Original Article

Relationship between Impaired Aldosterone Response to Adrenocorticotropic Hormone and Prevalence of Hemodialysis in Type 2 Diabetic Patients without Azotemia

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The present prospective observational study was designed to assess the prevalence of hemodialysis in type 2 diabetic patients with an impairment of plasma aldosterone responsiveness to adrenocorticotropic hormone (ACTH). Sixty seven patients (43 men and 24 women) were selected. The inclusion criteria were age <65 years; presence of normoalbuminemia (serum albumin >3.6 g/dl); and absence of azotemia (serum creatinine ≤1.2 mg/dl in males, and ≤1.0 mg/dl in females). Soluble $\alpha^{1\text{-}24}$-ACTH was injected intramuscularly in a single dose of 0.25 mg after overnight recumbency. The area under the aldosterone curve (aldosterone AUC) was calculated. The diabetic patients were divided into two groups according to the levels of aldosterone AUC. Patients with an aldosterone AUC in the range of 0–381 were considered poor responders (n=31) and those with an AUC of 397–1,007 were considered good responders (n=36). The follow-up was performed during a 144-month period. The end point of the study was the introduction of hemodialysis. A total of 14 patients (12 poor responders and 2 good responders; p<0.001) were introduced to hemodialysis. The prevalence of hemodialysis in the poor responders (5.74 per 100 patient-years) was significantly higher (p<0.001, log-rank test) than that in the good responders (0.68 per 100 patient-years). One possible explanation is that an inappropriate level of salt intake relative to the impaired plasma aldosterone control may have contributed to the high prevalence of risks and hemodialysis in the poor responders. (*Hypertens Res* 2005; 28: 21–26)

***Key Words*: aldosterone, hemodialysis, type 2 diabetes mellitus, adrenocorticotropic hormone**

Introduction

Diabetes mellitus is associated with an impaired response of plasma aldosterone to various stimuli, including postural change (*1*), furosemide administration (*2*), and angiotensin II (AII) (*3*). The impaired aldosterone response to these stimuli has been partly explained by concomitant renin deficiency (*3*, *4*). The plasma aldosterone response to adrenocorticotropic hormone (ACTH) in nonazotemic diabetes mellitus, however, remains a subject of controversy, with some studies reporting that it is altered (*3*, *5*) and others that it is unaltered (*2*, *5*–*7*). Since it is well known that the responses of the adrenal zona glomerulosa to ACTH are critically dependent on sodium intake or sodium balance and are enhanced by sodium restriction (*8*), this discrepancy may be partly due to a difference in sodium intake. We have previously shown that some nonazotemic type 2 diabetic subjects with normoreninemia have

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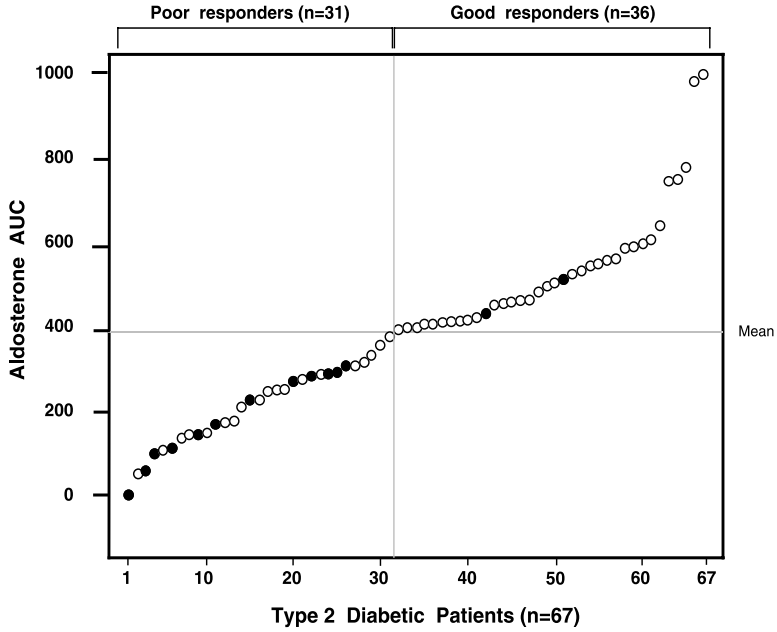

Fig. 1. *The scatter diagram of aldosterone AUC in 67 type 2 diabetic patients. The patients were divided into two groups according to the extent of the levels of aldosterone AUC (the mean level: 390.3 as a cut point): patients with a range of 0–381 of aldosterone AUC (poor responders;* n=*31) and patients with a range of 397–1,007 of aldosterone AUC (good responders;* n=*36). Closed circles indicate patients who were introduced to hemodialysis during the follow-up.*

a blunted responsiveness of plasma aldosterone to ACTH during moderate sodium depletion (*9*). However, the clinical significance of the blunted responsiveness of plasma aldosterone to ACTH is obscure. Plasma aldosterone level and sodium intake can affect renal function. Thus, the present prospective observational study was designed to assess the prevalence of hemodialysis in type 2 diabetic patients with an impairment of plasma aldosterone control.

