Original Article

Color Doppler imaging of the retrobulbar circulation and plasmatic biomarkers of vascular risk in age-related macular degeneration: A pilot study

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Purpose: To evaluate preliminarily and compare the level of plasmatic biomarkers of vascular risk in patients with and without exudative age-related macular degeneration (ARMD) and to relate it to vascular resistance alterations in the ophthalmic artery (OA), central retinal artery (CRA), posterior temporal ciliary artery (PTCA), and posterior nasal ciliary artery (PNCA). **Methods:** Color Doppler imaging of the OA, CRA, PTCA, and PNCA was performed in 30 eyes of 30 cataract patients (control group) as well as in 30 eyes of 30 patients with naive exudative ARMD (study group), measuring the peak systolic velocity, end-diastolic velocity (EDV), and Pourcelot resistive index (RI). Likewise, in both groups, a blood test was performed to determine the plasmatic levels of homocysteine, C-reactive protein (CRP), B12 vitamin, and folic acid. **Results:** A positive and significant correlation was found between the level of CRP and RI of the OA in the ARMD group ($r = 0.498$, $P = 0.005$), with an increased RI in all arteries compared to controls, although differences only reached statistical significance for the PTCA ($P = 0.035$). Likewise, a significantly lower EDV for the CRA was found in ARMD eyes compared to controls ($P = 0.041$). In the study group, significantly higher plasmatic levels of homocysteine ($P = 0.042$) and CRP ($P = 0.046$) were found. In contrast, no significant differences were found between groups in the levels of folic acid ($P = 0.265$) and B12 vitamin ($P = 0.520$). **Conclusion:** The decrease of the choroidal perfusion related to hyperhomocysteinemia, and increase in the CRP plasmatic levels may play an etiological role on the exudative ARMD. This should be investigated in future studies with larger samples of patients.

Key words: Age-related macular degeneration, B12 vitamin, color Doppler imaging, exudative age-related macular degeneration, folic acid, homocysteine

Age-related macular degeneration (ARMD) is the main cause of legal blindness in elderly people in developed countries.[1] Age is the main risk factor associated with this disease.[2] Although the pathogenesis of ARMD is not completely established, three different models of pathogenesis have been defined: oxidative stress, ischemia, and inflammation.[3] ARMD and atherosclerosis have been shown to share risk factors and pathogenic mechanisms, and this has led to the development of the hemodynamic[4] or vascular model[5] of ARMD. Several studies have found a relationship between ARMD, vascular risk factors (homocysteine, C-reactive protein [CRP], B12 vitamin, and folic acid) and a reduction in choroidal perfusion.[6,7] Specifically, some studies have suggested that a decrease in choroidal perfusion may play a role in the pathogenesis of exudative ARMD.[8]

The aim of the current study was to evaluate and compare, as in some previous studies, the level of plasmatic biomarkers of vascular risk (homocysteine, CRP, folic acid, and B12 vitamin) in a group of patients with exudative ARMD and in a control group but trying for the first time to our knowledge to relate these differences between groups to changes in vascular resistance measured by color Doppler imaging in the ophthalmic artery (OA), central retinal artery (CRA), posterior temporal ciliary artery (PTCA), and posterior nasal ciliary artery (PNCA). This preliminary evaluation was aimed at finding a trend of clinical relevance suggesting that a prospective comparative study in a larger sample size would provide significant findings.

Methods

Patients

A total of 60 eyes of 60 patients were enrolled in this prospective comparative pilot study. The control group included 30 eyes of 30 patients pending cataract surgery who were selected randomly. The group of cases (study group) included a total of 30 eyes of 30 patients with the diagnosis of naive exudative ARMD confirmed by fluorescein angiography and optical coherence tomography (OCT) analysis. All patients were

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Cite this article as: Rodrigo F, Ruiz-Moreno JM, García JB, Torregrosa ME, Segura JV, Piñero DP. Color Doppler imaging of the retrobulbar circulation and plasmatic biomarkers of vascular risk in age-related macular degeneration: A pilot study. Indian J Ophthalmol 2018;66:89-93.

properly informed of their inclusion in the study and signed an informed consent form. The study complied with the principles of the Declaration of Helsinki and was approved by the hospital ethics committee.

