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ORIGINAL ARTICLE

Comparative Evaluation of Levels of Vascular Endothelial Growth Factors in Aqueous Humor in Diabetic and Non-Diabetic Patients

Brijesha Dudhat[1], Reshma Ramakrishna[2], Dhairya Parikh[3], Shrikant Deshpande[4]

[1.3]PG Resident, [2]Lecturer, [4]H.O.D., Department of Ophthalmology, MGM Hospital, Kamothe, Navi Mumbai - 410209

Background: It is assumed, therefore, that the profile of the aqueous humor can afford useful information relating to inflammatory processes in the eyes. In patients with progressive diabetic retinopathy (PDR), neo-vascularization from the retina, ischemia, bleeding into the vitreous humor and retinal detachment due to vitreo retinal traction can eventually lead to blindness. Various cytokines have been identified as playing a role in diabetic retinopathy, including VEGF, TGF-b, IGF-1 and bFGF. **Aim of the study:** To comparatively evaluate the levels of vascular endothelial growth factors in aqueous humor in diabetic and non-diabetic patients. **Materials and methods:** 30 patients with 36 eyes scheduled for intraocular surgery were included in the study. 19 patients were males and 11 were females. The mean age of the patients was 62.32±12.04 years. 24 hours before the surgery, blood samples were taken from the patients for the evaluation of blood glucose, HbA1c, and C-reactive protein (CRP). Out of 30 patients, 21 patients were diabetic whereas 9 patients were non-diabetic. The subjects of the patients were grouped into two groups, Group A (diabetic patients) and Group B (non-diabetic patients). Group A consisted of 21 patients with 25 eyes in consideration for the study. Group B consisted of 9 with 10 eyes in consideration for the study. The samples of aqueous humor were collected just before the intraocular surgery under sterile conditions in the operating theatre. **Results:** The mean age of subjects in Group A was 63.21 ± 14.12 years and Group B was 65.23 ± 12.61 years. The level of Aqueous VEGF in aqueous humor in Group A was 212.3±52.3 pg/ml and in Group B was 68.12±31.2 pg/ml. The HnA1c level in the blood in Group A was 10.7 ± 3.1 % and Group B was 5.1 ± 1.3 %. The fasting glucose level in the blood of Group A was 182 ± 48.21mg/dL and Group B was 98.21 ± 7.2 mg/dl. The results with respect to aqueous VEGF, HbA1c, and fasting Glucose were statistically significant ($p<0.05$). **Conclusion:** Vascular endothelial growth factors in aqueous humor of diabetic patients were significantly elevated as compared to non-diabetic patients.

Keywords: aqueous humor, diabetes mellitus, diabetic retinopathy, intraocular surgery.

Corresponding author: Dr. Brijesha Dudhat, PG Resident, Department of Ophthalmology, MGM Hospital, Kamothe, Navi Mumbai – 410209, Maharashtra, India

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INTRODUCTION:

In the present world, it has been widely accepted that Diabetes mellitus (DM) is a major cause of avoidable blindness in both the developing and the developed countries. Patients with diabetic retinopathy (DR) are 25 times more likely to become blind than non-diabetics.[1]

Aqueous humor is located inside the anterior and posterior chambers of the eye and supplies nutrients to the non-vascularized cornea, lens and trabecular meshwork.[2] Certain physiological or pathological states of the retina can affect the composition of the aqueous humor and biomarkers associated with a number of pathological processes, including vascular diseases, arteriosclerosis, ischemia, necrosis and inflammation, can be detected in this fluid.[3, 4] It is assumed, therefore, that the profile of the aqueous humor can afford useful information relating to inflammatory processes in the eyes. In patients with progressive diabetic retinopathy (PDR), neovascularization from the retina, ischemia, bleeding into the vitreous humor and retinal detachment due to vitreoretinal traction can eventually lead to blindness. Various cytokines have been identified as playing a role in diabetic retinopathy, including VEGF, TGF-b, IGF-1 and bFGF.[5, 6] A growing body of evidence has demonstrated that VEGF is important in the pathogenesis of diabetic retinopathy. For example, elevated ocular VEGF levels in diabetic patients are correlated with the extent of retinopathy.[7,8] The Diabetes Control and Complications Trial found that chronic hyperglycemia was

a primary risk factor for the development of diabetic microangiopathy, including retinopathy. There is increasing evidence that advanced glycation end products (AGEs) are derived from non-enzymatic modification of proteins.[9-12]
So, the present study was planned to comparatively evaluate the levels of vascular endothelial growth factors in aqueous humor in diabetic and non-diabetic patients.

