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RETINAL VASCULAR DISEASES: THE RARE FORMS OF THROMBOPHILIA.

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Introduction

The blood supply of the eyes and the brain are in a very close embryologic/ontogenetic and morphologic relationship. The optic nerve head and retina circulation system site from the carotid artery. The cerebrospinal liquor is enclosed by the meningeal tissues, a layer of connective tissue also covering the optic nerve defining a closed space for it. Haemodynamic changes of the liquor can effectively influence the blood supply of the eye. The optimal balance of the arterial and venous circulation and the optimal level of intraocular/intracranial pressure are essential conditions for the sufficient blood circulation for the eyes and the brain as well. Any kind of pressure change can cause decreased perfusion in the eyes and/or in the brain leading to ischemic damages. Based on this close relationship in the circulation of the eye and brain, the condition of the blood vessels in the brain and other parts of the body can be predicted by the examination of the blood vessels in the fundus. Ischemic signs caused by the vascular disorders of the fundus leading to loss of vision, are good markers of intra/extra cranial circulation failure. The vascular retinal diseases are multifactorial disorders. The exact etiology and pathogenesis of these diseases are still unclear. This is one of the reasons why effective therapy to cure retinal, vascular diseases is still not available

Our study aimed to investigate the pathogenic effects of the widely accepted so-called acquired cardiovascular risk factors (hypertension, hypercholesterolemia) in parallel with the congenital thrombophilic risk factors. We predict that our increasing knowledge of the pathogenic and etiologic risk factors of retinal vascular disorders might help to define more effective therapy.

Specific aims:

The major goal of our retrospective analysis is to define the risk factors for the development of retinal vascular disorders.

Specific aim 1: Through the screening of thrombophilic factors we will determine their role in the development of thrombosis.

Specific aim 2: To determine the genetic background of thrombophilic factors and statistically analyse the mutational changes

Specific aim 3: To investigate whether anticoagulant therapy is warranted as a secondary prophylaxis.

Patients and methods:

Between 1997 and 2004 we have examined total of 221 patients including 81 age-and sex matched control patients in our Department with Division of Rare Diseases. Among these patients we have observed 36 patients with non-arteritic ischemic optic neuropathy (NAION), 80 central retinal vein occlusions (CRVO), and 24 retinal artery occlusions (RAO).

The mean age of the patients suffering from NAION (4 of them bilateral) was 65.9(±11.6) years, from CRVO (7 of them bilateral) was 59.2(±14.1) years and from RAO was 61.1(±12.3) years. The mean age control patients were 61.6(±12.6) years.

The criteria for the diagnosis of NAION patients were based on the following symptoms: impaired vision, the relative afferent pupillary reflex defects, characteristic visual field defects, fundus changes (swollen, pale optic disc with frame shape haemorrhages) and fluoresceine angiographic changes. Patients having systemic symptoms relevant to giant cell arteritis, as well as those having elevated C-reactive protein levels (0.5 mmol/l or more) or

erythrocyte sedimentation rates exceeding 33.0 mm/h were excluded from the study. None of the 36 NAION patients showed any of these differences, therefore histological analysis of the temporal artery was not performed. Brain magnetic resonance imagining (MRI) was used to identify and exclude demyelinisation disorders.

The diagnosis of CRVO was based on the loss of vision (best corrected visual acuity), on relative afferent pupillary reflex defects, on decreased visual field test results (when visual acuity made it possible), on pathological changes of fundus and fluoresceine angiographic differences. Ischemic and non ischemic CRVO were differentiated by considering all the symptoms and angiographic images of 30 papillary or larger non-perfuse areas characteristic to the ischemic form. We had 7 bilateral cases.

In case of the retinal artery occlusion (RAO) the diagnosis was based on the sudden loss of vision, on the abnormalities of relative pupillary reflex deficiencies, on pathological differences observed by funduscopy and FLAG. No bilateral cases were found.

The control group consisted of 81 age- and sex-matched patients who have previously undergone cataract surgery, and non of them showed any vascular changes of the fundus. The age of this group was 61.6(±12.6) years on average. Sex and age differences between the control and affected groups of patients were not significant. No patients have suffered of autoimmune diseases.

We have registered the previous diseases and medical therapy of our patients prior to their first ophthalmologic examination.