Methods

Subjects

Sixty seven patients (43 men and 24 women) were selected from 103 type 2 diabetic patients who underwent rapid ACTH test during their admission to Kanazawa Medical University Hospital between April 1988 and December 1989. Informed consent was obtained from each participant. The inclusion criteria were as follows: age <65 years; presence of normoalbuminemia (serum albumin >3.6 g/dl); and absence of azotemia (serum creatinine ≤ 1.2 mg/dl in males, and ≤ 1.0 mg/dl in females). We excluded patients who had severe complications such as congestive heart failure, liver cirrhosis, stroke, nephrotic syndrome, or acute inflammatory diseases.

The diagnosis of diabetic retinopathy was made by ophthalmoscopy and fluorescein angiography by an experienced ophthalmologist in our hospital. Diabetic neuropathy was defined as reduced tendon reflexes, especially the Achilles and patellar tendon reflexes, and abnormal electrodiagnostic studies (motor and/or sensory nerve conduction velocity). Diabetic patients were defined as having nephropathy when the urinary albumin excretion rate was more than 30 mg/24 h. Blood pressure (BP) was measured with the subject seated after an at least 5-min rest using a standard mercury sphygmomanometer and cuffs adapted to arm circumference. Hypertension was considered to be present in patients with systolic BP (SBP) >140 mmHg and/or diastolic BP (DBP) >90 mmHg on two different occasions, or in patients treated with antihypertensive drugs.

Rapid ACTH Test (ACTH Injection)

The diabetic patients had been on a sodium chloride (NaCl) intake of 150 mEq per day for a week before the ACTH injection. None of the patients took any medication for at least 1 week before the injection except for oral hypoglycemic agents or insulin. ACTH injection was started between 8 AM and 9 AM after overnight recumbency. Soluble $\alpha^{1\text{-}24}$-ACTH (Cortrosyn; Organon, West Orange, USA) was injected intramuscularly in a single dose of 0.25 mg. A blood sample for plasma aldosterone and cortisol assays was obtained before and 30 and 60 min after the injection, and was immediately centrifuged at 4°C. The plasma was frozen at -20°C until assayed.

To assess the integrated expression of plasma aldosterone response to ACTH injection in each subject, the area under the aldosterone curve (aldosterone AUC) was calculated by a trapezoidal rule (*10*). The area under the cortisol curve (cortisol AUC) was calculated in a similar manner.

Table 1. Baseline Clinical and Biochemical Characteristics

	Poor responders	Good responders
Number of patients	31	36
Age (years)	51±9	50±10
Male (*n* (%))	22 (71)	21 (58)
Duration of diabetes (years)	8.7±6.1	5.7±7.8
Body mass index (kg/m^2)	23.4±3.7	23.2±4.0
HbA1c (%)	9.9±2.5	9.1±2.8
Hypertensive patients (*n* (%))	9 (29)	6 (17)
Systolic BP (mmHg)	131±22	127±19
Diastolic BP (mmHg)	78±12	77±9
Treatment for diabetes (diet alone/OH/insulin)	3/16/12	11/14/11
Diabetic complications (Neu/Ret/Nep)	24/16/12	22/10/7
Serum sodium (mEq/l)	141±2	140±3
Serum potassium (mEq/l)	4.1±0.3	4.1±0.3
Serum albumin (g/dl)	4.0±0.3	4.1±0.3
Serum creatinine (mg/dl)	0.9±0.2*	0.8±0.2
Plasma renin activity (ng/ml/h)	1.1±0.9	1.1±1.0
Plasma aldosterone (ng/dl)	6.1±3.7	7.6±3.8
ACTH injection		
Aldosterone AUC	216±97$^{\#}$	541±152
Cortisol AUC	701±179	781±196
Urinary sodium (mEq/24 h)	115±30	122±33
Urinary potassium (mEq/24 h)	32±9*	39±13
Urinary albumin (mg/24 h)	231±324	119±240
Creatinine clearance (ml/min)	80.7±25.7	82.5±24.3

Data are means±SD, *n*, or *n* (%). BP, blood pressure; OH, oral hypoglycemics; Neu, neuropathy; Ret, retinopathy; Nep, nephropathy; AUC, area under the curve. *$p<0.05$ and $^{\#}p<0.001$ *vs.* those in the Good responders.

