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Association of ocular, cardiovascular, morphometric and lifestyle parameters with retinal nerve fibre layer thickness

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Data Availability Statement: The analysis presents clinical data of a large-scale population-based cohort with ongoing follow-up examinations. This project constitutes a major scientific effort with high methodological standards and detailed guidelines for analysis and publication to ensure scientific analyses on highest level. Therefore, data are not made available for the scientific community outside the established and controlled workflows and algorithms. To meet the general idea of verification and reproducibility of scientific findings,

Abstract

Background

Glaucoma is a neurodegenerative disease, leading to thinning of the retinal nerve fibre layer (RNFL). The exact influence of ocular, cardiovascular, morphometric, lifestyle and cognitive factors on RNFL thickness (RNFLT) is unknown and was analysed in a subgroup of the Gutenberg Health Study (GHS).

Methods

Global peripapillary RNFLT was measured in 3224 eyes of 1973 subjects (49% female) using spectral-domain optical coherence tomography (SD-OCT). The association of age, sex, ocular, cardiovascular, morphometric, lifestyle and cognitive factors on RNFLT was analysed using Pearson correlation coefficient and fitting a linear mixed model.

Results

In the univariable analysis highest correlations were found for axial length (r = -0.27), spherical equivalent (r = 0.24), and glaucoma (r = -0.15) (p<0.0001, respectively). Other significant correlations with RNFLT were found for age, sex, intraocular pressure, systemic hypertension and systolic blood pressure, previous eye surgery, cholesterol, homocysteine, history of coronary artery disease, history of myocardial infarction, apnoea, diabetes and alcohol intake, p<0.05, respectively. Body length,

we offer access to data at the local database in accordance with the ethics vote upon request at any time. The GHS steering committee, which comprises a member of each involved department and the head of the Gutenberg Health Study (PSW), convenes once a month. The steering committee decides on internal and external access of researchers and use of the data and biomaterials based on a research proposal to be supplied by the researcher. Interested researchers make their requests to the head of the Gutenberg Health Study (Philipp S. Wild; philipp.wild@unimedizin-mainz.de). More detailed contact information is available at the homepages of the GHS (www.gutenberghealthstudy.org) or the ophthalmic branch of the GHS (www.unimedizin-mainz.de/augenklinik/forschung/gutenberg-gesundheitsstudie.html).

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body weight, BMI, diastolic blood pressure, blood glucose, HbA1c, history of apoplexy, cognitive function, peripheral artery disease, tinnitus, migraine, nicotine intake, central corneal thickness, and pseudophakia were not significantly correlated with RNFLT. The regression model revealed a significant relationship between RNFLT and age in decades ($p<0.02$), spherical equivalent ($p<0.0001$), axial length ($p<0.0001$), glaucoma ($p<0.0001$), tinnitus ($p = 0.04$), apnoea ($p = 0.047$), homocysteine ($p = 0.05$) and alcohol intake >10g/d for women and >20g/d for men ($p = 0.02$). Glaucoma, apnoea, higher homocysteine, higher alcohol intake and higher axial length as well as age were related to decreased RNFLT while higher spherical equivalent or history for tinnitus were related to thicker RNFL.

Conclusion

RNFLT is related to age, ocular parameters and lifestyle factors. Considering these parameters in normative databases could improve the evaluation of peripapillary RNFLT. It is necessary to evaluate if a reduction of alcohol intake as well as the therapy of apnea or high homocysteine levels could positively influence RNFLT.

Introduction

Glaucoma is a neurodegenerative disease, leading to thinning of the retinal nerve fibre layer (RNFL) which can be measured by optical coherence tomography (OCT). In order to evaluate RNFL thickness measurements, other potential influencing factors should be known and taken into account.