MATERIALS AND METHODS:
The present study was conducted in the Department of Opthalmology of the medical institution. 30 patients with 36 eyes scheduled for intraocular surgery were included in the study. 19 patients were males and 11 were females. The mean age of the patients was 62.32±12.04 years. Twenty three eyes had undergone cataract surgery with phaco emulsification and thirteen eyes had undergone pars plana vitrectomy. The ethical approval for the protocol of the study was obtained from the ethics review committee of the institute. An informed written consent was obtained from the participating subjects after explaining them the procedure of the study.
24 hours before the surgery, blood samples were taken from the patients for the evaluation of blood glucose, HbA1c, and C-reactive protein (CRP). Out of 30 patients, 21 patients were diabetic whereas 9 patients were non-diabetic. The patients were cautiously inspected using slit-lamp prior to the surgery and thorough examination of fundus was done using cataract fundus lens. Before one week of the surgery, routine color fundus photographs, fluorescein angiograms (FFA), and macular scans with time-domain optical coherence tomography (OCT) were taken. The patients having histoy of previous intraocular surgery within six months, related retinal pathology, glaucomatous eyes were excluded from the study.
The subjects of the patients were grouped into two groups, Group A (diabetic patients) and Group B (non-diabetic patients). Group A consisted of 21 patients with 25 eyes in consideration for the study. Group B consisted 0f 9 with 10 eyes in consideration for the study. The non-diabetic patients in Group B act as control subjects.
The samples of aqueous humor were collected just before the intraocular surgery under sterile conditions in the operating theatre. The samples were collected in sterile tubes by using limbal anterior chamber puncture with a 27 gauge needle of a 1 ml insulin injector. Within few minutes of collection, the samples were stored at -80^{o}C. The evaluation of concentration of VEGF 165 isoform was done using enzyme-linked immunosorbent assay (ELISA) by using a kit for human VEGF. The linear range of detection was 5 - 1500 pg / ml for the assay.
The statistical analysis of the data was done using SPSS software (version 20.0) for windows. The groups were compared using Student's t-test and Chi-square test. A p-value <0.05 was predefined to be statistically significant.