Subclassification of the Subjects

The diabetic patients were divided into two groups according to their aldosterone AUC levels, using the mean level of 390.3 as a cut point. Poor responders ($n=31$) were those with aldosterone AUC in the range of 0–381, and good responders ($n=36$) were those with aldosterone AUC of 397–1,007.

Follow-Up and End Point Evaluations

After being discharged from the hospital, the diabetic patients regularly visited our outpatient clinic. The patients' medical records were reviewed intermittently to check for adverse events. The follow-up was performed over a 144-month period from 1988 to 2000. The end point of the study was the introduction of hemodialysis. The indication for and timing of the start of hemodialysis were decided by a experienced nephrologist in our hospital. Patients who were introduced to hemodialysis after 31 March 2000 were not included in the statistical analysis.

During the follow-up period, a total of 7 patients died. The causes of death were acute myocardial infarction ($n=2$), cerebral infarction ($n=2$), cerebral hemorrhage ($n=1$), cancer ($n=1$), and a traffic accident ($n=1$). In addition, 4 patients (2 poor responders and 2 good responders) were lost to follow-up midway through this study. Data from these patients were also withheld from the statistical analysis. Their clinical and biochemical data at the time of the final follow-up were based on medical records at their last visits to our outpatient clinic. During the follow-up period in both groups, the patients with chronic renal failure received standard treatment, including dietary counseling on low protein diet and low salt diet, administration of an oral absorbent, or subcutaneous injection of erythropoietin for renal anemia.

Laboratory Measurements

Plasma renin activity (PRA) and plasma aldosterone were measured by radioimmunoassay (RIA) using antisera obtained from Dainabot Radioisotope Institute (Tokyo, Japan) as previously described (*7*). Plasma cortisol was measured by RIA using its specific antiserum purchased from Endocrine Science (Tarzana, USA) (*7*). Serum and urinary concentrations of sodium, potassium, creatinine and serum albumin were measured on a sequential multiple analyzer plus computer (Technicon, New York, USA). Urinary albumin was determined with a Latex agglutination nephelometric immunoassay kit (Eiken, Tokyo, Japan). Hemoglobin A1c (HbA1c) was measured by the chromatographic method with a hemoglycosimeter auto-A1c-TM (Daiichi, Kyoto, Japan).

Statistical Analysis

Data are expressed as the mean±SD. Statistical analyses were performed using the StatView 5.0 statistical software package (SAS Institute, Cary, USA). As all data for the comparison of group means were almost normally distributed, Student's *t*-test was applied for data with equal variances; otherwise, Welch's test was applied for data with nonequal variances. For testing of frequency data in the two groups, the χ^2 test was used. The responses of plasma aldosterone and cortisol to ACTH injection were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Bonferroni's post hoc test. Renal survival curves were estimated using the Kaplan-Meier product-limit method, and were compared by log-rank test. The unadjusted relative risk was obtained from the Cox-Mantel's test. The prevalences of hemodialysis are presented as the number of events per 100 patient-years based on the ratio of the observed number of events to the total number of patient-years of exposure. Two-tailed values of $p<0.05$ were considered to indicate statistical significance.

Table 2. Clinical and Biochemical Characteristics at the Time of the Final Follow-Up

	Poor responders	Good responders
Number of patients	31	36
Systolic BP (mmHg)	142±21	136±18
Diastolic BP (mmHg)	83±12	80±12
HbA1c (%)	8.4±1.1	8.3±1.4
Serum potassium (mEq/l)	4.7±0.8$^{\#}$	4.1±0.3
Serum creatinine (mg/dl)	2.8±2.5†	1.1±1.1
Hypertension	21*	14
Antihypertensive medication (*n* (%))		
Calcium channel blockers	15 (71)	11 (79)
ACE inhibitors or ARB	11 (52)	8 (57)
Diuretics	11 (52)	6 (43)
α- or β-blockers	4 (19)	3 (21)

Data are means±SD, *n*, or *n* (%). BP, blood pressure; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker. *$p<0.05$, $^{\dagger}p<0.01$, and $^{\#}p<0.001$ *vs*. those in the Good responders.