Acir et al. could show that iron deficiency anaemia can lead to local RNFL thinning. [1] RNFL thinning was also shown to be positively correlated with increase in serum urea and creatinine levels in patients with diabetic retinopathy. [2] A reduction of lipoprotein lipase and accumulation of visceral fat were shown to be potential factors of retinal neurodegenerative disorders that decrease RNFL thickness. [3] Acer et al. demonstrated that migraine patients without aura had decreased peripapillary RNFL thickness in temporal and nasal-superior sectors compared with control patients.[4] Ferrandez et al. [5] found a decrease in RNFL thickness in patients with severe obstructive sleep apnoea which could be confirmed by Ozge et al. [6] Also, Alzheimer disease was found to lead to significant RNFL thinning. [7]

The above mentioned factors are only a potpourri of influencing factors and disorders that might lead to thinning of RNFL thickness. However, the exact influence of ocular, cardiovascular, morphometric and lifestyle factors on peripapillary RNFL thickness remains unknown.

It can be assumed that a person's individual RNFL thickness is influenced not only by a few but many factors. This makes the interpretation of an individual's measured RNFL values even more difficult and puts in question the use of small databases for RNFL thickness evaluation which do not take into account all these parameters.

To date there exist no studies which investigated comprehensively many associated parameters of RNFL thickness in an epidemiologic setting. It was the aim of the current study to evaluate the relationship of ocular, cardiovascular, morphometric and lifestyle factors with global RNFL thickness in a subgroup of the Gutenberg Health Study (GHS) and to provide a formula which allows the estimation of an individual's RNFL thickness based on these parameters.

Material and methods

Gutenberg Health Study

The Gutenberg Health Study (GHS) is a prospective, population-based, observational, single-center cohort study carried out in the Rhine-Main region of Western Germany (Rhineland-Palatinate). The GHS study sample is recruited from subjects aged between 35 and 74 years at the time of baseline examination (2007–2012). The sample was drawn randomly from local governmental registry offices, in which every resident is mandatorily registered, equally stratified by sex, residence (urban or rural) and for each decade of age. The present study analyses participants of the 5 year follow-up between April 2012 and December 2013.

The study protocol and study documents were approved by the local ethics committee of the Medical Chamber of Rhineland-Palatinate, Germany (reference number 837.020.07: original vote: 22 March 2007, latest update: 20 October 2015).

In accordance with the tenets of the declaration of Helsinki, written informed consent was obtained from all participants prior to entering the study.

An important feature of the GHS design is its interdisciplinary character including ophthalmological examinations, general and cardiovascular examinations, psychosomatic evaluations, laboratory tests, and biobanking for proteomic and genetic analyses. Five years after the baseline investigation, study participants are invited to participate in a follow-up visit (FU2) including the same series of investigations. All investigations are based on standard operating procedures (SOPs).

Cognitive Performance: The TOL test (Freiburg version) [8] was applied for the assessment of cognitive function. The test examines planning ability [9] and is a test of complex executive functions. The TOL performance is inked to fluid intelligence and strongly coupled with prefrontal functioning.[10]

Ophthalmological investigations include visual acuity testing and refraction with the Humphrey® Automated refractor/keratometer (HARK) 599™, slitlamp biomicroscopy (Haag-Streit BM 900®, Bern Switzerland), intraocular pressure measurement with a non-contact tonometer (NT 2000™, Nidek Co./Japan), non-contact central corneal thickness and keratometry measurement with the Pachycam™ (Oculus, Wetzlar/Germany), non-mydriatic fundus photography, and visual field testing using frequency-doubling technology perimetry with the Humphrey Matrix Perimeter (Carl Zeiss Meditec AG, Jena, Germany). [11] In April 2012 optical coherence tomography using the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) was added to the study protocol.

Optical coherence tomography

OCT is a non-contact, non-invasive imaging technique using light reflection in different retina levels to produce high-resolution, two-dimensional cross-sectional and 3D images.

In the current study, a circular scan was manually placed in the center of the optic disc while the eye tracking system was activated. The mean peripapillary RNFLT of the scans was estimated automatically by the software. The RNFL limits of the circle scans were automatically determined based on the software algorithms. This way, the retinal nerve fiber layer was automatically segmented in each image. These RNFL limits were then used to estimate global and sectoral values for the retinal nerve fiber layer thickness. The Spectralis OCT software, Heidelberg Eye Explorer, was used for the automatic segmentation of the RNFL and for the calculation of the RNFL thickness.

