Evaluation of Retinal Nerve Fiber Layer Thickness as a Possible Measure of Diabetic Retinal Neurodegeneration in the EPIC-Norfolk Eye Study

Sidra, Zafar[1], Kristen A Staggers[2], Jie Gao[3], Yao Liu[4], Praveen J Patel[5], Paul J Foster[5], Benjamin J Frankfort[3], Michael D Abramoff[6], Charles G Minard[2], Alasdair Warwick[7], Anthony P Khawaja[7*], Roomasa Channa[4*]

[1] Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD, USA

[2] Institute for Clinical and Translational Research, Baylor College of Medicine, Houston, TX, USA

[3] Department of Ophthalmology, Baylor College of Medicine, Houston, TX, USA

[4] Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, Madison, WI, USA

[5] NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust & UCL Institute of Ophthalmology, London, UK

[6] Department of Ophthalmology and Visual Sciences, University of Iowa, USA

[7]MRC Epidemiology Unit, University of Cambridge, Cambridge, UK

*RC and APK will be sharing senior authorship

Corresponding author: Roomasa Channa MD, Assistant Professor, Department of, Ophthalmology, UW-Madison, Madison, Wisconsin. Email: rchanna@wisc.edu

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Abstract

Background/Aims: Markers to clinically evaluate structural changes from diabetic retinal neurodegeneration (DRN) have not yet been established. To study the potential role of peripapillary retinal nerve fiber layer (pRNFL) thickness as a marker for DRN, we evaluated the relationship between diabetes, as well as glycemic control irrespective of diabetes status and pRNFL thickness.

Methods: Leveraging data from a population-based cohort, we used general linear mixed models (GLMM) with a random intercept for patient and eye to assess the association between pRNFL thickness (measured using GDx) and demographic, systemic, and ocular parameters after adjusting for typical scan score. GLMM were also used to determine: 1) the relationship between: a) glycated hemoglobin (HbA1c) irrespective of diabetes diagnosis and pRNFL thickness, b) diabetes and pRNFL thickness; and 2) which quadrants of pRNFL may be affected in participants with diabetes and in relation to HbA1c.

Results: 7,076 participants were included. After controlling for co-variates, inferior pRNFL thickness was 0.94 µm lower (95% CI: -1.28 µm, -0.60 µm), superior pRNFL thickness was 0.83 µm lower (95% CI: -1.17 µm, -0.49 µm), and temporal pRNFL thickness was 1.33 µm higher (95% CI: 0.99 µm, 1.67 µm) per unit increase in HbA1c. Nasal pRNFL thickness was not significantly associated with HbA1c (p=0.23). Similar trends were noted when diabetes was used as the predictor.

Conclusion: Superior and inferior pRNFL thinning was significantly thinner among those with higher HbA1c levels and/or diabetes, representing areas of the pRNFL that may be most affected by diabetes.

Precis

Our study showed that both diabetes and increasing HbA1c levels were independently associated with thinner superior and inferior pRNFL thickness measurements, after controlling for ocular and systemic confounders including glaucoma.

Introduction

Diabetic retinopathy (DR) is a leading cause of vision loss among working-age adults and represents a significant financial burden to healthcare systems worldwide.[1,2] The global prevalence of diabetes mellitus (DM) is projected to increase almost 54%, from 451 million in 2017 to 693 million in 2045.[3] The prevalence of DR is also projected to rise concurrently with the increase in DM prevalence. Thus, there is an urgent need to improve our understanding of the structural and functional changes that occur in DR, particularly for a relatively newly recognized condition known as diabetic retinal neurodegeneration (DRN).

While DR has classically been described as a retinal vasculopathy—with clinical guidelines for screening, classification, and management based on detecting and treating retinal vascular abnormalities—there is now increasing evidence from animal and human studies of an underlying neurodegenerative component.[4,5] These findings have spurred efforts that are underway to incorporate DRN into the DR severity score.[6] However, the best methods to quantify and monitor structural DRN have not been established, making it difficult to incorporate evaluation of DRN into routine clinical practice. One possible measure of DRN, peripapillary or macular RNFL thickness, has shown mixed findings in cross-sectional studies. Some studies report increased RNFL thickness,[7,8] others report decreased RNFL thickness,[9-14] and still others report no difference between participants with DM compared to controls.[15,16,17,18]

