**First Prospective Cohort Study of Diabetic Retinopathy from Sub-Saharan Africa**

*High Incidence and Progression of Retinopathy and Relationship to HIV Infection*

**Running Head: Diabetic Retinopathy in Sub-Saharan Africa**

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**Conflicts of Interest**

No conflicting relationship exists for any author

**Abstract**

**Purpose** To describe the prevalence, incidence and progression of retinopathy and to report associations with demographic, clinical and biochemical variables in people with diabetes in Southern Malawi.

**Design** Prospective cohort study of subjects attending primary care diabetes clinics.

**Methods** We performed the first prospective cohort study of diabetic retinopathy from Sub-Saharan Africa over 24 months. Subjects were systematically sampled from two primary care diabetes clinics. Visual acuity, glycemic control, blood pressure, HIV status, urine albumin–creatinine ratio, hemoglobin and lipids were assessed. Retinopathy was graded atan accredited reading centre using modified Wisconsin grading of four-field mydriatic photographs.

**Main Outcome Measures** Incidence of sight threatening retinopathy and progression of retinopathy by 2 steps on the Liverpool Diabetic Eye Study Scale

**Results** 357 subjects were recruited to the 24 month cohort study. At baseline 13.4% subjects were HIV-positive and 15.1% anemic. 2-year incidence of sight threatening diabetic retinopathy for subjects with level 10 (no retinopathy), level 20 (background) and level 30 (pre-proliferative) retinopathy at baseline was 2.7% (95% CI 0.1-5.3), 27.3% (16.4-38.2) and 25.0% (0-67.4), respectively. In a multivariate logistic analysis 2 step progression of diabetic retinopathy was associated with HbA1c (OR 1.27, 95%CI 1.12-1.45), baseline grade of retinopathy (1.39, 1.02-1.91) and HIV infection (OR 0.16, 0.03-0.78). At 2 years 17 subjects (5.8%) lost ≥15 letters.

**Conclusions** Incidence of sight threatening diabetic retinopathy was approximately 3 times that reported in recent European studies. The negative association of HIV infection with retinopathy progression is a new finding.

**Keywords**

Diabetes Mellitus; diabetes complications; diabetic retinopathy; HIV; anemia; Africa South of the Sahara.

**Abbreviations**

ACCORD Action to Control Cardiovascular Risk in Diabetes Study

ADA American Diabetes Association

ART Anti-retroviral therapy

BCVA Best corrected visual acuity

BMI Body mass index

BP Blood pressure

CI Confidence interval

CSME Clinically significant macular edema

DR Diabetic retinopathy

ETDRS Early treatment of diabetic retinopathy study

HbA1c Glycosylated hemoglobin

HDL High density lipoprotein

HIV Human immunodeficiency virus

HMa Hemorrhages and microaneurysms

IRMA Intra-retinal microvascular abnormalities

LDES Liverpool Diabetic Eye Study

LDL Low density lipoprotein

MDRS Malawi diabetic retinopathy study

NGSP National glycohemoglobin standardisation program

NPDR Non-proliferative diabetic retinopathy

OR Odds ratio

PDR Proliferative diabetic retinopathy

QECH Queen Elizabeth Central Hospital

STDR Sight threatening diabetic retinopathy

uACR urine albumin-creatinine ratio

VA Visual acuity

WHO World Health Organisation

ZCH Zomba central hospital

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**Introduction**

The International Diabetes Federation has estimated that the number of adults diagnosed with diabetes in Africa will increase from 12.1 million in 2010 to 23.9 million in 2030 [1]. The prevalence and incidence of sight-threatening diabetic retinopathy (STDR) in developed countries [2-4] and the association with systemic factors, including glycemic control [5,6] blood pressure [7] and blood lipid levels [8], are well documented. No cohort studies have investigated the determinants of severity and progression of DR in sub-Saharan Africa [9]. In this resource-poor setting, population-specific variables, such as a high burden of infectious disease (including HIV and malaria) and anemia, may affect the spectrum of pathology encountered.

