

Correlation of Retinal Vessel Analysis and Nerve Fiber Layer Thickness in Normal Tension Glaucoma

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Abstract

Background: To investigate the retinal vessel diameter and its possible relationship with the retinal nerve fiber layer (RNFL) thickness in normal tension glaucoma (NTG).

Methods: 86 NTG patients with detailed eye examination including retinal vessel analysis (DVA) and measurement of the RNFL thickness with scanning laser polarimetry (SLP; GDxVCC) were included in this retrospective study. The temporal retinal arteriolar diameters, temporal retinal venular diameters, vascular flicker response were compared with RNFL thickness and NFI (nerve fiber index).

Results: The flicker response of DVA was significantly reduced as compared to normal eyes. The diameters of the temporal retinal arteriolar vessels were significantly correlated with RNFL thickness ($P=0.0204$) and NFI of GDxVCC ($P=0.0021$). The diameters of the temporal retinal venular were significantly correlated with the NFI ($P=0.0298$).

Conclusion: Our results show that narrower arteriolar vessels are found in advanced NTG patients. These findings may be due to the decreased demand for retinal blood flow in damaged retina, but also may be one reason for the damage. Vessel analysis can contribute to the available tools predicting glaucomatous damage before visual field loss becomes manifest.

Keywords: Glaucoma; Dynamic vessel analysis (DVA); Retinal nerve fiber layer (RNFL); Autoregulation; Nerve fiber indicator (NFI); Scanning laser polarimetry.

Introduction

Normal tension glaucoma (NTG) is a disease that has shattered the traditional concept of the pathogenesis of glaucoma as a neuropathy of the optic nerve caused primarily by an elevated intraocular pressure (IOP). That outdated concept described IOP values above 21 mm Hg as pathological while an IOP below 19 mm Hg was considered normal. In its clinical appearance, NTG almost perfectly mimics primary open-angle glaucoma (POAG): normal angles on gonioscopy, cupping of the optic nerve, and visual field loss correlated with cupping that shows the progressive damage of the optic nerve [1]. In East Asia, NTG seems to be more frequent than POAG with high IOP [2] and it is certainly the most common form of glaucoma in Japan [3].

The fact that glaucomatous damage occurs and progresses despite IOP levels that have traditionally been regarded as "safe" has led to suspect other pathogenetic mechanisms, mainly vascular factors. Flammer et al. have described a reduction of ocular blood flow (OBF) caused by vascular dysregulation, a distinct pathological entity that leads to both low perfusion pressure and insufficient autoregulation, as a cause of glaucomatous optic neuropathy (GON). These disturbances of ocular blood flow tend to be more common in individuals with normal tension glaucoma and may lead to unstable ocular perfusion

and thereby to ischemia and reperfusion damage [4]. A clinical sign frequently associated with NTG is arterial hypotension, particularly nocturnal dips of blood pressure [5]. Twenty-four hour blood pressure (BP) monitoring in NTG patients revealed lower BP than in normals with the greatest differences occurring in nocturnal systolic and diurnal diastolic values [6].

Assessing ocular blood flow can prove crucial in diagnosing and possibly treating NTG, its measurement, however, is not easy. As Mi et al. [1] have pointed out there is so far not a single comprehensive technique to assess OBF. Different measurements provide different details of vascular parameters, and can be interpreted differently. Over the last couple of years dynamic vessel analysis (DVA) emerged as a method to gather objective and quantitative data on the efficiency and function (or dysfunction) of retinal vessels. The test uses a device called retinal vessel analyzer (RVA) which provides a fast and non-invasive evaluation of changes in retinal blood vessel diameter [7]. We have recently employed this technology to demonstrate a significantly reduced retinal vessel response to stimulation in eyes with POAG compared to healthy controls. We documented an increase of some vascular parameters after these eyes underwent trabeculectomy, pointing to a possible beneficial effect of this surgical intervention in improving vascular autoregulative reserves. DVA in the context of high-pressure glaucoma might be a helpful decision-making tool in favour of glaucoma surgery when a more profound IOP reduction is needed to enhance perfusion [8].

To assess glaucomatous damage, an analysis of the retinal nerve fiber layer (RNFL) is a valuable method. In a couple of studies, RNFL defects were discovered in more than 50% of eyes with glaucoma approximately 5-6 years before functional deficits became manifest in these patients' visual fields [9,10]. Scanning laser polarimetry has been proven a valuable tool to measure RNFL thickness and to document nerve fiber loss due to glaucoma [11].

