Bulum T., Blaslov K., Duvnjak L. (2014) ***Risk factors for development and progression of nonproliferative retinopathy in normoalbuminuric patients with type 1 diabetes.*** **Diabetes Research and Clinical Practice, 106 (3). pp. 555-9. ISSN 0168-8227**

http://www.elsevier.com/locate/issn/01688227

http://www.sciencedirect.com/science/journal/01688227

http://dx.doi.org/10.1016/j.diabres.2014.09.018

Risk factors for development and progression of nonproliferative retinopathy in normoalbuminuric patients with type 1 diabetes

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The Authors state that this manuscript has not been published previously and is not currently being assessed for publication by any journal other than the Diabetes Research and Clinical Practice. The authors disclose that they did not receive any financial support for the study. No proprietary interest is involved in the study.

ABSTRACT

Background: Previous studies have reported that retinopathy might be present already in normoalbuminuric state in type 1 diabetes mellitus (T1DM). The aim of this study was to evaluate the risk factors for development and progression of nonproliferative retinopathy (NPR) in normoalbuminuric T1DM.

Methods: A total of 223 T1DM with normal renal function and normoalbuminuria were included in this study and followed for 48 months. Photodocumented retinopathy status was made according to the EURODIAB protocol. Urinary albumin excretion rate (UAE) was measured from at least two 24-h urine samples. The possible risk factors for development or progression of NPR were examined in backward stepwise Cox's multiple regression analysis.

Results: The majority of patients (70%) had no retinopathy while 67 (30%) had NPR at baseline. Patients with NPR were older, had longer duration of diabetes, higher systolic blood pressure, BMI, resting heart rate, UAE and lower estimated glomerular filtration rate ($p \leq 0.04$ for all). After 48 months twenty-four patients (10.7%) developed NPR or progressed to proliferative retinopathy. Systolic blood pressure (HR 1.03, CI 1.01-1.05, p=0.02), UAE (HR 1.14, CI 1.07-1.21, $p<0.001$), and resting heart rate (HR 1.05, CI 1.01-1.09, p=0.006) were significantly associated with development or progression of NPR.

Conclusions: Our results suggest that retinopathy is present and may progress in T1DM even when coexisting renal disease is excluded. Normoalbuminuric T1DM require close monitoring for the early detection of retinopathy, especially if they have a higher UAE, systolic blood pressure and resting heart rate.

Keywords: type 1 diabetes, retinopathy, microvascular complications, albuminuria

INTRODUCTION

It is well established that 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]. Prospective studies identified poor glycemic control, duration of diabetes and blood pressure as most important risk factors for development of retinopathy [3-7]. Retinopathy and nephropathy are most important microvascular complications in patients with diabetes. It is assumed that retinopathy and nephropathy occurs at the same time and that the severity of retinopathy parallels the presence and severity on nephropathy in diabetes mellitus [8,9]. In addition, it is suggested that relationship between renal abnormalities and retinopathy are more frequent in patients with type 1 diabetes than type 2 diabetes [10]. Nephropathy also had a strong effect on the associations between risk factors like dyslipidemia and retinopathy in type 1 diabetes [11]. However, it is still controversial whether albuminuria is independently associated with the incidence of diabetic retinopathy because several studies demonstrated that albuminuria is not a risk factor for diabetic retinopathy and that retinopathy might be present already in normoalbuminuric state in patients with type 1 diabetes [5,7,12-14]. Moreover, up to 27% of patients with type 1 diabetes with advanced retinopathy may be normoalbuminuric and even with normal glomerular structural measures determined with renal biopsy [15]. In patients with type 2 diabetes it has been documented that albuminuria is a risk factor for retinopathy even in normoalbuminuric state [16,17].

The aim of this study was to evaluate the risk factors for development or progression of nonproliferative retinopathy (NPR) in normoalbuminuric patients with type 1 diabetes with glomerular filtration rate (GFR) > 60 ml min^{-1} 1.73m^{-2}.