Malawi (population 15.9 million) is one of the poorest countries in the world, with an annual per capita healthcare expenditure of US$77 [10]. The WHO Malawi national STEPwise survey estimated a prevalence of diabetes of 5.6% in adults 25–64 years, with a similar prevalence in rural and urban areas [11]. In 2007, our group performed a cross-sectional study using clinical ocular examination to assess grades of retinopathy in patients

attending the diabetes clinic at Queen Elizabeth Central Hospital, Blantyre [12,13]. We reported a high prevalence of sight-threatening and proliferative retinopathy: 19.6 and 5.7%, respectively. Because of these important findings, we performed the Malawi Diabetic Retinopathy Study (MDRS): a prospective, observational, cohort study of patients attending two hospital diabetes clinics over 24 months. The study aimed to describe the prevalence, incidence and progression of diabetic retinopathy in Southern Malawi and to investigate the determinants of retinopathy severity and progression in this population. Baseline data from the cohort has been published [14].

**Materials and Methods**

**Setting**

Queen Elizabeth Central Hospital (QECH) in Blantyre is the main teaching hospital in Malawi. It provides primary and secondary care to the urban and semi-urban population of greater Blantyre (approximately 1.0 million people, 50% adult), and tertiary care to the southern region. Zomba Central Hospital (ZCH) provides primary and secondary care to Zomba district. The diabetes clinics at QECH and ZCH are the only public sector diabetes clinics in Blantyre and Zomba with around 2000 and 250 registered patients, respectively. The clinics provide free consultation and monitoring (measurement of height and weight, blood pressure and fasting blood sugar). Medications regularly available free of charge are metformin, glibenclamide and insulin (lente and soluble) as well as a limited range of anti-hypertensives.

**Participants**

Patient selection has been described elsewhere [14]. Briefly, systematic random sampling was used to select subjects from the diabetes clinics at QECH and ZCH between December 2011 and May 2012. Patients attend these clinics for medical management of diabetes; no eye care is provided. The inclusion criterion was a diagnosis of diabetes according to American Diabetes Association criteria [15]. Exclusion criteria were age <18 years and diagnosis of gestational diabetes according to American Diabetes Association criteria. The diabetes clinics at QECH and ZCH provide predominantly primary diabetes care (primary care for diabetes is non-existent at health centre level). Central hospitals are tertiary centres which receive referral cases. In order to effectively exclude referral cases, patients living more than 60km from the clinic in question and those visiting the clinic for the first time were excluded from the study.

**Procedures**

Following assessment at baseline, subjects were recalled (by telephone or home visit) at 12 and 24 months. Clinical assessment of subjects in the MDRS has been described elsewhere [14]. Briefly, visual acuity (uncorrected and using pinhole) was measured as the number of letters read on a standard Early Treatment of Diabetic Retinopathy Study (ETDRS) chart. Moderate visual impairment (50 to 59 letters; equivalent to 6/24 Snellen) and severe visual impairment or blindness (<50 letters; equivalent to 6/36 or worse) were defined according to the WHO [16]. For each patient with corrected visual acuity in the better eye of < 80 letters, the primary cause of visual impairment was recorded by the examining clinician (PB). Subjects were classified as having hypertension according to the WHO definition [11]: taking anti-hypertensive medication, or systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥ 90 mmHg. All subjects were offered point-of-care testing for HIV (Malawian national protocol [17]) and hemoglobin level. Thresholds for anemia were set according to WHO guidelines: 130 g/l for men; 120 g/l for women [18]. Blood samples were assayed for putative biochemical risk factors: fasting glucose, triglycerides, LDL cholesterol, HDL cholesterol, serum creatinine, urine albumin–creatinine ratio and HbA1c.

