Resting Heart Rate Is Associated With Nonproliferative Retinopathy in Normoalbuminuric Type 1 Diabetic Patients

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Previous studies have reported that retinopathy might be already present in the normoalbuminuric state in type 1 diabetic patients. The aim of this study was to evaluate the prevalence and predictors of nonproliferative retinopathy in normoalbuminuric type 1 diabetic patients. The study included 312 normoalbuminuric type 1 diabetic patients with normal renal function before any interventions with statins, angiotensin-converting enzyme inhibitors, or angiotensin II receptor blockers. Diagnosis of nonproliferative retinopathy was made by fundoscopy after pupillary dilatation. Urinary albumin excretion (UAE) rate was measured from at least two 24-hour urine samples. Nonproliferative retinopathy was present in 36% of normoalbuminuric patients. Patients with nonproliferative retinopathy were older and had longer duration of diabetes, higher hemoglobin A_{1c}, daily insulin dose, and higher resting heart rate (RHR) ($P \leq .01$ for all). Patients in the 4th quartile of RHR were older and had longer duration of diabetes, higher hemoglobin A_{1c}, daily insulin dose, serum creatinine, UAE, and a significantly higher prevalence of nonproliferative retinopathy compared with subjects in the 2nd, 3rd, and 4th quartiles ($P < .05$). In logistic regression analysis, after adjustment for risk factors, higher RHR was significantly associated with risk of nonproliferative retinopathy in patients ($P < .001$), with odds ratios of 1.02 to 1.08. These data suggest that RHR is independently associated with nonproliferative retinopathy in normoalbuminuric type 1 diabetic patients. *J Clin Hypertens (Greenwich).* 2013;15:579–583. ©2013 Wiley Periodicals, Inc.

Identification of the determinants of the onset of early diabetic retinopathy is essential for reducing the morbidity and mortality associated with diabetes because diabetic retinopathy is one of the leading causes of visual impairment and blindness in patients with type 1 diabetes.[1] Diabetic retinopathy is also associated with mortality and cardiovascular disease incidence in diabetes.[2] Many studies have identified poor glycemic control, duration of diabetes, and blood pressure (BP) as important risk factors for development of retinopathy.[3–6] In addition, other factors including serum lipids and heart rate (HR) may affect the onset and progression of diabetic retinopathy.[6–8] Identification of potentially modifiable clinical parameters associated with development of retinopathy might be important for understanding and preventing retinopathy.

It is well known that microalbuminuria is a marker of higher cardiovascular risk as well as a predictive factor for diabetic complications such as diabetic nephropathy and retinopathy.[9] Moreover, microalbuminuria has been demonstrated to be an independent risk factor for the incidence of retinopathy in patients with type 1 diabetes.[10–12] It is assumed that retinopathy and nephropathy, as important microvascular complications in diabetes, occurs at the same time and that retinovascular pathology reflects renal disease.[13] However, several studies demonstrated that albuminuria is not a risk factor for diabetic retinopathy and that retinopathy might be already present in the normoalbuminuric state in type 1 diabetic patients.[5,14] Moreover, some patients with advanced retinopathy are normoalbuminuric and have normal glomerular morphology.[15]

It is important to establish risk factors of the onset of early diabetic retinopathy among individuals with normal renal function, because most studies have focused on the progression of established renal and ocular disease. The objective of this study, therefore, was to evaluate the associations of clinical and metabolic parameters, including duration of diabetes, body mass index (BMI), glucoregulation, serum lipids, BP, and resting HR, with nonproliferative retinopathy in normoalbuminuric type 1 diabetic patients with glomerular filtration rate (GFR) >60 mL/min/1.73 m^2.

