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Increased pregnancy-associated protein A in retinal vein occlusion

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Abstract. – OBJECTIVE: The aim of this study was to evaluate pregnancy-associated plasma protein A (PAPP-A) levels in patients with retinal vein occlusion (RVO), and to investigate its possible role as a predictive biomarker.

PATIENTS AND METHODS: The study included 26 patients with RVO and 30 age- and gender-matched healthy subjects as controls. PAPP-A levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit. The PAPP-A levels in patients with RVO were compared with those in the control group using the Mann-Whitney U test.

RESULTS: The mean serum PAPP-A levels were 1.27 ± 0.46 mIU/L (mean ± standard deviation) in the RVO group and 1.14 ± 0.11 mIU/L in the control group. There was a significant difference in PAPP-A levels between RVO patients and healthy subjects (p = 0.03). Moreover, in ROC analysis comparing the RVO patients and controls, a cutoff value of 1.126 (AUC: 0.669), specificity of 63.3% and sensitivity of 76.9% were calculated for the RVO patients (p = 0.03).

CONCLUSIONS: Our data seems to support the roles of both thrombosis and atherosclerosis in the development of RVO. It is possible that PAPP-A may be involved in the pathogenesis of venous thrombosis in the retina.

Key Words:
Retina vein occlusion, Pregnancy-associated plasma protein A, Atherosclerosis.

Introduction

Pregnancy-associated plasma protein A (PAPP-A) is a zinc-binding metalloproteinase. Fundamentally, it separates insulin-like growth factor-binding protein 4 (IGFBF-4) from insulin-like growth factor (IGF)[1]. PAPP-A was found in pregnant women's blood in high concentrations in 1974 and was then isolated, as it was thought to be one of the four placenta-based proteins. It was used as a serum biomarker in pregnancy for a long time. Following the reports of Lawrence et al[2] on PAPP-A being the IGFBP-4 proteinase associated with fibroblast culture media isolated IGF, it has been discovered that PAPP-A is not exclusively secreted during pregnancy, also be synthesized by a variety of cells in the body such as granulosa cells in the ovaries, fibroblasts, vascular smooth muscle cells, endothelial cells and osteoblasts[4]. From experiments conducted on animals and from histological studies, it has been found that PAPP-A plays a critical role in growth and development, is involved in many physiological/physiopathological processes involving regulation of the local IGF concentration, and is necessary for new vein formation after vascular injury, wound and broken bone healing[5,8]. In 2001, the clinical usage of high-level PAPP-A in atherosclerotic plaque ruptures in patients with acute coronary syndrome increased in the cardiovascular field[9]. In many studies[10,11] conducted to examine the potential role of PAPP-A in atherosclerosis, the raise of PAPP-A in blood levels during sudden cardiovascular events that would occur in stable coronary heart disease has been reported as significant.

Retinal vein occlusion (RVO) is a vascular retinal disease that is usually seen in older age, and it is the second most common problem after diabetic retinopathy[12]. RVO usually occurs due to pressure applied on the vein by the sclerosing artery at the crossing point with the vein, but the pathogenesis of the disease is yet to be completely clarified. RVO has been reported to be linked with hemostatic factors, hypertension, diabetes mellitus, dyslipidemia, pregnancy, oral contraceptive drugs, atherosclerosis and systemic inflammatory diseases[13,14]. Lately, in the public studies regarding RVO, it has been emphasized that among the usual cardiovascular risk factors that generally lead the list, atherosclerosis is highly related to the disease[15]. In the long-term

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follow-up results of the RVO patients, the atherosclerotic cardiovascular morbidity and mortality risks have shown a significant increase[16].

The aim of this study was to compare the PAPP-A levels in RVO patients to those in healthy individuals. To the best of our knowledge, this work is the first to evaluate the PAPP-A levels in RVO patients. The results of this study might enlighten etiopathogenesis in RVO development and make a contribution towards future investigations related to the potential treatment of retinal vascular diseases.

Patients and Methods

In this prospective research, patients who were diagnosed with any type of RVO between June 2015 and December 2015 were enrolled at Kocaeli Derince Education and Research Hospital. The control group consisted of age- and gender-matched healthy subjects with no preexisting ocular disease. All the participants underwent a full ophthalmologic examination including best-corrected visual acuity, intraocular pressure measurement, and biomicroscopic and funduscopic examinations at the beginning of the study. Fundus photography, fundus fluorescein angiography and optical coherence tomography were performed in the patients with RVO. The study was performed according to the Declaration of Helsinki, and it was approved by the Kocaeli University Ethics Committee.

Participants with diabetes mellitus, uncontrolled hypertension, infectious diseases, abnormal leukocyte count, malignancy, infectious diseases, liver or renal insufficiency, cerebrovascular disease or any cardiovascular disease, such as congestive heart failure and heart valve disease, which was being treated with an anticoagulant, were excluded. Patients and healthy subjects with any ophthalmic problems were also excluded.