In addition, while the relationship between glycated hemoglobin levels (HbA1c) and retinal vascular abnormalities in DR has been well-established, studies examining the impact of HbA1c on RNFL thickness in DM have been mixed. Some authors report a negative correlation between HbA1c levels and RNFL thickness,[11,19,20] others report no correlation [8,21,22] and still others report a positive correlation.[10] The variability in findings could have resulted from a failure to adjust for multiple ocular and systemic comorbidities. In addition, many of these prior studies were limited by small sample sizes. To the best of our knowledge, the Maastricht Study has been the only study to include over a 1,000 participants.[23] Furthermore, to fully evaluate the impact of glycemic levels on RNFL thickness, the association between HbA1c and RNFL thickness should be evaluated irrespective of having a formal diagnosis of DM because up to half of patients with DM[24] may be unaware that they have DM.

In this study, we address these inconsistencies in the literature by assessing relationships between peripapillary RNFL thickness and HbA1c in the EPIC-Norfolk Eye Study, a population-based prospective study designed to assess visual health. Our objectives were to: 1) evaluate the relationship between HbA1c levels on peripapillary RNFL (pRNFL) thickness irrespective of DM diagnosis; and 2) compare differences in pRNFL thickness among those with and without known DM, while controlling for ocular and systemic comorbidities.

Materials and Methods

Study Population

The European Prospective Investigation of Cancer (EPIC) is a pan-European longitudinal prospective cohort study that began in 1989 to investigate diet, nutritional, lifestyle and environmental factors influencing the incidence of cancer and other chronic diseases. EPIC-Norfolk was one of the UK centers. This center recruited a total of 30,445 participants aged 40 to 79 years between 1993 and 1997 (1st study visit). Within this longitudinal cohort, the EPIC-Norfolk Eye Study evaluated 8,623 participants who completed eye examinations between 2004 and 2011 (only during the 3rd study visit).

Assessment of Ocular Measures and Conditions

Measurements of pRNFL were performed using the GDxVCC (Carl Zeiss Meditec, Inc., Dublin, CA), without pupil dilation. First, a corneal scan was taken, followed by the pRNFL scan. The software automatically delineated an annulus centered on the optic disc, with an inner and outer diameter of 2.4 and 3.2 mm, respectively. Only scans with a quality score of at least 7 were included in the analyses, typical scan score was adjusted linearly as described previously.[25] Average pRNFL thickness measures, as well as the pRNFL thickness measures in each of the 4 quadrants (superior, inferior, nasal and temporal), were computed.

Monocular visual acuity (VA) was measured using a LogMAR (Logarithm of the Minimum Angle of Resolution) chart (Precision Vision, LaSalle, Illinois, USA) on a light box under standard illumination. Measurements of refractive error were obtained as spherical and cylindrical power values derived from an autorefractor (Auto-Refractor 500, Humphrey Instruments, San Leandro, CA). Biometry was conducted using non-contact partial coherence interferometry (IOLMaster V.4, Carl Zeiss Meditech Ltd, Welwyn Garden City, UK). For each eye, five measurements of axial length, three measurements

of corneal curvature and one measurement of anterior chamber depth were taken. Axial length measurements were repeated if any measurement had greater than 0.1 mm difference from the others.

Intraocular pressure (IOP) was measured on the first 443 participants using an AT555 Non-Contact Tonometer (Reichert, New York, USA). Three readings were taken for each eye, and measures were repeated if more than 5 mm Hg different from the other two. For all subsequent participants, IOP was measured using the Ocular Response Analyzer (ORA, Reichert, New York, USA; software V.3.01) which takes into account corneal biomechanical factors in measuring intraocular pressure. Three readings were taken in each eye and ORA measurements with a poor-quality pressure waveform were repeated.

We identified participants with glaucoma, a possible confounder in the evaluation of pRNFL thickness, based upon participant self-reported history of glaucoma or glaucoma medication use.

