Insulin Resistance as an Independent Predictor of Cardiovascular Mortality in Patients with End-Stage Renal Disease

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Abstract. Insulin resistance is closely associated with atherosclerosis and cardiovascular mortality in the general population. Patients with end-stage renal disease (ESRD) are known to have insulin resistance, advanced atherosclerosis, and a high cardiovascular mortality rate. We evaluated whether insulin resistance is a predictor of cardiovascular death in a cohort of ESRD. A prospective observational cohort study was performed in 183 nondiabetic patients with ESRD treated with maintenance hemodialysis. Insulin resistance was evaluated by the homeostasis model assessment method (HOMA-IR) using fasting glucose and insulin levels at baseline, and the cohort was followed for a mean period of 67 mo. Forty-nine deaths were recorded, including 22 cardiovascular deaths. Cumulative incidence of cardiovascular death by Kaplan-Meier estimation was significantly different between subjects in the top tertile of HOMA-IR (1.40 to 4.59) and those in the lower tertiles of HOMA-IR (0.28 to 1.39), and the hazard ratio (HR) was 2.60 (95% confidence interval [CI], 1.12 to 6.01; \( P = 0.026 \)) in the univariate Cox proportional hazards model. In multivariate Cox models, the positive association between HOMA-IR and cardiovascular mortality remained significant (HR, 4.60; 95% CI, 1.83 to 11.55; \( P = 0.001 \)) and independent of age, C-reactive protein, and presence of preexisting vascular complications. Further analyses showed that the effect of HOMA-IR on cardiovascular mortality was independent of body mass index, hypertension, and dyslipidemia. In contrast, HOMA-IR did not show such a significant association with noncardiovascular mortality. These results indicate that insulin resistance is an independent predictor of cardiovascular mortality in ESRD.

Materials and Methods

Study Design and Subjects

We recently reported a prospective, observational cohort study that evaluated the effects of metabolic changes on arteriosclerosis and prognosis in ESRD (The MAP ESRD Study) (26). The present study is a subanalysis of the MAP ESRD cohort that includes only 183 subjects with normal fasting glucose concentration. Table 1 summarizes baseline characteristics of the subjects. The original cohort consisted of 265 ESRD patients. They had been treated by regular hemodialysis for more than 3 mo at Inoue Hospital, Suita, Japan. The subjects were recruited from those who were dia-
Definition of Hypertension

BP was measured with a standard mercury sphygmomanometer and cuffs adapted to arm circumference after the subject had rested in the supine position for at least 5 min. The systolic and diastolic BP levels were taken as the points of appearance and disappearance of Korotkoff sounds, respectively. The average of three measurements was used for analysis. Presence of hypertension was diagnosed when a subject had one or more of the following criteria (29): (1) systolic BP of $\geq 140$ mmHg; (2) diastolic BP of $\geq 90$ mmHg; and (3) use of one or more antihypertensive drugs. According to the criteria, 148 out of the 183 subjects were diagnosed to have hypertension at baseline.

Definition of Dyslipidemia

Presence of dyslipidemia was diagnosed when a subject had one or more of the following criteria (28): (1) LDL cholesterol of $\geq 100$ mg/dl (2.58 mmol/L); (2) non-HDL cholesterol of $\geq 130$ mg/dl (3.36 mmol/L); (3) plasma triglycerides of $\geq 150$ mg/dl (1.69 mmol/L); (4) HDL cholesterol of $\leq 40$ mg/dl (1.03 mmol/L); and (5) use of one or more lipid-lowering drugs. According to the criteria, 138 out of the 183 subjects were diagnosed to have dyslipidemia at baseline.

Preexistence of Vascular Complications at Baseline

Presence of vascular complications was evaluated by clinical information regarding coronary, cerebral, and peripheral artery diseases and aortic aneurysm. Coronary artery disease was diagnosed when a subject had one or more of the following criteria: (1) past history of percutaneous coronary intervention or coronary artery bypass grafting; (2) presence of significant stenosis by coronary angiography; (3) presence of ST-T abnormalities on electrocardiogram associated with typical symptoms attributable to angina pectoris; and (4) use of one or more medications for coronary ischemia. Thirteen patients were diagnosed to have coronary artery disease. Cerebrovascular disease was diagnosed by past history that had been confirmed by positive findings of infarction or bleeding by x-ray computed tomography or magnetic resonance imaging. Seventeen patients had such past history. Peripheral artery disease was diagnosed in four patients with intermittent claudication and/or leg pain at rest when significant arterial stenosis was confirmed by angiography. Diagnosis of aortic aneurysm was made by x-ray computed tomography in one patient. At baseline, 31 patients had one or more of the above vascular complications.