To avoid the confounding effects of diurnal variation on the hemostatic system, sampling procedures were performed in the morning while the participants were in a fasting state. Venous blood samples were collected and placed at 4°C immediately following collection, and were subsequently centrifuged at 4°C at 4,000 r/min for 5 min to collect the serum. Serum aliquots were stored at −80°C until assayed, and each sample was used only once. Serum PAPPA concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ZenTech Kit, i Epoch MICROPLATE, (spectrophotometer) BioTech Instrument, Inc., Winooski, VT, USA).

Statistical Analysis

All statistical analyses were performed with using SPSS for Windows 18.0 software (SPSS Inc., Chicago, IL, USA). The values are presented as the mean ± standard deviation (SD). The data were compared between groups using the independent *t*-test and the Mann-Whitney test. The significance level was set at a $p < 0.05$. Receiver operating characteristic (ROC) analyses were used to identify the cutoff values and the specificity/sensitivity of the PAPP-A levels.

Results

A total of 56 participants were examined in the study, consisting of 26 patients with any type of RVO and 30 healthy subjects. The average age of the RVO group was 61.1 ± 16.4 years, and that of the control group was 62.3 ± 13.8 years. The overall female to male ratio was 14/12 in the RVO group and 16/14 in the control group. There were no differences attributable to age and sex in either of the groups ($p > 0.05$). The patients showed no significant differences with control subjects in levels of low density lipid and high density lipid, triglycerides, fasting blood sugar or body mass index at the time of the investigation. Baseline demographic and clinical characteristics are provided in Table I.

The mean PAPP-A values were 1.27 ± 0.46 mIU/L in RVO patients, and 1.14 ± 0.11 mIU/L in the control group. A significant difference was found in PAPP-A values between the groups ($p = 0.03$) (Table I). Also, in ROC analysis comparing the RVO patients and controls, cutoff value of 1.126 [area under the curve (AUC): 0.669], specificity of 63.3% and sensitivity of 76.9% were calculated for the RVO patients ($p = 0.03$) (Figure 1).

Discussion

PAPP-A, which was detected first in high concentrations in pregnant women's blood in 1974, has been found to be a useful biomarker for many years for detecting diseases such as preeclampsia, Down syndrome, etc. in the preg-

Table I. The clinical features and demographic data of groups.

	Retinal vein occlusion	Control group	*p*-value
Age (years)	61.1 ± 16.4	62.3 ± 13.8	0.58
Gender (female/male)	14/12	16/14	0.49
Body mass index (kg/m^2)	26.2 ± 0.8	27.4 ± 0.9	0.62
Triglyceride (mg/dl)	145.43 ± 41.7	152.19± 31.5	0.24
HDL cholesterol (mg/dl)	41.6 ± 5.5	43 ± 4.18	0.35
LDL cholesterol (mg/dl)	140.24 ± 38.7	132.27 ± 25.6	0.28
PAPP-A levels (mIU/L)	1.27 ± 0.46	1.14 ± 0.11	0.03

nancy period. With the discovery of the possibility of PAPP-A's significant role in atherosclerotic lesions in some clinical and histopathological studies, its usage in the cardiovascular field has increased[9,17]. PAPP-A is stimulated via macrophage-based pro-inflammatory cytokines, tumor necrosis factor-α and interleukin-1 , and it is synthesized intensely by fibroblasts and vascular smooth muscle cells[4,5,8]. These factors also form a part of the inflammatory response to other types of vascular and kidney damage. In the experiment conducted on animals, overexpression of PAPP-A has been shown in the smooth muscle arteries of the mice with atherosclerotic lesions[18]. Bayes et al[9] have asserted that PAPP-A exists intensely in the atherosclerotic cells in ruptured unstable plaques and extracellular matrix, as serum

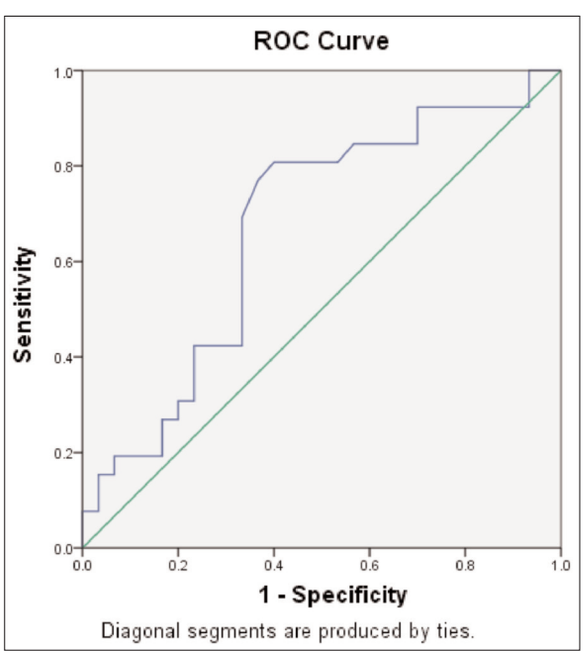

Figure 1. Receiver-operating characteristic curves for predictors of retinal vein occlusion. Area under the curve for the PAPP-As = 0.669, purple line.

levels show a significant increase in myocardial infarction and unstable angina, and that this could be a sign of acute coronary syndrome. They have argued that patients would be recognized before myocardial necrosis via the increase in PAPP-A level in the serum at the beginning of unstable angina. Many other studies[10,11,19] conducted to examine the potential role of PAPP-A in atherosclerosis have reported that PAPP-A is related to the increase in sudden cardiovascular events that would occur in stable coronary heart disease.