Study Outcomes

Our primary aims were to assess the association between pRNFL thickness and: 1) HbA1c levels (including those with and without DM) and 2) diagnosis of DM. HbA1c values from the 1st (1993-1997), 2nd (1998-2000) and 3rd (2004-2011) study visits were used to calculate the average HbA1c value for each participant. Individuals were considered to have DM if they met one of the following criteria: self-reported history of diagnosis of DM, use of DM medications or having an average HbA1c ≥ 6.5%. Individuals missing information regarding DM diagnosis, or medication use were excluded from the analyses evaluating DM, but included in the analyses evaluating A1c level.

Statistical Analysis

Descriptive statistics of participant characteristics were stratified by the presence or absence of DM. For continuous variables including body mass index (BMI), blood pressure measurements, cholesterol, and triglyceride level, the average of the 1st, 2nd, and 3rd study visits were used. Patient summary statistics were compared using the independent t-test, Wilcoxon rank sum, Fisher's exact or Chi-square test. To account for the correlation between eyes within participants, generalized estimating equations (GEE)

with a logit link and exchangeable correlation were used to test whether eye characteristics differed between those with and without DM.

General linear mixed models (GLMM) with a random intercept for patient and eye were used to assess the association between pRNFL thickness and demographic, systemic, and ocular parameters after adjusting for typical scan score. Factors found to be significantly associated with pRNFL or DM at the $p<0.05$ level were considered for inclusion in a GLMM that would be used to test whether pRNFL was associated with DM status or HbA1c after adjusting for significant factors. To avoid multicollinearity, we checked for correlation between co-variates so that variables found to be correlated ($r > \pm 0.5$) were not included in the same model.[26] These multiple GLMMs also included the interaction between DM/HbA1c and the location of GDx measurement to test whether the association between DM/HbA1c and pRNFL depended on the location of GDx measurement. Backwards elimination was used to find a more parsimonious model. GLMMs for average pRNFL thickness were also examined, and a sensitivity analysis was conducted excluding participants with glaucoma, eyes with refractive error more than ±6 diopters or axial length > 26 mm. Stata version 15 (StataCorp LP, College Station, TX) was used for all analyses

Ethics and Institutional Review Board Approval

The EPIC–Norfolk Eye Study was carried out according to the tenets of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (identifier, 05/Q0101/191) and East Norfolk and Waveney National Health Service Research Governance Committee (identifier, 2005EC07L). All participants gave written informed consent.

Results

Participant Characteristics

A total of 7,076 participants (12,555 eyes) had GDxVCC image quality score of over 7 and were included in the study. Among these participants, 419 had DM and 6,656 did not have DM, 1 patient was missing information regarding self-reported DM or use of DM medication. This participant was not included in the DM analysis as it was not clear whether or not the participant had DM, but was included in the analyses looking at HbA1c (Tables 1a and 1b). Briefly, participants with DM were older ($p<0.001$), more likely to be

men (p<0.001), had lower total cholesterol (p<0.001), and had lower Mini-Mental State Examination (MMSE) scores (p=0.004). Those with DM had a higher BMI (p<0.001), systolic blood pressure (p<0.001), diastolic blood pressure (p= 0.003), triglycerides (p<0.001), and HbA1c (p<0.001). They were also more likely to have had eye surgery (p<0.001), to take anti-anginal (p<0.001), lipid lowering (p<0.001) or anti-hypertensive medications (p<0.001).

Association Between pRNFL Thickness and DM Status or HbA1c Levels

Factors significantly associated with thicker pRNFL thickness after adjusting for only typical scan score included: increased axial length (p=0.001), higher MMSE scores (p=0.007) and level of education (p=0.002). Factors associated with a thinner pRNFL included: higher age (p<0.001), higher systolic blood pressure (p<0.001), previous eye surgery (p<0.001), self-reported glaucoma or glaucoma medication use (p<0.001), relative with eye disease (p=0.026), use of anti-anginal medications, (p=0.006), anti-hypertensives (p<0.001), DM medications (p=0.04), and lipid-lowering medications (p<0.001) (Table 2). When all of these factors were included in a GLMM, some were no longer significant, and parsimonious models for the relationships between pRNFL thickness and DM status and HbA1c levels, respectively, are shown in Table 3. None of the covariates included in the final model were correlated.