Exclusion criteria in both groups were glaucoma, diabetic retinopathy, arterial or venous occlusive vascular disease, optic neuropathy, macular dystrophy, intraocular inflammation, retinal detachment or previous vitreoretinal surgery, myopia magna (more than 7 D), and patients treated with antioxidants or multivitamin supplements. In control group, the presence of age-related maculopathy (ARM) or ARMD was an exclusion criterion.

Examination protocol

A complete ophthalmological examination was performed in all patients, which included best uncorrected and corrected visual acuity, refraction, slit-lamp biomicroscopy, fundus examination under pupil dilation, fluorescein angiography, and OCT examination. A detailed clinical history was performed, asking all patients about previous and current diseases as well as medical or surgical treatments. Furthermore, a basic blood test was performed that included the analysis of the plasmatic level of total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, CRP, homocysteine, folic acid, and B12 vitamin. This blood test was performed before cataract surgery in patients from the control group and before the injection of antiangiogenics in the study group. Each blood sample was frozen to −20°C immediately after its extraction. The measurement of the homocysteine level was performed at the end of the study.

Besides all these tests, a color Doppler imaging analysis was performed in all cases with the ecographer Aplio XV (Toshiba Medical Systems, Tokyo, Japan) using a linear (38 mm) multifrequency (6–11 Mhz, model PLT-704AT) transductor designed and developed to perform a peripheral vascular Doppler study and also a Doppler analysis of the superficial soft tissue. Specifically, the peak systolic velocity (PSV), the end-diastolic velocity (EDV), and the Pourcelot resistive index (RI) were determined in both groups for the OA, CRA [Fig. 1], PTCA [Fig. 2], and PNCA. The Pourcelot RI is a measure of the peripheral vascular resistance and is calculated as $RI = PSV − EDV/PSV$. This index reflects the resistance to arterial flow originated from the microvascular bed distal to the measurement site. The color Doppler imaging was done before cataract surgery in control group and before the injection of antiangiogenics in the group of cases. The color Doppler imaging measurement in the ciliary arteries was performed in the common trunk before their subdivision in multiples branches around the optic nerve.

Statistical analysis

Statistical analysis was performed with the software package SPSS for Windows version 21.0 (SPSS, Chicago, Illinois, EE.UU, USA). First, the Kolmogorov–Smirnov test confirmed that the data samples evaluated followed a normal distribution. For the comparison between groups, the unpaired Student's t-test was used. Furthermore, the relationship among different variables evaluated was assessed with the Pearson coefficient of correlation depending if the condition of normality could be assumed or not. Finally, Chi-square test was used for comparative analysis of qualitative variables. All statistical
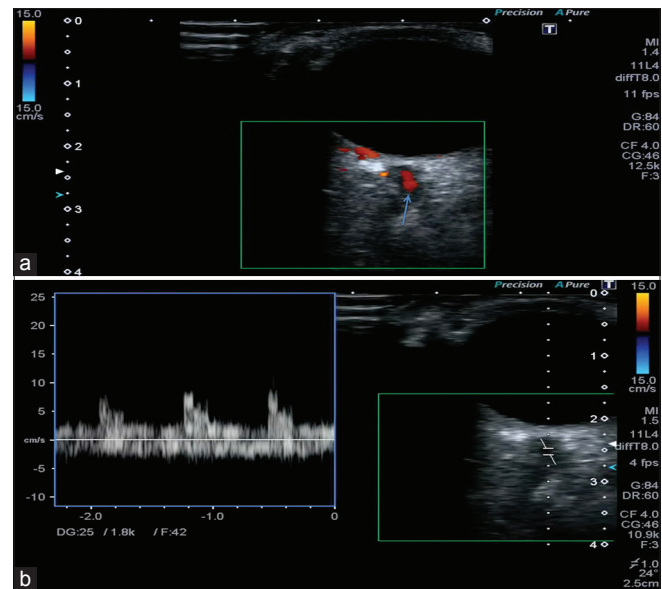

Figure 1: Example of the analysis of the Doppler ecography of the central retinal artery in one patient with age-related macular degeneration. (a) Doppler ecography in B mode of the orbit in which the central retinal artery that proceeds through the optic nerve can be seen. (b) Once the artery is identified, the function of spectral analysis is activated, which shows the flow on a time-scale

tests were 2-tailed, and $P < 0.05$ was considered statistically significant. A large effect size of 0.80, a significance level of 0.05, and a power of 80% were checked that was achieved with a minimum of 26 individuals per group.

