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Association of visual function and ganglion cell layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy

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Abstract

Diabetic retinopathy (DR) classically presents with micro-aneurysms, small haemorrhages and/or lipoprotein exudates. Several studies have indicated that neural loss occurs in DR even before vascular damage can be observed. This study evaluated the possible relationship between structure (spectral domain- optical coherence tomography) and function (Rarebit visual field test) in patients with type 1 diabetes mellitus and no or minimal diabetic retinopathy (DR). Results demonstrated loss of macular visual function and corresponding thinning of the ganglion cell layer (GCL) in the pericentral area of the macula of diabetic patients ($R_s = 0.65$, $p < 0.001$). In multivariable logistic regression analysis, GCL thickness remained an independent predictor of decreased visual function (OR 1.5, 95% CI 1.1 – 2.1). Early DR seems to include a neurodegenerative component.

Keywords

Diabetes Mellitus; Neurodegeneration; Ganglion cells; Visual function test; Retina

Introduction

One of the most frequent causes of blindness among adults in the Western world is diabetic retinopathy (DR) (Fong *et al.*, 2004). The clinical hallmarks of DR are primarily vascular, including microaneurysms, hemorrhages, capillary occlusions, and lipoprotein exudates. In addition to vascular changes, neurodegenerative changes have been described including neural apoptosis, loss of ganglion cell bodies, glial reactivity and reduction in thickness of the inner retinal layers in the earliest stages of DR (Abu-El-Asrar *et al.*, 2004; Antonetti *et*

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et al., 2006; Barber *et al.*, 1998; Barber, 2003; Barber *et al.*, 2005; Gardner *et al.*, 2002; Gastinger *et al.*, 2006; Gastinger *et al.*, 2008; Kern and Barber, 2008; Li and Puro, 2002; Martin *et al.*, 2004; Park *et al.*, 2003; Rungger-Brandle *et al.*, 2000). These findings of structural neuropathy may explain the neuroretinal functional deficits that are known in patients with diabetes, even before the presence of frank retinopathy. Several studies have shown electroretinogram abnormalities, loss of dark adaptation and contrast sensitivity and colour vision disturbances independent of vascular retinopathy (Bears, Jr. *et al.*, 2006; Bronson-Castain *et al.*, 2009; Di Leo *et al.*, 1992; Dosso *et al.*, 1996; Hardy *et al.*, 1992; Kurtenbach *et al.*, 2002; Lopes de Faria *et al.*, 2001; Ng *et al.*, 2008; Realini *et al.*, 2004).

Conventional threshold perimetry and visual function tests are insensitive measures of minor neuro-visual damage (Frisen and Quigley, 1984; Kerrigan-Baumrind *et al.*, 2000). The Rarebit technique, which includes Rarebit Perimetry (RBP) and the Rarebit Fovea Test (RFT), was developed to improve detection of subtle defects (Frisen, 2002). The Rarebit technique is based on the principle of detection of very small and bright stimuli. The small stimulus corresponds to half the minimum angle of resolution at the tested retinal location. The test avoids simultaneous stimulation of more than one receptive field, defined as the group of photoreceptors converging on the same ganglion cell (Fischer, 1973). In a previous study employing the Rarebit technique, Nilsson *et al.* detected foveal dysfunction in patients with diabetes mellitus type 1 without DR (Nilsson *et al.*, 2007).

With optical coherence tomography (OCT) the retinal thickness (RT) can be measured with high accuracy. The retinal thickness in diabetic patients with no or minimal DR is thinner than in normals. (Asefzadeh *et al.*, 2008; Bialosterski *et al.*, 2007; Bronson-Castain *et al.*, 2009; Browning *et al.*, 2008; Nilsson *et al.*, 2007; Oshitari *et al.*, 2009; Van Dijk *et al.*, 2009). The high resolution of spectral domain-OCT (SD-OCT) allows measurement of the thickness of all individual retinal layers (Garvin, 2008), especially if the layers are segmented automatically in three-dimensions (Garvin *et al.*, 2009). Results of a recent study showed that the decreased total RT in type 1 diabetic patients with minimal DR is predominantly caused by a thinning of the ganglion cell layer (GCL) in the pericentral area and retinal nerve fiber layer (RNFL) thinning in the peripheral area of the of the macula (Van Dijk *et al.*, 2010), i.e. both axons and nerve bodies are involved in thinning.

The purpose of the present study is to evaluate the hypothesis that GCL thickness measured with SD-OCT and the function of the macula tested with the Rarebit technique are associated in patients with type1 diabetes mellitus (DM) and no or minimal DR.