After controlling for covariates, including age, gender, education level, GDx typical scan score, axial length, self-reported glaucoma or glaucoma medication, having a relative with eye disease and taking anti-hypertensive medications, the change in thickness per unit increase in HbA1c varied depending on the location of the pRNFL thickness (p<0.001) (Figure 1). 5,642 participants (9,938 eyes) were included in this model. On average, inferior pRNFL thickness was 0.94 µm lower (95% CI: -1.28 µm, -0.60 µm), superior pRNFL thickness was 0.83 µm lower (95% CI: -1.17 µm, -0.49 µm), and temporal pRNFL thickness was 1.33 µm higher (95% CI: 0.99 µm, 1.67 µm) per unit increase in HbA1c. Nasal pRNFL thickness measurements were not significantly associated with HbA1c (p=0.23). HbA1c was not associated with average pRNFL thickness (p=0.16).

The trends in sectoral pRNFL thickness measurements observed with DM status were similar to those observed with increasing HbA1c levels (Table 4). 5,712 participants (10,064 eyes) were included in this model. After controlling for the covariates mentioned

above and also HDL, sectoral thickness measurements for those with DM were 1.57 µm lower for the inferior pRNFL (95% CI: -2.41 µm, -0.74 µm), 1.07 µm lower (95% CI: -1.91 µm, -0.23 µm) for superior pRNFL and 1.35 µm higher (95% CI: 0.52 µm, 2.19 µm) for temporal RNFL compared to those without DM. Nasal pRNFL thickness was not associated with DM (p=0.26). Average pRNFL thickness was 0.62 µm lower (95% CI: -1.22 µm, -0.01 µm) among DM participants compared to those without DM.

We performed a sensitivity analysis excluding participants with self-reported glaucoma or glaucoma medication, and eyes with refractive error (spherical equivalent) more than ±6D, or axial length > 26 mm as these are potential confounders that may affect pRNFL thickness measurements. After adjusting for covariates in this subset of 6,616 participants (12,034 eyes), the association between HbA1c and pRNFL thickness still depended on location (p<0.001). Average changes in thickness per unit increase in HbA1c at each location were similar to those found when all participants were included (Supplementary Table 1a). After adjusting for covariates, the association between DM status and pRNFL thickness depended on location (p<0.001). The differences in average thickness measurements between participants with and without DM after adjusting for covariates were similar to those found when including all participants (Supplementary Table 1b). In addition, average pRNFL thickness remained not associated with HbA1c (p=0.10) after adjusting for covariates. On average, pRNFL thickness was 0.65 microns lower (95% CI: -1.25, -0.05) in DM participants compared to those without DM after adjusting for covariates.

Discussion

Our analysis of data from the EPIC-Norfolk Eye study showed that DM status and HbA1c levels, irrespective of known diagnosis of DM, were significantly associated with pRNFL thinning in the inferior and superior quadrants. This finding held true after controlling for glaucoma in the multivariable model and also in sensitivity analyses excluding participants with self-reported glaucoma, extremes of refractive error, and high myopia. Interestingly, temporal pRNFL thickness was higher among participants with DM than without DM. This could be due to macular changes such as clinical or sub-clinical macular edema impacting thickness measurements in that location.

Several pathophysiological mechanisms have been implicated in DRN including chronic hyperglycemia, oxidative stress, glutamate excitotoxicity and accumulation of

advanced glycation end products.[4 27] These metabolic alterations have been shown to cause disruption of the retinal neurovascular unit, ultimately resulting in retinal neuronal apoptosis. Studies from streptozocin rat models of DM suggest that neuronal apoptosis and retinal thinning occur before the development of microaneurysms.[28] . The loss of neural tissue which manifests as structural changes on OCT or scanning laser polarimetry may explain why functional deficits are present in participants with DM, even before the onset of vascular lesions.[4]

Our findings agree with prior studies demonstrating thinning of the neuroretina among patients with DM. Sohn et al reported an average decrease in thickness of neuroretinal rim of 0.54 µm/year (RNFL 0.25 µm/year & ganglion cell/inner plexiform layer 0.29 µm/year) in people with DM and no/minimal DR[29]. Our study showed that inferior and superior pRNFL thickness decreased on average 0.94 µm and 0.83 µm respectively per unit increase in HbA1c level, independent of age and self-reported glaucoma. Together, these findings highlight that damage to the neuroretinal tissue may be cumulative. If DRN were to progress linearly at a rate of 0.54 µm/year as reported by Sohn et al, over 10 years, it would result in a neuroretinal loss of 5.4 µm, a similar magnitude of damage to that seen in severe glaucoma.[30] Whereas in glaucoma, this loss is closely monitored and managed, in DM this loss may slowly progress unnoticed as it is not routinely evaluated or treated in the clinical algorithms for management of DR. Moreover, retinal neurons are associated with a phenomenon called “metabolic memory,” where early hyperglycemia is still harmful, irrespective of whether later glycemic control is improved,[31] further highlighting the need for assessing and managing DRN early in the disease course.

