risque cardiovasculaire. Dans ce travail, nous nous proposons d’étudier l’association entre la cystatine C et le risque cardiovasculaire chez les diabétiques de type 2. Quarante-deux diabétiques type 2 ont été inclus dans ce travail, dont 27 avaient une pathologie cardiovasculaire. Le groupe témoin est constitué de 30 sujets indemnes de toute pathologie. La cystatine C, la créatinine, la microalbuminurie et la CRP ont été dosées par le Cobas 6000TM. Les concentrations de la cystatine C sont significativement plus élevées chez les patients ayant une atteinte cardiaque. Nous avons noté une différence significative de la cystatinémie entre les patients avec et sans atteinte cardiaque en fonction de l’albuminurie. Aucune différence significative de la cystatinémie en fonction du nombre d’artères atteintes n’a été notée. Une cystatinémie supérieure à 1,10 mg/L est associée à une augmentation significative du risque cardiovasculaire (OR = 45,52 ; IC 95 % 1,455 à 1242,827 et p = 0,029). Nos résultats suggèrent que la cystatine C est un marqueur potentiel des maladies cardiovasculaires.

Mots clés : cystatine C, diabète de type 2, pathologie cardiovasculaire, microalbuminurie

CVD is the main cause of death in diabetic adults. In deep, the World Health Organization (WHO) estimated at 3.4 million the death due to diabetes in 2004 [1]. These early deaths are engendered both by characteristics of diabetes and by its complications, particularly CVD. The estimation of the cardiovascular risk (CVR) was based on estimation of glomerular filtration rate (GFR). A recent marker, cystatin C, was identified as an independent risk factor of CVD [2]. Cystatin C is an endogenous marker that may be more
sensitive for detecting the debutante decrements in GFR [3]. Influence of age, sex, muscle mass and exercise on serum cystatin C level remain unclear [4-9]. Different studies showed that cystatin C is superior to serum creatinine or creatinine based estimating equations for prediction of all-cause mortality, cardiovascular (CV) events, and incident congestive heart failure in elderly subjects without CVD [2, 3]. Is cystatin C really an independent risk factor of CVD or its increase is associated with debutante renal disease? The present study was performed to evaluate cystatin C as a marker of CVD in patients with type 2 diabetes with and without diabetic nephropathy.

**Patients and methods**

**Patients selection**

Patients admitted to cardiology department in Fattouma Bourguiba University hospital, Monastir, for coronary angiography between September 2010 and April 2011 were eligible for participation. Inclusion criteria were: age ≥ 18 years and type 2 diabetes. Exclusion criteria were type 1 diabetes, pregnancy woman, child, renal failure and thyroid dysfunction. Of the 142 eligible patients, 42 were enrolled in the study: 7, 28 and 6 patients are respectively treated with antidiabetic treatment, association of antidiabetic and antihypertensive treatment and association of antidiabetic, antihypertensive and hypolipidemic treatment. Only one patient is treated with antidiabetic diet. The local ethics committee approved the study. The control group consisted of 30 healthy adults.

**Definition of cardiovascular diseases**

Patients with CVD were defined as patients who have a past history of angina, myocardial infraction, valvular disease, cardiomyopathy, arrythmia, peripheral arterial disease and/or cardiac failure. The number of the stenotic vessels was determined from the results of coronary angiography.

**Definition of diabetic nephropathy**

Our population was divided into three groups: normo, micro and macro-albuminuria patients respectively if the urinary albumin excretion (UAE) < 20, between 20 and 200 and > 200 μg/min.

**Biochemical measurements**

In all patients, a venous blood samples was obtained after 12 h fasting. UAE was measured by immunoturbidimetric assay from timed overnight urine collections. Cystatin C and CRP were measured by immunoturbidimetric assay, TSH and FT4 by electrochemiluminescence) and creatinine by the JAFFE compensated traceable to IDMS method on Cobas 6000TM (Roche Diagnostics). Estimated GFR was calculated by Modification of Diet in Renal Disease (MDRD) formula. Renal failure is defined as GFR ≤ 60 mL/min.

**Statistical analysis**

Continuous variables were presented as means ± standard deviation. Statistical analysis was performed using SPSS version 19.0. Student’s test for independent means was used for intergroup comparisons of quantitative variables. The level of significance was set at p < 0.05. A binary regression model was used with the aim of identifying associations between increase in cystatin C levels and CD. The cut off point was fixed at 1.10 mg/L which is associated to the best sensitivity and specificity. All factors presenting p < 0.25 were considered as covariables.