Blood Sampling and Assays

Blood was drawn in the morning after an overnight fast of at least 12 h before starting a dialysis session. Whole blood was used for hematocrit and hemoglobin A1c, EDTA-plasma for glucose, insulin, and lipids, and serum for other biochemical assays. Glucose was measured by a glucose oxidase method. Insulin was measured by radioimmunometric assay (Insulin RIA-BEAD II; Dinabot Co., Tokyo, Japan). Total cholesterol and triglycerides were measured enzymatically. HDL-cholesterol was measured after precipitating apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride. Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. LDL cholesterol was calculated according to the Friedewald formula. Other measurements were by routine methods.

Assessment of Insulin Resistance Using HOMA-IR

Insulin resistance was assessed using the homeostasis model assessment (HOMA-IR) originally described by Mathew et al. (30). HOMA-IR was calculated using the following formula:

$$\text{HOMA-IR} = \frac{\text{fasting glucose (mmol/L)} \times \text{fasting insulin (\muU/ml)}}{22.5}$$

Table 1. Characteristics of the cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>183</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55.4 $\pm$ 10.7</td>
</tr>
<tr>
<td>Duration of hemodialysis treatment (yr)</td>
<td>7.4 (0.17 to 21.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.0 $\pm$ 2.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>148 $\pm$ 24</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>84 $\pm$ 14</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>1011 $\pm$ 167</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.39 $\pm$ 1.01</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>113 (33 to 359)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.04 $\pm$ 0.31</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td>3.35 $\pm$ 0.99</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.71 $\pm$ 0.86</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>38.0 $\pm$ 2.81</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>26.9 $\pm$ 3.92</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>4.0 (1.0 to 53.0)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>4.26 $\pm$ 0.53</td>
</tr>
<tr>
<td>Fasting plasma insulin (µU/mL)</td>
<td>6.0 (2.0 to 20)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.16 (0.28 to 4.59)</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>38</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>21</td>
</tr>
<tr>
<td>Presence of hypertension (%)</td>
<td>81</td>
</tr>
<tr>
<td>Presence of dyslipidemia (%)</td>
<td>75</td>
</tr>
<tr>
<td>Presence of elevated C-reactive protein (%)</td>
<td>54</td>
</tr>
<tr>
<td>Presence of vascular complication (%)</td>
<td>17</td>
</tr>
</tbody>
</table>

*Continuous variables are summarized as mean ± SD, and median (range) is shown for variables with skewed distribution. Prevalence was reported as percentage. Definitions of hypertension, dyslipidemia, elevated C-reactive protein, and vascular complications are described in the text. HOMA-IR, insulin resistance by the homeostasis model assessment.*
HOMA-IR has close correlation with the insulin sensitivity index by the standard euglycemic hyperinsulinemic clamp as shown by Mathew et al. (30) and by us (31). This index can be applied to subjects with renal failure (32).

Outcome Data Collection
During the follow-up, one patient underwent renal transplantation. He was censored at the time of transplantation. Twelve patients were also censored when they moved away from Inoue Hospital. The outcome data of 170 out of the 183 patients could be obtained. At the end of the follow-up, 121 patients were confirmed to be alive on hemodialysis and 49 to be dead.

Date and cause of death were obtained by reviewing the hospital record forms. In the cases that moved away to other dialysis units but the follow-up could be continued, we reviewed the questionnaire forms filled by the attending physicians at the units. The 49 deaths during the follow-up included 22 fatal cardiovascular events: 2 deaths attributable to coronary heart disease, 5 to cerebrovascular disease, 8 to congestive heart failure, and 7 to sudden death. Sudden death was defined as a witnessed death that occurred within 1 h after the onset of acute symptoms and with no evidence of accident or violence. The 27 fatal noncardiovascular causes were cancer (n = 5), infectious disease (n = 8), and others (n = 14).

Statistical Analyses
Continuous variables were summarized as mean ± SD. Median (range) was given for duration of hemodialysis, triglycerides, C-reactive protein, fasting plasma insulin, and HOMA-IR because of their skewed distribution. Survival curves were estimated by the Kaplan-Meier method followed by log rank test. Prognostic variables for survival were first examined using the univariate Cox proportional hazards regression models, and significant variables were forced into the multivariate Cox model. P < 0.05 was considered significant. All these analyses were performed using statistical software (StatView 5; SAS Institute Inc., Cary, NC) for Windows personal computers.