SUBJECTS, MATERIALS AND METHODS

Study included 223 patients with diabetes mellitus type 1, defined as an onset of diabetes before the age of 35 years, positive autoantibodies and permanent insulin treatment initiated within 1 year of diagnosis, who were referred to tertiary care specialist diabetes clinic between January 2005 and December 2012. The majority of patients were referred to clinic by general practitioners requiring assistance with surveillance and management of chronic complications of diabetes. Other patients were referred from other specialty units within clinic. Eligible participants were at least 18 years old, minimum duration of type 1 diabetes of 1 year, no medical history of cardiovascular diseases or electrocardiogram (ECG) evidence of ischemic heart disease, absence of any systemic disease, and absence of any infections in the previous month.

All subjects were studied in the morning after an overnight fast. Basic anthropometric measurements were performed on all study subjects, including body mass index (BMI). Blood pressure was measured twice in the sitting position with a mercury sphygmomanometer and resting heart rate was determined using a standard 12-lead ECG, after a resting period of 10 minutes. Urinary albumin excretion (UAE) was measured from at least two 24-h urine samples and determined as the mean of 24-h urine collections. Normoalbuminuria was defined as a UAE<30 mg/24h. Those with microalbuminuria (UAE≥30<300 mg/24h) and macroalbuminuria (UAE≥300 mg/24h) were excluded from the study. 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 [18,19]. Those with impaired estimated GFR (less than 60 ml min^{-1} $1.73m^{-2}$) were excluded from the study. All subjects were confirmed to be free of urinary tract infections. Retinopathy was diagnosed by binocular indirect slit lamp fundoscopy and fundus

photography after mydriasis with eye drops containing 0.5% tropicamide and 5% phenylephrine. Color fundus photographs of two fields of both eyes were taken with a suitable 45° fundus camera (VISUCAM, Zeiss) according to the EURODIAB retinal photography methodology [20]. EURODIAB classification scheme was used because it uses two-field 45° fundus photography and standard photographs to grade retinal lesions [20]. In each patient the "worse" eye was graded for retinopathy using fundus photographs. Those with proliferative retinopathy at baseline were excluded from the study.

Fasting venous blood samples were collected in the morning between 08:00 and 09:30 hours after an overnight fast for the determination of HbA1c, total, LDL, HDL cholesterol, and triglycerides. Microalbumin and HbA1c was measured spectrophotometrically by turbidimetric immuno-inhibition (Olympus AU600, Beckman-Coulter, USA). Results of HbA1c (%) are expressed in the DCCT-equivalent. Glucose, cholesterol and triglycerides in serum were measured by an enzymatic colorimetric method (Olympus AU600, Beckman-Coulter, USA). Complete blood count was determined on an automatic blood counter (Advia 120, Siemens Diagnostic Solutions, USA).

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

Statistical analysis

Numeric variables are given as means (SD) or medians (ranges) depending on the normality distribution tested using Kolmogorov-Smirnov test, or percentages and absolute numbers for nominal variables. The unadjusted incidence rate was calculated by dividing the number of people with development or progression of NPR by the person years of follow up for the NPR development/progression and reported as events per 1000 persons for years of follow up. Follow up time was calculated from the beginning of the study to the first occurrence of that

complication to the end of the study for those who did not have that complication. We calculated adjusted incidence rate of NPR development/progression using a Poisson regression model adjusted for sex, age and duration of diabetes and expressed in events per 1,000 person per years of follow up.

Cox's proportional hazards multiple regression analyses were used to examine the baseline variables predictive of development or progression of NPR, taking in account of potential confounders and pre-existing retinopathy. Results are described as relative risk (hazard ratio). A p value of less than 0.05 was regarded as significant.

RESULTS:

Baseline clinical and metabolic characteristics of patients with NPR and those without retinopathy are presented in Table 1. Patients with NPR were older, had longer duration of diabetes, higher BMI, systolic blood pressure, resting heart rate, UAE and lower estimated GFR compared to patients without retinopathy ($p \leq 0.03$ for all).