**Results**

**Clinical and biological characteristics in our population**

Clinical characteristics of 42 type 2 diabetic patients are shown in table 1. The mean age in our population was 58.9 ± 11.1 years vs 53.6 ± 3.9 in control group; the median of the duration of diabetes was 5.0 ± 7.2 years. Patients with CVD had a duration of diabetes more important than those without CVD, and were the olders (p < 0.001 and p < 0.001 respectively).

**Variation of serum cystatin C concentration according to diabetic nephropathy**

A significant difference was found in serum cystatin C level between total patients and control group but no difference was found in creatinine concentration between the two groups. In both CVD group and non CVD group, no significant differences of cystatin C levels were found according to the diabetic nephropathy but a significant statistically increase of cystatin c levels was found in CVD group versus non CVD group (p = 0.001), especially in patients with normalbuminuria (p = 0.005) (table 2).

**Variation of serum cystatin C concentration according to the number of affected vessels**

No differences of serum cystatin C level were found according to number of vessels affected (table 3).

**Serum cystatin C level and risk factor for CVD**

Multivariate logistic regression analysis was performed to determine the association between the increase of cystatin
Table 1. Clinical and biological characteristics of the studied population.

<table>
<thead>
<tr>
<th></th>
<th>Total patients (n = 42)</th>
<th>CVD (n = 27)</th>
<th>Non CVD (n = 15)</th>
<th>Controls (n = 30)</th>
<th>P</th>
<th>P’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.9 ± 11.1</td>
<td>63.5 ± 8.6</td>
<td>50.7 ± 10.6</td>
<td>53.6 ± 3.9</td>
<td>&lt; 0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>2/40</td>
<td>0/27</td>
<td>2/13</td>
<td>20/10</td>
<td>0.054</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diabetes duration*</td>
<td>5.0 ± 7.2</td>
<td>10.0 ± 10.0</td>
<td>1.0 ± 2.0</td>
<td>—</td>
<td>&lt; 0.001</td>
<td>—</td>
</tr>
<tr>
<td>HTA (Y/N)</td>
<td>34/8</td>
<td>25/2</td>
<td>6-Sep</td>
<td>—</td>
<td>0.009</td>
<td>—</td>
</tr>
<tr>
<td>Coronary disease (Y/N)</td>
<td>23/19</td>
<td>23/4</td>
<td>0</td>
<td>—</td>
<td>&lt; 0.001</td>
<td>—</td>
</tr>
<tr>
<td>BMI</td>
<td>32.0 ± 7.3</td>
<td>32.5 ± 7.0</td>
<td>31.1 ± 7.9</td>
<td>26.6 ± 3.6</td>
<td>0.555</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Normoalbuminuria</td>
<td>30</td>
<td>19</td>
<td>11</td>
<td>—</td>
<td>0.308</td>
<td>—</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Serum cystatin C</td>
<td>1.16 ± 0.22</td>
<td>1.24 ± 0.36</td>
<td>1.01 ± 0.53</td>
<td>0.98 ± 0.12</td>
<td>0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRP*</td>
<td>7.1 ± 7.2</td>
<td>4.3 ± 7.2</td>
<td>8.9 ± 9.6</td>
<td>2.2 ± 3.0</td>
<td>0.32</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FT4</td>
<td>13.2 ± 2.37</td>
<td>13.1 ± 1.9</td>
<td>13.6 ± 3.1</td>
<td>—</td>
<td>0.52</td>
<td>—</td>
</tr>
<tr>
<td>TSH*</td>
<td>1.69 ± 1.83</td>
<td>1.53 ± 1.88</td>
<td>1.94 ± 2.38</td>
<td>—</td>
<td>0.15</td>
<td>—</td>
</tr>
<tr>
<td>Creatinine</td>
<td>85 ± 11</td>
<td>89 ± 10</td>
<td>80 ± 13</td>
<td>90 ± 12</td>
<td>0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>GFR calculated as MDRD formula</td>
<td>82 ± 15</td>
<td>76 ± 11</td>
<td>91 ± 17</td>
<td>79 ± 11</td>
<td>0.001</td>
<td>0.49</td>
</tr>
</tbody>
</table>

P: comparison between CVD and non CVD; p': comparison between total patients and controls; (Y: yes; N: no); * Median ± interquartile range; CVD: cardiovascular disease; BMI: body mass index; HTA: hypertension.

Table 2. Comparison of serum cystatin C levels among groups of nephropathy.