The results of our study have important implications for DM associated neurodegeneration. Recent studies have shown DM to be associated with abnormalities on brain MRI including regional reductions in brain volume in T1DM[32 33] and global brain atrophy in T2DM.[34] MRI imaging however more is expensive and time-consuming and less easily accessible than OCT. Hence, there may be a role for RNFL OCT imaging to serve as a potential biomarker for central nervous system (CNS) volume loss in the future, if not for clinical, then for research purposes. Moreover, our finding of decreased RNFL thickness among individuals with high HbA1c levels (irrespective of DM status) is similar to studies in the neurology literature. In these studies, high HbA1c levels were associated with increased rates of CNS neurodegeneration and decrease in memory

score among older adults without DM.[35 36] Proposed mechanisms underlying neuronal injury included increased formation of reactive oxygen species and advanced glycation end products in the setting of chronic hyperglycemia, which in turn promotes neuronal injury in the CNS.[35 36] We believe that a similar neurodegenerative mechanism also occurs in the eye, that eventually manifests as RNFL thinning.

Interestingly, the relationship between DM and open angle glaucoma remains an area of ongoing research. A recent UK Biobank analysis found that while DM resulted in increased corneal stiffness, true IOP was not higher in those with DM,[37] despite previous reports.[38-40] DM has also been shown not to be a risk factor for open-angle glaucoma in multiple population-based studies suggesting that the neurodegenerative changes observed in DM may be due to a distinct pathological process unrelated to glaucoma.[41] However, this area merits further research.

The effect of anti-hypertensive medications on neuronal health remains inconclusive. Preclinical models of neurodegenerative disease have demonstrated angiotensin converting enzyme inhibitors (ACEIs)[42] or beta-blockers[43] to be neuroprotective, whereas clinical studies primarily in the glaucoma literature have shown anti-hypertensive medication use to be both negatively and positively associated with glaucoma onset and/or progression.[44-46] Similar to the findings in our study, use of any anti-hypertensive medication was recently shown to be associated with thinner RNFL in a population-based analysis of the Singapore Epidemiology of Eye Diseases Study.[47] This association was most evident in participants using ACEIs or diuretics, and was independent of patient demographics, IOP and systemic risk factors including BP. An inverse U-shaped effect exists between BP status and structural OCT metrics, with both low and high BP associated with inner retinal layer thinning.[48] ACEIs and diuretics have been proposed to have differential effects on the ocular microvasculature structurally and functionally in terms of diurnal BP regulation compared to other classes of anti-hypertensive medications.[47] Moreover, only a small autoregulatory reserve is present in individuals with low BP, or in those with intensively treated arterial hypertension.[48] Low or unstable BP can subsequently result in low ocular perfusion pressure,[49] thereby increasing the risk of flow-mediated damage to RGCs. Considering that up to 40-60% of participants with DM may have concomitant hypertension,[50 51] the results of our study have important implications for the use of anti-hypertensive medications and retinal health, and highlight an area where further research is needed.

Our study has several strengths including using a population-based study design with a large sample size. Our analysis also accounted for a comprehensive panel of potential ocular and systemic confounders. This is important as the presence of confounders may either mask or potentiate the effect of DM or HbA1c levels on structural markers of DRN. We also report the impact of glycemic status, irrespective of known DM diagnosis, on RNFL thickness. Limitations of our study include that some participants may have had some level of DR which we are unable to account for as we did not have information regarding participants' DR status. Our study also uses GDxVCC which is an older imaging technology for measuring pRNFL thickness, which is now predominantly measured using spectral-domain optical coherence tomography (SD-OCT). However, studies have shown strong correlation between GDxVCC and SD-OCT measures of pRNFL thickness.[52 53]