Data acquisition was adjusted for the refraction of the examined eye. No pupil dilation was performed. The scan quality ranged from 0 (no signal) to 40 (excellent) and only high-quality

images (centered and well-focused optic disc with a signal strength > 15 dB) were selected for this study. All scans were quality controlled and manually checked before enrollment. Segmentations were manually re-adjusted when necessary. One scan was acquired for each eye of every subject.

Statistical analysis

Subject demographics, age, sex, ocular, cardiovascular, morphometric and lifestyle characteristics were described by mean, standard deviation, minimum and maximum, median and quartiles for quantitative variables and by absolute and relative frequencies for categorical variables.

The association with age, sex, ocular, cardiovascular, morphometric and lifestyle factors on RNFLT was analysed using Pearson correlation coefficient and a mixed linear model with RNFLT as endpoint and the above mentioned explanatory variables as covariates. In order to account for dependence between eyes within a subject, subject was included as a random effect into the model. This analysis essentially can be interpreted as linear regression which takes dependence into account.

We then analysed the best predictive model using linear models separately for right and left eyes with stepwise backward selection and Schwarz Bayesian criterion. All independently associated parameters, determined by this procedure, were then included into a final linear mixed model with global RNFL thickness as dependent variable.

In addition, we analysed the relationship between cognitive function and RNFLT using a mixed linear model adjusted for age and sex as covariates.

Because of the explorative character of the analysis, p-values should be interpreted as a continuous measure of strength of statistical evidence.

Results

3224 eyes of 1973 subjects (49% women) aged between 40 and 80 years were included in this study. Table 1 presents demographics of the study population and the distribution of all categorical variables. More than half of the subjects (51.9%, n = 1024) suffered from elevated blood pressure, 2.8% (n = 56) had a history of myocardial infarction and 2.6% (n = 51) reported a history of glaucoma out of whom 84% (n = 43) were treated with medication or surgery.

Table 2 presents further demographics of the study population and the distribution of quantitative variables including data for global retinal nerve fiber layer thickness (RNFLT).

Pearson correlation coefficients were calculated in order to analyze univariate associations between RNFLT and age, sex, ocular, cardiovascular, morphometric and lifestyle factors. Highest absolute correlations were found for axial length (r = -0.27), spherical equivalent (r = 0.24), and known glaucoma (r = -0.15) ($p<0.0001$, respectively). Other correlations with RNFLT were found for age (r = -0.12, $p<0.0001$), sex (r = 0.05, p = 0.0023), intraocular pressure (r = -0.07, p = 0.0002), hypertension (r = -0.05, p = 0.002) and systolic blood pressure (r = -0.07, p = 0.0002), previous eye surgery (r = -0.04, p = 0.03), cholesterol (-0.04, p = 0.03), homocysteine (r = -0.08, $p<0.0001$), history of coronary artery disease (r = -0.05, p = 0.01), history of myocardial infarction (r = -0.05, p = 0.01), apnoea (r = -0.04, p = 0.01), diabetes (r = -0.04, p = 0.03) and alcohol intake (r = -0.05), $p<0.05$, respectively. High alcohol intake (>10g/day in women and >20g/day in men) was correlated more strongly (r = -0.08, $p<0.0001$) than lower alcohol intake (<10 / 20g/day) (r = -0.05, p = 0.01).

Body size, body weight, BMI, diastolic blood pressure, blood glucose, HbA1c, history of apoplexy, peripheral artery disease, tinnitus, migraine as well as nicotine intake did not show correlation with RNFLT beyond chance. Neither did central corneal thickness, or pseudophakia.

Table 1. Demographics of the study population and distribution of categorical variables.