PATIENTS, MATERIALS, AND METHODS

This cross-sectional study included 312 euthyroid patients with type 1 diabetes mellitus, defined as an onset of diabetes before the age of 35 years, positive autoantibodies, and permanent insulin treatment initiated within 1 year of diagnosis. Initially, 476 patients, who were hospitalized for their comprehensive annual review, were screened. The study included patients with the following characteristics: 18 to 65 years of age, minimum duration of type 1 diabetes of 1 year, no medical history of cardiovascular diseases or electrocardiographic (ECG) evidence of ischemic heart disease, absence of any systemic disease, and absence of any infections in the previous month. Patients were excluded

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from the study if they took any of the following: lipid-lowering therapy, antihypertensive medications including angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers, and medications that might affect glucose metabolism such as glucocorticoids. Patients with microalbuminuria (urinary albumin excretion rate [UAE] ≥30<300 mg/24 hours), macroalbuminuria [UAE≥300 mg/24 hours]), impaired estimated GFR (<60 mL/min/1.73 m^2), and proliferative and laser-treated retinopathy were also excluded from the study. Euthyroidism was defined as thyroid-stimulating hormone (TSH), FT3, and FT4 within the normal reference range.

All patients were studied in the morning after an overnight fast. Basic anthropometric measurements were performed on all study patients, including BMI and waist to hip ratio (WHR). BP was measured on both arms twice in the sitting position with a mercury sphygmomanometer after a resting period of 10 minutes and expressed in mm Hg. HR was determined using a standard 12-lead ECG after a resting period of 10 minutes and expressed in beats per minute. UAE was measured from at least two 24-hour urine samples and determined as the mean of 24-hour urine collections. Patients performed collections on two consecutive days to minimize variability. Normoalbuminuria was defined as a UAE<30 mg/24 hours. Serum creatinine was measured in a fasting blood sample. Data on serum creatinine levels, age, sex, and race were used to calculate the estimated GFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, which has been shown to be accurate in determining renal function in diabetic patients with normal renal function.[16] All patients were confirmed to be free of urinary tract infections. Diagnosis of nonproliferative retinopathy was made by experienced ophthalmologists, who had no knowledge of the patient's clinical characteristics, by fundoscopy after pupillary dilatation. Retinopathy was classified into two categories: absent and nonproliferative (microaneurysms, intra-retinal hemorrhages, and/or hard exudates).

Fasting venous blood samples were collected in the morning between 8 AM and 9:30 AM after an overnight fast for the determination of hemboglobin A_{1c} (HbA_{1c}); total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol; triglycerides; and C-reactive protein (CRP). CRP, microalbumin, and HbA1c was measured spectrophotometrically by turbidimetric immunoinhibition. Results of HbA_{1c} (%) are expressed in the DCCT-equivalent. Glucose, cholesterol, and triglycerides in serum were measured by an enzymatic colorimetric method. TSH, FT3, and FT4 were determined by fluoroimmunoassay. Complete blood cell count was determined on an automatic blood counter.

The study protocol complies with the Declaration of Helsinki as well as with local institutional guidelines, and was approved by the local ethics committees.

Data are expressed as mean±standard deviation for normally distributed values, as median with range for non-normally distributed values, and as percentage. Differences between groups were examined, depending on the nature of the data, parametric (*t* test) or nonparametric tests (Mann-Whitney). To investigate the relationship between HR with clinical and metabolic parameters data were also stratified into quartiles of HR. Kruskal-Wallis test was used for calculating the significance of the trend for each variable among the different quartiles. Multivariate logistic regression models were used to assess associations of HR and risk of nonproliferative retinopathy, taking into account the potential of confounders. Level of statistical significance was chosen as α=0.05. Statistical analysis was performed by statistical package SPSS, version 17.0 for Windows (SPSS Inc, Chicago, IL).

RESULTS

The characteristics of the study patients are listed in Table I. The average age was approximately 34 years, most were not overweight and 51% were women. Mean/median values of BMI, WHR, systolic BP, diastolic BP, total and HDL cholesterol, and triglycerides were within the normal range for patients with diabetes, with slightly elevated levels of HbA_{1c} and LDL cholesterol. The majority of patients had no retinopathy (36.5% with nonproliferative retinopathy). The mean