Materials and Methods

Patients

Patients with type 1 DM were recruited from the outpatient clinic of the department of Internal Medicine at the Academic Medical Center (AMC University Hospital, Amsterdam, the Netherlands) for an ongoing longitudinal observational study. In September 2008 they were invited to participate in this observational cross-sectional study. Additionally, healthy age-matched individuals served as controls. The study adhered to the tenets of the Declaration of Helsinki, and Institutional Review Board approval was obtained at both the AMC and the University of Iowa. All subjects provided written informed consent.

DR status was evaluated by a retinal specialist through indirect funduscopy, slit-lamp stereo biomicroscopy and stereoscopic fundus photography. Patients were included if they were diagnosed with minimal or no DR. The definition of minimal DR was conform stage 2 of the International Clinical Diabetic Retinopathy Disease Severity Scale (Wilkinson *et al.*, 2003). Control subjects did not have a diagnosis of any ocular disease, diabetes or other systemic

disease, and were randomly recruited from accompanying persons of patients visiting the outpatient clinic of the department of Ophthalmology. Exclusion criteria were refractive errors over S+5, or under S-8 diopters, visual acuity below 20/25, significant media opacities, previous ocular surgery and a previous diagnosis of glaucoma, uveitis, or retinal disease.

Age, gender, duration since diagnosis of diabetes and serum glycosylated hemoglobin (HbA1c) at the time of the study examinations were gathered from the patient charts. Best corrected visual acuity was obtained conform the Early Treatment Diabetic Retinopathy Study, and recorded as Snellen equivalent. All subjects underwent Rarebit Perimetry and the Rarebit Fovea Test (Frisen, 2002). Finally, all subjects underwent papillary dilatation and an ophthalmic examination including slitlamp biomicroscopy with a handheld lens (SuperField; Volk Optical, Inc., Mentor, OH), OCT imaging (3D OCT-1000, Topcon Corporation, Tokyo, Japan) and stereoscopic fundus photography (TRC-50IX; Topcon Corporation, Tokyo, Japan).

Rarebit Perimetry and Rarebit Fovea Test

The RBP and the RFT form a computerized visual function test developed to detect subtle damage to the visual system (Frisen, 2002). The RBP evaluates the central 30° visual field, while the RFT evaluates the central 4° visual field. The test principle is to briefly (200 ms) present zero, one, or two, bright small (<0.5 min of arc) dots against a dark background in a completely dark room. Due to photopic luminance levels for both the fixation mark and the test targets, dark adaptation is not of influence for test results. The subjects are asked to focus on the fixation mark and meanwhile respond by clicking a mouse button once or twice when they detect one respectively two dots anywhere on the screen. The result of the Rarebit test is presented as mean hit rate (MHR). The MHR is a percentage of the stimuli seen by the subject of all presented stimuli. In this study we combined the RFT and RBP and present a combined MHR. The combined MHR is abnormal if below 95% (Malmer and Martin, 2005; Salvetat *et al.*, 2007).

Optical coherence tomography imaging and layer segmentation

OCT images of the subjects were obtained with SD-OCT (3D OCT-1000, Topcon Corporation, Tokyo, Japan) using the 3D volume scan protocol ($6 \times 6 \times 2.2 \text{ mm}^3$), consisting of 128 (y) by 512 (x) by 650 (z) voxels. From this volume, nine intraretinal surfaces defining 8 retinal layers were segmented automatically by our algorithm, which uses an extensively validated, robust fully three-dimensional graph search approach (Garvin *et al.*, 2009). The 8 layers were interpreted as follows (from inner to outer surface): 1/ retinal nerve fiber layer (RNFL), 2/ ganglion cell layer (GCL), 3/ inner plexiform layer (IPL), 4/ inner nuclear layer (INL), 5/ outer plexiform layer (OPL), 6/ outer nuclear layer (ONL) + inner segments (photoreceptors) (IS), 7/ outer segments (photoreceptors) (OS), 8/ retinal pigment epithelium (RPE) (Figure 1).

The pericentral area of the macula - a donut shaped ring centered on the fovea with an inner diameter of 1 mm - was defined by one of the authors (HvD), who was masked for the DR status and demographic features of the patients and controls. The mean thickness of each layer in the pericentral area was automatically calculated with the computer program ImageJ 1.41 (Abràmoff *et al.*, 2004).