<table>
<thead>
<tr>
<th></th>
<th>CVD (n = 29)</th>
<th>Non CVD (n = 18)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoalbuminuria</td>
<td>1.25 ± 0.19</td>
<td>1.00 ± 0.24</td>
<td>0.005</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>1.14 ± 0.16</td>
<td>1.10</td>
<td>0.834</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>1.31 ± 0.31</td>
<td>1.07</td>
<td>0.644</td>
</tr>
<tr>
<td>Total* (n = 37)</td>
<td>1.24 ± 0.19</td>
<td>1.01 ± 0.21</td>
<td>0.001</td>
</tr>
<tr>
<td>p’</td>
<td>0.6</td>
<td>0.91</td>
<td>—</td>
</tr>
</tbody>
</table>

CVD: cardiovascular disease; p: comparison between CVD and non CVD; p’: comparison between normoalbuminuria, microalbuminuria and macroalbuminuria. *Microalbuminuria was determined only in 37 patients.

C concentration (dependant variable > 1.10 mg/L which associated to the best sensitivity and specificity) and the risk of CVD.

HTA, treatment, diabetes duration, hypothyroidism and age were included in regression as independent variables. In the present study, we found that a cystatin C level > 1.10 mg/L was associated with increase of risk of CVD with significant difference (OR = 42.520 ; IC 95% 1.455 to 1242.827 and p = 0.029) (table 4).

**Discussion**

In adults with type 2 diabetes, CVD is the major cause of death. Pathophysiologic mechanisms remain unclear. Some risk factors such as dyslipidemia, hypertension, prothrombotic and proinflammatory factors are implicated in the development of atherosclerosis in type 2 diabetes patients. Different studies showed that cystatin C was independently associated with CVD [10].
Cystatin C is a cysteine protease inhibitor that is produced at a constant rate by all nucleated cells and freely filtered by glomeruli owing to its low molecular weight (13359 Daltons) [11, 12]. Since cystatin C is mainly reabsorbed and catabolized in the proximal tubule, its concentration depends mainly on the GFR [11-13]. In the present study, we found that a cystatin C level > 1.10 mg/L was significantly associated with increase of risk of CVD (OR = 42.520; IC 95% 1.455 to 1242.827 and p = 0.029).

In diabetic patients, our results are in agreement with other studies. In fact, Maah et al. [14] reported a statistically important relationship between serum cystatin C level and coronary artery disease (CAD) in type 1 diabetic patients. In deep, they showed, in their study including 652 patients with type 1 diabetes, that increasing serum cystatin C predicts progression of subclinical coronary atherosclerosis (SCA), even while adjusting for other CD risk factors (OR = 1.44; 95% CI 1.0 to 2.18 and p = 0.048). More, Lee et al. [15] showed that serum cystatin C is associated with the risk for CVD in patients with type 2 diabetes mellitus. In the same context, a recent study, including subjects with chronic kidney disease (CKD) in a cohort of 1,153 individuals with diabetes, showed that only cystatin C-based CKD definition was an independent risk predictor for CV events and indicated a potentially better clinical utility for CVR prediction than creatinine-based equations [16].

Other studies showed an association between the increase of serum cystatin C and CV events in other disease. In fact, Meng et al. [17] reported, in their study that included 724 patients with relatively normal renal function, that a higher level of serum cystatin C might be another independent risk factor for CV events. According to Koenig et al. [18], increased cystatin C levels are strongly and independently associated with future secondary CV events in 1033 patients with newly diagnosed coronary heart disease.

Ix et al. [19] showed that high cystatin C concentration predicts all cause of mortality, CV events and incident heart failure independently of traditional CV risk factors among ambulatory persons with CHD. Jemberg et al. [20] demonstrated that the risk of death during follow up increased with increasing levels of cystatin C in patients with acute coronary syndrome. Shilpak [21] showed that cystatin C is a best predictive factor in patients with heart failure.

In contrast with previous studies, Kim et al. reported no association between serum cystatin C and CD in diabetic patients [22]. In the present study, a significant difference of serum cystatin C level was found in diabetic patients with and without CVD in groups especially in group with normoalbuminuria. Therefore serum cystatin C level may be associated with CVD.

Ben Dhia et al. [23] showed, in their study including 83 patients with type 2 diabetes, that cystatin C is a good marker of incipient renal disease. In a recent study, Kim et al. [22] reported that serum cystatin C level was higher in patients with diabetic nephropathy, but no differences were found between CAD and non-CAD groups despite the presence of several common: size of sample, number of patients, methodology, and definition of diabetic nephropathy.

### Conclusion

Our results suggested that serum cystatin C concentrations are a potential marker for CVD in type 2 diabetic patients.

### Acknowledgments

The authors thank patients and control subject for their assistance in this study.

### Conflicts of interest:

none.

### References

1. WHO. Available at: http://www.who.int/mediacentre/factsheets/fs312/fr/index.html.