	N	%	Missing data (N; %)
Sex			
male	1006	51	1, 0.1
female	966	49	
Age decades			
40–49	463	23.5	
50–59	604	30.6	
60–69	534	27.1	
70–80	372	18.9	
Hypertension	1024	51.9	4; 0.2
History of myocardial infarction	56	2.8	1; 0.1
History of stroke	32	1.6	2; 0.1
History of coronary artery disease	96	4.9	1; 0.1
History of peripheral artery disease	83	4.2	1; 0.1
History of tinnitus	244	12.4	3; 0.2
History of migraine	534	27.1	2; 0.1
History of sleep apnea	161	8.2	1; 0.1
Ever smoked	1052	53.3	4; 0.2
History of diabetes	146	7.4	
Alcohol intake	1143	57.9	
$\geq$ 10g/d (women) / $\geq$ 20g/d (men)	509	25.8	
History of glaucoma	51	2.6	
Glaucoma therapy (medication or surgery)	43	2.2	
Pseudophakia	101	5.1	
Eye surgery	125	6.3	

N = number of cases

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In the multivariable model age, sex, spherical equivalent, axial length, history of glaucoma, history of tinnitus and high alcohol intake remained independently associated, while all other parameters were not related to global retinal nerve fibre layer thickness (Table 3).

After stepwise backward elimination, age, spherical equivalent, axial length and history of glaucoma remained in the model best predicting global retinal nerve fibre layer thickness (Table 4).

Included parameters were determined using stepwise backward selection to find the best model for prediction (based on Schwarz Bayesian criterion) of global retinal nerve fiber layer thickness.

Regarding cognitive function, we did not find an association between tower of London score and RNFLT (p = 0.50), adjusted for age and sex.

Discussion

As part of the population-based Gutenberg Health Study, we demonstrate that peripapillary RNFL thickness is linked to aging and biometric parameters, namely refraction and axial length. PRNFL thickness is lower in persons with glaucoma, and interestingly in persons with high alcohol intake.

More recently, several groups evaluated influencing factors on peripapillary RNFL thickness using different OCT devices. Leung et al. showed that high myopia is linked to thinner

Table 2. Demographics of the study population and distribution of quantitative variables.

	N	N miss	mean	SD	Min	Q1	Median	Q3	Max
Systemic parameters (persons)									
Body weight [kg]	1973	0	79.8	15.9	45.6	68.5	78.7	89.1	162.2
Body length [cm]	1973	0	170.4	9.3	137.0	163.4	170.2	177.0	198.5
BMI	1973	0	27.44	4.74	17.17	24.21	26.82	29.93	52.27
Cholesterol [mg/dL]	1968	5	222.7	42.0	70	194	220	248	419
HbA1c [%]	1965	8	5.66	0.58	4.5	5.3	5.6	5.9	11.6
Glucose [mg/dL]	1969	4	92.6	16.0	49	84	90	97	290
Homocysteine	1960	13	11.9	4.6	4.6	9.3	11.1	13.6	73.0
Ocular parameters (eyes)									
Spherical equivalent	3217	7	-0.29	2.14	-12.5	-1.13	-0.13	0.88	8.00
Intraocular pressure	3156	68	14.89	2.91	7.00	13.00	14.7	16.7	26.7
Axial length	2901	323	23.62	1.15	18.29	22.92	23.57	24.27	28.48
Central corneal thickness	2890	334	548.5	34.5	405	525	549	571	773
RNFLT global	3224	0	96.1	10.4	48	90	96	103	207

N = number of cases; N miss = number of missing cases, SD = standard deviation, Min = minimum, Q1 = quantile 1, Q3 = quantile 3, Max = maximum, BMI = body mass index, RNFLT = retinal nerve fiber layer thickness.