The purpose of this study is to investigate the retinal vessel diameter of eyes with normal tension glaucoma (NTG) and to evaluate a possible relationship of the retinal vessel diameter with the retinal nerve fiber layer (RNFL) thickness in these patients.

Methods

This retrospective study included one eye of 86 patients with newly diagnosed NTG from the glaucoma service of the University Hospital Essen. As a basic clinical evaluation, all patients underwent a standard ophthalmic examination including tests for the best-corrected visual acuity, slit-lamp inspection, Goldmann applanation tonometry, pachymetry, gonioscopy, funduscopy including optic disc evaluation, and white on white perimetry with Oculus Twinfield II. The diagnosis of glaucoma was established by typical visual field defects and optic disc damage. To ensure the diagnosis of NTG, in all patients, repeated intraocular pressure (IOP) measurements were recorded including daytime and nighttime on three consecutive days in the hospital. In NTG patients, untreated IOP was less than 22 mmHg.

As part of the basic glaucoma diagnostics all patients then underwent more detailed eye examinations: dynamic retinal vessel analysis (DVA) (details as described before [8]) and the assessment of the RNFL thickness with scanning laser polarimetry (GDxVCC). None of the patients had any antiglaucomatous medication at the time of the DVA examination. If necessary, this was started after this diagnostic procedure.

Dynamic vessel analysis (DVA) is a method to gather objective and quantitative data on the efficiency and function (or dysfunction) of retinal vessels. The test uses a device called retinal vessel analyzer (RVA) which provides a fast and non-invasive evaluation of changes in retinal blood vessel diameter. The RVA (Imedos, Jena, Germany) was developed to analyze large retinal vessels with diameters between 60 and 200 μm . The device takes video image sequences of the fundus under green monochromatic light using a special CCD camera fitted to a retinal camera. The possible time resolution is 25 measurements per second, the spatial resolution 180 μm in the vessel direction and $<1\mu\text{m}$ in the measurement direction [12]. The measuring principle of the RVA is basically less a direct assessment of the vessel's width but rather a measurement of the diameter of the column consisting of red blood cells that fills the artery or the vein. These blood cells absorb a certain amount of the light emitted by the fundus camera and reflected by different retina layers. Measurement algorithms of the RVA assess vessel diameters from brightness profiles and compensate for disturbances like shadowing structures and reflections on the vessel surface [7]. Static vessel analysis supplies objective and quantitative information about the actual vessel state and morphological changes respectively. Methods of dynamic vessel analysis, by comparison, assess functional parameters obtaining retinal image sequences, which record vessel responses to physiologic changes or artificial stimulation. The dynamic vessel analyzer, (Imedos, Jena, Germany) as an advancement of the RVA, is supposed to be a tool in the examination of eye diseases

like glaucoma and diabetic retinopathy and appears to be of value in the assessment of vascular risks and its progression in individuals. Its key function is a flicker module. During DVA measurements, RVA assessment of retinal vessel diameters before, during and after flicker light provocation of the retina is performed. The flicker light induces diameter changes on the retinal vessels which are recorded over time for all segments of the selected vessel. Flicker-evoked dilation of retinal arteries measured by the DVA has proven to be a parameter that is suitable as a functional parameter of the regulation ability of retinal arteries [13]. Nguyen et al. have demonstrated the high reproducibility of DVA for repeated measures over a short period of time on a study group of 33 healthy subjects. The authors reached the conclusion that such measurements may allow non-invasive quantification of endothelial function to study its association with systemic and ocular diseases [14].

Scanning laser polarimetry (SLP) was performed with a GDx VCC (Carl Zeiss Meditec, Dublin, California). We compared the temporal retinal arteriolar diameters, temporal retinal venular diameters as well as the vascular flicker response with RNFL thickness. Spearman correlation coefficient was used to analyse the correlations between the measurements. Results are given as mean \pm standard deviation. $P < 0.05$ was considered as the level of significance.

Results

Average age of the patients was 57.0 ± 15.0 years. Intraocular pressure at the time of the examination was 18.5 ± 1.8 mmHg without any antiglaucomatous medication. Mean defect in the perimetry was 0.6 ± 3.8 .

68 of the patients had a 24-hour blood pressure measurement in their history. In these patients minimal systolic blood pressure was 92.1 ± 11.9 mmHg and minimal diastolic blood pressure 48.0 ± 8.2 mmHg.

