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Albuminuria and hypertension are independently associated with circulating antipericyte autoantibodies in type 2 diabetic patients

Ramesh C. Nayak[a,b,*], Elizabet Agardh[c], Michael G.K. Kwok[a], Pamela J. Farthing-Nayak[a,b], Kristian Lynch[d], Carl-David Agardh[d]

[a]*Department of Ophthalmology, Tufts University School of Medicine, Tufts Center for Vision Research and New England Eye Center, Tufts-New England Medical Center, Box 8103, Boston, MA 02111, USA*
[b]*Department of Ophthalmology, University of Arizona College of Medicine, Tucson, AZ 85711, USA*
[c]*Department of Ophthalmology, University Hospital MAS, Malmö, S-205 02, Sweden*
[d]*Department of Endocrinology, University Hospital MAS, Malmö, S-205 02, Sweden*

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Abstract

Aims: To determine whether albuminuria, hypertension, or HbA_{1c} are independently associated with antipericyte autoantibodies (APAAs) in type 2 diabetes mellitus.

Methods: Two hundred ninety-nine subjects with different degrees of retinopathy according to the Early Treatment Diabetic Retinopathy Study Scale participated in this study. Albuminuria was defined as an albumin/creatinine ratio above the normal cutoff limit, that is, 2.0 g/mol for men and 2.8 g/mol for women. Hypertension was defined as a diastolic blood pressure more than 90 mm Hg, a systolic blood pressure more than 140 mm Hg, or pharmacological antihypertensive treatment. Serum APAAs were detected by immunofluorescence on tissue-cultured bovine retinal pericytes. Association analysis was performed using univariate and multivariate statistical tools.

Results: In type 2 diabetes, APAAs were independently associated with albuminuria (OR = 0.56; $P < .04$), hypertension (OR = 2.21; $P < .01$), as well as with proliferative retinopathy (OR = 0.39; $P < .01$).

Conclusions: The increased prevalence of APAA in patients with hypertension may suggest that these antibodies are related to tissue damage and repair and that the decline in frequency with albuminuria may serve as a marker for more advanced angiopathy. Future longitudinal studies are needed to determine whether the frequency of APAA is associated with the progression of angiopathy, and to determine the biological activity and antigens recognized by the antibody.

1. Introduction

Although it has been shown that tight metabolic control has beneficial effects on the development and progression of diabetic microangiopathy in type 1 [1] and type 2 [2] diabetes, the degree of blood glucose elevation does not account for all the risk for development and progression of the microvascular complications. The Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study reports documented that tight blood glucose control inhibits or retards the development and progression of microangiopathy in many patients. Thus, protracted exposure to elevated blood glucose is almost certainly the initiating factor for diabetic complications. However, a subpopulation in the DCCT did not benefit from tight control of blood glucose, and it could be seen that although glycemic exposure is the dominant predictor, the updated mean HbA_{1c} did not completely explain the risk of progression [3]. The UK Prospective Diabetes Study reported that a reduction of blood pressure by 10/5 mm Hg reduced the risk for and progression of microangiopathy [4]. However, the pathogenic mechanisms behind the development of diabetic angiopathy are not fully understood. Nephropathy is usually associated with severe retinopathy [5], although severe retinopathy is not always associated with signs of nephropathy [6] indicating partly different mechanisms.

* Corresponding author. Department of Ophthalmology, University of Arizona College of Medicine, Tucson, AZ 85711, USA. Tel.: +1 617 636 8103; fax: +1 617 636 4233.
E-mail addresses: rnayak@eyes.arizona.edu, rnayak@opal.tufts.edu (R.C. Nayak).

