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Higher levels of prorenin predict development of diabetic retinopathy in patients with type 2 diabetes

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Running title: Prorenin and development of diabetic retinopathy

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Abstract

Aims. To determine whether serum prorenin levels affect the development of diabetic retinopathy (DR) in type 2 diabetes.

Methods. Baseline serum prorenin levels were measured in 196 patients (85 men, 111 women) with type 2 diabetes without DR using the antibody-activating direct prorenin assay. The fundi were checked regularly. The participants were divided into two groups based on the serum prorenin levels (high and low). We used Kaplan-Meyer analysis to detect differences in the development of DR between the two groups within the same gender.

Results. Kaplan-Meyer analysis showed that men with a high serum prorenin level tended to develop DR earlier and more frequently than men with a low prorenin level (p=0.004 by the log rank test), however, there was no difference in the development of DR between high and low groups in women (p=0.58).

Conclusions. Serum prorenin levels in men with type 2 diabetes could be a new

prognostic indicator of the development of DR.

Keywords: diabetic retinopathy, prorenin, renin-angiotensin system, type 2 diabetes

Introduction

Diabetic retinopathy (DR) is still a major cause of blindness worldwide because the details of the mechanisms underlying the development and progression of DR are not fully understood. The role of the renin-angiotensin system (RAS) in the pathogenesis of DR has received a great deal of attention recently.[1-3] The Diabetic Retinopathy Candesartan Trials study, a recent representative trial, found that inhibiting the RAS using an angiotensin II type 1 receptor blocker (ARB) had a beneficial inhibitory effect on the development and progression of DR.[2,3] Interestingly, the local RAS is activated in various organs in diabetes; however, circulatory renin remains at the same level or decreases compared to that level in normal subjects,[4] a phenomenon referred to as “renin paradox”, as renin controls a rate-limiting step in the RAS. Recently, Satofuka et al. resolved this paradox in diabetes and showed that binding of prorenin to the (pro)renin receptor accelerates not only the local concentration of angiotensin II but also intracellular signaling but not via angiotensin II (Figure 1).[5] Prorenin is comprised of prosegment and mature renin and usually does not have any enzymatic activity. Normally, prorenin is converted into renin in a proteolytic manner (Figure 1a); however, in diabetes, binding of prorenin to the

(pro)renin receptor changes its structure and subsequently exposes its active site located in renin in a non-proteolytic manner (Figure 1b).[6] This newly identified mechanism of the local RAS is referred to as the receptor-associated prorenin system (RAPS), which has been reported to play a key role in diabetic nephropathy[7] and hypertensive organ damages[8,9] as well as DR.[5]

Our previous cross-sectional study demonstrated that serum levels of prorenin in patients with type 2 diabetes increase with the severity of DR, also indicating that prorenin might promote progression of DR.[10] Interestingly, the serum levels of prorenin in patients with diabetes and no DR are significantly higher than those in normal subjects, which might indicate that patients with diabetes with higher serum levels of prorenin may develop DR relatively earlier than patients with relatively lower serum levels of prorenin. Taken together, we speculated that prorenin level increases before DR development and that measuring prorenin might be a way to predict the development of DR. To our knowledge, no study has reported that the serum levels of prorenin increase before the development of DR in patients with type 2 diabetes.

Methods

One hundred ninety-six patients (85 men, 111 women) with type 2 diabetes mellitus without DR participated in this prospective cohort study. All patients satisfied the diabetes diagnostic criteria of the World Health Organization. Before the study enrollement, all patients received a detailed explanation of the study design and protocol and provided us written, informed consent. The procedure adhered to the tenets of the Declaration of Helsinki. We conducted this study in accordance with the guidelines approved by the ethics committee of our hospital.

One well-trained examiner (T.N.) performed the fundi examinations and was masked to the detailed patient information. The fundi were examined and photographed at least every 6 months. The exclusion criteria included any stage of DR, a previous intraocular surgery, uveitis, glaucoma, or other retinal diseases. We also confirmed that women were not pregnant at their enrollment, because pregnancy stimulates ovarian release of prorenin.[11]

After measuring the blood pressure and examining the fundi, blood samples were collected from all participants and the antibody-activating direct prorenin (AAD-PR) assay

was performed to determine the serum levels of prorenin. This assay was chosen because it is more sensitive and less time consuming than conventional methods. The details of the AAD-PR assay were reported previously.[12] Data except for the serum levels of prorenin are expressed as the means ± standard deviation, while prorenin as the median, since prorenin distributions showed wide variation. Based on the median serum prorenin levels (men: 224.3 pg/ml, women: 114.2 pg/ml), the participants were divided into two groups within the same gender, as groups with high and low prorenin values. To determine if the higher levels of prorenin promote the development of DR, we used Kaplan-Meyer analysis. Significant differences were confirmed by the log-rank test.