Scanning laser polarimetry

Average thickness of the retinal nerve fiber layer was 52.6 ± 8.8 μm and the NFI (nerve fiber index) 30.7 ± 19.9 .

DVA measurement

The initial baseline values of arterial and venous diameter was 108.6 ± 18.1 and 132.0 ± 20.1 respectively. The maximal dilation of the artery was $2.65 \pm 2.56\%$, the maximal constriction 0.09 ± 2.18 and the peak value 2.65 ± 3.15 . The maximum dilation of the vein was $4.23 \pm 2.12\%$. All these values of the flicker response are significantly lower ($P < 0.001$) than in normal healthy eyes. Figure 1 shows a typical plot of the dynamic vessel analysis of a NTG patient with a reduced flicker response curve.

The diameters of the temporal retinal arteriolar vessels were significantly correlated with RNFL thickness ($P = 0.0204$) and nerve fiber index (NFI) of GDxVCC ($P = 0.0021$) (Figure 2a; Table 1).

The diameter of the temporal retinal vein was significantly correlated with the NFI ($P = 0.0298$) (Figure 2b). There was no statistically significant correlation between temporal retinal venular diameter and RNFL thickness. Furthermore, no statistically significant correlation was found between flicker response (max dilation, max constriction, and peak) and RNFL thickness or NFI.