In conclusion, our population-based study found that both DM and HbA1c levels (irrespective of DM diagnosis) were independently associated with thinner pRNFL thickness in the superior and inferior quadrants after controlling for multiple ocular and systemic confounding factors. Anti-hypertensive medication use was also associated with thinner pRNFL. These findings have important clinical implications. First, there is a need to expand the classification of the effects of DM on the retina beyond vascular retinopathy by integrating methods that assess structural and functional integrity of the neuroretina (i.e. neurodegeneration). Secondly, considering the high prevalence of systemic hypertension among those with DM, more research is needed to better understand the relationship between use of anti-hypertensive medications, BP, and retinal health. Additional studies are also needed to study the impact of RNFL thinning on function and whether these changes in pRNFL thickness are also reflected in other retinal areas, such as the macular RNFL and ganglion cell-inner plexiform layer thickness. Our study provides important information contributing towards an improved understanding regarding how to quantify neurodegenerative changes in DM.

Footnotes

Contributors: SZ contributed to data interpretation, initial drafting, critical review and final revision of the manuscript; KS contributed to data analysis and interpretation, critical review and final revision of the manuscript; JG, YL, PJF, BJF, MDA, CGM, APK

contributed to data interpretation, critical review and final revision of the manuscript; RC contributed to developing the study idea, designing the study, conducting the study, data analysis and interpretation, critical review and final revision of the manuscript

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Competing interests: APK has acted as a consultant to Abbvie, Aerie, Google Health, Novartis, Reichert, Santen and Thea.

Patient consent for publication: Not required.

Ethics approval: The EPIC–Norfolk Eye Study was carried out according to the tenets of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research Ethics Committee (identifier, 05/Q0101/191) and East Norfolk and Waveney National Health Service Research Governance Committee (identifier, 2005EC07L). All participants gave written informed consent.

Data availability statement: Data are available for approved research (see https://www.epic-norfolk.org.uk/).

Conflict of Interest: Anthony P Khawaja has acted as a consultant to Abbvie, Aerie, Google Health, Novartis, Reichert, Santen and Thea. The remaining authors have no conflicts of interest to disclose

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Tables

Table 1a. Baseline patient characteristics in the EPIC-Norfolk Eye Study stratified by diabetes status

Characteristic	No Diabetes	Diabetes	P-value [a ,b, c]
Age (years), Mean (standard deviation)	68 (7.7) **N=6656*	70.8 (7.2) **N=419*	<0.001
Gender, n (%) Female	3762/6656 (56.5)	188/419 (44.9)	<0.001
Education [d], n (%) O-level or higher	4035/6656 (60.6)	218/419 (52)	0.001
Ethnic origin, n (%) White	6620/6640 (99.7)	416/418 (99.5)	0.38
Self-reported glaucoma or use of glaucoma medications, n (%) Yes	254/6656 (3.8)	16/419 (3.8)	>0.99
Previous eye operation, n (%) Yes	977/6121 (16)	87/381 (22.8)	0.001
Cataract surgery, n (%) Yes	4/6070 (0.1)	0/376 (0)	>0.99
Any treatment or medication for any eye condition, n (%) Yes	611/6108 (10)	38/376 (10.1)	0.93
Family member with eye disease, n (%) Yes	1535/5623 (27.3)	79/337 (23.4)	0.13
Anti-anginal medications [e], n (%) Yes	190/6656 (2.9)	36/419 (8.6)	<0.001
Lipid lowering medications [e], n (%) Yes	1344/6656 (20.2)	254/419 (60.6)	<0.001
Anti-hypertensive medications [e], n (%) No	2323/6656 (34.9)	283/419 (67.5)	<0.001
Parkinson's medications [e], n (%) No	27/6656 (0.4)	1/419 (0.2)	>0.99
Body mass index (BMI) (kg/m^2), Median (minimum, maximum)	25.7 (16.1, 56.2) **N=6655*	28.6 (17.1, 54.4) **N=419*	<0.001