The majority of patients (70%) had no retinopathy while 67 (30%) had NPR at baseline. The mean UAE was 9.6 mg/24h and GFR estimated by the CKD-EPI was 108 ml min^{-1} $1.73m^{-2}$ at baseline. After 48 months twenty-four patients (10.7%) developed NPR or progressed to proliferative retinopathy. From 156 patients free of retinopathy at the beginning of the study 15 (9.6%) progressed to NPR, while from 67 patients with NPR at the beginning of the study 9 (13.4%) progressed to proliferative retinopathy (Figure 1). The unadjusted incidence rate of retinopathy development/progression was 2.7 and the incidence rate adjusted for age, gender and disease duration was 1.904 (1.824-1.984) per 1,000 persons per year.

The possible risk factors for development or progression of NPR were examined in backward stepwise Cox's multiple regression analysis (Table 2). Systolic blood pressure, UAE and resting heart rate were significantly associated with development or progression of NPR. An increase in 1 mg/24h of UAE was associated with a risk of 14,3% (95% confidence interval 7-21%).

DISCUSSION

It is assumed that retinopathy and nephropathy, as most important microvascular complications in diabetes, occurs at the same time although previous studies documented that retinopathy might be present already in normoalbuminuric state in patients with type 1 diabetes [5,7,12,14]. The prevalence of NPR at the beginning of the study in our patients was 30%, which is much lower to the prevalence of retinopathy reported in previous studies including normoalbuminuric patients with type 1 diabetes [5,6, 21]. After 48 months of following, UAE, systolic blood pressure and resting heart rate were risk factors for development or progression of NPR in our normoalbuminuric patients with type 1 diabetes.

Resting heart rate is an independent predictor of all-cause death and major cardiovascular complications in subjects with and without diabetes [22]. Higher heart rate might promote higher UAE, indicating endothelial dysfunction, which are important factors in the development of retinopathy and nephropathy [23]. However, it seems that relation between resting heart rate and the risk of retinopathy is stronger than the association with nephropathy in diabetes [24]. In addition, it has been shown that resting heart rate is independently associated with retinopathy in patients with type 1 and 2 diabetes, even in those with normoalbuminuria [16, 24, 25].

Relationship between hypertension and retinopathy in type 1 diabetes is still controversial. It has been documented that hypertensive normoalbuminuric patients with type 1 diabetes had no higher prevalence of retinopathy compared to normoalbuminuric normotensive patients [26]. In addition, blood pressure is similar in normotensive normoalbuminuric patients with type 2 diabetes with and without retinopathy [27]. Another study of normoalbuminuric patients with type 1 diabetes found higher only night blood pressure in patients with retinopathy compared to those without retinopathy [5]. However, results from EURODIAB study found that diastolic blood pressure is significant risk factor

for retinopathy in type 1 diabetes after adjusting for UAE, while nighttime systolic 24-hour ambulatory blood pressure was associated with presence and severity of retinopathy in normotensive patients with type 1 diabetes without nephropathy [6, 28]. In our study only systolic blood pressure was associated with risk of progression of retinopathy, and the prospective UKPDS study also found higher relative risk for incidence of retinopathy with higher systolic blood pressure in type 2 diabetes [29]. However, methods used for diagnosis of blood pressure (single standard sphygmomanometer not 24-hour ambulatory blood pressure) may influence on final results in our investigation.

Of all factors studied, UAE has the strongest impact on retinopathy development and progression. UAE could predict the presence and progression of retinopathy even in normoalbuminuric patients with type 1 diabetes. It has been documented that some patients with advanced retinopathy have normal UAE and glomerular morphology determined with renal biopsy [15]. However, in most subjects a normal UAE does not imply normal renal function and does not exclude glomerular barrier damage because normoalbuminuric patients with type 1 diabetes and retinopathy have preclinical renal morphologic changes, higher urinary IgG2, IgG4 excretion and higher IgG2/creatinine and IgG2/IgG4 ratios compared to the patients without retinopathy [6, 21, 30]. Moreover, the cutt-off point for microalbuminuria is based on findings from studies in the 1980s in which the progression rate to nephropathy was very high in patients with values of UAE of 30 mg/24h [31]. In patients with type 2 diabetes it has been documented that UAE threshold of 10.7 mg/24, which are in normoalbuminuric range, could predict the increase risk for diabetic retinopathy and that the severity of retinopathy is aggravated with an increasing of UAE in normoalbuminuric range [17, 32]. In multiple regression analyses UAE was strongest variable that predict progression to incipient or progression to proliferative retinopathy confirming previous observations that

retinal microvascular abnormalities are associated with renal dysfunction independently of age, diabetes, hypertension and other risk factors [33].