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Table 1
Patient characteristics

(N = 293)	
Male (%)	59
Age (y)	64.4 ± 10.9
Age at diagnosis (y)	51.3 ± 11.5
Known diabetes duration (y)	13.0 ± 9.1
Systolic blood pressure (mm Hg)	148 ± 18
Diastolic blood pressure (mm Hg)	83 ± 9
Treatment for hypertension (%)	45
BMI (kg/m^2)	28.7 ± 4.6
HbA_{1c} (%)	7.1 ± 1.5
Plasma creatinine (μmol/L)	90 ± 40
Urinary creatinine (mmol/L)	7.8 (1.3-24.2)
Urinary albumin (g/L)	0.014 (0.001-5.920)
A:C ratio	2.1 (0.3-1286.0)

Results are given as percent, mean ± SD, or median (range) (urinary creatinine, albumin, and A:C ratio).

We have previously reported finding antipericyte autoantibodies (APAA) at high frequency in sera of type 1 [7] and type 2 [8] diabetic patients with nonproliferative retinopathy. We now report on the frequency of these autoantibodies in diabetic patients with and without albuminuria in relation to the degree of retinopathy.

2. Subjects, materials, and methods

2.1. Subjects

Two hundred ninety-nine subjects classified as having type 2 diabetes, that is, diabetes onset at or after 30 years of age (including 10 patients younger than 30 years but not treated with insulin and that were classified as having type 2 diabetes) and with different degrees of retinopathy attending the Department of Ophthalmology, Malmö University Hospital, Sweden, gave their informed consent to participate in the present study. The Ethics Committees of Malmö/Lund and Tufts-New England Medical Center approved the study. The investigations reported were carried out in accordance with the principles of the Declaration of Helsinki as revised in 1996 [9].

Subject characteristics are given in Table 1. Blood pressure was measured in the supine position after 5 minutes of rest using a mercury sphygmomanometer. Diastolic blood pressure was taken at Korotkoff's phase V. HbA_{1c} and plasma creatinine were measured in 287 patients. Urinary creatinine and albumin concentrations were measured on one morning urine sample, and the albumin/creatinine (A:C) ratio was calculated. A single void A:C ratio has been shown previously to correlate well to timed urinary collection [10-12]. Presence of albuminuria was defined as an A:C ratio above the normal cutoff value for men and women.

Twenty-four control sera from Red Cross donors reporting no family history of diabetes were used as negative controls. The characteristics of these donors were reported previously [7].

2.2. Analytical techniques

HbA_{1c} was analyzed with a high-performance liquid chromatography (VARIAN II Hemoglobin A_{1c} program, BioRad, Hercules, CA), reference range, 3.6% to 5.0% for healthy individuals younger than 50 years and 4.0% to 5.3% for individuals older than 50 years. Urinary creatinine was analyzed using a kinetic method (Synchron LX20, Beckman Coulter, Fullerton, CA), urinary albumin with nephelometry (Image, Beckman Coulter) or turbidimetry (Synchron LX20, Beckman Coulter), normal value less than 0.025 g/L, and normal values for urine albumin/urine creatinine ratio less than 2.0 g/mol for men and less than 2.8 g/mol for women. These cutoff levels are similar to those reported by others [11,12].

2.3. Ophthalmologic evaluation

After dilation of the pupils, stereo photographs were taken from 7 standard fields in each eye using a 30° fundus camera (Topcon Inc, Paramus, NJ; TRC-50). Grading was performed in a masked fashion. Retinopathy was graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) Scale [13]. Eyes treated with pan-retinal photocoagulation without signs of neovascularization were included in ETDRS level 61. The patients were characterized according to the retinopathy level in the worst affected eye. Due to cataract or insufficient pupil dilation, the quality of the photographs permitted grading of one eye only in 16

Table 2
APAA frequencies by ETDRS grade, albuminuria, and hypertension in patients with type 2 diabetes

		ETDRS grade					
		10-15		20-53		>53	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
No hypertension							
Albuminuria	No	17/31	55 (38-73)	9/17	53 (29-77)	1/2	50 (0-100)
	Yes	3/7	43 (6-80)	3/14	21 (0-42)	1/7	14 (0-40)
Hypertension							
Albuminuria	No	32/50	64 (51-77)	26/34	76 (62-90)	3/8	37 (4-71)
	Yes	11/21	52 (31-73)	25/41	60 (45-75)	8/29	27 (10-43)