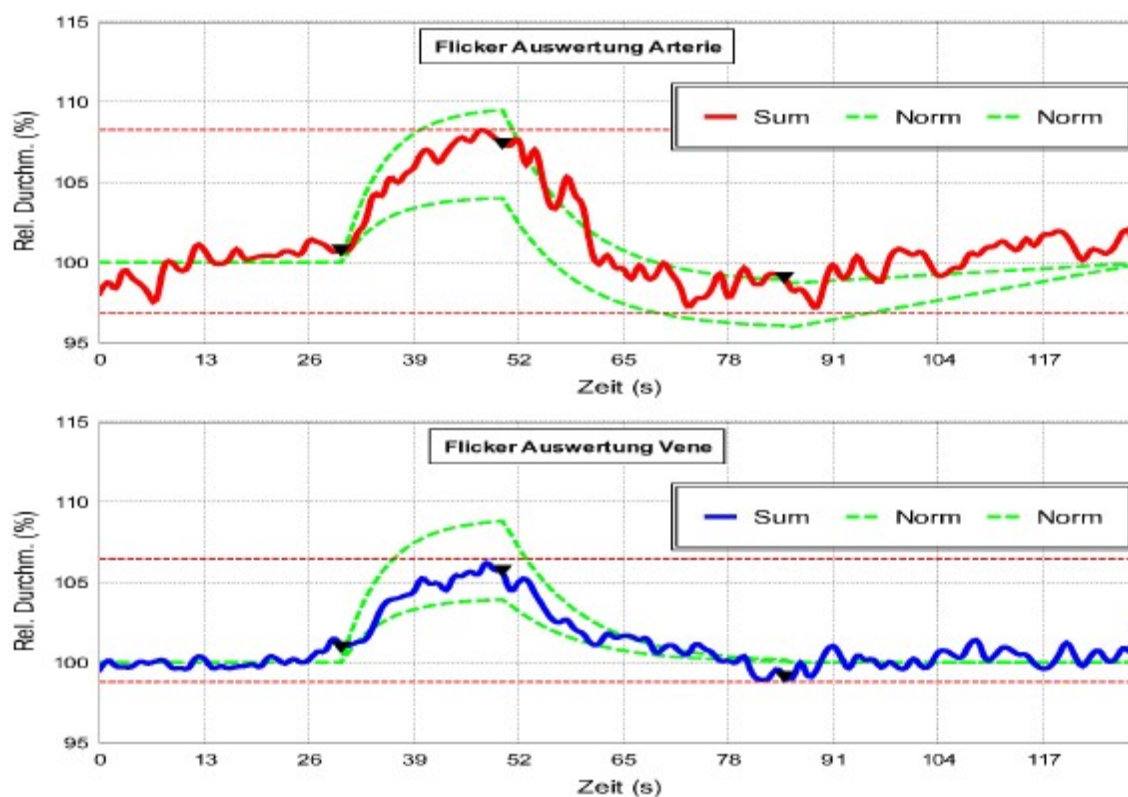


Figure 1: Typical plot of diameter of retinal vessels after flicker stimulation in a NTG eye (a) and healthy eye (b) as measured by dynamic vessel analysis (red: artery; blue: vein). The first 30 seconds show uninfluenced baseline data. The average of uninfluenced vessel diameter measurements is set 100 %. Higher values indicate dilation and lower values contraction as compared to the baseline vessel diameters. At the following flicker light provocation vessel diameters normally dilate in healthy eyes and after flicker stimulation they decrease. After recovery they reach baseline values again. 'Sum' is giving all measurements of one patient in their temporal sequence. The area between the two 'Norm' curves represents the normal range. In this typical plot of a NTG patient the vessel diameter remains uninfluenced by flicker (artery) or the dilation is significantly reduced (vein).

Spearman correlation	Correlation coefficient	p-values
Arterial vessel diameter vs RNFL	0.252	0.0204
Arterial vessel diameter vs NFI	-0.340	0.0021
Venular vessel diameter vs NFI	-0.243	0.0298
Arterial vessel diameter vs venular vessel diameter	0.343	0.0014
Arterial vessel dilatation vs venular vessel dilatation	0.220	0.0431
Arterial vessel dilatation vs peak value	0.766	<0.001
Arterial vessel contraction vs peak value	-0.449	<0.001
RNFL vs NFI	-0.770	<0.001

Table 1: Correlation of DVA and SLP parameters.

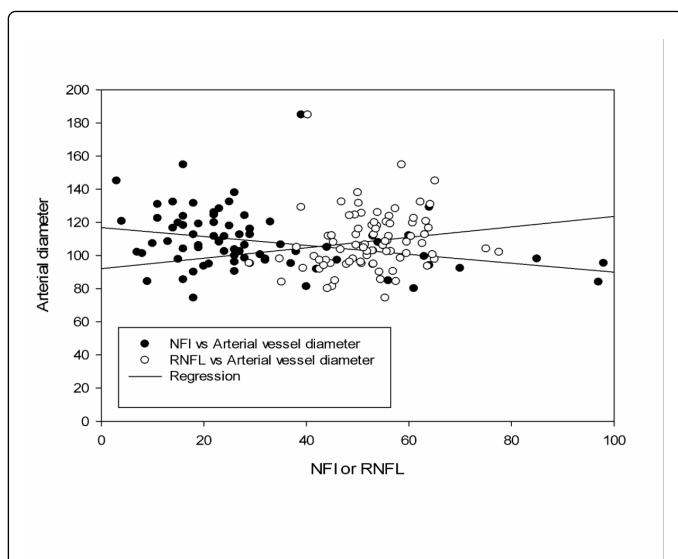


Figure 2a: Scatter plot and regression analysis of arterial vessel diameter (μm) and Nerve Fiber Index (NFI) or retinal nerve fiber layer thickness (RNFL; μm) showing a significant negative correlation between arterial diameter and NFI and a significant positive correlation between arterial diameter and RNFL ($P < 0.05$).

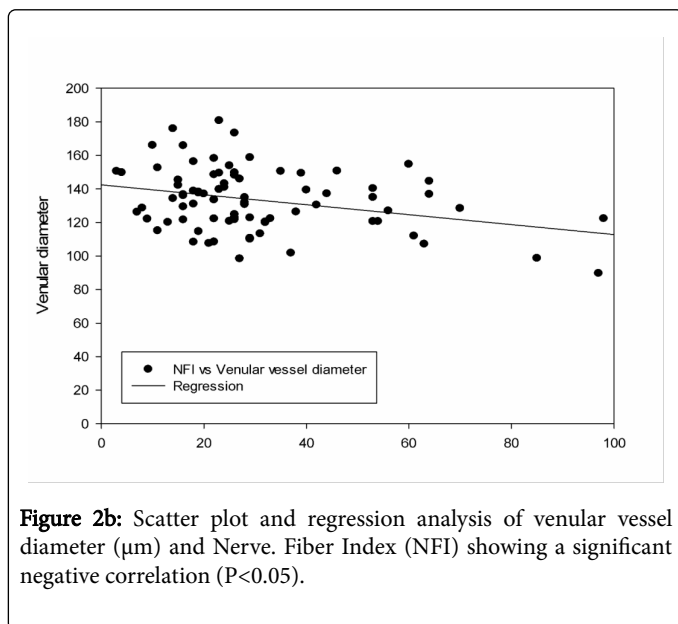


Figure 2b: Scatter plot and regression analysis of venular vessel diameter (μm) and Nerve. Fiber Index (NFI) showing a significant negative correlation ($P < 0.05$).