Systolic blood pressure (mmHg), Median (minimum, maximum)	132.5 (77.8, 216.2) *N=6656	138.2 (99.8, 178.2) *N=419	<0.001
Diastolic blood pressure (mmHg), Median (minimum, maximum)	79.7 (53.5, 113.5) *N=6656	81 (51.5, 121.3) *N=419	0.003
Cholesterol (mmol/l), Median (minimum, maximum)	5.8 (2.3, 11.3) *N=6537	5.4 (2.7, 9.3) *N=414	<0.001
HDL (mmol/l), Median (minimum, maximum)	1.4 (0, 3.2) *N=6533	1.2 (0.6, 2.3) *N=413	<0.001
LDL (mmol/l), Median (minimum, maximum)	3.6 (0, 8.7) *N=6528	3.1 (0, 5.9) *N=412	<0.001
Triglycerides (mg/dl), Median (minimum, maximum)	1.5 (0, 11.7) *N=6538	2.1 (0.6, 11.2) *N=414	<0.001
HbA1c (%), Median (minimum, maximum)	5.5 (3.2, 6.5) *N=6454	6.8 (3.5, 10.9) *N=412	<0.001
Short Mini Mental State Examination (MMSE), Median (minimum, maximum)	14 (0, 15) *N=6570	13 (2, 15) *N=412	0.004

[a] p-values for mean comparisons performed with t-test; [b] p-values for median comparisons using Two-sample Wilcoxon rank-sum (Mann-Whitney) test; [c] p-values calculated with exact testing for categorical variables when possible otherwise chi-square test; [d] O levels were taken at the age of 15/16 (generally at the end of compulsory schooling); [e] Self-reported medication use
*Due to missing values numbers for individual variables are presented
HDL=high density lipoprotein cholesterol, LDL=Low density lipoprotein cholesterol; HbA1c=Glycated Hemoglobin

Table 1b. Baseline ocular measures in the EPIC-Norfolk Eye Study stratified by diabetes status.

Ocular measures	No Diabetes	Diabetes	P-value [a]
GDx typical scan score, Median (minimum, maximum)	87 (0, 100) *N=11821	83 (0, 100) *N=733	0.37
Axial Length (mm), Median (minimum, maximum)	23.4 (16.1, 29.4) *N=10982	23.4 (18.1, 31.6) *N=678	0.93
Intraocular pressure, corneal compensated (mmHg), Median (minimum, maximum)	16.5 (4.3, 48.2) *N=10921	16.2 (6.7, 34.7) *N=667	0.70
Spherical Equivalent (Diopters), Median (minimum, maximum)	0.5 (-15.5, 7.4) *N=11732	0.4 (-7.3, 5.8) *N=727	0.76

[a] p-values from univariable generalized estimating equations; *Due to missing values numbers for individual variables are presented

Table 2. General Linear Mixed Model For pRNFL Thickness (adjusted for typical scan score)

	Coefficient	95% Confidence Interval		P-value
Age (10-year increase)	-1.31	-1.52	-1.10	<0.001
Axial length (mm)	0.28	0.12	0.43	0.001
IOP corneal compensated (mmHg)	-0.04	-0.08	0.00	0.063
Spherical equivalent (diopters)	-0.03	-0.11	0.05	0.452
Body mass index (kg/m^2)	-0.03	-0.07	0.02	0.23
Systolic blood pressure (mmHg)	-0.02	-0.03	-0.01	<0.001
Diastolic blood pressure (mmHg)	-0.02	-0.04	0.00	0.11
Cholesterol (mmol/L)	-0.01	-0.19	0.16	0.87
HDL (mmol/L)	0.02	-0.38	0.42	0.92
LDL (mmol/L)	0.04	-0.15	0.22	0.72
Triglycerides (mg/dL)	-0.12	-0.30	0.07	0.21

Short MMSE scores	0.13	0.04	0.22	0.007
Female	0.28	-0.03	0.60	0.080
O-level or higher education	0.52	0.20	0.84	0.002
Race/ethnic origin other than white	-1.19	-3.97	1.60	0.40
Glaucoma or glaucoma medication use	-4.60	-5.45	-3.74	<0.001
Previous eye operation	-0.83	-1.29	-0.37	<0.001
Cataract surgery	1.78	-4.87	8.44	0.60
Relative with eye disease	-0.44	-0.82	-0.05	0.026
Anti-anginal medications	-1.26	-2.15	-0.36	0.006
Diabetes medication	-0.87	-1.70	-0.04	0.040
Lipid lowering medications	-0.82	-1.20	-0.44	<0.001
Anti-hypertensive medications	-0.92	-1.25	-0.60	<0.001
Parkinson's medications	-1.26	-3.72	1.21	0.32