Results

A total of 30 eyes of 30 patients were included in both control and study groups, without significant differences between them in age (control: 75.33 ± 7.30 years, range 61–89 years; study: 78.60 ± 5.88 years, range 57–90 years) ($P = 0.061$). In study group, 22 females (73.3%) and 8 males (26.7%) were included whereas 14 females (46.7%) and 16 males (53.3%) were included in control group ($P = 0.064$). No statistically significant differences were found between control and study groups in the percentage of eyes with arterial hypertension ($P = 0.295$), hypercholesterolemia ($P = 1.00$), cardiovascular disease ($P = 0.671$), brain vascular disease ($P = 0.706$), and treatment with statins ($P = 1.00$). In the study group, all patients showed exudative ARMD in one eye and intermediate ARMD in the fellow eye. Concerning the eyes with exudative ARMD, 20 (66.6%) of them showed angiographic features compatible with occult exudative ARMD and 10 (33.3%) of them features compatible with classic exudative ARMD. In the control group, any eye of each patient showed signs compatible with ARMD or maculopathy associated with age.

Table 1 summarizes the results of the color Doppler imaging test in control and study groups. As shown, higher RI values were obtained in the group of cases for all the arteries evaluated, but the difference in RI among control and study groups was only statistically significant for PTCA ($P = 0.035$). Likewise, lower PSV and EDV were obtained in the study group for all the arteries evaluated except for OA, but differences among groups in EDV was only statistically significant for CRA ($P = 0.041$).
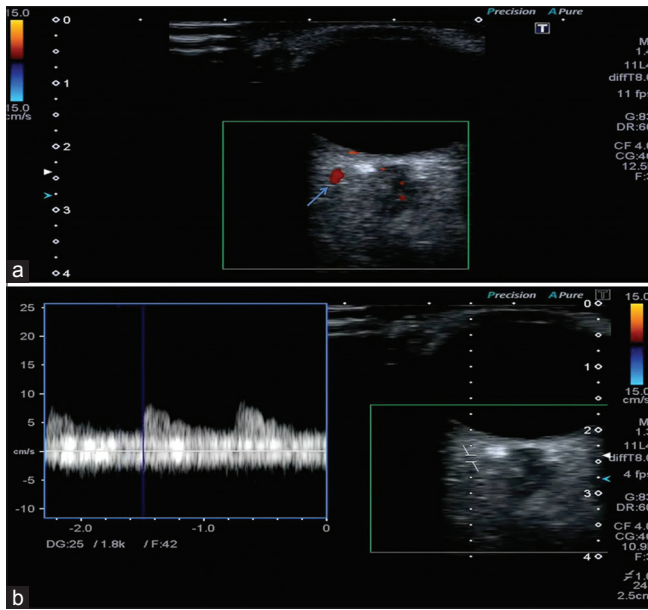
Figure 2: Example of the analysis of the Doppler ecography of the posterior temporal ciliary artery in one patient with age-related macular degeneration. (a) Doppler ecography in B mode of the orbit in which the posterior temporal ciliary artery can be seen. (b) Once the artery is identified, the function of spectral analysis is activated, which shows the flow on a time-scale

In control group, the level of folic acid correlated significantly with PSV of OA ($r = 0.399$, $P = 0.029$), PSV of PNCA ($r = 0.451$, $P = 0.012$), EDV of PNCA ($r = 0.438$, $P = 0.015$), PSV of PTCA ($r = 0.393$, $P = 0.031$), and EDV of PTCA ($r = 0.377$, $P = 0.040$). In study group, a positive and statistically significant correlation was found between the RI of OA and CRP ($r = 0.498$, $P = 0.005$) [Fig. 3]. Likewise, in this same group, a negative and statistically significant correlation was found between levels of homocysteine and B12 vitamin ($r = -0.549$, $P = 0.002$) [Fig. 4].

