



Research Paper

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

F.J. Pirie^{a,*}, S. Maharaj^a, T.M. Esterhuizen^b, I.M. Paruk^a, A.A. Motala^a^a Department of Diabetes and Endocrinology, University of KwaZulu-Natal, Nelson R Mandela School of Medicine, 419 Umbilo Road, Congella, Durban 4001, South Africa^b Programme of Bio & Research Ethics and Medical Law, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

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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. $8.8 \pm 1.7\%$, $p = 0.049$), serum creatinine (106.3 ± 90.2 vs. 75.2 ± 33.4 $\mu\text{mol/l}$), 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.

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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

* Corresponding author. Tel.: +27312604536; fax: +27312604774.

E-mail address: pirie@ukzn.ac.za (F.J. Pirie).

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 dipstick 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

Table 1

Clinical and laboratory characteristics of Indian and African subjects (*n* = 292)

	Indian N = 175	African N = 117	<i>p</i> -value
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

57.5 ± 12 years, mean duration of diabetes 14.48 ± 9.59 years and mean HbA_{1c} 9.06 ± 1.83%.

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/m², *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).

Table 2Clinical and laboratory characteristics of subjects with and without diabetic retinopathy (*n* = 292)

	Retinopathy N = 113 (39%)	No retinopathy N = 179 (61%)	<i>p</i> -value
Gender			
Male	34	50	0.6
Female	79	129	
Ethnic group			
Indian	72	103	0.2
African	41	76	
Age (years)	60.6 ± 9.6	55.4 ± 12.9	0.005
Duration diabetes (years)	18.5 ± 8.8	11.9 ± 9.2	<0.0001
Smoking (%)	58.1	41.9	0.046
Insulin therapy (%)	89	58	0.0001
Anti-hypertensive therapy (%)	96	80	0.0003
Lipid-lowering therapy (%)	70	56	0.023
Body mass index (kg/m ²)	31.6 ± 6.4	32.5 ± 6.3	0.2
Systolic BP (mm Hg)	136.7 ± 17.7	134.3 ± 17.03	0.2
Diastolic BP (mm Hg)	72.3 ± 9.5	76.7 ± 10.0	<0.0001
HbA _{1c} (%) [mmol/mol]	9.2 ± 1.8 [77]	8.8 ± 1.7 [73]	0.049
Creatinine (μmol/l)	106.3 ± 90.2	75.2 ± 33.5	<0.0001
Microalbuminuria (%)	38.5	61.5	0.001
Proteinuria (%)	71.9	28.1	0.001
Cholesterol (mmol/l)	4.6 ± 1.2	4.4 ± 0.9	0.1
Triglycerides (mmol/l)	2.1 ± 1.2	1.9 ± 1.6	0.042
HDL cholesterol (mmol/l)	1.1 ± 0.2	1.2 ± 0.3	0.3
LDL cholesterol (mmol/l)	2.3 ± 0.9	2.3 ± 0.7	0.5

One hundred and seventy nine subjects were treated with statins (either simvastatin or atorvastatin), of whom 79 (44%) had DR and 100 (56%) had no DR. Only 6 subjects were treated with fibrates (1 African and 5 Indian subjects, all treated with bezafibrate).

When risk factors for DR were stratified according to ethnicity, there was no difference between African and Indian subjects.

Genetic data was available for 288 subjects (117 with DR and 171 with no DR). As shown in Table 3, none of the polymorphisms studied were associated with the presence of DR. Furthermore haplotypes of *NOS3* were not found to be significantly different between those with and those without DR. Although the prevalence of polymorphisms at each of the five loci was found to vary between African and Indian subjects, analysis of *NOS3* and *VEGF* gene polymorphisms within each ethnic group separately also failed to show any association with the presence of DR.

In logistic regression analysis, duration (years) of diagnosed diabetes (OR 1.05, 95% CI 1.01–1.08, *p* = 0.006), presence of proteinuria (OR 4.15, 95% CI 1.7–10.1, *p* = 0.002) and the use of insulin therapy (OR 3.4, 95% CI 1.6–7.1, *p* = 0.001) was associated with DR. There was no association with BMI, blood pressure, lipid levels or *NOS3* or *VEGF* genotype.

Discussion

The current study has shown that subjects with DR were older, had longer duration diagnosed diabetes, higher HbA_{1c}, higher serum creatinine and prevalence of proteinuria, more often treated with anti-hypertensive therapy and lipid-lowering therapy and more were smokers. The study failed to show an association with polymorphisms in *NOS3* or *VEGF* genes.

Meta-analysis of the prevalence and risk factors for DR has shown that the overall global prevalence of any DR in subjects aged 20–79 years is 34.6% and that of PDR is 6.96% [1]. This report, however, did not include any studies from Africa. The prevalence of any DR in the current study (39%) is similar to the global estimate, although more subjects with PDR were identified in the current study (15.8%). Another study in an African population, conducted in

Malawi, reported a similar overall prevalence of any DR to the current study (32.5%) [10]. The prevalence of retinopathy in the Diabcare Africa Study was lower than that recorded both in the current study and the Malawian study although the incomplete ascertainment of data (51% of subjects had an eye examination in the preceding 12 months), suggests that the prevalence may have been under-estimated [2].

The findings in the current study concur with the global meta-analysis that major risk factors for DR include diabetes duration and HbA_{1c}. However, although HbA_{1c} was significant in bivariate analysis, it failed to achieve significance in multivariate analysis when controlled for other risk factors. This may be partly accounted for by the cross-sectional nature of this study. The current study failed to confirm an association between DR and blood pressure and this is possibly explained by the higher proportion of those with DR receiving anti-hypertensive therapy. The measured blood pressures therefore, reflect the efficacy of drug therapy, thereby mitigating the influence of recorded blood pressure on DR. In addition, although data on the specific type of anti-hypertensive therapy were not collected, differences in the use of ACE-inhibitors and other anti-hypertensive agents may also partly account for this observation.