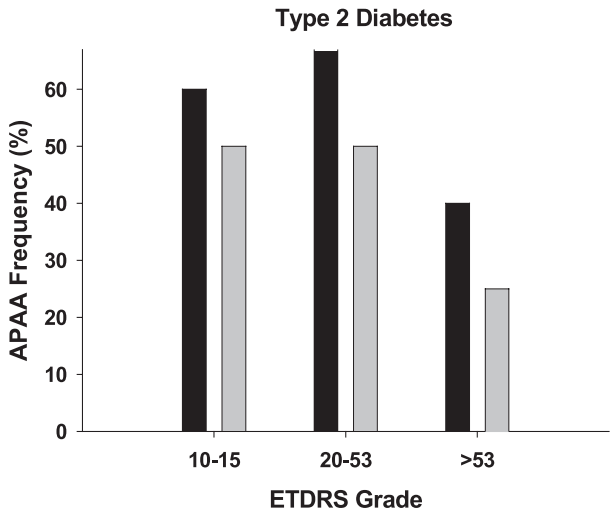

Fig. 1. Histogram of ETDRS grade-specific APAA frequency in type 2 diabetic patients with (gray bars) and without (black bars) albuminuria.

patients, and grading of any eye was not possible in 7 patients.

2.4. Indirect immunofluorescence assay

Antipericyte autoantibodies were detected on bovine retinal microvascular pericytes [14] as previously described [7,8].

2.5. Statistical analysis

Statistical analysis of the APAA prevalence was carried out using logistic regression analysis. The association between clinical parameters (independent variables/predictors) and prevalence of APAA (dependent variable) were first analyzed individually and then with an adjustment for all other predictors. Two hundred fifty-seven patients with complete data on all measured variables were included. To increase power to detect associations, age at onset, HbA_{1c}, body mass index (BMI), and diabetes duration were left as continuous variables. Results are reported as odds ratios (OR) and 95% confidence intervals (95% CI). Two sample *t* tests tested for differences in means among independent groups of patients, whereas differences in medians were tested using Wilcoxon two-sample test. χ^2 Tests examined for differences in proportions. Calculations were performed using the statistical package Splus for windows version 6.1 (Insightful Corp, Seattle, Wash).

3. Results

3.1. Diabetes and APAA

No difference in APAA frequencies was found between males and females. Age at diagnosis and known duration of diabetes were both associated with presence of APAA. Antibodies were present in 13/40 (33%) of type 2 diabetic patients diagnosed before 40 years of age, in 42/83 (51%) when diagnosed between 40 and 50 years, and in 102/168 (61%) when diagnosed after 60 years of age (trend; $P < .01$). In addition, APAA positivity was more common in patients recently diagnosed [<5 years; 58/82 (71%)] compared with patients who had had the disease for a longer period [>5 years; 99/209 (47%); $P = .0005$]. Body mass index and

Table 3
Unadjusted and adjusted odds ratios (95% CI) of A:C ratio, retinopathy grade, and hypertension with presence of APAA in patients with type 2 diabetes

Predictor	Type 2 diabetes (n = 257)			
	Unadjusted		Adjusted	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex				
Females[a]	1.19 (0.72, 1.96)	.50	0.91 (0.51, 1.60)	.74
Age at diagnosis	1.03 (1.01, 1.06)	<.01	1.02 (0.99, 1.04)	.19
Known duration	0.96 (0.94, 0.99)	<.01	0.98 (0.95, 1.02)	.29
BMI	1.03 (0.97, 1.09)	.27	1.02 (0.96, 1.09)	.57
HbA_{1c}	0.86 (0.73, 1.02)	.08	0.95 (0.78, 1.15)	.61
Hypertension[b]				
Yes	1.70 (0.99, 2.92)	.06	2.21 (1.19, 4.12)	.01
Increased A:C ratio[c]				
Yes	0.47 (0.28, 0.77)	<.005	0.56 (0.31, 0.98)	.04
Proliferative retinopathy[d]				
Yes	0.27 (0.14, 0.55)	<.001	0.39 (0.18, 0.83)	.01

