EXTENDED REPORT

Serum prorenin levels and diabetic retinopathy in type 2 diabetes: new method to measure serum level of prorenin using antibody activating direct kinetic assay

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Br J Ophthalmol 2005;**89**:871–873. doi: 10.1136/bjo.2004.056580

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Accepted for publication 1 December 2004

Aim: To investigate the serum levels of prorenin and its correlation with the severity of diabetic retinopathy (DR).

Methods: 248 patients with diabetes and 108 control subjects were divided into four groups: no-DR (n = 146), no proliferative diabetic retinopathy (no-PDR) (n = 78), PDR (n = 24), and controls (n = 108). Serum levels of prorenin from all subjects were measured using the new antibody activating direct kinetic (AAD-PR) assay. The serum prorenin levels were compared among the groups.

Results: The serum levels of prorenin in the control, no-DR, no-PDR, and PDR groups, respectively, were 109.1 (66.1), 194.6 (160.4), 271.5 (220.3), and 428.4 (358.4) pg/ml (mean (SD)). Prorenin in the PDR group was remarkably high compared with the control and no-DR groups ($p<0.0001$) and with the no-PDR group ($p=0.002$). Serum levels of prorenin increased with increasingly severe retinopathy. No correlation was found between the prorenin level and the duration of disease or HbA_{1c}.

Conclusions: The serum levels of prorenin in patients with PDR were found to be markedly high using the AAD-PR assay. Increased levels of prorenin in diabetes may have an important role in the pathogenesis of DR.

Diabetic retinopathy (DR) is a major cause of blindness worldwide. Although strict glycaemic control is thought to be essential to prevent the occurrence of DR,[1] some cases unfortunately develop advanced proliferative diabetic retinopathy (PDR).[2] In fact, because it is difficult to confirm if diabetic microangiopathy including retinopathy is progressive or not, a useful predictor that is well correlated with the occurrence of diabetic microangiopathy is needed to prevent the development of diabetic microvascular complications.

Prorenin is an inactive precursor of renin. The circulating prorenin level is five to 10 times higher than the active form of renin. Although little is known about the biological function of prorenin, it reportedly increases in diabetes and is associated with the occurrence of DR and nephropathy.[3–5] Furthermore, in adolescents with diabetes, higher serum levels of prorenin occur several years before diabetic nephropathy[6–8] and retinopathy.[9] This modulation of prorenin in diabetes indicates that prorenin is involved in the occurrence and the progression of diabetic microangiopathy. Although measuring prorenin seems to be a good method to determine if diabetic microangiopathy is present or not, the method of measuring prorenin in previous reports has been complicated. Until recently, the level of prorenin was determined by measuring the total renin level and subtracting the active rennin level.[10–12] Total renin was measured after activating inactive prorenin by trypsin or non-proteolytically. At the same time, active renin was measured independently, and the difference in the levels between total renin and active renin was defined as the prorenin level.

A new method called the antibody activating direct prorenin (AAD-PR) assay, developed by Suzuki *et al*,[13] enables direct measurement of the concentration of prorenin using an antibody to the prorenin profragment, which detects prorenin in serum and confirms the complex to the prorenin. This complex has renin-like activity—that is, the ability to convert angiotensinogen to angiotensin I. The generated angiotensin I is measured with the enzyme linked immunosorbent assay. The prorenin level can be calculated by the amount of generated angiotensin I. The AAD-PR assay was reported to have higher sensitivity than previous methods.[14]

In this study, we focused on the relation between the serum levels of prorenin and the severity of DR. We measured serum levels of prorenin in patients with type 2 diabetes and estimated the clinical implication of prorenin in DR using the AAD-PR assay.

PATIENTS AND METHODS

In all, 248 patients with diabetes and 108 control subjects from Asahikawa Medical College Hospital were included. The control subjects had a normal examination that included urinalysis, blood chemistry, and blood pressure measurement and had never had type 2 diabetes. Patients with diabetes who were followed by physicians at Asahikawa Medical College Hospital all satisfied the criteria for diagnosis of diabetes by the World Health Organization. The subjects received a detailed explanation of the aims of the study and provided informed consent. This study protocol was reviewed by the ethics committee of our institution. All procedures adhered to the tenets of the Declaration of Helsinki.

