

## Research Article

### Aqueous VEGF and IL-6 Levels in Diabetic Retinopathy

Shashi Ahuja<sup>1\*</sup>, Renuka Srinivasan<sup>1</sup>, Praveen Kumar S<sup>1</sup>, Medha R<sup>2</sup> and Ananthanarayanan PH<sup>2</sup>

<sup>1</sup>Department of Ophthalmology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India

<sup>2</sup>Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India

#### Abstract

##### Aims

To study Vascular Endothelial Growth Factor (VEGF) and Interleukin-6 (IL-6) levels in aqueous humor of diabetic patients undergoing cataract surgery. To study the predictive value of aqueous VEGF and IL-6 levels in Diabetic retinopathy.

##### Materials and methods

Non diabetic controls (group A), patients of diabetes without retinopathy (group B) and those with diabetic retinopathy (group C) undergoing cataract surgery were recruited in the study. Detailed ophthalmic evaluation was done and diabetic retinopathy changes were documented. Intraoperatively approximately 0.2 ml of aqueous was withdrawn and frozen for analysis. Commercially available ELISA kits were standardized and used. ANOVA test was done to correlate the VEGF and IL6 levels among the three groups.

##### Results

60 non diabetic controls, 53 diabetics without retinopathy and 27 diabetics with retinopathy were assessed. IL-6 levels in non diabetic controls were average 41.88 pg/ml while in diabetics without retinopathy, it was an average of 161.65 pg/ml and in diabetics with retinopathy, average value of 310 pg/ml ( $p < 0.0001$ ) was obtained. VEGF levels in non diabetic controls were an average of 130.69 pg/ml, in diabetics without retinopathy, an average of 131.79 pg/ml and in diabetics with retinopathy, an average of 397.6 pg/ml ( $p < 0.0001$ ) was obtained. In group C, there were fifteen patients with mild retinopathy, six with moderate and four with severe non-diabetic retinopathy while two patients had proliferative diabetic retinopathy. The IL6 and VEGF levels did not have statistically significant correlation with the severity of diabetic retinopathy ( $p = 0.2035$ ).

\*Corresponding author: Shashi Ahuja, Department of Ophthalmology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-605006, India, Tel: +91 9176790793; Email: drshashiahuja@gmail.com

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#### Conclusion

There is an increase in the IL6 and VEGF levels in diabetic patients as compared to non diabetic controls. Further, the increase in IL 6 and VEGF is more in diabetics with retinopathy. IL6 and VEGF levels may have a predictive value in assessing the risk for developing diabetic retinopathy.

#### Introduction

Diabetes has reached epidemic proportions. There are 150 million diabetics worldwide with one third in developing countries. India has approximately 30 million diabetics and is ahead of China and USA. The prevalence of diabetic retinopathy among diabetics in India is 25% [1].

Many cytokines are implicated in the pathogenesis of diabetic retinopathy. Vascular Endothelial Growth Factor (VEGF) is an endothelial cell mutagen and vasopermeability factor [2-4]. Interleukin 6 (IL-6) by inducing the expression of VEGF increases angiogenesis and vasopermeability [2,5]. The VEGF and IL-6 levels are elevated in both vitreous and aqueous humor of patients with diabetes especially in the presence of proliferative diabetic retinopathy [2,6]. The major stimulus for VEGF secretion is retinal hypoxia [4]. The local secretion of VEGF in intraocular fluids is reported to be higher in patients with diabetic retinopathy than their serum levels [6,7]. Its activity as vascular permeability factor matches perfectly with the findings of leaky vessels in diabetic retinopathy [4]. Aqueous VEGF levels have been reported to increase by two folds in patients with non-proliferative diabetic retinopathy [6]. IL-6 level in the vitreous has been correlated with VEGF levels in diabetic macular edema and in various stages of diabetic retinopathy [7-10]. The aqueous and plasma levels of IL-6 have not been correlated with VEGF and severity of diabetic retinopathy. Hence our study paves the way in finding the marker which may help us to predict the risk for developing diabetic retinopathy. Early detection of the risk for proliferative retinopathy, especially in patients undergoing cataract surgery (which may accelerate the progression of retinopathy), may help to plan on close follow-up of these patients and early treatment preventing blinding complications of diabetic retinopathy.

