Hypothalamic-pituitary-adrenal axis activity is associated with the prevalence of chronic kidney disease in diabetic patients

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Abstract. Progression of chronic kidney disease (CKD) in diabetic patients can occur through enhanced hypothalamic-pituitary-adrenal (HPA) axis activity. The purpose of our study was to determine whether HPA axis activity influences the prevalence of CKD in patients with type 2 diabetes mellitus. Seventy-seven diabetic patients (mean age, 60 years) were enrolled. CKD was defined by K/DOQI criteria, and serum cortisol level was measured after the 1 mg overnight dexamethasone suppression test (F-DST). F-DST values were significantly negatively correlated with estimated glomerular filtration rate (eGFR), and significantly positively correlated with cystatin C level and spot urine albumin to creatinine ratio in simple and multiple regression analyses. The subjects were divided into 3 groups (low, middle, and high) according to the F-DST, and the odds for CKD were 8.7-fold (95% confidence interval 2.56 to 29.6, \( P=0.01 \)) and 12.5-fold (95% confidence interval 3.3 to 47.9, \( P<0.001 \)) higher in subjects in the middle and high groups than those in the low group, respectively. In multivariate regression analysis, subjects in the middle group and high group (compared to those in the low group) had 13.0-fold (95% confidence interval, 2.9 to 58.8 and \( P=0.001 \)) and 14.7-fold (95% confidence interval, 2.8 to 78.5 and \( P=0.002 \)), respectively, higher risk for CKD. In conclusion, F-DST values have a relationship with decreased eGFR and increased cystatin C or albumin excretion involved in CKD, and enhanced HPA axis activity may be an independent risk factor for CKD in patients with type 2 diabetes mellitus.

Key words: Hypothalamic-pituitary-adrenal axis, Hypercortisolism, 11β-hydroxysteroid dehydrogenase type 2, Diabetic kidney disease

CHRONIC KIDNEY DISEASE (CKD) is defined as abnormal renal function or morphology, especially associated with proteinuria and/or decreased estimated glomerular filtration rate (eGFR) [1]. Risk factors for CKD include aging, diabetes mellitus, obesity, hypertension, and smoking habit [2-5]. Remarkably, diabetes mellitus is well known to cause CKD, described as diabetic nephropathy, which occurs in up to 50% of diabetic patients [6]. Therefore, the identification of risk factors for diabetic nephropathy is of crucial importance, and the prevention of diabetic nephropathy should be a common goal of many countries worldwide.

Based on some clinical evidences, cortisol has also drawn a high degree of attention as one of the risk factors for CKD. It is reported that 84.6% of patients with Cushing’s syndrome, who presented with autonomous cortisol production, had increased urinary albumin excretion (UAE) [7]. Additionally, UAE declined profoundly, independent of changes in blood pressure and plasma glucose levels, in all these patients after amelioration of hypercortisolism [7]. Patients having metabolic syndrome with hypercortisolism displayed more microalbuminuria than did those without hypercortisolism, even though there were no differences in blood pressure, plasma glucose, and serum lipids between the two groups [8]. These clinical evidences indicate that endogenous hypercortisolism caused by enhanced hypothalamic-pituitary-adrenal (HPA) axis activity itself could increase UAE and eventually lead to CKD independent of blood pressure and metabolic factors such as plasma glucose and serum lipids.

The mineralocorticoid receptor (MR) is activated
mainly by aldosterone and has an important role in the development of renal disease, including CKD, in humans [9-12]. MR stimulation causes progression of renal diseases by inducing glomerular podocyte injury or mesangial cell proliferation as well as by mediating blood pressure changes due to renal Na\(^+\) reabsorption [9, 10]. Podocytes line the urinary side of the glomerular basement membrane and function as a fine filter contributing to ultimate size-selectivity and providing permeability to molecules smaller than albumin [13]. Podocyte injury causes various glomerular diseases indicating proteinuria and diabetic nephropathy [14]. Oxidative stress, generated by MR function in cultured podocytes [15], subsequently induces podocyte apoptosis and injury under diabetic conditions [11]. MR action also enhances mesangial cell proliferation, which is an essential component of glomerulonephritis, by activating mitogen-activated protein kinase 1/2, cyclin D1, and cyclin A [12].