Statistical analysis

For the statistical analyses SPSS 16.0.2 for Windows (SPSS, Chicago, IL) was used. An independent t-test was used to assess differences in mean age and MHR between diabetic patients and controls. A Chi-square test was used to compare distribution of gender between

patients and controls. Mean HbA1c, age, duration of diabetes and mean pericentral GCL thickness of diabetic patients with subnormal MHR and diabetic patients with normal MHR were compared using the independent t-test. The presence or absence of DR was compared between patients with subnormal and normal MHR using the Chi-square test. The possible correlation between MHR and pericentral GCL and INL thickness was analyzed using the Spearman rank test. Multivariable logistic regression analyses were performed to identify independent predictors of subnormal MHR. Confidence intervals were computed at the $p = 0.05$ level.

Results

Thirty-two patients type 1 diabetes with no or minimal DR and 38 controls were included. There was no significant difference in age and gender between patient groups and controls. Patients were in reasonable glycemic control (mean HbA1c = 8.1%; SD = 1.4).

The mean MHR of patients with DM and controls were 93.5 ± 5.3 and 97.1 ± 2.8 , respectively. The mean MHR of the patient group was significantly decreased compared with the control group (95% CI of the difference 1.6 – 5.6).

The subjects with subnormal MHR had significantly longer duration of DM and were older than subjects with normal MHR (see Table 2). HbA1c did not differ significantly between the groups. Although the number of subjects with minimal DR differed substantially between the patient group with subnormal MHR and normal MHR this difference was not significant (Chi-square, $p = 0.08$). The patients with subnormal MHR had significantly lower pericentral GCL and INL thickness (see Table 3). The remaining retinal layers did not differ significantly between patients with normal and subnormal MHR.

In the population of 32 DM patients, both the pericentral GCL thickness ($R_s = 0.65$, $p < 0.001$) and the pericentral INL thickness ($R_s = 0.41$, $p = 0.02$) showed a significant correlation with the MHR. However in logistic regression analysis with GCL and INL thickness as variables, the GCL thickness was the strongest independent predictor of subnormal MHR (OR 1.37, 95% CI 1.05 – 1.79) compared to the INL thickness (OR 0.99, 95% CI 0.72 – 1.37). Subsequently the GCL thickness remained the only independent predictor of subnormal MHR (OR 1.5, 95% CI 1.1 – 2.1) in a second multivariable logistic regression analysis with GCL thickness, duration of DM, DR status, HbA1c and age as variables. Duration of DM (OR 1.0, 95% CI 0.8 – 1.1), DR status (OR 0.7, 95% CI 0.04 – 10.9), HbA1c (OR 2.5, 95% CI 0.8 – 7.6) and age (OR 0.9, 95% CI 0.8 – 1.0) had no predictive value for subnormal MHR. A scatter-plot of GCL thickness versus MHR is shown in Figure 2.

Discussion

These results support our hypothesis that GCL thickness as measured with SD-OCT and the macular sensitivity as tested with the Rarebit technique are associated. In fact, decreased GCL thickness in the pericentral area of the macula correlates highly with MHR, and is the only significant predictor of subnormal MHR, independent of other known variables. To our knowledge, this study is the first to describe the possible link between structure and function in type 1 diabetes patients.

The results support the early neurodegenerative effect of diabetes on the retina. The ganglion cells seem to be most vulnerable to diabetes related degeneration (Abu-El-Asrar *et al.*, 2004; Antonetti *et al.*, 2006; Barber *et al.*, 1998; Barber, 2003; Barber *et al.*, 2005; Gardner *et al.*, 2002; Gastinger *et al.*, 2008; Kern and Barber, 2008; Martin *et al.*, 2004). This study shows a decreased MHR in diabetes patients due to loss of receptive fields which

is possibly caused by apoptosis of ganglion cells throughout the retina. However, the question remains whether the function decrease is directly and solely caused by the ganglion cell loss or whether the ganglion cell loss is a proxy for a more general neuronal loss. In this study the INL was also significantly decreased in thickness. All retinal neurons - photoreceptors, bipolar cells, horizontal cells, amacrine cells and ganglion cells - are involved in the transmission of visual signals to the brain (Masland, 2001). Degeneration of the neuroretinal system is associated with architectural defects such as loss or disconnection of retinal ganglion cells or upstream connections. The results of the Rarebit test in type 1 diabetes patients indicate that such architectural defects may produce the small visual field defects as observed in this study (Frisen, 2002).