Unadjusted analysis examines association between each predictor and APAA using univariate regression models.
Adjusted analysis examines association between predictors and APAA in a multivariate regression model.
[a] Reference group = males.
[b] Reference group = diastolic blood pressure <90 mm Hg, systolic blood pressure <140 mm Hg, and no blood pressure treatment.
[c] Reference group = normal A:C ratio.
[d] Reference group = proliferative retinopathy (ETDRS grade > 53).

HbA_{1c} were not related to the presence of APAA. There was no difference in APAA frequency between patients with and without insulin treatment. In 4 patients, APAA results were missing.

3.2. Hypertension and APAA

In all, 130 patients had pharmacological treatment for hypertension. Antipericyte autoantibodies were found in 77/130 (59%) of treated patients, but the type of drug used was not related to the presence of antibodies. High systolic blood pressure (>140 mm Hg) was seen in an additional 76 patients, with APAA present in 41/76 (54%), and one additional patient had high diastolic blood pressure (>90 mm Hg) and was also positive for APAA. Of the remaining 86 patients with no evidence of hypertension, only 45% were found to have APAA.

3.3. Albuminuria and APAA

The frequency distribution of APAA in patients with and without albuminuria is shown according to retinopathy grade and hypertension in Table 2. At each retinopathy grade, the APAA frequency was lower in patients with albuminuria (Fig. 1). This difference was still present when patients had hypertension. In patients with no evidence of hypertension, only albuminuria was associated with lower APAA frequency ($P = .02$).

3.4. Independent predictors of APAA

Albuminuria remained independently associated with APAA after adjusting for proliferative retinopathy (OR = 0.59, $P = .048$) and hypertension (OR = 0.54, $P = .023$). This association did not change when sex, age at diagnosis, duration of diabetes, BMI, and HbA_{1c} were also adjusted for (Table 3). Only hypertension was also found to be independently associated with APAA.

4. Discussion

We have previously reported a decreased frequency of APAA in proliferative diabetic retinopathy, in both type 1 [7] and type 2 [8] diabetes. In the present study, we demonstrated an independent negative association between the frequency of APAA and albuminuria and an independent positive association between the frequency of APAA and hypertension in type 2 diabetic patients.

4.1. Albuminuria

In type 1 diabetes, albuminuria is usually a sign of diabetic nephropathy and almost always associated with retinopathy [15]. In type 2 diabetes, however, microalbuminuria is present in 20% to 30% of all patients, and although associated with nephropathy, it is also associated with hypertension, endothelial dysfunction, and other features of the insulin resistance syndrome, thus, also reflecting a generalized abnormality of vascular function [16]. We have previously reported that APAA frequency declines with duration of diabetes raising the possibility that the apparent decline in APAA frequency with increasing albuminuria is actually associated with duration and not albuminuria (duration and albuminuria are confounded). In patients diagnosed with type 2 diabetes at a similar age, known duration of diabetes is not associated with APAA after adjusting for albuminuria. Without adjustment for age at onset, known duration of diabetes and albuminuria are both associated with APAA. This is because many of the recently diagnosed patients are in their 50s and are more likely to have APAA.

4.2. Hypertension

We found an association between hypertension and antibody prevalence in type 2 diabetes using multivariate analysis suggesting that antibody prevalence is related to hypertension. Hypertension may be one of several factors that act to alter pericyte physiology and function resulting in a more activated phenotype that expresses antigens recognized by circulating APAA. The vascular stretching effects of increased blood pressure [17] may cause pericyte activation and phenotypic changes that reflect pericyte migration and biosynthetic activity as a response to injury.