Not only aldosterone but also cortisol has high affinity for MR, despite circulating levels of plasma cortisol being 100 to 1000 times higher than those of aldosterone, with levels of plasma free cortisol likely being higher. However, only aldosterone, but not cortisol, can act within target organs selectively, because 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) that is co-localized with MR converts active cortisol to inactive cortisol [16, 17]. Importantly, 11β-HSD2 mRNA and protein were detected in human glomerular podocyte [18]. Additionally, the level of mRNA expression and activity of renal 11β-HSD2 have been found to decrease in diabetic rats [19], and diabetic patients have lower 11β-HSD2 activity defined as serum cortisone and cortisol [20]. Thus, decreased activity of 11β-HSD2 in the diabetic state does not render cortisol inactive, and thereby leads to a mineralocorticoid-like action due to cortisol in the kidney.

Collective findings from clinical and basic researches indicate that CKD could be induced by relatively excess cortisol, which acts as a mineralocorticoid on MRs via decreased 11β-HSD2 activity in diabetic patients. Therefore, we hypothesized that higher endogenous cortisol secretion could lead to progression of CKD through conditions such as proteinuria and decreased eGFR in patients with type 2 diabetes mellitus. The aim of our study was to determine the association of cortisol levels after 1-mg overnight dexamethasone suppression test (DST) with CKD parameters in patients with type 2 diabetes mellitus.

Materials and Methods

Subjects

This study was carried out on 125 consecutive in-patients with type 2 diabetes mellitus, recruited at Hiroshima University Hospital. The study was approved by the Ethics Committee of Hiroshima University, and informed consent was obtained from all subjects. Selection criteria were as follows: no signs or symptoms of hypercortisolism including moon face, striae rubrae, hypertrichosis, skin atrophy, and buffalo hump; and/or no acute illness, cancer, inflammatory disease, psychiatric disease including depression, alcoholism; and no current use of glucocorticoid therapy. Patients diagnosed with slowly progressive insulin-dependent diabetes mellitus after admission were excluded. Finally, 77 patients with type 2 diabetes mellitus were enrolled in our study.

The diagnosis of CKD was defined by the presence of spot urine albumin to creatinine ratio (ACR) >30 mg/gCre or estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m\(^2\) based on the K/DOQI criteria [6, 21]. Subjects with systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg, and/or use of antihypertensive treatment were defined as hypertensive [22]. Dyslipidemia was defined as triglycerides ≥150 mg/dL, low-density lipoprotein cholesterol ≥140 mg/dL, or high-density lipoprotein cholesterol <40 mg/dL [23]. Subjects receiving antidyslipidemic treatment were also considered as having dyslipidemia.

Protocol and laboratory measurements

On the day of admission, each subject underwent a physical exam, blood pressure measurement in a sitting position, and urinalysis for evaluation of spot ACR. Blood samples were drawn in the morning after overnight fasting. Serum cystatin C and eGFR were used as markers of renal function impairment.

The DST was performed to evaluate HPA axis activity as we previously reported [24]. Briefly, patients took 1-mg dexamethasone at 2300 h at 7 days after admission, and fasting cortisol levels (F-DST) were assessed at 0700 h the next morning. When F-DST was above the cut-off value of 1.8 μg/dL, we consecutively measured diurnal variation of cortisol and ACTH, and cortisol and ACTH after corticotrophin-releasing hormone (CRH) test, as reported previously [25, 26]. Furthermore, patients with basal ACTH levels lower than 10 pg/mL underwent abdominal computed tomog-
rathy (CT). Those with basal ACTH levels higher than 10 pg/mL underwent abdominal CT, magnetic resonance imaging (MRI) scan of the pituitary region, and 8-mg overnight DST to evaluate whether those who had incomplete suppression of F-DST were affected by subclinical hypercortisolism (SH) caused by adrenal adenomas or pituitary adenomas.

Serum cortisol was measured using the ECLusys 2010 cortisol assay (ECL; Roche Diagnostics Co., Germany), and plasma ACTH was measured using an immunoradiometric assay by ACTH IRMA “MITSUBISHI” (Mitsubishi Chemical Medience Co., Tokyo, Japan). Serum cystatin C and urinary albumin were measured using N Latex Cystatin C (Dade Behring, Deerfield, IL, USA) and N Antiserum to Human Albumin (Siemens Healthcare Diagnostics, Deerfield, IL, USA), respectively. Serum and urinary creatinine were measured using an enzymatic assay (AUTO L “MIZUHO” CRE kit; Mizuho Medy Co., Tosu, Japan).