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pRNFL.[12] Oner et al. found a relationship between myopic refraction and thinner and average pRNFL thickness using the RTVue OCT (Optovue, Fremont, CA). In addition, longer axial length was linked to thinner pRNFL measurements and the authors suggest including axial length as parameter in normative databases.[13] Lee et al. evaluated the intra-individual influence of refractive power and compared the influence of different soft contact lenses on pRNFL measurement.[14] They reported a lower pRNFL thickness with increasing myopia and vice versa. In a small cohort, Schuster et al. reported an association between thinner pRFNL and myopia in otherwise healthy eyes using Topcon 3D-OCT 2000 (Tokyo, Japan).[15] Our study confirms these findings showing an independent association of thinner pRNFL with myopia and longer axial length using Spectralis-OCT. Whether these findings are due to ocular magnification, as discussed by Khan et al. using a Cirrus HD OCT-system [16], is still controversial. In contrast to the Cirrus HD OCT-system, the Spectralis-OCT system do not report peripapillary retinal nerve fiber layer thickness measured with with a fixed absolute scan diameter (i.e. 3.46mm), but on a 12˚ diameter.

There are few studies evaluating the influence of systemic factors on pRNFL thickness. Khawaja et al showed an association of thinner pRNFL thickness as measured with a GDxVCC device with a higher body mass index.[17] A Chinese population-based study in non-glaucomatous subjects showed age and axial length as associated factors for peripapillary RNFL thickness determined with SD-OCT, while systemic parameters (arterial blood pressure, body mass index, HDL and LDL cholesterol, triglycerides) except age did not have an impact.[18] Similar, Schuster et al. did not find any classical systemic cardiovascular risk factors to be associated with pRNFL thickness in apparently healthy subjects after correction for ocular parameters.[15] Our study confirms these findings on basis of a population-based study setup and therefore risk of selection bias is low.

Interestingly, we found an association of thinner peripapillary RNFL thickness and risky alcohol intake according to WHO definition ($\geq$ 10g/d (women) / $\geq$ 20g/d (men)). Similarly, Khawaja et al. found a univariate association between alcohol intake and peripapillary RNFL thickness as assessed with GDxVCC device, but could not confirm it after adjustment for

Table 3. Multivariable linear regression model to estimate associations with retinal nerve fiber layer thickness in the Gutenberg Health Study.

Effect	Estimate	p-value
Intercept:		
Male, 40–49 years	125.74	
Male, 50–59 years	124.63	
Male, 60–69 years	121.83	
Male 70–80 years	121.18	
Female, 40–49 years	127.73	
Female, 50–59 years	124.92	
Female, 60–69 years	123.85	
Female 70–80 years	119.59	
Spherical equivalent (per dioptre)	1.33	**<0.0001**
Intraocular pressure (per mm Hg)	-0.08	0.28
Axial length (per mm)	-1.29	**<0.0001**
Central corneal thickness (per μm)	0.0001	0.99
Pseudophakia (yes)	-0.31	0.90
Eye surgery (yes)	2.24	0.31
Self-reported glaucoma (yes)	-7.69	**<0.0001**
Systolic blood pressure (mm Hg)	0.0006	0.98
Diastolic blood pressure (mm Hg)	-0.02	0.65
History of myocardial infarction	-1.04	0.53
History of stroke	-0.35	0.85
History of coronary artery disease	-0.07	0.96
History of peripheral artery disease	-0.70	0.54
History of tinnitus	1.38	**0.04**
History of migraine	0.07	0.90
History of apnoea	-1.70	0.05
Body length (per cm)	0.02	0.52
Body weight (per kg)	0.03	0.07
Cholesterol (per mg/dl)	-0.0008	0.89
HbA1c (per mg/dl)	-0.01	0.98
Glucose (per mg/dl)	0.006	0.73
Homocysteine (per mg/dl)	-0.10	0.05
ever smoked (yes)	-0.45	0.34
Diabetes (yes)	-1.28	0.20
Alcohol (yes)	0.12	0.83
Alcohol >10g/day (female) / >20g/day (male)	-1.46	**0.02**

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covariates.[17] The intake of alcohol seems not only to have an impact on neuronal degeneration, but also on neuronal development. A recent study of patients with fetal alcohol syndrome showed that these have a decreased peripapillary RNFL thickness.[19] These patients did not show temporal RNFL thinning, in contrast to case reports of tobacco-alcohol-induced toxic optic neuropathy.[20]

Apart from technical examinations, fundus examination of RNFL maintained that several systemic factors such as older age, male sex, hyperglycemia and dyslipidemia, had an influence on RNFL visibility. [21] High arterial blood pressure and higher concentration of low-density lipoproteins were associated with localized RNFL defects.[21]

Table 4. Best fitting model to predict global retinal nerve fiber layer thickness in the Gutenberg Health Study.