Results
Distribution of HOMA-IR
HOMA-IR showed a skewed distribution with a median of 1.16 (Figure 1). Because of the non-normal distribution, HOMA-IR was entered as a categorical variable instead of a continuous one in the Cox proportional hazards model below.

Univariate Association between Mortality and HOMA-IR
In a preliminary analysis in which the subjects were divided into tertiles according to HOMA-IR, those in the top tertile (HOMA-IR, 1.40 to 4.59) had a significantly higher risk of cardiovascular death compared with those in the middle tertile (HOMA-IR, 0.89 to 1.39; HR, 3.39; 95% CI, 1.09 to 10.52; P = 0.035), whereas the risk did not differ between the lowest (HOMA-IR, 0.28 to 0.88) and middle tertiles (HR, 1.64; 95% CI, 0.46 to 5.81; P = 0.444), suggesting that there was a threshold level of HOMA-IR in relation to cardiovascular mortality. Therefore, the risk was calculated between the top and the combined lower two tertiles (Table 2). In such analyses, the patients in the top tertile of HOMA-IR showed a significantly higher risk of cardiovascular mortality (HR, 2.61; 95% CI, 1.12 to 6.01; P = 0.026). Figure 2 shows the survival curves estimated by the Kaplan-Meier method.

Univariate Association between Mortality and Other Covariates
Table 2 shows univariate association between mortality and other covariates. Among these, greater age, elevated C-reactive protein, presence of vascular complications, and increased HOMA-IR were found to be significant univariate predictors of cardiovascular mortality. In contrast, significant univariate predictors of noncardiovascular mortality were greater age, lower serum albumin, elevated C-reactive protein, and male gender. HOMA-IR was not associated with noncardiovascular mortality.

Independent Predictors of Cardiovascular Mortality
Independent predictors of cardiovascular mortality were identified by the multivariate Cox models (Table 3). The first model, including the four significant univariate predictors, indicated that HOMA-IR and three other covariates were significant and independent predictors of cardiovascular mortality. To examine whether the effect of HOMA-IR on cardiovascular mortality was independent of body mass index, hypertension, and dyslipidemia, each of the three factors was added to the model as the fifth covariate (model 2, 3, and 4). HOMA-IR remained significant and independent of these covariates.

Discussion
Insulin resistance plays an important role in clustering risk factors of atherosclerosis such as hypertension, dyslipidemia, and abnormal glucose metabolism. Many (5–17) but not all studies (33,34) in the general population show positive association of indices of insulin resistance with arterial wall changes, coronary artery disease, and cardiovascular mortality. In the present study, we revealed for the first time that HOMA-IR, an index of insulin resistance, was an independent predictor
Clustering of risk factors synergistically increases the risk of atherosclerosis (35). The current hypothesis of “multiple risk factor syndrome” is that insulin resistance is the important mechanism for the clustering of hypertension, dyslipidemia, and abnormal glucose metabolism (2–4,36). We therefore expected that the association between HOMA-IR and cardiovascular mortality would be dependent on these risk factors and that the association would become insignificant when these individual risk factors were included in the multivariate Cox model. However, the association between HOMA-IR and cardiovascular mortality remained significant and independent of these major risk factors. There are several possibilities to explain this observation. First, because the Cox analysis evaluated the independent effect of individual risk factors on cardiovascular mortality, the synergistic effect of these risk factors may have been reflected on the significant association with insulin resistance, a common basis of the multiple risk factors. Second, HOMA-IR may represent insulin resistance–associated risk factors that were present in renal failure but not included in the model, such as increased plasminogen-activator inhibitor 1 (37,38), hyperhomocystinemia (39,40), and the small-dense LDL phenotype (36,41). Third, although all subjects showed normal fasting glucose, it is possible that those with increased HOMA-IR had impaired glucose tolerance, a risk factor for cardiovascular mortality (42).