Both foveal dysfunction and GCL thinning in patients with type 1 diabetes have been described previously (Nilsson *et al.*, 2007; Van Dijk *et al.*, 2009; Van Dijk *et al.*, 2010). In glaucoma, previous studies attempted to understand how functional losses may relate to structural damage. Glaucoma is an optic neuropathy characterized by a loss of retinal ganglion cells leading to characteristic changes in the structure of the optic disc and in loss of visual fields, as measured with standard automated perimetry. Recent studies detected macular thinning of the ganglion cell layer in glaucoma patients using SD-OCT. The location of ganglion cell loss colocalized with the visual field loss. The most severe loss of ganglion cells was seen in patients with the most severe loss of visual field loss. Significant thinning of the GCL could be detected in pre-perimetric glaucoma patients. These studies confirm the relationship between structural loss of ganglion cells and functional loss (Hood and Kardon, 2007; Strouthidis *et al.*, 2006; Tan *et al.*, 2008; Tan *et al.*, 2009; Wang *et al.*, 2009). In patients with DM the loss of ganglion cells is much less pronounced, and in general will not lead to frank visual field loss. The Rarebit technique tests visual function differently and is sensitive to local loss of single receptive fields.

The present study has some pitfalls. The grading of the severity of DR was done by a single reader through ophthalmologic examination and evaluation of a single set of stereoscopic central retinal photographs, instead of the gold standard, i.e. 7 field stereoscopic fundus photography assessment by independent trained graders (Early Treatment Diabetic Retinopathy Study Research Group, 1991). However, the patients definitely did not have advanced retinopathy, indicating that the results do apply to the earliest stage of DR.

Type 2 diabetes is often subclinical. It cannot be excluded that some controls actually had diabetes type 2 and elevated blood sugar levels because the HbA1c of the normal subjects was not known. The prevalence of diabetes at this age is however low. The presence of undiagnosed diabetes would most likely lead to underestimation of the difference in retinal layer thickness between patients and controls instead of overestimation.

Furthermore, the number of included patients in this study is low. Due to low power it can not be ruled out that some of the variables in the logistic regression model would have been significantly correlated with the decrease of MHR if the number of patients was higher. In a previous study concerning GCL thinning in diabetic patients, the presence of early vasculopathy was the most important explanatory variable for the observed decrease in GCL thickness. The same study showed that the duration of DM was significantly correlated with GCL thickness (Van Dijk *et al.*, 2010). Studies by Nilsson *et al.* and Salvetat *et al.* both previously showed that age correlated negatively with MHR (Nilsson *et al.*, 2006; Salvetat *et al.*, 2007). However the fact that in this study solely the GCL remains an independent predictor in the multivariable logistic regression model despite the small number of included patients shows that the correlation between the GCL thinning and the visual function is strong.

In summary, this study demonstrates subtle loss of macular visual function and corresponding loss of thickness of the GCL in the pericentral area of the macula in type 1 diabetic patients. These results support the concept that early DR includes a neurodegenerative component. Though larger studies are needed, the hypothesis of diabetes causing a retinal neuropathy independent of vascular retinopathy is intriguing, and potentially links retinal neuropathy to other diabetic neuropathies.

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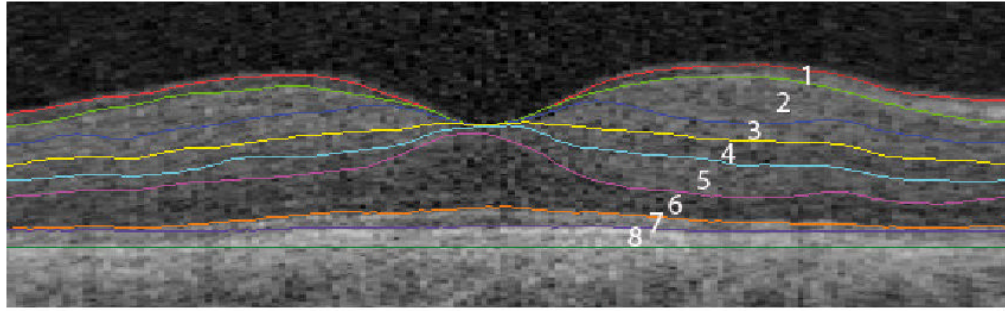


Figure 1.

The intraretinal surfaces in a macular B-scan as indicated by the colored lines and the corresponding retinal layers in figures. The retinal surfaces are segmented fully automatically using an inherently 3D approach.

1/ retinal nerve fiber layer, 2/ ganglion cell layer, 3/ inner plexiform layer, 4/ inner nuclear layer, 5/ outer plexiform layer, 6/ outer nuclear layer + inner segments, 7/ outer segments, 8/ retinal pigment epithelium.