TABLE I. Clinical and Metabolic Characteristics of All Patients

Variable	Value
Age, y	34 (18–65)
Sex, male/female, %	49/51
Duration of diabetes, y	12 (1–42)
BMI, kg/m^2	24 (15–37)
Waist to hip ratio	0.81±0.06
Hemoglobin A_{1c}, %	7.2±1.6
SBP, mm Hg	120 (80–180)
DBP, mm Hg	80 (50–110)
Pulse, beats per min	72 (44–114)
Total cholesterol, mmol/L	5.0±0.8
LDL cholesterol, mmol/L	2.8±0.7
HDL cholesterol, mmol/L	1.7±0.4
Triglycerides, mmol/L	0.91 (0.3–4.1)
Serum creatinine, μmol/L	70±14
eGFR, mL/min/1.73 m^2	107±15
Urinary albumin excretion, mg/24 h	10.7 (1.7–29.8)
Nonproliferative retinopathy, %	36.5
TSH, mU/L	2.3±0.9
FT3, pmol/L	5.3±1.0
FT4, pmol/L	14±2.5
C-reactive protein, mg/L	0.9 (0.1–15.9)
Daily insulin dose, IU/d	41 (8–96)

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TSH, thyroid-stimulating hormone.

GFR estimated by the CKD-EPI was 107 mL/min/1.73 m^2.

Clinical and metabolic characteristics of patients with and without nonproliferative retinopathy are presented in Table II. Patients with nonproliferative retinopathy were older and had longer duration of diabetes, a higher daily insulin dose, and higher HR ($P \leq .01$ for all). BMI, WHR, systolic and diastolic BP, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, serum creatinine, estimated GFR, CRP, TSH, and UAE levels did not differ between two groups. We also explored the relationship between clinical and metabolic characteristics of patients among those in the 2nd, 3rd, and 4th quartiles of HR compared with those in quartile 1 (<68 beats per minute) (Table III). Stratifying clinical and metabolic characteristics of patients for degree of HR, trends across quartiles for age, sex, duration of diabetes, triglycerides, serum creatinine, daily insulin dose, CRP, and UAE were statistically significant. Patients in the 4th quartile of HR had significantly higher prevalence of nonproliferative retinopathy compared with patients in the 2nd, 3rd, and 4th quartiles ($P < .001$).

In logistic regression analysis, higher HR was significantly associated with risk of nonproliferative retinopathy in our patients ($P < .001$), with odds ratios of 1.02 to 1.06 (Table IV, model A). The odds ratio for HR was attenuated slightly but remained strongly significant after adjustment for age, sex, duration of diabetes, HbA_{1c}, BMI, systolic and diastolic BP, total cholesterol, serum creatinine, eGFR, UAE, daily insulin dose, WHR, and CRP (Table IV, models B and C).

TABLE II. Clinical and Metabolic Characteristics of Patients Without and With NPR

	Without NPR (n=198)	With NPR (n=114)	*P* Value
Age, y	31 (18–65)	39 (21–60)	<.001
Sex, male/female	99/99	54/60	.4
Duration of diabetes, y	7 (1–35)	18 (1–42)	<.001
BMI, kg/m^2	23 (17–34)	24 (15–37)	.3
Waist to hip ratio	0.81±0.07	0.82±0.07	.08
Hemoglobin A_{1c}, %	7.2±1.5	7.8±1.6	<.001
SBP, mm Hg	120 (90–180)	120 (80–180)	.2
DBP, mm Hg	80 (60–110)	80 (50–110)	.6
Pulse rate, beats per min	70 (44–102)	78 (54–114)	<.001
Total cholesterol, mmol/L	4.8±0.9	5.0±0.8	.06
LDL cholesterol, mmol/L	2.7±0.8	2.8±0.7	.1
HDL cholesterol, mmol/L	1.6±0.4	1.7±0.3	.2
Triglycerides, mmol/L	0.91 (0.3–4.1)	0.93 (0.3–4.1)	.2
Serum creatinine, μmol/L	71±14	68±13	.07
eGFR, mL/min/1.73 m^2	112±15	106±14	.09
UAE, mg/24 h	10.3 (1.7–29.8)	11.8 (2.8–29.8)	.05
TSH, mU/L	2.4±1.1	2.1±0.8	.1
C-reactive protein, mg/L	0.8 (0.1–15.9)	1.1 (0.1–12.5)	.2
Daily insulin dose, IU/d	39 (8–82)	44 (16–96)	.01

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NPR, nonproliferative retinopathy; SBP, systolic blood pressure; TSH, thyroid-stimulating hormone; UAE, urinary albumin excretion rate.