Although the pathogenesis of DR is multi-factorial, hyperglycemia is a major driver of the process, leading to alteration in capillary permeability and density [11–13]. The current study has shown a relationship between DR and hyperglycemia in that those with DR had longer duration of disease, higher HbA_{1c} and more subjects with DR were treated with insulin than those without DR. The latter observation is probably a reflection of longer diabetes duration with greater reduction in beta-cell function necessitating treatment advancement to insulin therapy. This is, however, speculative, as data on indices of beta-cell function were not collected.

Table 3Prevalence of *NOS3* and *VEGF* gene polymorphisms in subjects with and without diabetic retinopathy (*n* = 288)

Polymorphism	Retinopathy (N = 117)	No retinopathy (N = 171)	<i>p</i> -value
	N (%)	N (%)	
<i>NOS3</i> gene			
rs61722009			
aa	17 (10)	8 (7)	0.313
ab	64 (37)	49 (44)	
bb	89 (51)	50 (45)	0.215
rs2070744			
TT	117 (67)	69 (62)	0.257
TC	51 (29)	39 (35)	
CC	6 (4)	3 (3)	0.514
T	285 (82)	176 (80)	0.650
C	63 (18)	44 (20)	
rs1799983			
GG	137 (79)	81 (73)	0.329
GT	35 (20)	28 (25)	
TT	2 (1)	2 (2)	0.952
G	309 (89)	190 (86)	0.317
T	39 (11)	32 (14)	
<i>VEGF</i> gene			
rs35569394			
Deletion	86 (49)	51 (46)	0.651
Insertion/deletion	65 (37)	48 (43)	
Insertion	23 (13)	12 (11)	0.675
rs2010963			
GG	83 (48)	61 (55)	0.283
CG	79 (45)	42 (38)	
CC	12 (7)	8 (7)	0.891
G	245 (70)	164 (74)	0.422
C	103 (30)	58 (26)	

Previous studies have reported nephropathy to be associated with DR [11] and the current study found proteinuria to be more frequent in subjects with DR, whereas microalbuminuria was more frequent in those without DR. The association between proteinuria and DR is substantiated by the finding of higher serum creatinine in subjects with DR, suggesting perhaps more renal involvement (nephropathy). By contrast, some studies have found an association between microalbuminuria and DR [14]. A recent Chinese study found microalbuminuria to be associated with an increased risk of severe or proliferative retinopathy [15]. The reason for a higher frequency of microalbuminuria in subjects without DR in the current study is not known. It is possible that subjects with DR had longer duration of disease and more often had established nephropathy (shown by a greater frequency of proteinuria), whereas those without DR and shorter duration of disease, had markers of incipient nephropathy.

The Hoorn study found a positive relationship between cholesterol and triglyceride levels and DR in older subjects with type 2 diabetes [16]. The global meta-analysis also found an association between serum cholesterol and DMO [1]. The current study failed to show an association between total cholesterol, LDL cholesterol or HDL cholesterol and DR. However, lipid-lowering therapy was used more frequently in those with DR. Whether the efficacy of lipid-lowering therapy altered the relationship between serum cholesterol and DR is speculative. The finding that subjects with DR had higher triglyceride levels than those without DR is possibly a reflection of poorer metabolic control in those with DR.

Several inflammatory mediators have been shown to play a role in the development of DR, including VEGF, nitric oxide, tumor necrosis factor alpha and interleukin-1 beta [17]. The importance of the role of VEGF in the development of DR is underscored by the efficacy of anti-VEGF intra-ocular therapy in the management of DR and particularly in the management of DMO [12,18]. Furthermore, genetic factors have been shown to have an independent effect on the development of DR and polymorphisms in a number of candidate genes, including *VEGF*, *NOS3* and aldose reductase (*AKR1B1*) have been studied [7]. A recent meta-analysis showed that the rs2010963 (-634 G to C) *VEGF* polymorphism was associated with DR in subjects with type 2 diabetes [19]. In a separate study, no significant association was found between the rs25648, rs1570360, rs3095039, rs35569394 and rs699947 *VEGF* polymorphisms and DR [7]. Some studies have shown a relation between *NOS3* polymorphisms and DR, although meta-analysis has excluded most of the polymorphisms studied in this gene [7,20]. In agreement with the generally negative studies of candidate genes in DR, the current study failed to show a relationship between three *NOS3* polymorphisms and two *VEGF* gene polymorphisms and DR. Although an independent genetic risk for the development of DR may exist, the effect size is likely to be small and require large study numbers to demonstrate positive associations.

Strengths of the current study include a multi-ethnic population, allowing comparison between groups and the inclusion of gene polymorphisms in the determination of factors associated with DR. Limitations include the relatively small numbers of subjects as well as the method of determination of DR; retinal photography may have improved the diagnostic assessment of DR.

In addition the findings, in this clinic-based study, may not be able to be extrapolated to the general population in view of the possibility of referral bias.

In conclusion, the current study has shown that in subjects with type 2 diabetes in KwaZulu-Natal, the well-established risk factors for DR are affirmed and a number of candidate genes failed to show an association with DR. These findings suggest that focus on control of hyperglycemia, blood pressure and serum lipids should remain the cornerstone of therapy, aiming at preventing the development of DR and other diabetes complications.

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