Table 2. Univariate association between individual covariates and mortality by the Cox proportional hazards model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiovascular</th>
<th>Noncardiovascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 1 yr)</td>
<td>1.12 (1.06 to 1.169)</td>
<td>1.08 (1.04 to 1.15)</td>
</tr>
<tr>
<td>Serum albumin (per 1 g/L)</td>
<td>0.94 (0.81 to 1.09)</td>
<td>0.75 (0.65 to 0.86)</td>
</tr>
<tr>
<td>Hematocrit (per 1%)</td>
<td>1.00 (0.90 to 1.20)</td>
<td>1.06 (0.96 to 1.17)</td>
</tr>
<tr>
<td>Body mass index (per 1 kg/m²)</td>
<td>0.96 (0.80 to 1.15)</td>
<td>0.87 (0.73 to 1.03)</td>
</tr>
<tr>
<td>Duration of hemodialysis (short-term versus long-term)</td>
<td>2.44 (0.99 to 5.99)</td>
<td>1.74 (0.81 to 3.75)</td>
</tr>
<tr>
<td>Elevated C-reactive protein (presence versus absence)</td>
<td>4.96 (1.67 to 14.57)</td>
<td>2.74 (1.20 to 6.27)</td>
</tr>
<tr>
<td>Gender (female versus male)</td>
<td>0.44 (0.190 to 1.01)</td>
<td>0.46 (0.22 to 0.98)</td>
</tr>
<tr>
<td>Vascular complications (presence versus absence)</td>
<td>6.33 (2.73 to 14.67)</td>
<td>2.03 (0.82 to 5.06)</td>
</tr>
<tr>
<td>Hypertension (presence versus absence)</td>
<td>1.65 (0.49 to 5.59)</td>
<td>1.16 (0.44 to 3.07)</td>
</tr>
<tr>
<td>Dyslipidemia (presence versus absence)</td>
<td>1.11 (0.41 to 3.00)</td>
<td>0.64 (0.29 to 1.43)</td>
</tr>
<tr>
<td>Smoking (smoker versus nonsmoker)</td>
<td>1.43 (0.56 to 3.66)</td>
<td>1.14 (0.46 to 2.82)</td>
</tr>
<tr>
<td>HOMA-IR (top versus lower 2 tertiles)</td>
<td>2.60 (1.12 to 6.01)</td>
<td>0.95 (0.42 to 2.17)</td>
</tr>
</tbody>
</table>

P values by log rank test.

Table 2. Univariate association between individual covariates and mortality by the Cox proportional hazards model

Duration of hemodialysis was categorized into short-term (<7.4 yr) and long-term (7.4 yr or longer) according to the median level. C-reactive protein level was categorized into normal (<4.0 mg/L) and elevated (4.0 mg/L or higher) on the basis of the median level. HOMA-IR levels for the top tertile and the lower 2 tertiles were 1.40 to 4.59 and 0.28 to 1.39, respectively. The table gives hazards ratios (95% confidence intervals) with statistical significance (P values).

**Figure 2.** Kaplan-Meier curves showing association between HOMA-IR and outcome. Survival curves were estimated by the Kaplan-Meier method and compared between the subjects with and without increased HOMA-IR of ≥1.40 or higher. P values by log rank test.

of cardiovascular mortality in a cohort of nondiabetic ESRD patients.

Clustering of risk factors synergistically increases the risk of atherosclerosis (35). The current hypothesis of “multiple risk factor syndrome” is that insulin resistance is the important
Insulin resistance may develop in the presence of inflammation. Also, chronic inflammation has been shown to be an independent predictor of cardiovascular mortality (44,45). Therefore, there was a possibility that the observed association between increased HOMA-IR and cardiovascular mortality was mediated by chronic inflammation. However, this study showed that the effect of HOMA-IR was independent of C-reactive protein. In addition, there was no significant correlation between HOMA-IR and C-reactive protein levels ($r_c = -0.057; P = 0.358$ by Spearman’s rank correlation). These data clearly indicate that insulin resistance and chronic inflammation independently affect cardiovascular mortality in the ESRD population.

What causes insulin resistance in ESRD? Physical inactivity would be one of the possible explanations (46). In addition, cytokines secreted by adipocytes (adipocytokines) play important roles in insulin resistance in obese subjects; another possibility may therefore be increased adipocytokine levels in uremic plasma. Adipocytokines that can induce insulin resistance include tumor necrosis factor-α (TNF-α) (47,48) and leptin (49,49). Plasma concentrations of these molecules are increased in patients with renal failure (50–52). Therefore, although ESRD subjects are not necessarily obese, the increased cytokine levels may be one of the causes for “insulin resistance syndrome without obesity.” Clearly, further studies are needed to prove such a hypothesis.

There are a few limitations in this study. First, the number of fatal events was relatively small, and statistical power may not be large enough to detect important cardiovascular risk factors such as hypertension and dyslipidemia. Second, this study included only subjects treated in morning dialysis sessions. This may affect the generalizability of the findings, because a recent study (53) showed that morning-shift hemodialysis patients survived significantly longer than afternoon-shift patients.

In conclusion, the present study revealed that insulin resistance, as assessed by HOMA-IR, was an independent predictor of cardiovascular mortality in a cohort of nondiabetic ESRD patients. Insulin resistance is a modifiable risk factor; reduction of insulin resistance may therefore be a new target in treating these patients.

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