Results

Table shows the baseline characteristics of the participants. Using unpaired t-test, we compared the groups with the low serum prorenin levels to those with the high serum prorenin levels to detect any significant differences in age, observation period, systolic blood pressure, diastolic blood pressure, and HbA1c within the genders. There was a significant difference in the systolic blood pressure in women. The systolic blood pressure

was higher in the group with low prorenin values than in that with high prorenin values. The parameters measured except for the systolic blood pressure did not differ significantly between the two groups within the genders. Figure 2 shows the results of Kaplan-Meyer analysis in the men and women. Men with a high prorenin value with type 2 diabetes tended to develop DR earlier and more frequently than those with a low prorenin value ($p=0.004$ by the log-rank test). However, there was no significant ($p=0.58$) difference in the development of DR between the groups of women with the high and low values of prorenin and type 2 diabetes.

Discussion

The current study clearly demonstrates that men with type 2 diabetes with a high serum level of prorenin developed DR earlier than those with a low serum level of prorenin. Kordonouri et al. reported that an increase in the serum levels of total renin precedes development of DR in patients with type 1 diabetes.[13] Especially in patients with diabetes, the increase in total renin is assumed to result from increased prorenin, because total renin is comprised of renin and prorenin, and renin fundamentally stays the same

level or decreases in diabetes.[4] Therefore, it follows that the prorenin levels in patients with type 1 diabetes increase before DR develops as well.

In the current study, we did not detect an obvious correlation between the serum levels of prorenin and the development of DR in women. We analyzed the data separately in men and women, since a gender difference in serum prorenin levels was reported previously.[12] The current study also found apparent differences in the serum prorenin levels between genders, with the median of serum levels of prorenin in men (224.3 pg/ml) significantly higher than in women (114.2 pg/ml) ($p=1.3x10^{-8}$ by the Mann-Whitney test). Furthermore, we performed chi-square test to see whether the serum levels of prorenin fundamentally affect the development of DR, showing a P value of 0.02 in men and 0.45 in women. This result also indicates that the serum levels of prorenin are well correlated with the development of DR in men. Higher levels of prorenin may be attributed to androgen-induced release of prorenin from the kidney[14], because, even in healthy subjects, the serum levels of prorenin in men are statistically significantly higher than those in women[12]; however, the gender difference in the effect of prorenin on the development of DR should be studied further.

Angiotensin II is a strong vasoconstrictor and growth factor that induces various molecules including vascular endothelial growth factor, monocyte chemotactic protenin-1, and interleukin-6.[15,16] These molecules also play an important role in the pathogenesis of DR.[5] In fact, angiotensin-converting (ACE) inhibitors and ARBs suppress the development and progression of DR not only by lowering blood pressure but also through organ protective effect.[1-3] Furthermore, many animal studies have suggested strongly that ACE inhibitors and ARBs suppress the retinal pathology including retinal inflammation in STZ-induced diabetic models[17-19] and retinal neovascularization in oxygen-induced retinopathy models.[20,21] Taken together, it is likely that local activation of RAS by the angiotensin II-dependent pathway may be essential to generate the pathological changes in DR. However, some degree of the importance of local activation of RAS by the angiotensin II-independent pathway recently was elucidated.[5] In fact, knockout mice without AT1R could not completely abolish the changes in the retinal cytokines in diabetes.[5] Instead, administration of the handle region peptide, which interrupts binding of prorenin to the (pro)renin receptor, completely inhibited these alterations.[5] These results indicated that local activation of the RAS in diabetes depends greatly on the RAPS. Even

though it is unknown whether angiotensin II-independent or dependent pathway is more important for the pathogenesis of DR, prorenin is essentially a key factor in both pathways in the RAPS in diabetic organ damages.

In conclusion, the current results showed that men with type 2 diabetes who have a higher serum level of prorenin tend to develop DR more often and faster than those with a lower serum prorenin level. Measurement of serum levels of prorenin in men with type 2 diabetes without DR may be a clinically valuable way to predict the development of DR. More studies are needed to determine the effect of the gender difference in prorenin on the development of DR.

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Legends

Table

Patient characteristics in the groups of men and women with high and low serum levels of prorenin. * Continuous values are expressed as the means ± standard deviation (SD). ** Serum levels of prorenin are expressed as the median (inter-quartile). † Significant ($p < 0.05$) vs. low group in women.

Figure 1

Comparison of the renin-angiotensin system (RAS)(a) and the receptor-associated prorenin system (RAPS)(b).

Figure 2

Kaplan-Meyer analysis for the development of DR in men (a) and in women (b).

(black line: high group. gray line: low group)