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Aqueous levels of VEGF correlate with retinal non-perfusion areas in patients with diabetic macular edema and macular edema secondary to central retinal vein occlusion

Korelacja stężenia VEGF w cieczy wodnistej z obszarem niedokrwienia siatkówki u pacjentów z cukrzycowym obrzękiem plamki i obrzękiem plamki w przebiegu zakrzepu żyły środkowej siatkówki

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Abstract:

Aim: To evaluate the association between the level of vascular endothelial growth factor in the aqueous humor and the size of capillary non-perfusion areas in patients with macular edema secondary to retinal vein occlusion and diabetic retinopathy.

Material and methods: The study group consisted of 24 patients (24 eyes) at the age of 55–78 years, with diffuse macular edema secondary to retinal vein occlusion and diabetic retinopathy. The control group consisted of 26 subjects aged 55–87 years who were admitted for scheduled cataract surgery. The VEGF aqueous humor levels, retinal thickness using optical coherence tomography, as well as the size of non-perfusion areas measured on fluorescein angiography images were evaluated in each enrolled subject.

Results: The vascular endothelial growth factor aqueous humor levels were found to be significantly higher in patients with macular edema as compared to controls ($p = 0.0002$). In the diabetic macular edema and retinal vein occlusion group, the concentration of vascular endothelial growth factor in aqueous humor positively correlated with the extent of non-perfusion areas measured on fluorescein angiograms ($Rs = +0.45$, $p = 0.02$;). Multivariate analysis of patients and controls performed using the general linear model, adjusted for age, sex, intraocular pressure and the presence of diabetes, revealed that macular edema was an independent factor associated with higher aqueous VEGF concentrations ($ß = +0.74$, $p = 0.0012$).

Conclusions: Macular edema secondary to either retinal vein occlusion or diabetic retinopathy is associated with the increased levels of vascular endothelial growth factor in the aqueous humor. Therefore, the management of patients with macular edema secondary to retinal vein occlusion or diabetic retinopathy should aim at reducing the ocular vascular endothelial growth factor concentrations, especially in the presence of capillary non-perfusion areas.

Key words: vascular endothelial growth factor, diabetic macular edema, retinal vein occlusion.

Abstrakt:

Cel: ustalenie zależności między obecnością obrzęku plamki i wielkością stref bezprzepływowych siatkówki a poziomem naczyniowo-śródbłonkowego czynnika wzrostu w cieczy wodnistej u pacjentów z obrzękiem plamki na tle zakrzepu żyły siatkówki i retinopatii cukrzycowej.

Materiał i metody: grupę badaną stanowiło 24 pacjentów (24 oczy) z rozlanym obrzękiem plamki w przebiegu zakrzepu żyły środkowej siatkówki i retinopatii cukrzycowej w wieku 55–78 lat. Grupę kontrolną stanowiło 26 pacjentów w wieku 55–87 lat. U pacjentów wykonano: badanie poziomu naczyniowo-śródbłonkowego czynnika wzrostu w cieczy wodnistej metodą immunoenzymatyczną, pomiar grubości siatkówki w optycznej koherentnej tomografii oraz badanie angiografii fluoresceinowej z oceną rozległości obszarów niedokrwienia siatkówki.

Wyniki: stwierdzono statystycznie istotnie wyższy poziom naczyniowo-śródbłonkowego czynnika wzrostu u pacjentów z obrzękiem plamki w stosunku do wartości tego parametru u osób z grupy kontrolnej niezależnie od przyczyny obrzęku ($p = 0{,}0002$).

Stwierdzono pozytywną korelację między nasileniem zaburzeń perfuzji w angiografii fluoresceinowej a poziomem naczyniowo-śródbłonkowego czynnika wzrostu u pacjentów z badanej grupy (R = +0,45; p = 0,02). W analizie wieloczynnikowej obrzęk plamki był niezależnym od płci, wieku, ciśnienia wewnątrzgałkowego i cukrzycy czynnikiem związanym z wyższym poziomem naczyniowo-śródbłonkowego czynnika wzrostu w cieczy wodnistej (ß = +0,75; p = 0,0012), a u pacjentów z obrzękiem obecność zaburzeń perfuzji była niezależnym od płci, wieku, ciśnienia wewnątrzgałkowego i cukrzycy czynnikiem związanym z wyższym poziomem naczyniowo-śródbłonkowego czynnika wzrostu w komorze przedniej oka (ß = +0,45; p = 0,02).