DISCUSSION

Previous studies documented that microalbuminuria is independently associated with development of retinopathy in patients with type 1 diabetes.[10–12] However, retinopathy might be already present in the normoalbuminuric state in type 1 diabetic patients.[5,14] The present study demonstrates that a higher resting HR is associated with a higher risk of nonproliferative retinopathy in patients with type 1 diabetes. The prevalence of nonproliferative retinopathy in our patients was 36%, which is much lower than the prevalence of retinopathy reported in previous studies, including normoalbuminuric patients with type 1 diabetes.[5,17] Patients with nonproliferative retinopathy had significantly higher metabolic risk factors including age, duration of diabetes, HbA_{1c}, and resting HR. However, in multiple logistic regression analysis, only resting HR was independently related to nonproliferative retinopathy, even after adjusting for glycemic control and other risk factors.

HR is a strong and independent predictor of all-cause death and major cardiovascular complications including myocardial infarction, heart failure, cardiac death, and stroke in patients with and without diabetes.[18,19] It is also documented that higher HR is associated with increased prevalence and severity of microalbuminuria, and that HR and microalbuminuria were each independently predictive of the composite endpoint of all-cause death, myocardial infarction, and stroke.[20,21] In addition, it has been shown that HR is independently associated with retinopathy in type 2 diabetic patients, even in those with normoalbuminuria.[8,22,23] Little is known about the relationship between HR and incident retinopathy in normoalbuminuric type 1 diabetic patients. Two groups of our patients divided according to presence of retinopathy were similar regarding sex, BMI, systolic and diastolic BP, and serum lipids, but with higher UAE in those with higher HR. Higher HR might promote higher UAE, indicating endothelial dysfunction and proatherosclerotic activity, which are important factors in the development of retinopathy and progression of nephropathy.[24] However, it seems that relation between resting HR and the risk of retinopathy is stronger than the association with nephropathy.[8] Higher HR may also increase perfusion pressure that could contribute to diabetic retinopathy in patients with nonproliferative retinopathy.[25] Higher resting HR is also associated with increased visceral adipose tissue, which is associated with microvascular dysfunction.[26,27]

Higher HR could also indicate autonomic dysfunction in patients with retinopathy and poorer autoregulation of retinal vessel, because major clinical manifestation of autonomic neuropathy include resting tachycardia, and autonomic neuropathy is associated with an increased prevalence of microvascular compli-

TABLE III. Quartiles of Resting Heart Rate

	First Quartile (<68) (n=75)	Second Quartile (69–78) (n=81)	Third Quartile (79–89) (n=73)	Fourth Quartile (>90) (n=83)	*P* Value for Trend
Age, y	31 (18–57)	34 (19–60)	37 (19–65)	33 (19–55)	.03
Sex, male/female	48/27	40/41	31/42	34/49	.01
Duration of diabetes, y	6 (1–28)	12 (1–38)	10 (1–42)	14 (1–35)	.001
BMI, kg/m^2	24 (18–34)	24 (17–31)	24 (19–32)	23 (15–37)	.7
Hemoglobin A_{1c}, %	7.1±1.4	7.0±1.5	7.3±1.4	8.2±1.8	<.001
SBP, mm Hg	120 (90–150)	120 (95–170)	122 (80–150)	123 (90–180)	.1
DBP, mm Hg	80 (60–100)	80 (60–110)	80 (60–110)	80 (50–95)	.1
LDL cholesterol, mmol/L	2.8±0.7	2.9±0.8	2.9±0.8	2.7±0.7	.2
HDL cholesterol, mmol/L	1.7±0.3	1.6±0.3	1.8±0.3	1.7±0.4	.1
Triglycerides, mmol/L	0.9 (0.4–2.5)	1.0 (0.3–4.1)	1.0 (0.3–2.8)	1.2 (0.4–3.9)	.01
Serum creatinine, μmol/L	76±16	71±12	70±13	67±13	.005
eGFR, mL/min/1.73 m^2	106±16	108±14	105±15	110±17	.1
UAE, mg/24 h	8.8 (2.4–29.7)	9.6 (4.1–29.8)	10.1 (1.7–28.9)	14.2 (2.8–29.8)	<.001
NPR, yes/no	15/60	31/50	22/51	46/37	<.001
TSH, mU/L	2.3±0.0	2.3±0.9	2.4±1.1	2.3±1.0	.8
C-reactive protein, mg/L	0.7 (0.1–9.7)	0.8 (0.1–6.2)	0.9 (0.1–8.9)	1.4 (0.1–15.9)	.003
Insulin dose, IU/d	38 (14–71)	41 (8–88)	40 (13–82)	49 (16–96)	.002