Statistical analysis
Quantitative data were expressed as mean ± S.D., and qualitative variables were expressed as percentages. Parameters that failed to show normal distributions, such as duration of diabetes and basal ACTH, were analysed by non-parametric analyses (Kruskal-Wallis test). Simple and multiple regression analyses were conducted to evaluate the relationships of eGFR, cystatin C, or ACR with F-DST. We performed logistic regression analysis to assess the independent contribution of F-DST to CKD. In logistic regression analysis, we divided subjects into 3 groups according to F-DST, with nearly equal number of subjects per group. Subjects with F-DST in the lowest tertile (F-DST ≤0.6 μg/dL) were defined as the low group, those with F-DST in the middle tertile (0.6 < F-DST ≤1.0 μg/dL) as the middle group, and those with F-DST in the highest tertile (F-DST >1.0 μg/dL) as the high group. P-values less than 0.05 were considered statistically significant. Statistical analyses were performed using the software package SPSS (version 12.0; Chicago, IL, USA).

Results
Fifty men (64.9%) and 27 women (35.1%) were included in this study. The characteristics of the study subjects were as follows: age, 60.0 ± 14.6 years; body mass index (BMI), 25.6 ± 4.4; duration of type 2 diabetes mellitus, 12.8 ± 10.3 years; and HbA1c, 10.1 ± 2.0%. Sixty-seven subjects (87.0%) and 64 subjects (83.1%) had hypertension and dyslipidemia, respectively. Forty-five smokers (58.4%) were included in this study. Basal cortisol and ACTH were 14.3 ± 4.5 μg/dL and 50.0 ± 29.2 pg/mL, respectively.

Three subjects had serum cortisol levels above 1.8 μg/dL after 1 mg overnight DST. These subjects were further examined according to our protocol (see methods), and no patient showed adrenal cortisol-producing tumors or pituitary ACTH-producing tumors.

F-DST significantly negatively correlated with eGFR ($\beta$=-0.441, $P<0.001$), and significantly positively correlated with cystatin C ($\beta$=0.441, $P<0.001$) and ACR ($\beta$=0.374, $P=0.001$) in simple regression analysis (Fig. 1). The relationships between F-DST and eGFR, cystatin C, or ACR were retained after adjusting for parameters affecting renal function or ACR, such as sex, age, BMI, HbA1c, duration of diabetes mellitus, hypertension, and smoking (eGFR, $\beta$=-0.263, $P=0.012$; cystatin C, $\beta$=-0.342, $P=0.003$; ACR, $\beta$=0.306, $P=0.018$). Basal serum cortisol or plasma ACTH levels in the morning after overnight fasting had no relation with eGFR, cystatin C, or ACR (data not shown).

We divided subjects into 3 groups according to F-DST, with nearly equal number of subjects per group to assess the odds ratio of CKD. There were significant differences in age and smoking history among the groups (Table 1). A logistic regression analysis revealed that subjects in middle and high groups had 8.7-fold (95% confidence interval, 2.6 to 29.6 and $P=0.010$) and 12.5-fold (95% confidence interval, 3.3 to 47.9 and $P<0.001$) higher odds ratio for CKD than the low group, respectively (Fig. 2A). In a multivariate regression analysis, after adjustment by age, sex, BMI, HbA1c, duration of type 2 diabetes mellitus, prevalence of hypertension, and smoking status, subjects in the middle group had 13.0-fold increased risk (95% confidence interval, 2.9 to 58.8 and $P=0.001$) while those in the high group had 14.7-fold increased risk (95% confidence interval, 2.8 to 78.5 and $P=0.002$) for CKD compared to subjects in the low group (Fig. 2B).

Discussion
In the present study, F-DST showed a strong association with decreased eGFR and increased cystatin C or ACR involved in CKD progression in type 2 diabetic patients. Moreover, our findings demonstrated that high F-DST was an independent risk factor for CKD.
The present study and previous reports suggest that HPA axis activation influences CKD progression via MR stimulation induced by low activity of 11β-HSD2 in diabetic patients. Atherosclerosis may also participate in the progression of CKD related to the activation of HPA axis, because glomerulosclerosis and arteriosclerosis occur commonly together [28, 29]. It has been demonstrated that the MR/11β-HSD2 system exists in non-epithelial cells of these patients.