Conclusions

Our study in NTG patients shows a reduced flicker response in DVA and a positive correlation between arterial vessel diameter assessed by DVA and the retinal nerve fiber layer thickness as measured by scanning laser polarimetry. We also could demonstrate a negative correlation between arterial and venous vessel diameter and the nerve fiber index.

The nerve fiber index (NFI) has proven to be a good discriminating parameter in scanning laser polarimetry and contributes to the high value of the GDxVCC as a reliable tool to allow an easy discrimination

between healthy eyes and those that have suffered glaucomatous damage to their RNFL; the sensitivities of the NFI for correctly identifying glaucoma patients with mild, moderate, and severe damage were demonstrated to be 83.8%, 92.9%, and 90.1%, respectively [15]. Dynamic Vessel Analysis, on the other hand, has emerged as a helpful tool in gaining insights into the dynamics of retinal circulation and to determine its alterations in a number of diseases [16]. This holds true for diabetes where the severity of the disease was associated with diminished response rates to stimulation [17] and where in some cases the vessels exhibited abnormal reaction even before retinopathy was manifest [18]. DVA has also emerged in the diagnostics of glaucoma as well as in the monitoring of the disease under therapy - we have for instance been able to reveal a significant recovery of the regulative capacity of retinal arteries by using DVA measurements after an IOP-lowering laser surgical intervention (CPC) in glaucoma patients [19] while a "standard" pharmacotherapy of glaucoma did not influence the small retinal vessels significantly according to DVA evaluation [20].

Combining both methods-the one measuring damage to the nerve fiber layer, the other to demonstrate abnormalities in small retinal blood vessels-we were able to show that narrower arteriolar vessels are found in advanced NTG patients. Our findings support the study by Kim et al. who measured-though not stimulated with flicker light as we did-the retinal vessel diameter (measured by means of an image analysis software) and RNFL thickness (assessed by OCT) in 67 untreated patients with NTG and in 48 healthy subjects. They were able to show an association between the appearance of the vessels and the location of glaucomatous damage: the mean diameter of the temporal retinal vessels in the quadrants with RNFL defects was significantly smaller in patients with NTG than in those with quadrants without RNFL defects and in control subjects [21]. These findings are in line with other studies that found a strong association between decreased peripapillary arteriole diameter and visual field defects in the corresponding hemifield in eyes with POAG [22]. It has been hypothesized that the decrease of retinal vessel diameter is a response by autoregulatory mechanisms to a reduced demand for oxygen in a RNFL that has suffered substantial ganglion cell loss [23-25].

There are more recent studies that raised the possibility of vessel morphology and function acting as a predictor or a risk indicator in normal tension glaucoma like, for instance, Pinto et al. who demonstrated a decreased flow velocity in the retinal veins of glaucoma patients and registered a lack of spontaneous vein pulsation (SVP) which they considered of particular importance in NTG patients, where it may be associated with more advanced functional damage [26].

All this fits in our current concept of normal tension glaucoma. There is no doubt that many though probably not all NTG patients are suffering from a disturbed blood flow and that a common cause for a disturbed OBF autoregulation are primary vascular dysregulations (PVD) which are frequently observed in normal tension glaucoma patients [27]. Flammer has postulated that even with normal IOP and regular blood pressure, OBF may be impaired in patients with PVD due to a disturbed autoregulation [28]. This syndrome of a distinct complex of vascular dysregulations that lead to a cluster of symptoms and signs in healthy people as well as in those with manifest glaucoma is now called Flammer syndrome. Among its different ocular signs is a reduced capacity to autoregulate OBF and an increased stiffness of retinal vessels. It has been recognized that patients who develop glaucomatous damage despite a normal IOP or patients with

progressing glaucomatous damage despite well-controlled IOP very often suffer from Flammer syndrome [29].

Our findings, in conclusion, of narrowed arteriolar vessels in NTG may be due to the decreased demand for retinal blood flow in damaged retina, but also may be one reason for the damage. Because of the limited number of patients in our study it is of course not possible to make a conclusive claim. But our results at least add to the evidence that examining the morphology and the function of retinal vessels in glaucoma can - with the established knowledge that morphological changes precede functional damage usually by years in GON - contribute not only to the diagnosis but also add a valuable element of prediction to the management of NTG patients.

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