HDL=high density lipoprotein cholesterol, LDL=Low density lipoprotein cholesterol, MMSE= Mini Mental State Examination; pRNFL=peripapillary retinal nerve fiber layer thickness

Table 3: Results From Two General Linear Mixed Models With pRNFL Thickness As The Dependent Variable

	Model 1 with HbA1c as the independent variable (5,642 participants; 9,938 eyes)					***Model 2 with Diabetes as the independent variable (5,712 participants; 10,064 eyes)***			
	Coefficient	**95% Confidence Interval**		**P-value**		**Coefficient**	**95% Confidence Interval**		**P-value**
Location				<0.001	**Location**				<0.001
Inferior	Reference				Inferior	Reference			
Nasal	-30.62	-32.98	-28.26	<0.001	Nasal	-24.31	-24.55	-24.06	<0.001
Superior	-0.70	-3.06	1.66	0.56	Superior	-0.08	-0.33	0.16	0.51
Temporal	-47.57	-49.93	-45.21	<0.001	Temporal	-35.17	-35.41	-34.92	<0.001
HbA1c (%)	-0.94	-1.28	-0.60	<0.001	**Diabetes**	-1.57	-2.41	-0.74	<0.001
HbA1c - location interaction				<0.001	**Diabetes – location interaction**				<0.001
HbA1c * Inferior	Reference				Diabetes * Inferior	Reference			
HbA1c * Nasal	1.15	0.72	1.57	<0.001	Diabetes * Nasal	1.09	0.04	2.13	0.041
HbA1c * Superior	0.11	-0.31	0.53	0.61	Diabetes * Superior	0.51	-0.54	1.55	0.34
HbA1c * Temporal	2.27	1.85	2.69	<0.001	Diabetes * Temporal	2.93	1.88	3.98	<0.001
GDx typical scan score	-0.16	-0.16	-0.15	<0.001	**GDx typical scan score**	-0.16	-0.16	-0.15	<0.001
Axial length (mm)	0.17	0.05	0.29	0.006	**Axial length (mm)**	0.18	0.06	0.30	0.004
HDL (mmol/L)					**HDL (mmol/L)**	-0.36	-0.70	-0.01	0.043
Glaucoma	-3.65	-4.33	-2.96	<0.001	**Glaucoma**	-3.74	-4.42	-3.06	<0.001
Relative with eye disease	-0.59	-0.87	-0.31	<0.001	**Relative with eye disease**	-0.60	-0.87	-0.32	<0.001
Anti-hypertensive medication	-0.34	-0.60	-0.07	0.014	**Anti-hypertensive medication**	-0.34	-0.61	-0.08	0.012
Age (10-year increase)	-1.14	-1.32	-0.96	<0.001	**Age (10-year increase)**	-1.12	-1.30	-0.94	<0.001
Female	0.29	0.04	0.55	0.026	**Female**	0.42	0.14	0.70	0.004
O-level or higher education (reference no)	0.30	0.05	0.56	0.021	**O-level or higher education** (reference no)	0.32	0.06	0.57	0.017

HDL = high density lipoprotein cholesterol; HbA1c= Glycated Hemoglobin; pRNFL=peripapillary retinal nerve fiber layer

Table 4. Average difference in peripapillary RNFL (pRNFL) thickness at each location among participants with and without diabetes (DM) after adjusting for co-variates

	Average pRNFL thickness (DM)	**Average pRNFL thickness (Non-DM)**	**Average difference**	**95% Confidence Interval for the average difference**		**p-value**
Average pRNFL thickness of all four quadrants (um)	56.01	56.63	-0.62	-1.22	-0.01	0.046
pRNFL thickness of **inferior** quadrant (um)	64.74	66.31	-1.57	-2.41	-0.74	<0.001
pRNFL thickness of **nasal** quadrant (um)	41.52	42.01	-0.49	-1.32	0.35	0.26
pRNFL thickness of **superior** quadrant (um)	65.16	66.23	-1.07	-1.91	-0.23	0.013
pRNFL thickness of **temporal** quadrant (um)	32.50	31.15	1.35	0.52	2.19	0.002

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