Multivariable mixed linear regression model		Estimate (in µm)	Standarderror	Pr > |t|
Reference: 70–80 years:		121	5.2	
Age 40–49 years	40–49	6.6	0.7	< .0001
Age 50–59 years	50–59	4.3	0.7	< .0001
Age 60–69 years	60–69	2.1	0.7	0.0018
Spherical equivalent (per dpt)		1.3	0.1	< .0001
Axial length (per mm)		-1.2	0.2	< .0001
Self-reported glaucoma (yes)		-7.4	1.4	< .0001

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We did not find an association between cognitive function and RNFL thickness which is in contrast to several other studies. Oktem et al. found an association between cognitive functioning measured with mini mental state examination (MMSE) test and RNFL thickness,[22] as did Khawaja et al. using the short form of MMSE.[23] These tests examine different characteristics of cognitive functioning compared to the tower of London test as a planning ability test. A recent meta-analysis [24] analyzed the relationship of RNFL thickness with Alzheimer's disease and mild cognitive impairment and found substantial heterogeneity between the studies due to methodology. High-quality studies indicated that global RNFL thickness was thinner in subjects with Alzheimer's disease or mild cognitive impairment compared to normal controls. Apparently, thinning of the RNFL is linked to neurodegenerative processes which are only marginally represented in the population-based sample.

Although our study population was randomly drawn from the population, all participants underwent standardized ophthalmic and cardiovascular examinations and reports on a relatively large sample size, it has several limitations. First, we did not analyze all participants of the cohort, but a subsample, as sufficient image quality was not always available. Therefore, we performed an item-nonresponder analysis and found to have included a comparable sample with slightly younger age. In addition, our population-based study has a response rate of 84% and therefore our underlying cohort might not perfectly reflect the general population with an age range of 40 to 79 years. We did not measure the size of the optic disc and therefore cannot control for this parameter in our analysis. In contrast to other devices, Spectralis-OCT performs a peripapillary circle with a diameter of 12˚, which is less influenced by the optical biometry compared to circles with an absolute diameter (i.e. 3.4 mm).

In conclusion, our study analyzed associated factors with RNFL thickness readings using spectral-domain OCT. Our analysis found age, refraction and axial length as major influencing factors, while cardiovascular parameters (body weight, arterial blood pressure, biochemical parameters, history of stroke or myocardial infarction) did not reach significance. High alcohol intake was associated with thinner RNFL. A prospective study comparing patients with and without alcohol use is needed to show the impact of alcohol on RNFLT. A marginal association with history of tinnitus was found, which further studies need to support for the interpretation of RNFL thickness readings. Current data indicates that clinical evaluation of RNFL thickness is independent of cardiovascular parameters, but refraction and axial length has to be kept in mind when interpreting RNFL data.

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References

1. Acir NO, Dadaci Z, Cetiner F, Yildiz M, Alptekin H, Borazan M. Evaluation of the peripapillary retinal nerve fiber layer and ganglion cell-inner plexiform layer measurements in patients with iron deficiency anemia with optical coherence tomography. Cutan Ocul Toxicol. 2016; 35(2):131–6. https://doi.org/10.3109/15569527.2015.1067228 PMID: 26293666.
2. Srivastav K, Saxena S, Mahdi AA, Kruzliak P, Khanna VK. Increased serum urea and creatinine levels correlate with decreased retinal nerve fibre layer thickness in diabetic retinopathy. Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals. 2015; 20(6–7):470–3. Epub 2015/10/17. https://doi.org/10.3109/1354750x.2015.1094142 PMID: 26474118.
3. Shiba C, Shiba T, Takahashi M, Hori Y, Maeno T. Relationships among serum lipoprotein lipase mass, visceral fat, and retinal nerve fiber layer thickness. Graefe's archive for clinical and experimental

ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie. 2015; 253(11):1883–8. Epub 2015/01/01. https://doi.org/10.1007/s00417-014-2898-6 PMID: 25550098.

