Relaxin Is an Independent Risk Factor Predicting Death in Male Patients With End-Stage Kidney Disease

Berthold Hocher, MD; Reinhard Ziebig, PhD; Rolfdieter Krause, MD; Gernot Asmus, MD; Hans-H. Neumayer, MD; Lutz Liefeldt, MD; Johannes-Peter Stasch, PhD

**Background**—Patients with end-stage kidney disease (ESKD) have a reduced life expectancy mainly as the result of cardiovascular diseases. Relaxin has been implicated in the pathogenesis of cardiovascular diseases. We analyzed the impact of relaxin on death in patients with ESKD.

**Methods and Results**—Patients (n = 245; 122 women, 123 men) on long-term hemodialysis were followed for 1140 days for death. Blood samples for analysis of relaxin, C-reactive protein, Troponin T, cholesterol, HDL, brain natriuretic peptide, and albumin were taken at study entry. Survival was compared by the Kaplan-Meier method and Cox regression analysis. One hundred seven patients died during the observation period; 66 died of cardiovascular diseases and 28 died of infectious diseases. Elevated serum relaxin concentrations (greater than median) predicted death in male but not in female patients with ESKD: All-cause death (men: relative risk, 2.63; 95% CI, 1.34 to 5.12; P = 0.005; women: relative risk, 0.671; 95% CI, 0.26 to 1.56; P = 0.324).

**Conclusions**—Relaxin is an independent risk factor predicting death in male patients with ESKD on chronic hemodialysis.

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**Key Words:** cardiovascular diseases ▪ kidney ▪ risk factors ▪ mortality

Injections of serum from pregnant rabbits induced relaxation of the interpubic ligament of guinea pigs. The hormone responsible for this action was named relaxin. Corpus luteum, decidua, and placenta secrete this hormone during pregnancy. Relaxin has many important roles in pregnancy, including softening effects on connective tissue, reducing uterine contractility, and control of mammary gland growth and differentiation.

Recently, however, it was recognized that relaxin also plays a role in the cardiovascular system. Patients with chronic heart failure have increased myocardial relaxin gene expression and elevated plasma relaxin concentrations. Relaxin stimulates cardiac ANP secretion and increases coronary blood flow through a nitric oxide–mediated mechanism. It was furthermore shown that relaxin is a vasodilator of small systemic resistance arteries. Relaxin is also involved in the regulation of cardiac and renal collagen synthesis.

We analyzed whether relaxin is a mortality risk factor in patients with end-stage kidney disease (ESKD). Patients with ESKD have a substantially reduced life expectancy. The mortality risk is about 50% higher in elderly patients with ESKD and even much more in younger patients with ESDK as compared with age-matched persons without renal disease.

**Methods**

The 245 patients (122 women, 123 men) were recruited from two dialysis centers (KfH Dialysezentrum-Neukölln, Berlin, and KfH Dialysezentrum-Moabit, Berlin). The patients are dialyzed at least 3 times weekly in 4- to 6-hour sessions, sufficient to provide an urea kinetic on dialysis (KT/V) of at least 1.3. The included patients represent all patients being on stable hemodialysis in these centers without actual health problems. All patients were included in the study in March 2000 and followed for 1140 days. The following patients characteristics were documented: age, gender, body mass index, cause of ESKD, time on dialysis, diabetes, hypertension, and coronary heart disease (patients with a history of myocardial infarction, coronary heart disease confirmed by heart catheterization, or typical stable angina).

Time points and cause of death were documented. Blood samples were taken before start of hemodialysis at study entry. The study was approved by the local ethics committee.

Albumin, cholesterol, HDL, C-reactive protein (CrP), and Troponin T (TnT) were measured by standardized methods. Relaxin and brain natriuretic peptide (BNP) concentrations were analyzed with the use of a commercial ELISA (Immundiagnostik GmbH, Bensheim, Germany), according to the manufacturer’s instructions.

Considering the gender-dependent synthesis of relaxin, Kaplan-Meier analysis and Cox regression analysis were done for women and men separately. We included relaxin a priori and factors known to have an influence on the end point of death in patients with ESKD (age, time on dialysis, diabetes, preexisting coronary heart disease, TnT, CrP, albumin, total cholesterol, HDL cholesterol, and BNP).

**Conclusions**—Relaxin is an independent risk factor predicting death in male patients with ESKD on chronic hemodialysis.

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TABLE 1. Clinical Data and Serum Parameters at Study Entry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>245</td>
</tr>
<tr>
<td>Age</td>
<td>63.5±5.8</td>
</tr>
<tr>
<td>Gender, male/female, n</td>
<td>123/122</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>84</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>157</td>
</tr>
<tr>
<td>Hypertension</td>
<td>220</td>
</tr>
<tr>
<td>Smoker</td>
<td>101</td>
</tr>
<tr>
<td>Time on dialysis, y</td>
<td>5.0±4.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.2±4.6</td>
</tr>
<tr>
<td>Serum albumin, g/dL</td>
<td>3.74±0.34</td>
</tr>
<tr>
<td>CrP, mg/dL</td>
<td>1.26±1.30</td>
</tr>
<tr>
<td>TnT, μg/L</td>
<td>0.125±0.449</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>203.2±57.27</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>42.5±12.3</td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>14.4±35.4</td>
</tr>
<tr>
<td>Relaxin, pg/mL</td>
<td>33.8±39.3</td>
</tr>
</tbody>
</table>

All continuous parameters are given as mean±SD.