Table 2 summarizes the results of the blood test in the two groups of the current pilot study. As shown, significantly higher levels of homocysteine were found in the study group compared to the control group (15.57 ± 4.65 µmol/l vs. 18.67 ± 6.68 µmol/l, $P = 0.042$) as well as significantly higher levels of CRP (0.39 ± 0.26 mg/dl vs. 0.97 ± 1.52 mg/dl, $P = 0.046$). In contrast, no statistically significant differences between groups were found in the levels of total cholesterol ($P = 0.669$), HDL ($P = 0.254$) and LDL cholesterol ($P = 0.440$), folic acid ($P = 0.265$), and B12 vitamin ($P = 0.520$).

Discussion

Age is still the main risk factor for ARMD,[2] and aging is associated with some vascular changes, including progressive reduction of the choroidal thickness,[8] decrease in the density and diameter of the intravascular light of the choriocapillaris,[9] decrease of choroidal blood flow in the foveal area,[10] decrease of the ocular pulsatile blood flow,[11] reduction of the diameter of retinal arteries and veins,[12] and reduction of the neurogenic control of the choroidal blood flow.[13] All these changes promote an increase of the vascular resistance and a decrease of the ocular blood flow at a choroidal level. Although the etiopathogenesis of the ARMD is not completely clarified, some vascular risk factors and the atherosclerosis have been

Table 1: Ecography Doppler examination results in the control group and group of cases of the current study

Variable, mean±SD range	Control group (n=30)	Study group (n=30)	P
PSV OA (cm/s)	38.41±13.78 18.70-68.70	44.61±18.27 17.70-98.10	0.143
EDV OA (cm/s)	8.41±4.03 2.40-19.40	8.91±5.48 2.10-24.10	0.693
RI OA	0.78±0.07 0.62-0.94	0.80±0.08 0.65-0.95	0.218
PSV CRA (cm/s)	14.08±6.25 6.60-31	13.11±3.88 4.40-18.90	0.473
EDV CRA (cm/s)	4.06±2.01 1.90-8.70	3.22±0.86 2.20-5.50	0.041
RI CRA	0.70±0.11 0.27-0.87	0.73±0.08 0.43-0.84	0.181
PSV PNCA (cm/s)	14.77±8.15 6.50-40.40	13.96±4.44 7-23.40	0.633
EDV PNCA (cm/s)	4.22±2.31 2.40-11.40	4.09±1.40 2.40-7.30	0.793
RI PNCA	0.69±0.07 0.51-0.81	0.70±0.05 0.57-0.79	0.724
PSV PTCA (cm/s)	14.32±7.58 7-40	14.00±5.75 5.30-31	0.856
EDV PTCA (cm/s)	4.33±2.19 2.20-9.70	3.66±1.22 2.10-6.30	0.147
RI PTCA	0.69±0.06 0.52-0.81	0.72±0.06 0.60-0.84	0.035

PSV: Peak systolic velocity, EDV: End-diastolic velocity, RI: Pourcelot resistive index, OA Ophthalmic artery, CRA: Central retinal artery, PTCA: Posterior temporal ciliary artery, PNCA: Posterior nasal ciliary artery, SD: Standard deviation

related to the development of ARMD.[6,7] Our hypothesis is that a relationship between vascular resistance and the presence of plasmatic biomarkers of vascular risk would provide an additional support to the vascular theory of the pathogenesis of ARMD. Specifically, if a positive trend is found in our pilot study, it may be the initial point for the development of a prospective comparative study in a larger sample size, including also the analysis of additional vascular biomarkers, such as matrix metalloproteinase-9 (MMP-9), which is an elastin-degrading enzyme that may have a direct correlation with the vascular changes evaluated by color Doppler imaging.