Wnioski: obrzękowi w plamce związanemu z przebytym zakrzepem lub retinopatią cukrzycową towarzyszy podwyższony poziom naczyniowo-śródbłonkowego czynnika wzrostu w cieczy wodnistej. U pacjentów z obrzękiem plamki w przebiegu zakrzepu i/lub retinopatii cukrzycowej wskazane jest stosowanie terapii obniżającej poziom naczyniowo-śródbłonkowego czynnika wzrostu, zwłaszcza w przypadku obecności widocznych stref pozbawionych perfuzji włośniczkowej.

Słowa kluczowe: czynnik wzrostu śródbłonka naczyniowego, cukrzycowy obrzęk plamki, zakrzep żyły siatkówki.

Introduction

Macular edema (ME) is defined as an accumulation of fluid within the retinal space, as well as swelling of the Muller cells in the macular area. ME, which develops secondarily to many ocular diseases, such as diabetic retinopathy (DR), retinal vein occlusion (RVO) and ocular inflammation, is a common cause of vision loss (1). The pathogenesis of ME is known to be very complex. Vascular dysfunction, disrupted blood-retinal barrier and several inflammatory mediators, including vascular endothelial growth factor (VEGF), have been proposed as possible underlying mechanisms and causative factors of ME. Retinal hypoxia has also been implicated in the pathogenesis of ME secondary to DR and RVO. Hypoxia causes increased expression of VEGF, which is a potent inducer of vascular permeability, shown to cause vascular leakage (2).

Vascular endothelial growth factor promotes proliferation, migration and survival of vascular endothelial cells, increases vascular permeability, dilates blood vessels as well as attracts endothelial cell precursors and monocytes. It is soluble and can therefore be quantified in ocular fluid compartments. The volume of aqueous humor is approximately 250 μl and the amount of samples to be obtained by anterior chamber paracentesis is only 100–200 μl. Thus, it is recommendable to choose an assay, which yields the most precise results based on a small volume sample, in order to evaluate the soluble molecule count in the aqueous humor. The multiplex bead array assay (MBAA) is a promising technique that can quantify molecules in small sample volumes. For this reason, MBAA is useful for the angiogenic factor assay in aqueous humour. This technology has already been used for the measurement of cytokines and growth factors in plasma, vitreous and aqueous humor samples (3).

In this paper, we investigated the association between the aqueous levels of VEGF in patients with diabetic macular edema (DME) and macular edema secondary to RVO using MBAA and determined the correlation of the discussed the data with clinical characteristics, such as the size of non-perfusion area on fluorescein angiograms (FA) and the severity of macular edema measured using optical coherence tomography (OCT).

Material and methods

We enrolled patients with ME eligible for intravitreal triamcinolone administration and divided them into two groups: DME and macular edema secondary to retinal vein occlusion (RVO-ME). The inclusion criteria in DME group were: (1) central subfield macular thickness (CSMT) 250 μm or greater in optical coherence tomography (OCT); (2) recent onset of ME (within 3 months), and (3) non-proliferative DR. The inclusion criteria in RVO-ME group were: (1) CSMT 250 μm or greater on OCT; (2) recent onset of ME (within 3 months), and (3) any previous history of retinal disease.

The exclusion criteria for this study were as follows: (1) active neovascular disease; (2) vitreomacular traction seen in OCT; (3) history of ocular inflammation and vitreoretinal disease, (4) macular focal/grid laser photocoagulation within 3 months prior to surgery, (5) ocular diseases other than DR, RVO or cataract. Informed consent was obtained from each patient. This study was performed in accordance with the Helsinki Declaration, and approved by the Institutional Review Board of the Pomeranian Medical University.

Macular thickness was measured using optical coherence tomography (Stratus OCT, Zeiss Humphrey System, Dublin, California, USA) before collecting aqueous humor samples. A macular profile was acquired for the central 6.0 mm zone using fast macular scan protocol. Central macular thickness (CMT) measured automatically by the in-built retinal border detection algorithm was used for statistical analysis.

Fluorescein angiography (FA) was performed upon initial DR or RVO diagnosis using a digital fundus camera (Zeiss FF450 Plus IR, Carl Zeiss Meditec AG, Jena, Germany). The capillary non-perfusion area was outlined manually on the combined FA image. Based on FA our patients were categorized into 3 subgroups: 0 – without any visible areas of capillary non perfusion; 1 – local capillary non perfusion occupying less than 25% of retinal surface; 2 – extensive capillary non perfusion occupying more than 25% of retinal surface.

The aqueous humor samples of diabetic patients and patients with RVO were collected directly before the intravitreal injection of triamcinolone acetonide or cataract surgery. The non-diabetic subjects, with no signs of RVO, admitted for the scheduled cataract surgery, provided control aqueous samples harvested at the beginning of cataract surgery.