Our results showed that F-DST was implicated in the prevalence of CKD in type 2 diabetic patients. Since mRNA expression levels and activity of renal 11β-HSD2 are decreased in hyperglycemia [19, 20], the effects of HPA axis on CKD progression might be reinforced in patients with type 2 diabetes. Several reports have elucidated mineralocorticoid action on renal dysfunction via MR at glomerular podocyte [9, 11, 12, 15, 27]. The present study and previous reports suggest that HPA axis activation influences CKD progression via MR stimulation induced by low activity of 11β-HSD2 in diabetic patients. Atherosclerosis may also participate in the progression of CKD related to the activation of HPA axis, because glomerulosclerosis and arteriosclerosis occur commonly together [28, 29]. It has been demonstrated that the MR/11β-HSD2 system exists in non-epithelial cells of these patients.

Table 1 Clinical characteristics of the subjects divided by F-DST

<table>
<thead>
<tr>
<th>Low (F-DST ≤ 0.6)</th>
<th>Middle (0.6 &lt; F-DST ≤ 1.0)</th>
<th>High (1.0 &lt; F-DST)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>28</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>F-DST (μg/dL)</td>
<td>0.51 ± 0.09</td>
<td>0.89 ± 0.20</td>
<td>1.41 ± 0.35</td>
</tr>
<tr>
<td>Male/Female (%)</td>
<td>16/12 (57.1/42.9)</td>
<td>22/5 (81.5/18.5)</td>
<td>12/10 (54.5/45.5)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.4 ± 16.3</td>
<td>63.3 ± 12.8</td>
<td>65.5 ± 9.8</td>
</tr>
<tr>
<td>BMI</td>
<td>24.7 ± 3.9</td>
<td>25.3 ± 4.1</td>
<td>27.1 ± 5.1</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>11.4 ± 10.1</td>
<td>12.8 ± 11.2</td>
<td>14.7 ± 9.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.8 ± 1.6</td>
<td>10.4 ± 2.4</td>
<td>10.0 ± 1.7</td>
</tr>
<tr>
<td>Hypertension</td>
<td>24 (85.7%)</td>
<td>23 (85.2%)</td>
<td>20 (90.9%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>24 (85.7%)</td>
<td>20 (74.1%)</td>
<td>20 (90.9%)</td>
</tr>
<tr>
<td>Smoking (never/ex/current)</td>
<td>16/11/11</td>
<td>6/10/11</td>
<td>10/6/6</td>
</tr>
<tr>
<td>Basal cortisol (μg/dL)</td>
<td>13.5 ± 4.3</td>
<td>14.2 ± 5.2</td>
<td>15.4 ± 3.7</td>
</tr>
<tr>
<td>Basal ACTH (pg/mL)</td>
<td>47.6 ± 20.0</td>
<td>55.3 ± 34.9</td>
<td>46.6 ± 31.7</td>
</tr>
<tr>
<td>CKD</td>
<td>6 (21.4%)</td>
<td>19 (70.4%)</td>
<td>17 (77.3%)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD. P values were determined by χ² test, ANOVA, or Kruskal-Wallis test. F-DST, serum cortisol level after overnight 1mg dexamethasone suppression test; BMI, body mass index; CKD, chronic kidney disease.

Fig. 1 The association between serum cortisol level after overnight 1 mg dexamethasone suppression test (F-DST) and chronic kidney disease (CKD) parameters. A. estimated glomerular filtration rate (eGFR), B. cystatin C, C. albumin-creatinine ratio (ACR).
cells including the vasculature, and activation of MR in the vasculature is also proinflammatory and proatherogenic [30, 31]. A study on Apoe¬/¬/11β-HSD2¬/¬ double-knockout (Eb/2) mice suggested that loss of function of 11β-HSD2 led to striking atherogenesis in Eb/2 mice, mediated by activation of nonrenal MR mainly by glucocorticoids [32]. Therefore, decreased systemic 11β-HSD2 activity seen in type 2 diabetic patients may similarly accelerate atherogenesis, leading to atherosclerosis and eventually CKD, all the more so with hypercortisolism.