More significant plasmatic levels of homocysteine in patients with exudative ARMD compared to controls were found in our study, whereas the levels of folic acid and B12 vitamin did not differ significantly among control and ARMD groups. This is consistent with the results of previous studies supporting the hypothesis that an excess of homocysteine and a deficit of folic acid and B12 vitamin in plasma are associated to an increased risk of ARMD.[7] The hyperhomocysteinemia[14] and the increase in the levels of PCR[15] have shown to generate a very significant increase of the plasmatic levels of asymmetric dimethylarginine, which is an endogenous inhibitor of the nitric oxide synthase (NOS) enzyme,[16] with the potential of leading to an endothelial vascular dysfunction. It should be considered that the choroidal blood flow is very dependent on vasoactive substances, such as the nitric oxide (vasodilator) and the endothelin-1 (vasoconstrictor).[17] This increase in the
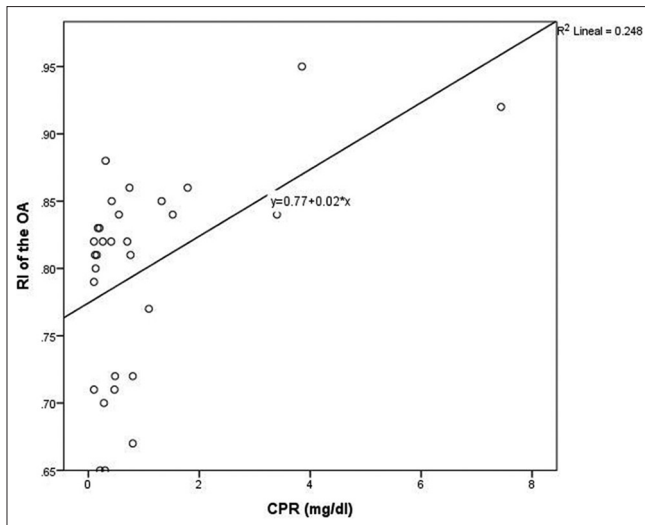
Figure 3: Scattergram showing the relationship among levels of C-reactive protein and the Pourcelot resistive index of the ophthalmic artery in study group

Figure 4: Scattergram showing the relationship among levels of B12 vitamin and homocysteine in study group

Table 2: Blood test results in the control group and group of cases of the current study

Variable, mean±SD range	Control group (n=30)	Study group (n=30)	P
Age (years)	75.3±7.3 61-89	78.6±5.9 57-90	0.061
Homocysteine (4-15, 4) (µmol/l)	15.57±4.65 9.20-26.8	18.67±6.68 33.80-15.57	0.042
Total cholesterol (50-200 mg/dl)	198.63±50.15 122-314	193.87±34.41 131-261	0.669
HDL cholesterol (45-65 mg/dl)	55.27±14.51 31-92	60.20±18.40 8-90	0.254
LDL cholesterol (0-160 mg/dl)	117.33±39.49 60-202	109.80±35.47 50-202	0.440
CRP (0-1 mg/dl)	0.39±0.26 0.1-1.20	0.97±1.52 0.1-7.44	0.046
Folic acid (3-15 ng/ml)	8.65±4.62 2.5-20	7.46±3.46 2-20	0.265
B12 Vitamin (210-920 pg/ml)	293.80±116.84 116-550	314.00±124.78 79-626	0.520

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CRP: C-reactive protein, SD: Standard deviation

plasmatic levels of asymmetric dimethylarginine due to the hyperhomocysteinemia leads to less availability of nitric oxide and then to an increase of the response to vasoconstriction, an increase of the muscle tone, and consequently to an increase of vascular resistance. This factor would be responsible for the generation of a reduction of the choroidal perfusion. Keles *et al*.[16] found a significant increase in the levels of homocysteine in patients with exudative ARMD, as in our study, but also in the levels of asymmetric dimethylarginine as well as a significant reduction of the levels of nitric oxide and NOS enzyme. According to these results, the authors concluded that the decrease in the levels of nitric oxide may have a role on the pathogenesis of ARMD. Besides homocysteine, in our sample, significantly higher plasmatic levels of CRP were
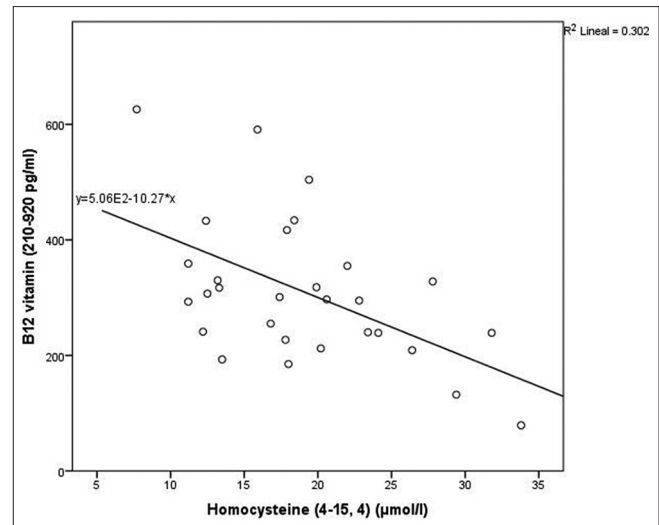
found in the group of eyes with exudative ARMD compared to controls, which is in agreement with other studies finding a similar association.[18] This suggests the presence of a potential inflammatory mechanism associated with the ARMD.[18]