All the patients of our affected and control groups were examined complete ophthalmologic examination including the best corrected visual acuity, visual field test (when visual acuity made it possible) with Goldmann perimetry, IOP measurement using Goldmann applanation tonometry, slit lamp biomicroscopy and funduscopy - using 90 D aspheric Volk lens -, plus fluoresceine angiography. The risk factors (hypertension, diabetes mellitus, ischemic heart

disease, arteriosclerosis and smoking) of each patient have also been recorded/documented. In addition to the routine laboratory investigations the level of serum cholesterol and triglyceride were measured, and a haemostasis screening test (prothrombine time, thrombine time, activated partial thromboplastine time) was performed. The starting date of the first ophthalmologic symptoms was noted and six weeks later the blood samples were taken to evaluate thrombotic risk factors: levels of PC and PS, AT activity, APC resistance (Leiden mutation), FII G20210A allele, fibrinogen level, ACL (antiphospholipid) antibodies, elevated levels of factor VIII, plasminogen and Lp(a). The level of factor VIII was considered to be elevated only in that case if it was found to be increased in three consecutive measurements made at 6-week intervals after the initial one. For the determination of decreased levels of PC, PS, and AT we have followed the same protocol. In order to rule out false-positive results and acquired elevation of parameters we performed parallel measurements of CRP and vWAg levels. Control examinations were dated 6 weeks, 3 to 6 months, and yearly after the initial one. The total follow up time was between 6 months to 6 years.

Laboratory methods

All the laboratory analysis was performed at the Department of Clinical Biochemistry and Molecular Pathology.

Fibrinogen levels were determined according to the Clauss method. PC and PS activities were measured using coagulation tests (Diagnostica Stago, Asnieres, France). AT and plasminogen were determined by chromogenic assays (Diagnostica Stago, Asnieres, France). FVIII was determined using a clotting assay. All these determinations were performed in an STA-Compact coagulometer (Diagnostica Stago, Asnieres France). FV Leiden mutation and FII G20210A allele were detected using a real-time polymerase chain reaction (RT-PCR)

method according to Bertina. Determination of the APC-R ratio was achieved by the measurement of two different activated partial thromboplastine time (APTT). One measurement represented the plasma with APC, the other one without APC. The ratio of these two measurements is called the APC ratio what we have considered positive at the level of 2 or higher. In positive cases the existence of the Leiden-mutation was always justified by molecular genetic methods. The potentially abnormal DNA sequences were amplified by polymerase chain reaction (PCR). The PCR products were digested with restriction endonuclease (MnII), and the restriction fragments were separated by agars gel electrophoresis. PCR method was used for the identification of the FII G20210A allele. Enzyme-linked immunosorbent assay (Selisa, Cambridge Life Sciences, U K) was performed to determine the antibody against anticardiolipin (ACL). Lp(a) concentration was measured using a chemical method in an Integra 700 automat (Roche Diagnostics, Mannheim, Germany). Homocysteine levels were determined by combinated enzymatic and fluorescent polarisation immune method. In this procedure first all the homocysteine content of the samples is converted to S-adenosyl homocysteine (SAH). This product competes for the binding to an antibody with a manufacturer labelled SAH analogue. The amount of the bided fluorescent analogue was measured by polarizing immune method.

Statistical analysis

The homogenous distribution of age and gender among patients of retinal vascular diseases and control subjects were confirmed by Mann-Whitney test respectively. The thrombophilic and cardiovascular risk factors were analysed by logistic regression odds ratios with 95% confidence intervals. The statistical analysis was performed with SPSS Windows 11.5 version.

Results

The mean age of the patients with NAION was 65.9(±11.6) years; they comprised 23 males and 13 females. The CRVO patients of 35 males and 45 females with the mean age of 59.7(±14.05) years. The mean age of the 16 male and 12 female patients with CRAO was 61.12(±12.3) years, while that of the control group was 61.6(±12.6) years, with a male/female ratio of 53/28. The age and sex differences between the control and affected groups of patients were not significant (p_1=0.71, $p_{2=}$0.11, p_3=0.79). In the NAION group, logistic regression odd ratio calculations for cardiovascular risk-factors like diabetes mellitus (OR: 5.2, 95% CI: 1.22-22.13, p=0.026) and hypercholesterolemia (OR: 1.54, 95% CI: 1.08-2.19, p=0.015) proved to be significant. Patients with FV (Leiden mutation) (OR: 5.5, 95% CI: 1.53-19.6, p=0.009), Lp(a) (OR: 1.01, 95% CI: 1.0-1.003, p=0.027), hyperhomocysteinaemia (OR: 0.43, 95%CI: 0.14-0.55, p=0.000) and hyperfibrinogenemia (OR: 1.91, 95%CI: 1.20-3.06, p=0.095) showed significant differences in the level of thrombophilic factors.

In the group of patients with CRAO only the incidence of hypertension (OR: 0.33, 95%CI: 0.13-0.97, p=0.014) as an average risk factor showed significant difference, but prothrombotic factors such as hyperfibrinogenaemia (OR: 2.9, 95%CI: 1.29-6.57, p=0.010) and the presence of FV (Leiden mutation) (OR: 3.9, 95%CI: 1.43-10.96, p=0.008) increased the chances of developing this disease.

The incidence of CRVO is significantly higher in cases of ischemic heart disease (OR: 5.3, 95%CI: 1.9-14.78, p=0.001) and hyperfibrinogenaemia (OR: 2.19, 95%CI: 0.87-5.53, p=0.09).