Retinopathy and maculopathy were classified by feature-specific grading using definitions established in the Liverpool Diabetic Eye Study [19] (Online Appendix Figure 1). Dual grading of digital photographic images of four 45° standard fields [19] was performed by accredited graders at the Liverpool Reading Centre. STDR was defined as any of the following: moderate pre-proliferative retinopathy or worse (level 40-71+); macular exudates in a circinate pattern or within one disc diameter of the foveal centre or clinically significant macular edema (CSME: ETDRS definition [20])(level 3–4: sight-threatening maculopathy); or other diabetes-related retinal vascular disease: central or branch retinal artery occlusion, central or branch retinal vein occlusion. Subjects who met thresholds for either scatter or macular laser treatment, were treated by one ophthalmologist (PB). Threshold for scatter laser treatment was the ETDRS ‘4-2-1’ rule (4 quadrants of hemorrhages/microaneurysms ≥ standard 2A, *or* 2 quadrants of venous beading ≥ standard 6A, or 1 quadrant of intraretinal microvascular abnormalities ≥ standard 8A). Threshold for macular laser was CSME as defined in the ETDRS (20) and visual acuity less than 85 ETDRS letters. The majority of deaths in Malawi are not registered. The relatives of deceased subjects were visited at home by a study nurse in order to confirm the death. Death was recorded if confirmed by a first degree relative or ‘Traditional Authority’ (village leader in rural districts).

**Statistical analysis**

Grades of retinopathy were calculated by patient according to the worse or only gradeable eye. Visual acuity data were investigated by patient according to the better eye. The primary outcome was progression of DR by 2 or more steps on the LDES severity scale (equates to either 1 step progression in both eyes *or* 2 step progression in one eye). We constructed a multiple logistic regression model (backwards stepwise with probability of removal of 0.2) to determine the odds ratio (OR) and 95% CIs for 2 step progression in association with an initial 12 variables: time since diagnosis of diabetes, type of diabetes, baseline grade of DR, mean HbA1c (mean of measurement at baseline, 12 and 24 months), sBP, urine albumin creatinine ratio (uACR), hemoglobin, high density lipoprotein (HDL) cholesterol, triglycerides, HIV status, age, and scatter laser treatment any time between baseline and 24 months. Descriptive analysis showed that uACR did not demonstrate a linear association with probability of 2 step progression; a logarithmic transformation (base 10) was more suitable. All tests were two-sided and a p value <0.05 was taken to indicate statistical significance. All calculations were performed using STATA version 12 (StataCorp, College Station, TX, USA). The study was approved by the University of Liverpool Research Ethics Committee and the University of Malawi College of Medicine Research Ethics Committee. All participants gave written informed consent.

**Results**

Of 357 subjects recruited 322 were seen for at least one further study visit and are included in the progression analysis below. 313 (88%) and 295 (83%) were assessed at 12 and 24 months, respectively (Online Appendix Figure 2). Median time to follow-up was 2.0 years (IQR 1.9-2.1). Baseline characteristics of subjects seen at 24 months and those who were not seen are shown in Table 1. 50 subjects (14.0%) were HIV positive (48 at baseline and 2 new diagnoses during the study). Incidence of death in the MDRS cohort at 24 months was 8.0% (95% CI 5.1-10.9; n=357; Life table method). Incidence of death amongst HIV positive subjects was 18.1% (7.4-28.8; n=50). Death during the MDRS was associated with STDR (OR 2.51; 95% CI 1.15-5.48; p=0.02; univariate analysis), PDR (OR 6.47; 2.51-16.7; p=0.0001), HIV (OR 3.72; 1.54-9.00; p=0.003) and moderate visual impairment (OR 8.21; 2.48-27.1; p=0.001). Two (or more) step progression (from baseline) was observed either at 12 or 24 months in 69 subjects (21.4%; 95% CI 16.9-25.9); three (or more) step progression in 30 subjects (9.3%; 6.1-12.5)(this analysis includes both subjects with no retinopathy and those with retinopathy at baseline) . Of 225 subjects without STDR at baseline 23 (10.2%; 6.3-14.2) developed the condition during the study (Table 2 and Online Appendix Tables 1, 2 and 3; Figure 1). Of 26 subjects with level 60 DR or above at baseline, at 24 months the following grades of DR were recorded: 7 subjects <level 60; 4 subjects level 60; 5 subjects >level 60. 8 subjects had died and 2 were lost to follow-up. Online Appendix Table 4 details the number of subjects who were listed for, started and completed a course of laser treatment during the course of the MDRS.