4. Acer S, Oguzhanoglu A, Cetin EN, Ongun N, Pekel G, Kasikci A, et al. Ocular pulse amplitude and retina nerve fiber layer thickness in migraine patients without aura. BMC ophthalmology. 2016; 16:1. Epub 2016/01/06. https://doi.org/10.1186/s12886-015-0180-2 PMID: 26728474; PubMed Central PMCID: PMCPMC4698917.
5. Ferrandez B, Ferreras A, Calvo P, Abadia B, Marin JM, Pajarin AB. Assessment of the retinal nerve fiber layer in individuals with obstructive sleep apnea. BMC ophthalmology. 2016; 16:40. Epub 2016/04/20. https://doi.org/10.1186/s12886-016-0216-2 PMID: 27090783; PubMed Central PMCID: PMCPMC4835866.
6. Ozge G, Dogan D, Koylu MT, Ayyildiz O, Akincioglu D, Mumcuoglu T, et al. Retina nerve fiber layer and choroidal thickness changes in obstructive sleep apnea syndrome. Postgraduate medicine. 2016; 128 (3):317–22. Epub 2016/02/27. https://doi.org/10.1080/00325481.2016.1159118 PMID: 26918297.
7. Gunes A, Demirci S, Tok L, Tok O, Demirci S. Evaluation of retinal nerve fiber layer thickness in Alzheimer disease using spectral-domain optical coherence tomography. Turkish journal of medical sciences. 2015; 45(5):1094–7. Epub 2016/01/08. PMID: 26738353.
8. Kaller CP, Unterrainer JM, Kaiser S, Weisbrod M, Aschenbrenner S. Tower of London—Freiburg version. Mödling: Schuhfried.2012.
9. Shallice T. Specific impairments of planning. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 1982; 298(1089):199–209. Epub 1982/06/25. PMID: 6125971.
10. Nitschke K, Kostering L, Finkel L, Weiller C, Kaller CP. A Meta-analysis on the neural basis of planning: Activation likelihood estimation of functional brain imaging results in the Tower of London task. Human brain mapping. 2017; 38(1):396–413. Epub 2016/09/16. https://doi.org/10.1002/hbm.23368 PMID: 27627877.
11. Hohn R, Kottler U, Peto T, Blettner M, Munzel T, Blankenberg S, et al. The ophthalmic branch of the Gutenberg Health Study: study design, cohort profile and self-reported diseases. PloS one. 2015; 10 (3):e0120476. Epub 2015/03/17. https://doi.org/10.1371/journal.pone.0120476 PMID: 25775251; PubMed Central PMCID: PMCPmc4361691.
12. Leung CK, Mohamed S, Leung KS, Cheung CY, Chan SL, Cheng DK, et al. Retinal nerve fiber layer measurements in myopia: An optical coherence tomography study. Investigative ophthalmology & visual science. 2006; 47(12):5171–6. Epub 2006/11/24. https://doi.org/10.1167/iovs.06-0545 PMID: 17122099.
13. Oner V, Aykut V, Tas M, Alakus MF, Iscan Y. Effect of refractive status on peripapillary retinal nerve fibre layer thickness: a study by RTVue spectral domain optical coherence tomography. The British journal of ophthalmology. 2013; 97(1):75–9. Epub 2012/11/13. https://doi.org/10.1136/bjophthalmol-2012-301865 PMID: 23143906.
14. Lee J, Kim NR, Kim H, Han J, Lee ES, Seong GJ, et al. Negative refraction power causes underestimation of peripapillary retinal nerve fibre layer thickness in spectral-domain optical coherence tomography. The British journal of ophthalmology. 2011; 95(9):1284–9. Epub 2010/10/20. https://doi.org/10.1136/bjo.2010.186536 PMID: 20956274.
15. Schuster AK, Fischer JE, Vossmerbaeumer C, Vossmerbaeumer U. Determinants of peripapillary retinal nerve fiber layer thickness regarding ocular and systemic parameters—the MIPH Eye&Health Study. Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie. 2016; 254(10):2011–6. Epub 2016/07/30. https://doi.org/10.1007/s00417-016-3422-y PMID: 27468713.
16. Adam RS, Pavlin CJ, Ulanski LJ. Ultrasound biomicroscopic analysis of iris profile changes with accommodation in pigmentary glaucoma and relationship to age. American journal of ophthalmology. 2004; 138(4):652–4. Epub 2004/10/19. https://doi.org/10.1016/j.ajo.2004.04.048 PMID: 15488798.
17. Khawaja AP, Chan MP, Garway-Heath DF, Broadway DC, Luben R, Sherwin JC, et al. Associations with retinal nerve fiber layer measures in the EPIC-Norfolk Eye Study. Investigative ophthalmology & visual science. 2013; 54(7):5028–34. Epub 2013/07/04. https://doi.org/10.1167/iovs.13-11971 PMID: 23821204; PubMed Central PMCID: PMCPMC3726240.
18. Cheung CY, Chen D, Wong TY, Tham YC, Wu R, Zheng Y, et al. Determinants of quantitative optic nerve measurements using spectral domain optical coherence tomography in a population-based sample of non-glaucomatous subjects. Investigative ophthalmology & visual science. 2011; 52(13):9629–35. Epub 2011/11/01. https://doi.org/10.1167/iovs.11-7481 PMID: 22039236.
19. Menezes C, Ribeiro I, Coelho P, Mateus C, Teixeira C. Pattern of Retinal Nerve Fiber Layer Thickness Loss in Fetal Alcohol Syndrome: A Spectral-Domain Optical Coherence Tomography Analysis. Acta medica portuguesa. 2016; 29(4):254–60. Epub 2016/06/29. https://doi.org/10.20344/amp.6871 PMID: 27349777.