Results

ACTH injection resulted in significant increases in plasma cortisol and aldosterone after 30 and 60 min ($p<0.001$) in 67 type 2 diabetic patients (data not shown). Figure 1 shows the scatter diagram of aldosterone AUC in the 67 type 2 diabetic patients divided into two groups (poor and good responders) according to their aldosterone AUC levels. As shown in Fig. 1, a total of 14 patients (closed circles; 12 poor responders and 2 good responders; $p<0.001$, χ^2 test) were introduced to hemodialysis during the follow-up period (mean: 89.9 ± 43.6 months, $n=67$).

Table 1 presents the baseline clinical and biochemical characteristics in the two groups. The levels of aldosterone AUC and urinary potassium excretion rate in the poor responders were significantly lower ($p<0.001$ and $p<0.05$, respectively) than those in the good responders. Serum creatinine levels in the poor responders were significantly higher ($p<0.05$) than those in the good responders. There were no significant differences in other continuous variables, including age, duration of diabetes, body mass index (BMI), HbA1c, SBP, DBP, serum sodium, serum potassium, serum albumin, plasma renin activity, basal plasma aldosterone, cortisol AUC, urinary sodium excretion rate, urinary albumin excretion rate, or creatinine clearance between the two groups. Also, there were no significant differences in categorical variables, including gender, the prevalence of hypertension, treatment for diabetes, and diabetic complications between the two groups.

Table 2 presents the clinical and biochemical characteristics at the time of the final follow-up in the two groups. Although the mean levels of SBP tended to be higher in the poor responders, there were no significant differences in SBP, DBP, or HbA1c between the two groups. The mean levels of serum potassium and creatinine in the poor responders were significantly higher ($p<0.001$ and $p<0.01$, respectively) than those in the good responders. Although the prevalence of hypertension at the time of the final follow-up was significantly higher in the poor responders ($p<0.05$) than in the good responders, there were no significant differences between the two groups in the prevalence of antihypertensive medication use at the time of the final follow-up.

Table 3. The Rates of the Introduction of Hemodialysis

	Poor responders	Good responders
Number of patients	31	36
Duration of follow-up (months)	81±41	98±45
Prevalence of hemodialysis (per 100 patient-years)	5.74*	0.68

Data are mean±SD or *n*. *$p<0.001$ *vs*. those in the Good responders.

There was no significant difference in the duration of follow-up between the two groups (Table 3). The prevalence of hemodialysis in the poor responders was significantly higher ($p<0.001$, log-rank test) than that in the good responders (Table 3). The Kaplan-Meier renal survival curves of the poor and good responders showed a significant difference in the renal survival rate ($\chi^2=12.749$; $p<0.001$ by log-rank test; Fig. 2). The unadjusted relative risk for the poor responders was 9.1-fold higher than that of the good responders ($p<0.05$; Cox-Mantel's test).

Discussion

The present results indicate a high prevalence of hemodialysis in nonazotemic type 2 diabetic patients with impaired responsiveness of plasma aldosterone to ACTH.

Sodium balance is an important factor in conditioning aldosterone responsiveness to various stimuli (*11*). In addition, exchangeable sodium has been shown to be increased in nonazotemic diabetes mellitus (*1*, *2*). In the present study, there were no significant differences in creatinine clearance or 24-h urinary excretion of sodium between the poor responders and the good responders. It is thus unlikely that the degree of sodium balance was different between the two groups. We previously reported (*12*) that aging causes a reduced aldosterone secretory response to ACTH. In the present study, however, all the diabetic subjects were less than 65 years old at the baseline period, and the levels of mean age were similar between the two groups. In addition, there were no significant differences in baseline serum potassium concentration or PRA, both known stimuli of aldoste-rone production, or in cortisol AUC between the two groups. Taken together, these observations indicate that the results of the rapid ACTH test

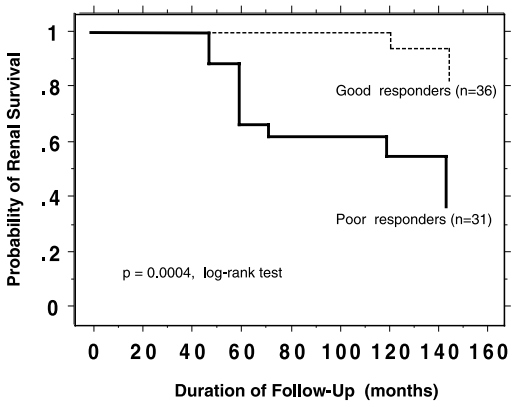

Fig. 2. *Kaplan-Meier renal survival curves in the poor (*n=*31) and good (*n=*36) responders.*