4.3. Proliferative retinopathy

It is unclear why the APAA prevalence is low in proliferative retinopathy. One possible explanation could be that all the pericytes have been destroyed and consequently there is no longer any antigenic stimulation to drive antibody secretion. Another possible explanation is that as a proangiogenic environment develops in the tissue, cytokines may be produced, which negatively regulate immunoglobulin synthesis/secretion in addition to their angiogenic effects. Alternatively, these cytokines could down-regulate pericyte autoantigens resulting in a loss of antigenic stimulation of immunoglobulin-producing lymphocytes.

4.4. Interactions among nephropathy, hypertension, and retinopathy

It has previously been reported that about 30% of type 1 diabetic patients with proliferative retinopathy have no signs of diabetic nephropathy, suggesting the possibility of partly different pathogenic mechanisms [18]. In type 2 diabetic patients, on the other hand, it cannot be excluded that the decline in autoantibody prevalence in the presence of albuminuria, which remained after adjustment for all other predictors including diabetic retinopathy, could be due to interactive effects of diabetic nephropathy on progression of diabetic retinopathy. Glycated hemoglobin is a risk factor in the absence but not in the presence of nephropathy [19], and blood pressure may relate to the concordance between retinopathy and nephropathy as it has been implicated in the development of these complications [20]. Chavers et al [21] and others [22-24] suggest that

hypertension is a major mediator of the renal-retinal link. In the study by Chavers et al, 89% of hypertensive subjects had retinopathy and 93% with nephropathy and hypertension also had advanced retinopathy. The Epidemiology of Diabetes Complications Study reported that although hypertension was associated with subsequent development of proliferative retinopathy, this was only true in subjects with nephropathy [19]. Furthermore, blood pressure levels were not higher in subjects who developed proliferative retinopathy without nephropathy. The importance of hypertension in explaining the renal-retinal link was confirmed by the 4-year incidence from the Epidemiology of Diabetes Complication study [25].

4.5. Functional significance

The decline of an autoantibody with disease progression is not unique to diabetic microangiopathy. Cardiac autoantibodies in dilated cardiomyopathy become undetectable with disease progression [26]. The reason for the decline in these cardiac autoantibodies is unknown. However, positive antibody status at diagnosis was associated with milder symptoms, and when patients who had persistently detectable antibodies at follow-up were compared with those who had lost these markers, antibody persistence was associated with absence of clinical deterioration. Similarly, there is a decline in islet cell autoantibody titer in long-standing type 1 diabetes [27] that probably reflects decreasing beta cell mass due to autoimmune destructive mechanisms. The presence of islet cell autoantibodies at diagnosis of type 1 diabetes is associated with diminished residual beta cell function, whereas the absence of islet cell autoantibodies at diagnosis of type 1 diabetes reflects a slower beta cell destructive process and a longer duration of preclinical disease [28]. Thus, the key to understanding the clinical significance of antibody decline lies in knowledge of the functional role of the antibody. In dilated cardiomyopathy and diabetic microangiopathy, the presence of the autoantibody is associated with less severe disease, whereas in type 1 diabetes, it is associated with more rapidly progressing disease. In the latter case, the autoantibodies may play a direct role in beta cell dysfunction [29].

The increased frequency of APAA in our patients with hypertension suggests that these antibodies are related to tissue damage and repair and that the decline in frequency with proliferative retinopathy may serve as a marker of impending proliferative retinopathy. Our finding that albuminuria is associated with a lower APAA frequency may be useful for molecular studies of interactions between albuminuria, as a marker of endothelial dysfunction, and development of vascular disease in diabetes.

4.6. Summary

In summary, in type 2 diabetic patients, APAA are negatively associated with albuminuria and proliferative retinopathy but positively associated with hypertension.

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