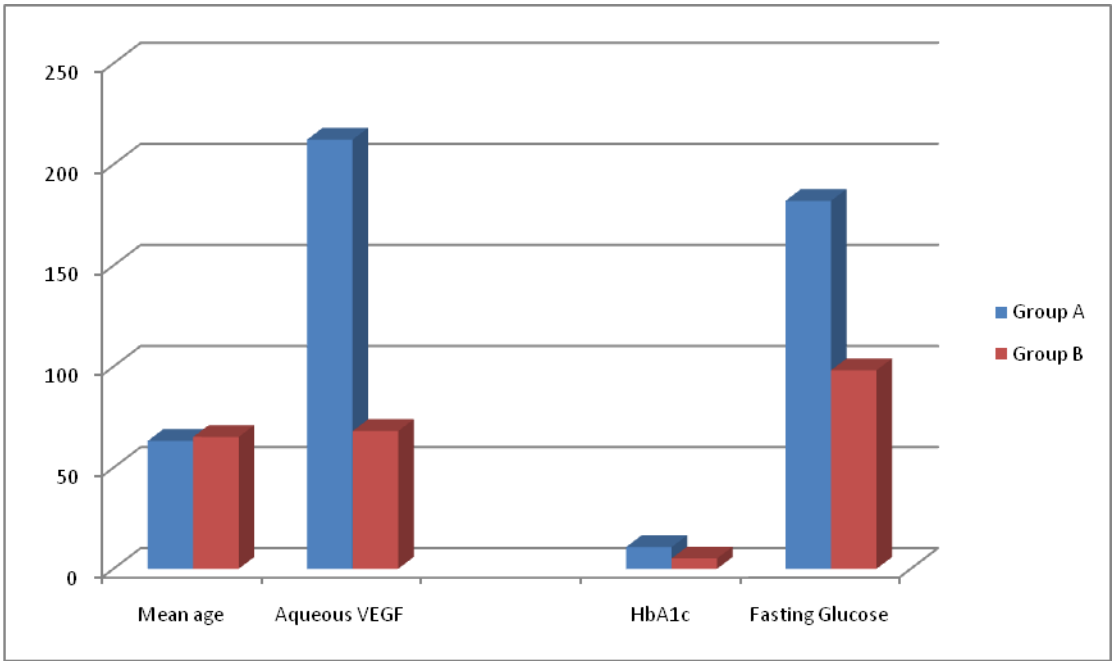
RESULTS:
In the present study, 30 patients with 36 eyes in the consideration were included. The patients were scheduled for intraocular surgery. The patients were grouped into two groups; Group A (diabetic) with 21 patients (25 eyes in consideration for present study) and Group B (non-diabetic) with 9 patients (10 eyes in consideration for present study)**.** **Table 1** shows the comparative analysis of various variables of Group A and Group B. The mean age of subjects in Group A was 63.21 ± 14.12 years and Group B was 65.23 ± 12.61 years. The level of Aqueous VEGF in aqueous humor in Group A was 212.3±52.3 pg/ml and in Group B was 68.12±31.2 pg/ml. The HnA1c level in the blood in Group A was 10.7 ± 3.1 % and Group B was 5.1 ± 1.3 %. The fasting glucose level in the blood of Group A was 182 ± 48.21mg/dL and Group B was 98.21 ± 7.2 mg/dL. The results with respect to aqueous VEGF, HbA1c, and fasting Glucose were statistically significant (p<0.05) [**Fig 1].**

DISCUSSION:
The present study was conducted to compare the levels of vitreous levels of vascular endothelial growth factor in diabetic and non-diabetic patients. The results of present study showed that the vitreous levels of vascular endothelial growth factor in diabetic patients were significantly increased as compared to non-diabetic patients. Also, HbA1c levels and blood glucose levels were significantly elevated in diabetic patients as compared to non-diabetic patients. The results were consistent to similar studies conducted by other authors. Funatsu H et al investigated whether vitreous levels of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) are related to diabetic macular edema (DME). Thirty-six patients with DME, 6 diabetic patients without retinopathy, and 13 patients with nondiabetic ocular disease were included in the study.

Table 1: Comparative analysis of various variables of Group A and Group B

Variables	Group A	Group B	p-value
Mean age (years)	63.21 + 14.12	65.23 ± 12.61	0.41
Aqueous VEGF (pg/ml)	212.3±52.3	68.12 ± 31.2	0.02
HbA1c (%)	10.7 ± 3.1	5.1 ± 1.3	0.032
Fasting Glucose	182 ± 48.21	98.21 ± 7.2	0.041

Figure 1: Graphical representation of comparative analysis of various variables of Group A and Group B



