Journal of Clinical & Translational Endocrinology 1 (2014) e9–e12

Contents lists available at ScienceDirect

Journal of Clinical & Translational Endocrinology

journal homepage: www.elsevier.com/locate/jcte

Research Paper

Retinopathy in subjects with type 2 diabetes at a tertiary diabetes clinic in Durban, South Africa: Clinical, biochemical and genetic factors

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ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form
12 December 2013
Accepted 13 December 2013
Available online 21 January 2014

Keywords:
Type 2 diabetes
Retinopathy
Genetics
Africa

ABSTRACT

Aim: To determine the prevalence of clinical and laboratory variables and genetic polymorphisms in association with diabetic retinopathy (DR) in subjects with type 2 diabetes attending a tertiary referral diabetes clinic in Durban, South Africa.
Methods: Cross-sectional study on 292 Indian and African patients with type 2 diabetes (71.5% women). The presence of DR was determined by direct ophthalmoscopy. Clinical and laboratory data were collected and polymorphisms in the *NOS3* (rs61722009, rs2070744, rs1799983) and *VEGF* (rs35569394, rs2010963) genes were determined.
Results: DR was present in 113 (39%) subjects. Those with DR were older (60.6 ± 9.6 vs. 55.4 ± 12.9 years, $p = 0.005$), had longer duration diabetes (18.5 ± 8.8 vs. 11.9 ± 9.2 years, $p < 0.0001$), higher HbA_{1c} (9.2 ± 1.8 vs. 8.8 ± 1.7%, $p = 0.049$), serum creatinine (106.3 ± 90.2 vs. 75.2 ± 33.4 μmol/l), triglycerides (2.1 ± 1.2 vs. 1.9 ± 1.6 mmol/l, $p = 0.042$), proteinuria (72% vs. 28%, $p = 0.001$), and used more insulin (78% vs. 39% $p = 0.0001$), anti-hypertensive (95% vs. 80%, $p = 0.0003$) and lipid-lowering therapy (70% vs. 56%, $p = 0.023$). There was no association between DR and any of the *NOS3* or *VEGF* gene polymorphisms studied, although there were ethnic differences. After adjustment, diabetes duration (OR 1.05, 95% CI 1.01–1.08), presence of proteinuria (OR 4.15, 95% CI 1.70–10.11) and use of insulin therapy (OR 3.38, 95% CI 1.60–7.12) were associated with DR.
Conclusion: Hyperglycemia, duration of diabetes and proteinuria are associated with DR in Indian and African patients in South Africa, whereas *NOS3* and *VEGF* gene polymorphisms were not associated with DR.

Introduction

Diabetic retinopathy (DR) is an important cause of visual loss and the progressively increasing global prevalence of diabetes suggests that there will be increasing numbers of persons affected by this complication. In terms of the global epidemiology of DR, a recent pooled data analysis, including an excess of 22,000 subjects from 35 different studies, reported that the age-standardized prevalence of any DR in subjects aged 20–79 years is 34.6%, proliferative diabetic retinopathy (PDR) 6.96% and diabetic macular edema (DMO) 6.81% [1]. This analysis did not include any studies from Africa and data on DR in African populations are sparse [2–4]. A systematic review of diabetes in sub-Saharan Africa between 1999 and 2011 reported that the prevalence of DR varied from 7% to 63% [3]. A previous study of African and Indian subjects with long-duration diabetes in KwaZulu-Natal, South Africa, showed that of 179 subjects with type 2 diabetes, 64.5% had DR, 47.9% non-proliferative DR only, 20.8% PDR and 26% had undergone laser photocoagulation therapy [5]. In this study, the subjects with DR had higher systolic blood pressure and longer diabetes duration than those without DR.

The major risk factors for the development of DR include disease duration, degree of hyperglycemia, hypertension, obesity, dyslipidemia and genetic factors [6]. A number of candidate genes have been shown to be associated with DR, including polymorphisms in the endothelial nitric oxide synthase (eNOS) gene, the vascular endothelial growth factor (VEGF) gene, the aldose reductase (*AKR1B1*) gene and the angiotensin converting enzyme gene [7]. Furthermore, some studies have shown the intron 4a allele of the eNOS gene may be associated with a reduced risk of DR [8]. However, the influence of risk factors on the development of DR has not been studied in detail in populations in Africa. The aim of the current study was to examine the association between modifiable and non-modifiable risk factors and the development of DR in a group of

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2214-6237
http://dx.doi.org/10.1016/j.jcte.2013.12.002

subjects with type 2 diabetes attending a referral diabetes clinic in KwaZulu-Natal, South Africa.