20. Moura FC, Monteiro ML. Evaluation of retinal nerve fiber layer thickness measurements using optical coherence tomography in patients with tobacco-alcohol-induced toxic optic neuropathy. Indian journal of ophthalmology. 2010; 58(2):143–6. Epub 2010/03/03. https://doi.org/10.4103/0301-4738.60087 PMID: 20195038; PubMed Central PMCID: PMCPMC2854446.

21. Zhang Y, Xu L, Zhang L, Yang H, Wang YX, Jonas JB. Ophthalmoscopic assessment of the retinal nerve fiber layer. The Beijing Eye Study. PloS one. 2013; 8(4):e62022. Epub 2013/05/03. https://doi.org/10.1371/journal.pone.0062022 PMID: 23637954; PubMed Central PMCID: PMCPmc3639254.

22. Oktem EO, Derle E, Kibaroglu S, Oktem C, Akkoyun I, Can U. The relationship between the degree of cognitive impairment and retinal nerve fiber layer thickness. Neurological sciences: official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 2015; 36(7):1141–6. Epub 2015/01/13. https://doi.org/10.1007/s10072-014-2055-3 PMID: 25575807.

23. Khawaja AP, Chan MP, Yip JL, Broadway DC, Garway-Heath DF, Luben R, et al. Retinal Nerve Fiber Layer Measures and Cognitive Function in the EPIC-Norfolk Cohort Study. Investigative ophthalmology & visual science. 2016; 57(4):1921–6. Epub 2016/04/20. https://doi.org/10.1167/iovs.16-19067 PMID: 27092718; PubMed Central PMCID: PMCPMC4849871.

24. Wang M, Zhu Y, Shi Z, Li C, Shen Y. Meta-analysis of the relationship of peripheral retinal nerve fiber layer thickness to Alzheimer's disease and mild cognitive impairment. Shanghai archives of psychiatry. 2015; 27(5):263–79. Epub 2016/03/16. https://doi.org/10.11919/j.issn.1002-0829.215100 PMID: 26977124; PubMed Central PMCID: PMCPMC4764001.