After vitreous fluid samples were obtained at vitreo retinal surgery, VEGF and PEDF levels in the vitreous fluid were measured by enzyme-linked immunosorbent assay. The vitreous level of VEGF was significantly higher in patients with DME than in nondiabetic patients and diabetic patients without retinopathy. Conversely, the vitreous level of PEDF was significantly lower in patients with DME than in nondiabetic patients and diabetic patients without retinopathy. The vitreous level of PEDF did not correlate significantly with that of VEGF. The vitreous level of VEGF was significantly higher in patients with hyperfluorescent DME than in those with minimally fluorescent DME. Conversely, the vitreous PEDF level was significantly lower in patients with hyperfluorescent DME than in those with minimally fluorescent DME. Vitreous levels of VEGF and PEDF were related to the retinal thickness at the central fovea. Their retrospective study suggested that VEGF and PEDF have an independent association with vascular permeability in the eye and on the DME, and they recommended that prospective validation of our findings be undertaken to confirm these observations. Salom D et al determined the level of vascular endothelial growth factor A (VEGF-A) in aqueous humors of patients with retinitis pigmentosa (RP). Aqueous humor was collected from 16 eyes of 16 patients with RP. The level of VEGF-A was determined with a commercially available enzyme-linked immunosorbent assay kit. The control group comprised 16 aqueous samples from 16 patients about to undergo cataract surgery and without any other ocular or systemic diseases. The concentration of VEGF-A in aqueous humor was markedly lower in patients with RP than in control subjects. The level of VEGF-A was 94.9 ± 99.8 (mean ± SD) pg/mL in eyes with RP and 336.5 ± 116.8 pg/mL in the eyes of the control group. The authors concluded that in patients with RP, the concentration of VEGF-A in aqueous humors is lower than in non-RP subjects. The lack of angiogenic actions attributed to VEGF-A may explain some of the clinical manifestations of this disease, such as narrowing and fibrotic degeneration of retinal blood vessels.[13, 14]

Endo M et al used enzyme-linked immunosorbent assays to determine the levels of VEGF, non-CML AGE and CML in the aqueous humor and serum of 82 Japanese patients with type 2 diabetes and 60 non-diabetic subjects. VEGF, non-CML AGE, and CML concentrations in aqueous humor and serum were then compared with the severity of diabetic retinopathy. Immunohistochemical detection analysis of non-CML AGE and CML was also performed using retinal tissues from patients with progressive diabetic retinopathy. Aqueous levels of VEGF, non-CML AGE and CML increased along with the progression of diabetic retinopathy compared to age-matched controls. After coagulation therapy, the VEGF, non-CML AGE, and CML levels were significantly reduced. Immunostaining showed diffuse co-localization of non-CML AGE and CML around microvessels and in the glial cells of proliferative membranes from patients with progressive diabetic retinopathy. These findings suggested that glycation and glycoxidation reactions (or oxidation, as revealed by CML) may contribute to both the onset and progression of diabetic retinopathy. Shimura M et al compared the effect of an intravitreal injection of bevacizumab, an anti-vascular endothelial growth factor (VEGF) antibody, with that of triamcinolone acetonide, a corticosteroid for reduction of

diabetic macular edema (DME). Twenty-eight eyes of 14 patients with bilateral DME participated in this study. In each patient, one eye received an intravitreal injection of 4 mg triamcinolone acetonide and the other eye received 1.25 mg bevacizumab. The clinical course of best-corrected visual acuity (VA) with a logarithm of the minimum angle of resolution chart and averaged foveal thickness using optical coherence tomography was monitored for up to 24 weeks after the injection. Before the injection, foveal thickness and VA were 522.3 +/- 91.3 microm and 0.64 +/- 0.28 microm in the triamcinolone-injected eye, and 527.6 +/- 78.8 microm and 0.61 +/- 0.18 microm in the bevacizumab-injected eye, respectively; there was no significant difference between the eyes. One week after the injection, both eyes showed significant regression of macular edema. The triamcinolone-injected eye showed significantly better results than the bevacizumab-injected eye. However, both eyes showed the recurrence of macular edema with time, even at 24 weeks. Triamcinolone kept better results than bevacizumab. The authors concluded that with the generally used concentration, intravitreal injection of triamcinolone acetonide showed better results in reducing DME and in the improvement of VA than that of bevacizumab, suggesting that the pathogenesis of DME is not only attributable to VEGF-dependency, but is also attributable to other mechanisms suppressed by corticosteroid.[15,16]

CONCLUSION:

From the results of present study this can be concluded that vascular endothelial growth factors in aqueous humor of diabetic patients were significantly elevated as compared to non-diabetic patients.

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