The specimens were immediately transferred into sterile plastic tubes and stored at -70°C until assayed. VEGF concentration was quantified in aqueous humour by multiplex fluorescent bead-based immunoassays (Luminex Corporation, Austin, TX, USA) using commercial Human Cytokine/Chemokine Magnetic Bead Panel (Merck Millipore, Billerica, MA, USA). 25 μL of each standard, control and samples were added to the plate together

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with multiplex antibody capture bead solution, and the plate was incubated with shaking at 4°C overnight. Next day, each well was washed with 200 μL Wash Buffer twice by using hand-held magnet. 25 μL of detection antibody cocktail was pipetted to each well and the plate was sealed and incubated at room temperature for 1 hour on a shaker. Afterwards, 25 μL of streptavidin-phycoerythrin mixture was added to the plate and incubated with agitation for 30 minutes at RT in dark. Finally, after washing, the microspheres in each well were re-suspended in 150 μL Sheath Fluid and shaken at room temperature for 5 minutes. The plate was then read and analyzed on the Luminex analyzer and the analyte concentration was determined from five different standard curves showing the MFI (Median Fluorescence Intensity) vs. protein concentration.

Since the distributions of most quantitative variables differed significantly from a normal distribution (as assessed by the Shapiro-Wilk's test), non-parametric tests were used. The Mann-Whitney U test was used for comparing the quantitative and rank variables between groups. The strength of association between quantitative and rank variables was measured with Spearman rank correlation coefficient (Rs). Fisher's exact test was used for comparing qualitative variables between groups. A general linear model (GLM) adjusted for age, sex, intraocular pressure and the presence of diabetes was used in order to determine the status of the aqueous humour VEGF level as an independent risk factor associated with retinal edema. The p-value <0.05 was considered statistically significant.

Results

The characteristics of the patients and controls are summarized in Table I. The VEGF aqueous humour levels were found to be significantly higher in patients with macular edema as compared to the control subjects ($p = 0.0002$). Multivariate analysis of patients and controls performed in the general linear model, adjusted for age, sex, intraocular pressure and the presence of diabetes, revealed that macular edema was an independent factor associated with higher aqueous VEGF concentrations ($ß = +0.74$, $p = 0.0012$). Aqueous humor levels of VEGF were significantly elevated in patients with DME (median 112 pg/ml) as compared to the control subjects (57.5 pg/ml) ($p = 0.001$, Fig. 1). Accordingly, we found that the aqueous humor VEGF levels were significantly higher in the RVO group than in controls (median: 136.5 vs. 57.5 pg/ml, respectively; $p = 0.01$).

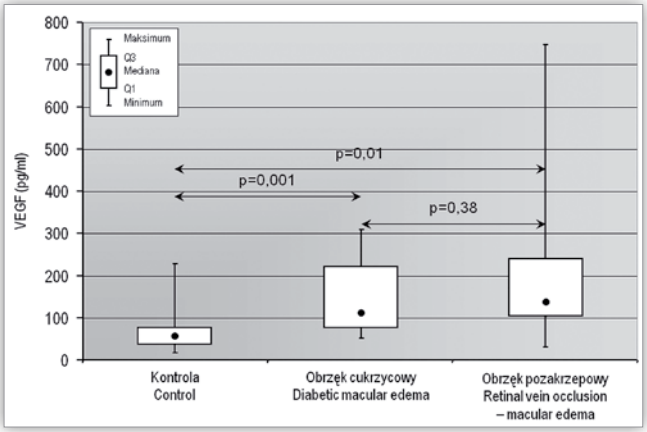

Fig. 1. Aqueous humour VEGF concentrations in the study groups. The values are expressed in pg/ml. Q_1–Q_3 – upper and lower quartiles.

Ryc. 1. Stężenie VEGF w cieczy wodnistej u pacjentów z badanych grup. Wartości wyrażono w pg/ml. Q_1–Q_3 – kwartyle górny i dolny.

Most importantly, the aqueous humor VEGF levels positively correlated with the extent of non-perfusion areas as measured on fluorescein angiograms in the DME and RVO groups ($Rs = 0.45$, $p = 0.02$). This finding implies that the widespread retinal ischaemia was associated with higher VEGF concentration in aqueous humor. This relationship remained significant after adjustment for age, sex, intraocular pressure and the presence of diabetes in the linear regression analysis ($ß = +0.45$, $p = 0.04$). No correlation was observed between the aqueous VEGF levels and central retinal thickness measured using the OCT ($p = 0.45$).