In conclusion, our results suggest that retinopathy is present and may progress in patients with type 1 diabetes even when coexisting renal disease is excluded. This points to the need for close monitoring of normoalbuminuric patients with type 1 diabetes aimed at early detecting, preventing or limiting the progression of NPR, especially in patients with higher UAE, systolic blood pressure and higher resting heart rate. However, this study used a convenience sample of patients with type 1 diabetes recruited at one of their regularly scheduled diabetes appointment and it is possible that the results may not generalize to all patients with type 1 diabetes.

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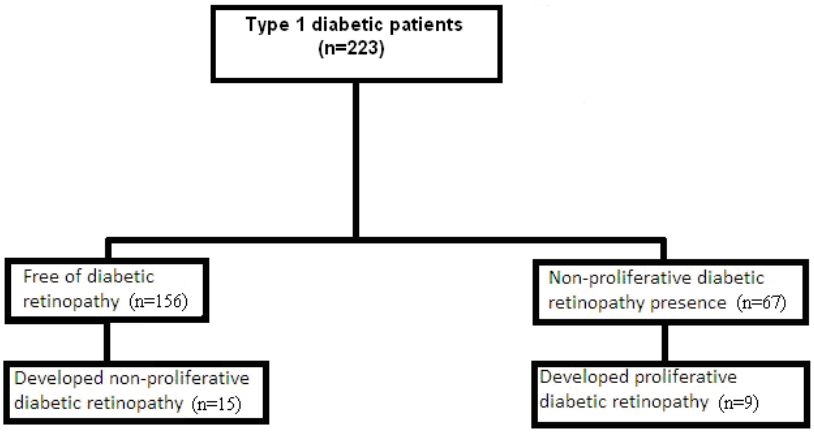

Figure 1. Number of patients that developed nonproliferative retinopathy or progressed to proliferative retinopathy during follow up

Table 1: Baseline clinical and metabolic characteristics of patients without and with nonproliferative retinopathy (NPR)

	without NPR (n=156)	with NPR (n=67)	P
Age (years)	39 (20-65)	49 (24-67)	<0.001
Sex (m/f)	83/73	34/33	0.7
Duration of diabetes (years)	14±8	24±9	<0.001
Body mass index (kg/m^2)	24 (18-38)	25 (19-35)	0.04
HbA1c (%)	6.9±1.4	7.2±1.5	0.2
SBP (mmHg)	120 (80-180)	130 (95-160)	0.03
DBP (mmHg)	80 (60-110)	80 (50-110)	0.5
Heart rate (beats/min)	70 (51-98)	74 (44-111)	0.001
LDL cholesterol (mmol/L)	2.8±0.7	3.0±0.9	0.2
HDL cholesterol (mmol/L)	1.7±0.4	1.7±0.4	0.9
Triglycerides (mmol/L)	0.98±0.6	1.13±0.7	0.07
Serum creatinine (µmol/L)	70±11	71±13	0.4
eGFR (ml min^{-1} 1.73m^{-2})	108±13	101±17	0.001
UAE (mg/24h)	7.8 (2.3-25.5)	10.3 (1.3-29)	0.006
Smoking (yes/no, %)	55/101	26/41	0.6

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion rate.

Table 2: Cox's multiple regression analysis model by means of baseline risk factors for development or progression of diabetic retinopathy

Variable	Relative risk (95% CI)	P value
Age	1.02 (0.95-1.09)	0.5
Duration of diabetes	0.98 (0.92-1.05)	0.7
Body mass index	0.95 (0.84-1.07)	0.4
Systolic blood pressure	1.03 (1.01-1.05)	0.02
Resting heart rate	1.05 (1.01-1.09)	0.006
Pre-existing retinopathy	0.67 (0.20-2.19)	0.5
Urinary albumin excretion	1.14 (1.07-1.21)	< 0.001
Estimated GFR	1.01 (0.97-1.06)	0.4