Patients and methods

Consecutive patients with type 2 diabetes attending a specialist referral diabetes clinic at Inkosi Albert Luthuli Central Hospital, Durban, South Africa, were enrolled. All patients attending the clinic were enrolled and there were no exclusion criteria. Each patient underwent clinical assessment including anthropometry and blood pressure measurement. Direct ophthalmoscopy through a dilated pupil was conducted by three experienced diabetologists. In subjects with proliferative DR, confirmation by an ophthalmologist was obtained. Retinopathy was graded as non-proliferative or proliferative. Non-proliferative changes included the presence of microaneurysms, venous beading, hard exudates, cotton wool spots and intra-retinal hemorrhages. Proliferative changes included neovascularization, intra-vitreous hemorrhages and fibrous retinal detachment [9]. Macular edema was not assessed. All patients provided written informed consent and the study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal. Venous blood was collected for measurement of creatinine, glycated hemoglobin (HbA_{1c}), lipid levels and for extraction of genomic DNA. All subjects had spot urine collected for the measurement of albumin and creatinine excretion and determination of urine albumin–creatinine ratio (ACR).

Assay methods

Creatinine was measured by the picric acid method, HbA_{1c} by high performance liquid chromatography, cholesterol by enzymatic method, using cholesterol esterase and cholesterol oxidase conversion, followed by Trinder endpoint, triglycerides by the Fossati three step enzymatic reaction with Trinder endpoint and high density lipoprotein (HDL) by enzymatic reaction. Low density lipoprotein (LDL) was calculated with the Friedewald equation. Urine albumin excretion was measured by polyethylene glycol-enhanced immunoturbidometric assay. Microalbuminuria was defined as ACR 2.5–25 mg/mmol creatinine in males and 3.5–35 mg/mmol creatinine in females. Proteinuria was defined as persistent dipstix positivity.

DNA was extracted from peripheral blood leucocytes using an in-house method involving sucrose lysis, phenol-chloroform extraction and ethanol precipitation.

Polymerase chain reaction (PCR) and gel electrophoresis was used to define the number of tandem repeat sequences in intron 4 (rs61722009) of the eNOS gene (*NOS3*). Allelic discrimination with real-time PCR (Taqman 3100, ABI) was used to determine the prevalence of -786 C/T (rs2070744) and 894 G/T (rs1799983) polymorphisms in the *NOS3* gene and -634 G/C (rs2010963) and -2549 insertion-deletion (rs35569394) polymorphisms in the *VEGF* gene.

Statistical methods

In bivariate analysis of risk factors associated with retinopathy, Mann Whitney tests were used for ordinal data, Pearson's chi square tests for categorical data and independent *t*-tests for quantitative normal data. Binary logistic regression analysis was used to determine factors associated with DR after adjustment for confounding. Statistical analysis was performed with SPSS version 15.

Results

The total study group included 292 subjects (Indian 175; African 117). Of these, 71.5% ($n = 208$) were women. The mean age was 57.5 ± 12 years, mean duration of diabetes 14.48 ± 9.59 years and mean HbA_{1c} 9.06 ± 1.83%.

Table 1
Clinical and laboratory characteristics of Indian and African subjects ($n = 292$)