ACL (antiphospholipid) antibodies were found only at one of the patients, who suffered of bilateral CRVO and did not have any other risk factor. In this patient the presence of the ACL antibody could have been responsible for the development of the disease. Statistical analysis could not be performed for other thrombophilic factors such as decrease in PC and PS activities, presence of FII 20210A allele and increase in FVIII, because thrombophilic changes could be observed only in 5 cases of the affected and non in the control patients.

Hypertension can play a serious role in the development of retinal vascular diseases because, this risk factor caused significantly elevated incidence of the disease in all the 3 groups of patients.

In the six years of patient follow-up, bilateral NAION developed in 4 cases and bilateral CRVO in 7 cases. Bilateral CRAO did not develop during this period. If both cardiovascular and thrombophilic risk factors are considered, each patient with bilateral retinal vascular disease had at least two but usually more (up to 8) different risk factors present at the same time. The simultaneous presence of multiple risk factors seems to contribute to the development of retinal vascular disease of the fellow eye. Substantially high level of Lp(a) can by itself significantly contribute to the patomechanism of the vascular disorders of the retina. (14 out of all the patients had higher Lp(a) level than 1000mg/l).

Discussion

Retinal vascular diseases are multifactorial disorders, causing serious and irreversible unilateral or bilateral visual impairment. These disorders are closely associated with cardiovascular risk factors, with changes in the local environment in

the eye and with thrombophilic factors as well. The etiology of the retinal vascular diseases shows many similarities, but their patomechanisms are different.

In the present study we found statistically higher incidence of cardiovascular and thrombophilic risk- factors in cases of retinal vascular disorders. In all three groups of patient hypertension, DM and elevated lipid levels could be detected more frequently. The etiologic role these factors are widely accepted as they can have a high impact in the patomechanism of not only the retinal vascular disorders, but also in other cardiovascular diseases.

Among the thrombophilic factors in cases APC-R (Leiden) mutation, elevated levels of Lp(a), hyperhomocysteinaemia had significantly higher incidence. In cases of CRVO and CRAO thrombophilic risk factors had a higher rate than in the control group. FV (Leiden) mutation and hyperfibrinogenaemia also showed higher incidence. Our results suggest that thrombophilic factors can play a significant role in the development of retinal vascular disorders.

The presence of APA could be detected only in one 50 years old mail patient with bilateral CRVO. He had no other risk factors, therefore only the presence of APA could be the cause of his disease.

According to Talks et al., in addition to hypercholesterolemia and smoking, high fibrinogen levels may also play a role in the development of NAION.

Tekeli et al. observed the decrease of PC more frequently in OVCR. However they failed to detect changes in the level of PS and AT. Higher incidence of APC-R (Leiden) mutation was published in cases of CRVO, but other authors could not prove the same.

These studies suggest that thrombophilic factors play a decisive role in the vascular pathology of the fundus, however additional large scale studies are required.

Our studies also indicate that investigations of accompanying diseases such as hypertension, diabetes mellitus, hyperlipidemia are also needed. Cessation of smoking will lower the risk factors.

In the case of thrombophilia causal therapy is also available. It has been proved, that in the case of homocysteinaemia the stop of smoking together with the administration of vitamins B_6, B_{12} and folic acid are beneficial.

In case of acut fundus vascular pathology (CRVO, CRAO) the introduction of fibrinolytic therapy is highly recommended. Unless otherwise contradicted, the introduction of thrombolytic therapy is indicated within 6 hours after the thrombosis occurred.

The thrombolytic therapy requires emergency room setting and professionals.

If the introduction of the thrombolytic therapy is not viable at the initial six hours, than antithrombotic or antiplatelet therapy is recommended.

Thrombophylic screening is recommended for patients under the age of 50 with bilateral fundus vascular pathology or with familiar history of thrombosis. In these cases the screening of family members should be performed.

The results of the above described screenings will identify the laboratory tests that can contribute to an efficient preventive care.

Publication related to the thesis

1. Nagy V, Facsko A, Takacs L, Balazs E, Berta A, Balogh I, Edes I, Czuriga I, Pfliegler G, Activated Protein C Resistance in Anterior Ischemic Optic Neuropathy. Acta Ophthalmol Scand 2004; 82(2):140-143.

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2. Nagy V, Facskó A., Balázs E., Pfliegler G., Kerényi A., Berta A, Az aktivált protein-C rezisztencia és az anterior ischaemiás opticus neuropathia közötti kapcsolat. Szemészet 2002; 139:51-54.

3. Nagy V, Steiber Z, Takács L et al. Thrombophilic screening for nonartritic ischemic optic neuropathy. Graefe's Arch Clin Exp Ophthalmol (közlésre elfogadva)

(Impact factor:1.27)