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NPR, nonproliferative retinopathy; SBP, systolic blood pressure; TSH, thyroid-stimulating hormone; UAE, urinary albumin excretion rate.

TABLE IV. Multivariate Logistic Regression Analysis of Resting Heart Rate With Development of Nonproliferative Retinopathy in Normoalbuminuric Type 1 Diabetic Patients

Independent Variable	Model A	Model B	Model C
Heart rate	1.04 (1.02–1.06)[a]	1.05 (1.02–1.07)[a]	1.04 (1.01–1.08)[a]

Data are expressed as odds ratios (95% confidence intervals) from separate models. Model A, crude; model B, adjusted for age, sex, duration of diabetes, body mass index; and model C, adjusted for age, sex, duration of diabetes, body mass index, waist to hip ratio, hemoglobin A_{1c}, systolic blood pressure, diastolic blood pressure, total cholesterol, serum creatinine, estimated glomerular filtration rate, urinary albumin excretion rate, daily insulin dose, and C-reactive protein. [a]*P*<.001.

cations in diabetes.[28,29] In addition, increasing impairment in autoregulation of retinal blood flow is associated with increasing grades of diabetic retinopathy.[30] Moreover, we previously documented in a population of normoalbuminuric and normotensive adults with type 1 diabetes that autonomic neuropathy was related to retinopathy.[31] Type 1 diabetic patients with reduced baroreflex sensitivity, an early marker of autonomic impairment, have increased prevalence of laser-treated retinopathy.[32] However, it has been shown that cardiac autonomic neuropathy is an independent risk factor for cardiovascular morbidity and mortality in type 1 diabetic patients with nephropathy, but not in those with normoalbuminuria.[33]

Previous studies documented impact of systolic and diastolic BP on diabetic retinopathy in type 1 diabetes.[5,6] However, after controlling for risk factors, associations were attenuated and of borderline statistical significance. Moreover, it has been documented that hypertensive normoalbuminuric type 1 diabetic patients had no higher prevalence of retinopathy compared with normoalbuminuric normotensive patients indicating that hypertension per se is not associated with increased retinopathy.[34] In addition, in normoalbuminuric type 2 diabetic patients, BP is not a risk factor for development of retinopathy.[23,24] This may indicate that HR has a closer association with retinopathy that BP. However, methods used for diagnosis of nonproliferative retinopathy and BP may have had an influence on the final results in our investigation.

STUDY LIMITATIONS

The present study has a number of potential limitations. First, our study was cross-sectional, which limited our ability to infer a causal relation between resting HR and risk for the development of retinopathy. Second, we did not use ambulatory BP measurement, which has been shown to be more useful than causal or office BP measurement.[35] BP measured once during the day at the time of a clinic examination may overestimate levels in patients with the white-coat syndrome or underestimate levels in those who are nondippers. Third, retinal photography is a more sensitive method to detect nonproliferative retinopathy than fundoscopy used in this study.[36] Fourth, our analyses were based on a single measurement of HR and BP that may not reflect the relation over time.

CONCLUSIONS

We have shown that elevated resting HR is associated with nonproliferative retinopathy in normoalbuminuric type 1 diabetic patients. The mechanisms relating resting HR and nonproliferative retinopathy in type 1 diabetes are not clear. The most obvious explanation is that resting HR indicates sympathetic nerve activation and autonomic dysfunction, which are associated with an increased prevalence of microvascular complications in diabetes. However, the overall impact of resting HR on retinopathy in our study was fairly small. Whether the detection of elevated resting HR in normoalbuminuric type 1 diabetic patients has predictive value for development of retinopathy needs to be assessed in further follow-up studies.

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