In univariate analysis 2 step progression of retinopathy was positively associated with duration of diabetes, baseline grade of DR, scatter laser treatment, HbA1c and uACR and negatively associated with HIV infection. Higher mean HbA1c and higher baseline grade of retinopathy were risk factors for 2 step progression in multivariate analysis. HIV infection was negatively associated with progression of DR (Table 3). This association may have been influenced by the high mortality in subjects with HIV and diabetes. We therefore performed a sensitivity analysis. The univariate association of HIV infection with a composite variable of either 2 step progression at 24 months or death during the study was not statistically significant (OR 0.84, 95%CI 0.41-1.75; p=0.64). HIV was not selected by a stepwise procedure for a multiple logistic regression model with the composite term as the dependent variable (data not shown).

127 subjects (43.0%) lost 5 or more ETDRS letters over the course of the study of which 17 subjects (5.8%) lost 15 or more letters. The most common primary causes of visual loss for the 127 subjects who lost five or more letters were DR (38.6%) cataract (16.5%), and both DR and cataract (3.9%). Incidence at 24 months of developing ‘moderate visual impairment’ (50 to 59 letters; equivalent to 6/24 Snellen) or ‘severe visual impairment or blindness’ (<50 letters; equivalent to 6/36 or worse) was 0.9% (0-2.0) and 1.5% (0.2-2.8), respectively (Life table method; n=322).

**Figure 1** Incidence at 2 years of sight threatening diabetic retinopathy (STDR) and proliferative diabetic retinopathy (PDR; level 60+) and of 2 (or more) step and 3 (or more) step progression on the LDES scale for subjects in the MDRS 24 month cohort with level 10 (n=177), level 20 (n=94), level 30 (n=25), level 40 (n=26) and level 50 (n=8) retinopathy at baseline. Error bars indicate 95% CI. Classes STDR, PDR, 2 step progression and 3 step progression are not exclusive i.e. a single subject can develop STDR and PDR and progress by 2 steps on the LDES scale.

**Discussion**

We report the first prospective longitudinal study of DR from sub-Saharan Africa. Incidence at 24 months of any DR (new DR) was 38.0%. Incidence of STDR for those with no (level 10), background (level 20) and mild pre-proliferative (level 30) retinopathy at baseline was 2.7%, 27.3% and 25.0%, respectively. Higher HbA1c and higher baseline grade of DR were risk factors for progression of retinopathy in multivariate analysis. HIV was negatively associated with progression. Despite the availability of laser treatment, 17 subjects (5.8%) had moderate visual loss (lost 15 or more letters) during the two years of the study.

Few high quality cohort studies are available for comparison from the African or Asian continents. In Mauritius researchers followed up a population based study performed in 1992 [21] with a survey of diabetes complications in 1998 [22]. The 6 year incidence of DR and PDR in subjects with diabetes but no DR in the first survey was 23.8% and 0.4%, respectively. The 6 year incidence of PDR in subjects with mild non-proliferative diabetic retinopathy (NPDR) (equivalent to level 20 in LDES grading) and moderate NPDR (equivalent to LDES level 30 or level 40) was 5.2% and 29.4%, respectively. Compared to recent studies of European screening programmes, in the MDRS 2 year progression to STDR from no DR (level 10) and from background DR (level 20) was approximately 3 times (2.7% vs estimates between 0.5% and 0.8% [2,23,24,25]) and 2.5 times higher (27.3% vs estimates between 6.4 [25] and 11.2% [2]), respectively.