Several histopathologic, epidemiological and biological studies[20,21] have been held on RVO pathogenesis, and various mechanisms have been suggested to explain the development of RVO. RVO pathogenesis occurs as a consequence of Virchow's triad, which is defined as the artery pressure in the arterio-venous junction, degenerative changes in the vessel wall and abnormal hematological factors. The pressure on the crossing point of the retinal vein due to atherosclerosis in the retinal artery plays an essential role in the pathogenesis of RVO[22]. In the Atherosclerosis Risk in Communities and the Cardiovascular Health Studies, correlations have been reported between RVO and hypertension, diabetes mellitus, dyslipidemia, smoking, hemostatic factors, obesity and carotid artery plaques that include the classic cardiovascular risk factors[13,14]. The connection between RVO and systemic atherosclerosis has been emphasized in these studies. In other studies, some diseases that cause atherosclerosis have been correlated with RVO[15,23].

Our paper revealed statistically significant higher PAPP-A values in the RVO group than the healthy control group. We predicted an elevation in serum PAPP-A values in the RVO patients before starting the study, since inflammation forms in the choroid and retina layers and wound healing phases occurred afterwards. However, we did not expect a statistically significant higher value

in PAPP-A, because we had thought that the inflammation effect in this process might have been less and local.

Martin et al[24] have observed that patients who are admitted to the clinic with RVO show high cardiovascular mortality and morbidity rates in their long-term follow-ups and that RVO could be an anticipatory sign. There is no clear evidence regarding the simultaneous existence of coronary artery disease during admittance with RVO; however, RVO may be the first sign of atherosclerosis development. Atherosclerosis plays an important role in the pathogenesis of various ischemic vascular diseases such as stroke and coronary artery disease. Macrophages, fibroblasts, vascular smooth muscle cells and endothelial cells are the primary cellular components of atherosclerosis[25]. Inflammatory agents stimulate these cells and implement PAPP-A formation and its involvement in the process[4,10]. Some publications[21,26] show an increase in systemic inflammatory cytokines such as IL-6 and tumor necrosis factor-α in RVO. Since atherosclerotic plaques contain common protein structures with inflammation and immune-mediated processes, chronic inflammation and ischemia may be some of the means by which RVO develops. The relationship between PAPP-A and atherosclerotic pathogenesis was the basis of this study, as well as eliciting the link between RVO and PAPP-A, and determining whether PAPP-A could serve as a biomarker and a prognostic factor in patients with RVO.

The methodology used in determining the PAPP-A level is another issue that should be addressed. In 2005, Qin et al[27] asserted that PAPP-A in the circulation in acute coronary syndrome does not form covalent bonds with the eosinophilic major basic protein. Therefore, in pregnancy, the dominantly present complex form of PAPP-A is measured, while in cardiac patients, PAPP-A in free form is determined in the serum. In our work, free PAPP-A levels were measured with the assay that we have used. The withdrawal of blood with heparin in studies of PAPP-A of coronary artery and renal artery disease is a drawback for those studies, because heparin application elevates the level of PAPP-A[28]. Patients who use heparin or other anticoagulants have not been included.

There are some limitations to our study; the number of patients included is limited; studies carried out with a larger population may confirm the results. Furthermore, different results might be obtained by making RVO sub-class comparisons with a larger number of patients and creating gender and age differentiations. Including different biomarker measurements in addition to the PAPP-A, may enrich the results and provide more distinctive results. In the future studies, intraocular vitreous samples could be examined, in addition to the sera prepared from the patients. Data showing the measurement of PAPP-A concentrations in serum and vitreous might be useful in the development of new medications for the treatment of retinal vascular disorders.

Conclusions

PAPP-A values in our study were significantly just as high in RVO patients as in those with coronary artery and other similar vascular occlusions. This result corroborated the existing information on patients with RVO being more prone to cardiovascular diseases, especially cardiac diseases that develop on the basis of atherosclerosis. PAPP-A can be used as a biomarker for RVO, just like a coronary artery and other similar vascular occlusions. Additionally, in order to lower the number of unwanted cardiovascular events in the future, a biomarker, which will be used on high-risk atherosclerosis, might be more beneficial in comparison to new markers that would be used after myocardial necrosis, has developed.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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