	Indian	African	*p*-value
	$N = 175$	$N = 117$	
Gender			
Male	61 (35)	25 (21)	0.02
Female	114 (65)	92 (79)	
Age (years)	56.5 ± 12.0	59.2 ± 11.6	0.054
Age at diagnosis (years)	41.5 ± 12.0	45.4 ± 9.5	0.003
Duration diabetes (years)	14.9 ± 10.1	13.8 ± 8.9	0.4
Smoking (%)	29 (16.4)	4 (3.4)	0.002
Insulin therapy (%)	124 (71)	83 (71)	0.9
Anti-hypertensive therapy (%)	149 (85)	108 (92)	0.1
Statin therapy (%)	133 (76)	56 (47.9)	<0.0001
Body mass index (kg/m^2)	30.0 ± 5.3	35.7 ± 6.5	<0.0001
Systolic BP (mm Hg)	133 ± 15	138 ± 20	0.033
Diastolic BP (mm Hg)	74 ± 10	77 ± 10	0.025
HbA_{1c} (%) [mmol/mol]	9.0 ± 1.7 [75]	9.2 ± 2.0 [77]	0.2
Creatinine (μmol/l)	84 ± 47	92 ± 83	0.4
Microalbuminuria (%)	65 (37)	33 (28.2)	0.2
Proteinuria (%)	21 (12)	11 (9.4)	0.5
Cholesterol (mmol/l)	4.6 ± 1.1	4.3 ± 1.0	0.019
Triglycerides (mmol/l)	2.3 ± 1.8	1.7 ± 0.8	0.0003
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.2 ± 0.3	0.3
LDL cholesterol (mmol/l)	2.4 ± 1.0	2.3 ± 0.9	0.3

African subjects were older at diagnosis (45.4 ± 9.5 vs. 41.5 ± 12.0 years, $p = 0.003$), had a higher BMI (35.7 ± 6.5 vs. 30.0 ± 5.3 kg/m^2, $p < 0.0001$), higher SBP (138 ± 20 vs. 133 ± 15 mm Hg, $p = 0.033$), higher DBP (77 ± 10 vs. 74 ± 10 mm Hg, $p = 0.025$), lower total cholesterol (4.3 ± 1.0 vs. 4.6 ± 1.1 mmol/l, $p = 0.019$), lower triglycerides (1.7 ± 0.8 vs. 2.3 ± 1.8 mmol/l, $p = 0.0003$) and fewer were treated with statins (47.9% vs. 76%, $p < 0.0001$) (Table 1). Fewer of the African subjects were current smokers (3.4% vs. 16.4%, $p = 0.002$). There was no difference between African and Indian subjects for age, number on anti-hypertensive therapy, number on insulin therapy, HbA_{1c}, HDL cholesterol, LDL cholesterol, use of fibrates, serum creatinine and prevalence of microalbuminuria and proteinuria.

DR (non-proliferative and proliferative) was present in 113 (39%) of the subjects. Non-proliferative DR was present in 67 (23%) and PDR in 46 (15.8%) subjects. There was no difference in the prevalence of either non-proliferative or proliferative DR between African and Indian subjects (Africans, non-proliferative DR 19.7%, PDR 14.5%; Indians, non-proliferative DR 23.4%, PDR 16.0%, $p = 0.996$).

Subjects with any DR were compared to those with no DR for clinical, laboratory and genetic variables. Table 2 shows the clinical and biochemical variables. Both groups had a similar gender distribution and ethnic variation. When compared with subjects without DR, those with DR were older (60.6 ± 9.6 vs. 55.4 ± 12.9 years, $p = 0.005$) and had longer duration diagnosed diabetes (18.5 ± 8.8 vs. 11.9 ± 9.2 years, $p < 0.0001$). A higher proportion used insulin therapy (78% vs. 39%, $p = 0.0001$) and anti-hypertensive therapy (95% vs. 80%, $p = 0.0003$) and there were more smokers (58% vs. 42%, $p = 0.046$).

Subjects with DR had higher HbA_{1c} (9.2 ± 1.8 vs. 8.8 ± 1.7% [77 vs. 73 mmol/mol], $p = 0.049$) and serum creatinine (106.3 ± 90.2 μmol/l vs. 75.2 ± 33.4 μmol/l, $p < 0.0001$). Microalbuminuria was present in fewer subjects with DR (38.5% vs. 61.5%, $p = 0.001$) whereas overt proteinuria was present in more subjects with DR (72% vs. 28%, $p = 0.001$).

Serum triglyceride levels (2.1 ± 1.2 vs. 1.9 ± 1.6 mmol/l, $p = 0.042$) were higher in subjects with DR. There was no difference in total, HDL or LDL cholesterol, although more subjects with DR were treated with lipid-lowering therapy (70% vs. 56%, $p = 0.023$).