The analysis of the vascular resistance by color Doppler imaging revealed that an increased RI was present in the group of eyes with exudative ARMD for all the arteries evaluated although only differences among control and ARMD groups reached statistical significance for the PTCA. Likewise, a decrease in PSV and EDV was detected in all arteries evaluated except for the OA in the group of eyes with exudative ARMD, but differences among control and ARMD groups were only statistically significant for the EDV of the CRA. These results support the hypothesis that an excess of homocysteine in exudative ARMD is generating changes in the ocular blood flow, leading to an increase of the vascular resistance. Other previous studies have evaluated some parameters characterizing the ocular blood flow in ARMD patients, obtaining results that are consistent with ours.[19] Metelitsina *et al*.[19] conducted a prospective study including 135 patients with ARMD and found that those patients developing a neovascular membrane had a significantly lower choroidal flow in the foveal area at the beginning of the study than those that did not develop a neovascular membrane during the follow-up. These authors assessed specifically the relative foveolar choroidal blood velocity, volume, and flow in 135 patients with ARMD, at baseline and then annually with laser Doppler flowmetry. They concluded that the decrease of the choroidal flow in the foveal area may precede the development of a neovascular membrane in ARMD and may have a role on such development. Boltz *et al*.[20] conducted a prospective study in 41 eyes with neovascular membrane in one eye and concluded that a low choroidal perfusion was a risk factor for the development of neovascular membrane in the other eye. In our study, we have detected which specific arteries show an increased vascular resistance in eyes with exudative ARMD as well as how this increased resistance is correlated with the increased levels of some plasmatic biomarkers for vascular risk. A positive and statistically significant correlation was found between the RI

of OA and CRP ($r = 0.498$), indicating that the higher the level of CRP, the higher was the vascular resistance in the OA. This suggests a potential role of CRP levels in a potentially stronger ocular vascular resistance in eyes with ARMD. This should be confirmed in future studies with larger sample sizes and also including additional plasmatic biomarkers, as previously mentioned. The correlations between homocysteine levels and color Doppler imaging parameters did not reach statistical significance. This may be due to the limitation in sample size.

In terms of limitations of the current study, the sample size and the observational design of the study should be mentioned. Many patients with exudative ARMD could not be included in our pilot study as they were treated with antioxidants. Future prospective and interventional studies with larger sample sizes would be necessary to confirm the real role of the vascular alterations and vascular risk factors on the pathogenesis of ARMD. It should be considered that this pilot study was aimed at finding any trend of clinical relevance suggesting that a prospective comparative study in a larger sample size would provide significant findings to provide a stronger support to the vascular theory of ARMD.

Conclusion

Our patients with exudative ARMD seem to have a general vascular disorder in all arteries evaluated and this vascular compromise might be aggravated due to the increase in the plasmatic levels of homocysteine and CRP that would lead to a worsening of the vascular changes associated to age. Therefore, the decrease of the choroidal perfusion related to the hyperhomocysteinemia, and the increase in the plasmatic levels of CRP may have an etiological role on the exudative ARMD. This should be confirmed in future studies with larger patient samples.

Conflicts of interest

There are no conflicts of interest.

References

1. Rohrschneider K. Blindness in Germany – Comparison between retrospective data and predictions for the future. Ophthalmologe 2012;109:369-76.

2. Smith W, Assink J, Klein R, Mitchell P, Klaver CC, Klein BE, *et al.* Risk factors for age-related macular degeneration: Pooled findings from three continents. Ophthalmology 2001;108:697-704.

3. Yonekawa Y, Miller JW, Kim IK. Age-related macular degeneration: Advances in management and diagnosis. J Clin Med 2015;4:343-59.