were comparable between the two groups. Thus, the significantly low aldosterone AUC levels in the poor responders implied that there was an abnormality of plasma aldosterone control in this group. The finding of a significantly low urinary potassium excretion rate may support this possibility. These findings are consistent with our previous report (*9*) that some nonazotemic type 2 diabetic patients with normoreninemia have a blunted responsiveness of plasma aldosterone, but not of plasma cortisol, to ACTH during moderate sodium depletion. Multiple defects in the renin-aldosterone axis with normal glucocorticoid function can also occur in normokalemic diabetic patients (*1*, *7*). These include a diminished aldosterone response with a normal renin response (*1*, *6*). Tuck *et al*. (*3*) observed the blunted plasma aldosterone response to ACTH in 5 diabetic patients with renal insufficiency, whereas Beretta-Piccoli *et al*. (*2*) reported the normal response both in 6 diabetic patients with mild nephropathy and in 12 diabetic patients without complications. The discrepancy of the plasma aldosterone response to ACTH in diabetic patients may thus be partly due to the difference in the patient populations studied. The clinical significance of the blunted responsiveness of plasma aldosterone to ACTH, however, has been obscure. Thus, the present prospective observational study was designed to assess the prevalence of hemodialysis in type 2 diabetic patients with an impairment of plasma aldosterone control.

The acceleration of the renin-angiotensin system has been suggested to be associated with a faster rate of decline in renal function (*13*, *14*). In the present study, however, the levels of basal plasma aldosterone, PRA and 24-h urinary excretion of sodium were similar between the two groups. Moreover, at the time of the final follow-up, there were no significant differences in the prevalence of antihypertensive medication use or HbA1c between the two groups. Nevertheless, a significantly high prevalence of hemodialysis was observed in the poor responders when compared with that in the good responders. In the baseline period, although there were no significant differences in the duration of diabetes, the prevalence of hypertension and nephropathy, or the urinary albumin excretion rate, the levels of these parameters tended to be higher, and serum creatinine levels were significantly higher in the poor responders than in the good responders. Moreover, at the time of the final follow-up, the levels of both SBP and DBP tended to be higher, and the serum creatinine levels and the prevalence of hypertension were significantly higher in the poor responders than in the good responders. Since these parameters are known to be associated with the progression of diabetic nephropathy (*15*), these risks may be associated with the high prevalence of hemodialysis observed in the poor responders. In the present study, because of a small sample size of diabetic patients studied, an adjustment for known prognostic and predictive factors (duration of diabetes, serum creatinine, SBP and DBP, treatment for diabetes, diabetic complications, antihypertensive medication, urinary albumin excretion rate) may lower a power of multivariate analysis. It is thus uncertain, based on the results of the present study, whether or not aldosterone AUC is an independent predictor of hemodialysis.

In the present study, however, the important question is that of why these risks were observed in the poor responders. It has been reported that the proximal tubule renin-angiotensin system is activated in early diabetes (*16*, *17*). In addition, the concept of "tissue aldosterone" has been proposed (*18*). It is, however, unclear whether there was a significant difference in locally produced AII or aldosterone between the two groups in the present study. A direct stimulatory action of aldosterone on the production of intact type IV collagen was reported in cultured mesangial cells (*19*). Type IV collagen is a main constituent of the network structure of the glomerular basement membrane (GBM) as a size barrier (*20*), and has been reported to be glycosylated in a hyperglycemic state (*21*). A glycosylated type IV collagen has been reported to accumulate without enzymatic decomposition, resulting in destruction of the network structure (*22*). Thus we cannot exclude the possibility that chronic aldosterone deficiency is disadvantageous for the maintenance of homeostasis in diabetic patients with nephropathy. On the other hand, it has been suggested that circulating aldosterone and excess salt are closely related to a renal damage (*18*, *23*). In addition, it has been reported that aldosterone-induced organ damage was not observed in animals fed a low-salt diet (*24*). Moreover, studies have strongly suggested that aldosterone-induced organ damage may occur even when the plasma aldosterone level is within normal range, if the plasma aldosterone levels and salt intake are imbalanced (*18*, *25*). In the present study, since the sodium balance was similar between the two groups, whereas plasma aldosterone control was impaired in the poor responders, as discussed above, it is possible that an inappropriate salt status may have existed in the poor responders. Thus, although the precise mechanism(s) remain uncertain, one possibility is that an inappropriate salt intake relative to an impaired plasma aldosterone control may have contributed to the high prevalence of risks and hemodialysis in the poor responders in our study.

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