Cutoff values for known risk factors were determined as recently described.9 The relaxin cutoff value was calculated by using the median of all relaxin values. We additionally used relaxin as a continuous variable in a separate series of Cox regressions. We calculated in this series the mortality risk increase per additional 5 pg/mL relaxin. All data were analyzed with the use of SPSS for Windows, Version 11.5.

Results

The underlying renal diseases are as follows: Sixty-five of the 245 patients had diabetic nephropathy, 38 patients had hypertensive nephrosclerosis, 30 patients had chronic glomerulonephritis, 28 patients had autosomal dominant polycystic kidney disease, and 20 patients had analgesic nephropathy. The remainder had various rare kidney diseases. In 40 patients, the underlying renal disease was unknown. The patient characteristics are given in Table 1. No patient was lost to follow-up. One hundred seven patients died; in 70 cases, patients died of cardiovascular disease, including 12 with acute myocardial infarction, 18 of sudden cardiac death, and 29 of chronic heart failure. The highest relaxin concentrations were seen in patients who died later of myocardial infarction. However, the differences in this subgroup as compared with survivors were not significant, most probably because of the low number of cases. (data not shown). Infectious diseases were the second most common cause of death (27 cases). Five patients died of cancer. The 5 remaining death cases were related to accidents, hyperkalemia, and in 1 case the cause of death was unknown.

The median plasma relaxin concentration was 28.8 pg/mL (range, 0 to 240 pg/mL) in the 245 patients with ESKD. Kaplan-Meier survival curves indicate that relaxin predicts all-cause and cardiovascular death in male but not female patients with ESKD (data not shown).

Cox regressions performed with the median of all measured relaxin values in the entire study population as a cutoff for relaxin (Tables 2) revealed that relaxin is a predictor of all-cause as well as cardiovascular death in male patients with ESKD. Calculating the risk increase per additional 5 pg/mL relaxin, using the same cofactors as in the initial Cox regressions (age, time on dialysis, diabetes, preexisting coronary heart disease, TnT, CrP, albumin, total cholesterol, HDL cholesterol and BNP), revealed again that relaxin is an all-cause mortality risk factor for men (all-cause mortality risk per 5 pg/mL relaxin for men: 1.077; 95% CI, 1.015 to 1.1143; P=0.014; all-cause mortality risk per 5 pg/mL relaxin for women: 1.032; 95% CI, 0.962 to 1.103; P=0.350). For cardiovascular death of men, there was a trend for increased mortality risk per 5 pg/mL relaxin steps (P=0.08). In female patients with ESKD, relaxin had no significant impact (P=0.66) on cardiovascular death. This latter analysis suggests that there is a linear relation between mortality risk and increasing relaxin values for men at least for all-cause death.

Discussion

This study demonstrates that elevated plasma relaxin concentrations are independently associated with all-cause and cardiovascular death in male but not female patients with ESKD.

In an earlier study,9 we demonstrated that risk factors predicting death in our cohort such as diabetes, elevated TnT, and low albumin are similar to those detected in other studies,7,8,10 indicating that our present findings are of general impact in patients with ESKD.

Our finding of a gender-dependent association of elevated plasma relaxin concentrations with death is based on several independent statistical approaches: (1) Kaplan-Meier survival curves showed only an impact of relaxin on death in male patients with ESKD. (2) Two independent sets of Cox regressions revealed that relaxin has a gender-dependent impact on death in patients with ESKD. We first performed Cox regression analysis by using the median of all measured relaxin values as cutoff. The principally similar finding was seen in Cox regressions using the mortality risk increase per additional 5 pg/mL relaxin.

It is also noteworthy that only male relaxin knockout mice have development of cardiac alterations (increased left ventricular collagen synthesis and impaired left ventricular function).3 The molecular pathways explaining the gender dependency remains to be clarified. Relaxin effects are—at least partially—mediated by an increased NO synthesis.3 Considering that NO synthesis is higher in female subjects,11,12 potential cardiovascular relaxin effects might be masked in female patients with ESKD. We furthermore suggest that elevated plasma relaxin concentrations in patients with ESKD, both male and female, could be a compensatory phenomenon to counteract developing cardiac dysfunction and coronary artery disease. Female patients probably are more sensitive to relaxin and could actually benefit from relaxin’s cardiovascular actions, whereas male patients could be less sensitive than female patients to relaxin and have little or no cardiac protection from relaxin. In support of that notion (compensatory increased synthesis of relaxin in the course of chronic heart damage in uremic patients with ESKD) is the observation that plasma relaxin and left ventricular relaxin mRNA are elevated in patient with chronic heart failure.9 Adding BNP, a parameter that describes heart failure in patients on hemodialysis,13 to the Cox regression...
models has no major impact on relaxin mortality risk factors or probability values. This suggests that relaxin is not mainly reflecting heart failure in male patients with ESKD. This hypothesis is supported by the finding that there was a trend toward highest relaxin concentrations in patients who died later of myocardial infarction but not of heart failure.

The present study also indicated that BNP is a predictor of death only in male patients with ESKD. The underlying molecular pathways are again unknown. However, it is of note that in the general population, elevated plasma BNP was associated reflecting heart failure in male patients with ESKD. This hypothesis is supported by the finding that there was a trend toward highest relaxin concentrations in patients who died later of myocardial infarction but not of heart failure.

Acknowledgments

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References

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