Differences between our study and recent European work are likely to reflect disparities in diabetic care, ethnicity, access to health services and presence of comorbidities. The high crude mortality rate (8% over 2 years) in our cohort is comparable with data from Tanzania. In a prospective cohort study, McLarty et al [26] reported 5 year mortality of 40.5% in those with insulin dependent diabetes and 19.0% in subjects with non-insulin dependent diabetes. In contrast the UKPDS [27] reported all-cause mortality across all study participants at mean 10.0 years follow-up to be 17.9%. In the more recent ACCORD study [28] at mean 3.5 years follow-up all-cause mortality was 4.5%. The association of death in the MDRS with STDR suggests poor glycemic control and presence of other complications of diabetes. The high mortality indicates the need for improved diabetes care in sub-Saharan Africa, but in the context of our study is an important cause of data censoring. As diabetes care improves the prevalence of retinopathy may (paradoxically) go up due to case survival.

We report a negative association between DR progression and HIV infection: a novel finding. An important potential confounder of this relationship is early diagnosis of diabetes in HIV positive subjects already attending health facilities. Reduced progression may have been influenced by high mortality in subjects with HIV and diabetes removing subjects whose DR would have otherwise progressed. This possibility is supported by our sensitivity analysis: progression of retinopathy was not associated, in univariate or multivariate analysis, with a composite variable of either 2 step progression at 24 months or death during the study. Both HIV infection and anti-retroviral therapies are associated with a vasculopathy which manifests as increased cardiovascular and cerebrovascular risk [29,30]. Low grade proteinuria is highly prevalent in HIV positive patients taking ART and is more common in persons with concomitant diabetes [31]. A real negative association between HIV and DR is biologically plausible but this finding should be treated with caution.

In the MDRS 24 month cohort study lower hemoglobin was associated with presence of STDR at baseline (reported elsewhere [14]) but not with progression of DR. Cross sectional (but not cohort) studies have demonstrated an association between presence of DR and anemia in India [32-34] and China [35]. Potential confounders of the association between hemoglobin and retinopathy are socioeconomic and nutritional status and decreased erythropoietin production due to diabetic nephropathy. A plausible mechanism for the relationship is impaired oxygen delivery. Whether treatment of anemia reduces diabetic microvascular complications is not known. Iron supplementation has significant potential drawbacks in diabetes: both high iron level and iron supplementation have been associated with gestational diabetes [36,37]. To our knowledge no studies from Africa have reported longitudinal visual acuity (VA) data in subjects with diabetes. While the MDRS was an observational cohort study, subjects could take up medical interventions with potential for improvement of vision including laser photocoagulation and cataract surgery. Without these interventions visual loss is likely to have been greater.

We recognise the limitations of our clinic-based study. Barriers to attendance include transportation costs, competing economic tasks (planting and harvesting staple crops), and ignorance of health services. Patients who do not attend clinics may be less likely to be diagnosed with diabetes or to comply with therapy. Conversely those with established complications may be more likely to attend clinics and participate in research studies. The MDRS included few subjects with diet controlled diabetes because very few of these attend the diabetic clinic [14]. While some patients travel long distances to attend clinics, rural subjects are likely to represent a selected sub-group of the rural diabetes population. The prevalence of diabetes in Africa is increasing rapidly and there is an urgent need for service provision. Future studies must provide an evidence base for prevention, early detection and management programmes for DR in the region. Our findings represent a baseline against which the efficacy and cost-effectiveness of such interventions can be judged.

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analysis, decision to publish, or preparation of the manuscript.

**Duality of interest**

We declare no competing interests.

**Contribution statement**

PIB, MGF, GM, TA and SPH designed the 24 month prospective cohort study. PIB analysed the data with advice from MGF. GM and NAVB contributed to interpretation of results. PIB collected the data and wrote the report. All authors commented on and approved the report. PIB is guarantor.

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