4. Friedman E. A hemodynamic model of the pathogenesis of age-related macular degeneration. Am J Ophthalmol 1997;124:677-82.

5. Friedman E, Krupsky S, Lane AM, Oak SS, Friedman ES, Egan K, *et al.* Ocular blood flow velocity in age-related macular degeneration. Ophthalmology 1995;102:640-6.

6. Bhutto IA, Baba T, Merges C, Juriasinghani V, McLeod DS, Lutty GA. C-reactive protein and complement factor H in aged human eyes and eyes with age-related macular degeneration. Br J Ophthalmol 2011;95:1323-30.

7. Gopinath B, Flood VM, Rochtchina E, Wang JJ, Mitchell P. Homocysteine, folate, Vitamin B-12, and 10-y incidence of age-related macular degeneration. Am J Clin Nutr 2013;98:129-35.

8. Yun C, Ahn J, Kim M, Hwang SY, Kim SW, Oh J. Ocular perfusion pressure and choroidal thickness in early age-related macular degeneration patients with reticular pseudodrusen. Invest Ophthalmol Vis Sci 2016;57:6604-9.

9. Chirco KR, Sohn EH, Stone EM, Tucker BA, Mullins RF. Structural and molecular changes in the aging choroid: Implications for age-related macular degeneration. Eye (Lond) 2017;31:10-25.

10. Xu W, Grunwald JE, Metelitsina TI, DuPont JC, Ying GS, Martin ER, *et al.* Association of risk factors for choroidal neovascularization in age-related macular degeneration with decreased foveolar choroidal circulation. Am J Ophthalmol 2010;150:40-7.e2.

11. Sandhu R, Sivaprasad S, Shah SP, Adewoyin T, Chong NV. Pulsatile ocular blood flow in asymmetric age-related macular degeneration. Eye (Lond) 2007;21:506-11.

12. Leung H, Wang JJ, Rochtchina E, Tan AG, Wong TY, Klein R, *et al.* Relationships between age, blood pressure, and retinal vessel diameters in an older population. Invest Ophthalmol Vis Sci 2003;44:2900-4.

13. Fitzgerald ME, Tolley E, Jackson B, Zagvazdin YS, Cuthbertson SL, Hodos W, *et al.* Anatomical and functional evidence for progressive age-related decline in parasympathetic control of choroidal blood flow in pigeons. Exp Eye Res 2005;81:478-91.

14. Pelikant-Malecka I, Sielicka A, Kaniewska E, Smolenski RT, Slominska EM. Endothelial toxicity of unusual nucleotide metabolites. Pharmacol Rep 2015;67:818-22.

15. Joshi MS, Tong L, Cook AC, Schanbacher BL, Huang H, Han B, *et.al.* Increased myocardial prevalence of C-reactive protein in human coronary heart disease: Direct effects on microvessel density and endothelial cell survival. Cardiovasc Pathol 2012;21:428-35.

16. Keles S, Ates O, Kartal B, Alp HH, Ekinci M, Ceylan E, *et al.* Evaluation of cardiovascular biomarkers in patients with age-related wet macular degeneration. Clin Ophthalmol 2014;8:1573-8.

17. Cabrera AP, Bhaskaran A, Xu J, Yang X, Scott HA, Mohideen U, *et al.* Senescence increases choroidal endothelial stiffness and susceptibility to complement injury: Implications for choriocapillaris loss in AMD. Invest Ophthalmol Vis Sci 2016;57:5910-8.

18. Colak E, Kosanovic-Jakovic N, Zoric L, Radosavljevic A, Stankovic S, Majkic-Singh N. The association of lipoprotein parameters and C-reactive protein in patients with age-related macular degeneration. Ophthalmic Res 2011;46:125-32.

19. Metelitsina TI, Grunwald JE, DuPont JC, Ying GS, Brucker AJ, Dunaief JL. Foveolar choroidal circulation and choroidal neovascularization in age-related macular degeneration. Invest Ophthalmol Vis Sci 2008;49:358-63.

20. Boltz A, Luksch A, Wimpissinger B, Maar N, Weigert G, Frantal S, *et al.* Choroidal blood flow and progression of age-related macular degeneration in the fellow eye in patients with unilateral choroidal neovascularization. Invest Ophthalmol Vis Sci 2010; 51:4220-5.