Other molecular mechanisms have been proposed for progression of CKD in a diabetic state. High extracellular glucose activates a number of signaling cascade at the cellular level via GLUT1-facilitated transport of glucose. They sequentially cause accumulation of extracellular matrix protein in the mesangial space with mesangial expansion, thickening of the glomerular and tubular basement membranes, and tubulointerstitial fibrosis [33-35]. Importantly, the activation of the MR cascade related with the HPA axis may lead to progression of diabetic nephropathy, independent of the general mechanisms for CKD due to diabetes mellitus.

Although the HPA axis defined as F-DST showed suppressive revels in most of the patients, the cortisol levels related with the prevalence of CKD. We need to consider the mechanisms that the low cortisol levels induced CKD prevalence. First, the sensitivity of dexamethasone for glucocorticoid receptor in pituitary gland might be influenced under diabetic condition in patients with higher F-DST, because the expressions of glucocorticoid receptor in rat brain were regulated by high-sucrose consumption [36]. Although 1mg dexamethasone is enough to suppress ACTH secretion from pituitary gland, un-suppressive ACTH under diabetic condition might potentiate slight cortisol secretion and influence the CKD status. Second, some ligands instead of ACTH might stimulate the cortisol secretion in adrenal gland, because it is well known that ectopic or aberrant receptors are expressed in zona fasciculata [37]. Therefore, the cortisol secretion via ectopic or aberrant receptors by some ligands might influence the progression of CKD.

MR antagonists may be useful to prevent CKD in patients with type 2 diabetes mellitus, especially in the presence of hypercortisolism. Recent reports analyzed 9 trials suggest that addition of MR antagonists to angiotensin-converting enzyme (ACE) inhibitor or
angiotensin receptor blocker (ARB) therapy has beneficial effects on proteinuria in diabetic patients [38]. In diabetic rodent models, the beneficial effects of MR antagonists on renal injury have also been reported [39, 40]. The Food and Drug Administration has placed a contraindication on the use of eplerenone, an aldosterone blocker that selectively blocks the MRs and not glucocorticoid, progesterone, androgen receptors, in type 2 diabetes mellitus and microalbuminuria because of potential adverse effects such as hyperkalemia. In selected patients, especially those with hypercortisolism, large clinical prospective studies should be performed to elucidate the effects of MR antagonists on renal injury.

In the present study, we could not find patients with SH caused by adrenal or pituitary adenomas, despite reports of a relatively high prevalence of SH in patients with type 2 diabetes mellitus [25, 27]. This may be attributed to the characteristics of study participants, such as race, levels of glycemic control, and duration of diabetes mellitus, which differed from those in previous studies. Diabetes mellitus resulting from SH may be cured after correction of SH. F-DST is an index not only to evaluate the risk for CKD but also to screen for SH and initiate further examination for its cause. Therefore, it would be very meaningful to perform DST in patients with type 2 diabetes mellitus.

It has been reported that the elderly subjects, who have lower renal function, show comparable levels of F-DST with the younger subjects [41]. On the other hand, the patients with severe chronic renal failure or hemodialysis were known to have lower clearance of cortisol and higher F-DST compared to normal subjects [42]. Chronic renal failure attenuate the expression of 11β-HSD2, however serum cortisol levels were not induced by lower 11β-HSD2 activity due to chronic renal failure [20]. Importantly, cortisol is basically metabolized by glucuronate conjugation in the liver, and the excretion of free cortisol through kidneys represents only 1% of the total cortisol secretion [43]. The patients with mild CKD in our study would not have impaired cortisol clearance, however we need further surveys which focused on the clearance or DST in mild CKD patients without diabetes mellitus.

The present study has several limitations. This study was cross-sectional; hence, we could not establish a causal relationship between F-DST and CKD. Although ACR is recommended to measure several times because of the fluctuation, we just assessed from single urine sample. However, F-DST correlated with other CKD parameters such as eGFR and Cystatin C, and thus F-DST might be associated with ACR. Moreover, study subjects were limited only to 77 Japanese patients with type 2 diabetes mellitus; therefore, a large-scale study is required to examine whether similar results will be obtained across different populations.

In conclusion, we determined that HPA axis activity defined as DST is strongly associated with decreased eGFR and increased cystatin C or ACR involved in CKD, and that F-DST is an independent risk factor for CKD in patients with type 2 diabetes mellitus. Screening using F-DST might be useful in assessing patients at risk for developing CKD. Finally, MR antagonists may have beneficial effects on the prevention of CKD.

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None.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