The subjects were divided into four groups: patients without DR (no-DR group), those with retinopathy but no proliferative DR (no-PDR group), patients with proliferative DR (PDR group), and controls. The characteristics of these groups are shown in table 1. Sera were obtained from all subjects and then treated as described by Kawazu *et al* to measure the serum levels of prorenin.[14] The distribution of serum prorenin levels in the four groups was compared using one way of analysis variance and Scheffe's test. A p value of

Abbreviations: AAD-PR, antibody activating direct prorenin; DR, diabetic retinopathy; PDR, proliferative diabetic retinopathy; RAS, renin-angiotensin system

Table 1 Characteristics of the study groups (SD)

	Control	No-DR	No-PDR	PDR
Number (male/female)	108 (62/46)	146 (70/76)	78 (42/36)	24 (13/11)
Age (years)	55.9 (15.5)	58 (13.3)	60.0 (13.3)	56.1 (10.1)
Duration of diabetes (years)		8.1 (6.9)	13.3 (7.2)	14.5 (8.4)
HbA_{1c} (%)		7.5 (1.7)	8.2 (1.8)	7.6 (1.3)
Systolic blood pressure (mm Hg)	124.0 (11.5)	123.2 (15.3)	131.2 (16.0)	134.0 (14.2)
Diastolic blood pressure (mm Hg)	75.8 (3.7)	72.5 (4.6)	70.9 (9.7)	75.5 (9.9)

0.05 or lower was considered significant. The Pearson correlation coefficient (r) was calculated to determine whether there were close associations among the variables.

RESULTS

The characteristics of the subjects are shown in table 1. There is no statistical difference between males and females, which has been reported to affect the serum levels of prorenin.[14] Systolic blood pressure and diastolic blood pressure were not significantly different among the four groups. The distribution of the serum levels of prorenin in the four groups is shown in figure 1. The serum levels of prorenin in the control, no-DR, no-PDR, and PDR groups were 109.1 (66.1), 194.6 (160.4), 271.5 (220.3), and 428.4 (358.4) pg/ml (mean (SD)), respectively. The serum levels of prorenin were markedly higher in the PDR group than in the control and no-DR groups ($p<0.0001$, Scheffe's test) and the no-PDR ($p = 0.002$) group. The serum levels of prorenin were higher with increasingly severe retinopathy. No significant correlation was found between the serum prorenin level and disease duration ($r = 0.17$, $p = 0.04$) or the HbA_{1c} level ($r = 0.05$, $p = 0.56$).

DISCUSSION

In this study, we evaluated the serum levels of prorenin in patients with diabetes using the newly developed AAD-PR assay. This study showed that the serum levels of prorenin in patients with diabetes with PDR were remarkably higher than in the normal healthy subjects, patients without DR, and those with no PDR. The serum concentration of prorenin in patients with diabetes was higher than in control subjects, and a high serum concentration of prorenin in patients with diabetes increased with increasingly severe retinopathy. These results supported previous reports that had shown the clinical implication of prorenin in the occurrence and the development of diabetic microangiopathy.[3–9 15]

Recent studies investigated the relation between the concentration of prorenin and the occurrence or the development of DR.[3–9 15] Franken *et al* reported that a high plasma prorenin level is associated with DR, particularly PDR.[4] Makimattila *et al* reported that the serum total renin level increased and was a useful marker of activity and the severity of DR.[15] Total renin is composed of renin and prorenin, and 90% of total renin is prorenin.[16] The active renin level in diabetes does not increase.[17 18] An increase in the total renin level was thought to be the result of the increased level of prorenin in diabetes. These reports showed the close relation between the concentration of prorenin and the severity of DR[4 15] and supported our results. Although those previous reports showed higher levels of prorenin in diabetes with retinopathy, the conventional measurement method was more complicated and less sensitive for determining the concentration of prorenin than the AAD-PR assay.[14]

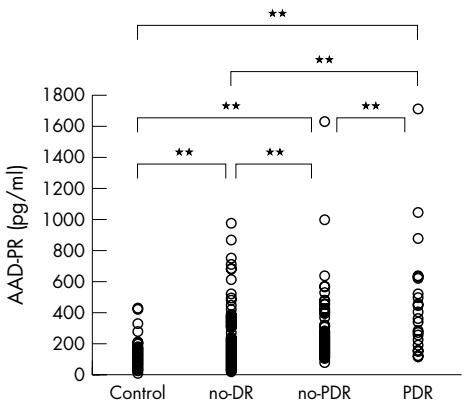

Figure 1 The distribution of serum levels of prorenin; *p<0.05, **p<0.01.