	Cukrzycowy obrzęk plamki/ Diabetic macular edema (DME)	Obrzęk plamki w przebiegu zakrzepu żyły środkowej siatkówki/ Macular edema secondary to retinal vein occlusion (RVO-ME)	Grupa kontrolna/ Control group	p*
Liczebność/ Number of subjects	16	8	26	-
Płeć (mężczyźni/ kobiety)/ Sex (male/ female)	12 /4	5 / 3	10 / 16	0.06
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Wiek pacjenta (lata)/ Patient's age (years)	67.9 ± 5	70.9 ±5	74.5±5	0.03
	%	**%**	**%**	
Jaskra/ Glaucoma	0	12.5	15.4	0.26
Nadciśnienie tętnicze/ Hypertension	75	50	73.1	0.40
Choroba niedokrwienna serca w wywiadzie/ History of ischemic heart disease	18.8	0	0	0.03
Udar mózgu w wywiadzie/ History of stroke	12.5	25	7.7	0.41

Tab. I. Clinical characteristics of the study groups.

Tab. I. Charakterystyka kliniczna pacjentów z poszczególnych grup.

Discussion

Vascular endothelial growth factor has long been implicated in promoting neovascularization. Various ocular tissue cells – most notably the retinal pigment epithelium, choroidal fibroblasts, ganglion cells and astrocytes – were shown to produce VEGF. Its levels are significantly elevated in ischaemia and hypoxia. In diabetes, VEGF along with other factors such as platelet derived growth factor (PDGF) or insulin-like growth factor (IGF-1) is believed to play the most prominent role in angiogenesis (5). Similarly VEGF also plays a key role in the pathogenesis of macular edema in retinal vein occlusion (RVO). Due to its solubility VEGF is readily identified in intraocular fluids. Therefore, its concentrations in aqueous humor have been studied in different diseases. In macular edema secondary to RVO and in diabetic macular edema, VEGF levels in aqueous humor have been found to be elevated (4, 5). According to Selim et al., the aqueous VEGF level had a strong correlation with the severity of diabetic retinopathy along with a statistically insignificant difference in macular edema (5). VEGF levels in aqueous humor and in the vitreous of diabetic patients undergoing panretinal photocoagulation for proliferative retinopathy tend to decrease after treatment (6). According to Noma et al., aqueous levels of VEGF correlated with the severity of macular edema (7, 8). Our findings are consistent with those reports, as we demonstrated the aqueous humor VEGF levels to be significantly elevated in the RVO group and DME group, as compared to controls.

The aim of our study was also to evaluate the association between the extent of capillary non-perfusion and the VEGF levels in the aqueous humor in patients with DME and ME secondary to RVO. We found that VEGF levels positively correlated with the extent of retinal ischemia, as measured on fluorescein angiograms. Remarkably, other researchers observed similar associations (8–10), which indicates that ocular VEGF concentration contributes to the progression of retinal ischemia. Indeed, preclinical studies in primates showed that intraocular injections of VEGF resulted in leukostasis and progressive retinal ischemia (10). Thus, it seems reasonable to assume that high VEGF levels promote the development and deterioration of retinal non-perfusion in a positive feedback loop. Importantly, it has been documented that monthly injections of ranibizumab reduced progression of retinal non-perfusion and significantly decreased the incidence of retinal non-perfusion in patients with RVO (11). Similarly, monthly injections of ranibizumab slowed retinal capillary closure in patients with DME (12). Thus, it appears that VEGF inhibition prevents progression of ischemia and improves retinal perfusion.

Interestingly, we did not find any correlation between the aqueous VEGF levels and central retinal thickness. Our findings are consistent with those of Fujikawa et. al. (13). We believe, that this lack of correlation can be attributed to variable locations of RVO within the retina in our patient group, as well as subretinal fluid accumulation within the macula stimulated by other inflammatory cytokines.

The limitation of this study was the impossibility to harvest vitreous samples from our study group so as to compare the aqueous and vitreous VEGF levels. However, several studies documented that aqueous levels of VEGF correlated with its concentrations in vitreous (14, 15). Thus, we assume that aqueous levels of VEGF reflect the vitreous levels of the molecule.

Conclusion

In conclusion, current study demonstrated significant elevation of aqueous VEGF levels in patients with macular edema as compared to controls, and a significant correlation of aqueous VEGF levels with the size of the retinal non-perfusion area. It remains to be seen based on future studies whether aqueous humor or vitreous levels of VEGF and the area of capillary non perfusion can be used as markers to predict the clinical course and treatment outcomes in macular edema secondary to diabetes and RVO.

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