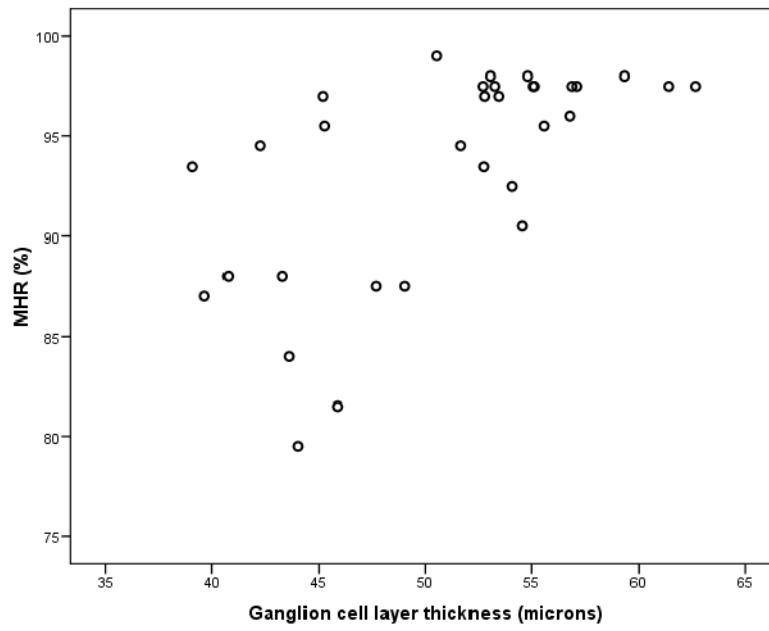


Figure 2. Scatter-plot of pericentral GCL thickness versus MHR in type 1 diabetes patients

Table 1

Demographics of patients with type 1 diabetes and controls.

Parameters	DM (n=32)	Controls (n=38)
Age (yrs)	34 ± 10	35 ± 11
Gender (M:F)	12 : 20	19 : 19
HbA1c (%)	8.1 ± 1.4	-

Values are the mean ± standard deviation for all subjects in each group. DM, diabetes mellitus; -, not performed; HbA1c, glycosylated hemoglobin; MHR, mean hit rate.

Duration of diabetes, age, HbA1c and retinopathy status in patients with type 1 diabetes with subnormal MHR compared to patients with normal MHR.

Table 2

Parameters	Subnormal Rarebit (n=14)	Normal Rarebit (n=18)	Mean difference	95% CI of the difference	
				Lower	Upper
Duration of DM (yrs)	24.6 ± 9.9	16.7 ± 5.3	7.9	2.3	13.4
Age (yrs)	39.8 ± 9.3	30.1 ± 7.9	9.6	3.4	15.8
HbA1c (%)	7.8 ± 1.3	8.4 ± 1.5	-0.59	-1.6	0.5
Minimal DR (%)	64%	33%	NA	NA	NA

Values are the mean ± standard deviation. CI, confidence interval; DM, diabetes mellitus; DR, diabetic retinopathy; HbA1c, glycosylated hemoglobin.

Table 3

Mean layer thickness measurements (microns) of the individual intraretinal layers in the pericentral area of the macula in patients with type 1 diabetes with subnormal Rarebit MHR compared to patients with normal Rarebit MHR.

Parameters	Subnormal Rarebit (N=14)	Normal Rarebit (N=18)	Mean difference	95% CI of the difference	
				Lower	Upper
RNFL	20.3 ± 1.8	21.4 ± 1.9	1.1	-2.4	0.3
GCL	46.3 ± 5.4	54.5 ± 4.6	8.2	-11.8	-4.6
IPL	37.0 ± 4.4	38.1 ± 2.9	1.1	-3.8	1.4
INL	37.3 ± 4.7	40.8 ± 3.4	3.5	-6.4	-0.6
OPL	29.9 ± 4.8	28.3 ± 2.7	-1.6	-1.1	4.4
ONL + IS	86.9 ± 13.0	93.3 ± 9.0	6.4	-14.4	1.5
OS	27.2 ± 2.0	26.2 ± 1.7	-1.0	-0.2	2.4
RPE	38.6 ± 1.8	39.6 ± 1.4	1.0	-2.1	0.2

Values are the mean ± standard deviation for all subjects in each group. The bold values indicate a statistically significant difference between patients with normal and subnormal rarebit Test. DR, diabetic retinopathy; CI, confidence interval; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segments; OS, outer segments; RPE, retinal pigment epithelium.