In the present study, we showed that there was no close relation between the serum levels of prorenin and HbA_{1c} or duration of diabetes. Franken *et al* reported that the plasma concentration of prorenin was not correlated with HbA_{1c} and the duration of diabetes.[5] On the other hand, Makimattila *et al* reported that the serum concentration of total renin was correlated with HbA_{1c}.[15] Luetscher *et al* also demonstrated a positive correlation between HbA_{1c} and the plasma concentration of prorenin.[3] HbA_{1c} and the duration of diabetes are key risk factors for diabetic microangiopathy and are thought to be associated with the occurrence of DR.[1 19] Although HbA_{1c} is an important indicator for determining the degree of glycaemic control in diabetes, this is not sufficient to be associated with the occurrence and the severity of DR.[20] Higher serum levels of prorenin in diabetes might be more appropriate for estimating the occurrence and the severity of DR than HbA_{1c}. In this study, the duration of diabetes was longer in patients with PDR than other patients who had no retinopathy or in whom retinopathy was not proliferative; however, there was no close relation between the serum levels of prorenin and the duration of diabetes. Duration, as mentioned previously, is also an important key factor for the occurrence of DR,[19] but it does not seem to affect the serum concentration of prorenin.

In this study, we did not measure renin at the same time to determine if the serum level of renin in diabetes increased or not. Renin is well known to be a key enzyme in the cleavage of angiotensinogen to angiotensin I, and this reaction is a rate limiting step to generate angiotensin II in the renin-angiotensin system (RAS). Previous reports showed that the concentration of renin in diabetes does not increase,[21] although RAS has been implicated in the pathogenesis of DR.[3–5 15 22–25] The fact that renin does not increase in diabetes seems to be a discrepancy, but RAS is activated in diabetes. Our study, as other previous reports showed,[3–5 13 26] might indicate the involvement of increased prorenin in the development of DR. In addition, as mentioned previously, the plasma concentration of prorenin precedes the occurrence of diabetic nephropathy by several years.[7 8] Increasing prorenin in diabetes may trigger microangiopathy and promote the development of diabetic microangiopathy through the activation of RAS.

Recently, prorenin was reported to have enzymatic activity that generated angiotensin I and activated the RAS thorough the generation of angiotensin II.[26–28] Prorenin is composed of two components, the profragment of prorenin and mature renin. Suzuki *et al* demonstrated that prorenin has a key region in its profragment for non-proteolytic activation with protein interaction.[26] Furthermore, Nguyen *et al*, who investigated the renin/prorenin receptor, showed that the prorenin binding this receptor activated the conversion of angiotensinogen to angiotensin I. High levels of this receptor mRNA were detected in the heart, brain, and placenta and lower levels in the kidney and liver.[27] Recently, Ichihara *et al* proved the non-proteolytic activation of prorenin and the presence of renin/prorenin receptor in the kidney using streptozotocin induced diabetic rats. They reported that the interference of prorenin with peptide which inhibits an interaction of prorenin with renin/prorenin receptor, inhibited the local generation of angiotensin II and improved diabetic nephropathy in streptozotocin induced diabetic rats.[18] Angiotensin I generated with non-proteolytic activation of prorenin is transformed to angiotensin II by soluble or endothelium specific angiotensin converting enzyme. Angiotensin II exhibits pathological effects in the retina in diabetes through binding angiotensin II type 1 receptor, which is thought to be the most important receptor of all subtypes to exhibit the physiological and pathological effects. Angiotensin II is associated with overexpression of some angiogenic factors—that is, vascular endothelial growth factor (VEGF),[29–33] and angiopoietin 2.[34] VEGF and angiopoietin 2 have a crucial role in the development of retinal neovascularisation,[35–38] a main feature of PDR. Taken together, it is possible that a high concentration of prorenin in patients with diabetes activates the local RAS in the eyes through its binding renin/prorenin receptor and promotes the pathogenesis of DR through the generation of angiotensin II.

In this study, we evaluated the serum levels of prorenin in patients with type 2 diabetes with a newly developed method, the AAD-PR assay. The serum levels of prorenin in patients with PDR were markedly high. High levels of prorenin in diabetes increase with increasingly severe retinopathy. We showed that a high concentration of circulating prorenin may be involved in the pathogenesis of DR. Further prospective study is needed to investigate the relation between modulation of the serum levels of prorenin and the severity of DR, and in turn, whether patients without DR with a higher level of prorenin will develop retinopathy.

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The authors have no proprietary interest in any aspect of this report.

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