#### Objectives

The present study was undertaken to measure aqueous VEGF and IL-6 levels in eyes of diabetics with and without retinopathy undergoing cataract surgery and to assess the predictive value of aqueous VEGF and IL-6 levels in diabetic retinopathy.

#### Materials and Methods

The study was conducted at a tertiary care hospital in south India. Institute ethics committee approval was taken. Patients aged 40 years or above undergoing cataract surgery were recruited in the study. Patients with other retinal diseases, those with pseudoexfoliation, presence of uveitis and those on immunosuppressant therapy were excluded from the study. Study subjects were divided into three groups- Group I included non-diabetic subjects, Group II were

	Group 1 (Non diabetic control)	Group 2 (Diabetic patients without retinopathy)	Group 3 (Diabetics with retinopathy)	Total
Number of subjects	60	53	27	140
Mean age	55	55	59	55.8
Males	31	25	15	71
Females	29	28	12	69
Mean IL-6 levels average (pg/ml)	42.01	161.66	310.98	139.67
Mean VEGF levels (pg/ml)	130.69	131.69	397.6	182.54

Table 1: Comparison of three group patients.

diabetics without retinopathy while group III included subjects with retinopathy.

Demographic data was noted. A detailed ophthalmic evaluation was performed including dilated fundus evaluation to stage diabetic retinopathy. The severity of diabetic retinopathy was graded based on Early Treatment of Diabetic Retinopathy Study (ETDRS) criteria.

In diabetic patients serum blood glucose levels, glycosylated Hemoglobin (HbA1c) and lipid profile were assessed. All patients underwent extracapsular cataract extraction. Intraoperatively 0.2 ml aqueous humor (which otherwise is lost during any intraocular surgery) was withdrawn in a tuberculin syringe using 26 gauze needle, before entering the anterior chamber. Extracapsular cataract extraction with posterior chamber intraocular lens implantation was done. The aqueous sample collected was immediately transferred and stored at -80°C until assay of VEGF and IL-6 levels was done.

Enzyme-linked Immunosorbent Assay (ELISA) of aqueous humor samples was performed to quantify the levels of VEGF with the Human VEGF ELISA Kit (Ray Biotech Inc., Norcross, GA, United States of America), which was designed to measure VEGF level with a sensitivity of less than 10 pg/ml. The intra-assay Co-efficient of Variation (CV) was <10% and inter-assay CV was <12%. Each sample was assayed in duplicate and mean of the measurements was taken to increase validity of the assay.

Enzyme-linked Immunosorbent Assay (ELISA) of aqueous humor samples was performed to quantify the levels of IL-6 with the Avibion Human IL-6 ELISA kit (Orgenium Laboratories, Helsinki, Finland), which was designed to measure IL-6 level with a sensitivity of less than 2 pg/ml. The intra-assay CV was  $\leq 9.4\%$  and inter-assay CV was  $\leq 8.6\%$ . Each sample was assayed in duplicate and mean of the measurements was taken to increase validity of the assay. The association of aqueous VEGF and IL6 levels to the stage of diabetic retinopathy was assessed using ANOVA test.

## Results

A total of 140 patients were recruited in our study. The group 1 (non-diabetic controls) included 60 patients, group 2 (diabetics without retinopathy) included 53 patients while 27 were recruited into the group 3 (diabetics with retinopathy). The mean age was 55 in groups 1 and 2, while it was 59 in group 3. There were 71 males and 59 female patients in the study. Comparison of the three groups is shown in table 1.

The duration of diabetes mellitus in group 2 varied from one month to 27 years with an average of 6.07 years. In group 3, duration of diabetes varied from 15 days to twenty years with an average of 7 years. The levels of IL-6 had a negative correlation with the increase in duration of diabetes in both group 2 and 3, though it was not

statistically significant ( $p=0.0805$  for group 2 and  $p=0.0266$  for group 3). In group 2, Spearman  $r=-0.2422$  with 95% confidence interval of -0.4874 to 0.03827 while in group 3, Spearman  $r=-0.3168$  with 95% confidence interval of -0.5551 to -0.03051. Aqueous VEGF levels showed a negative correlation on Spearman correlation analysis and was statistically significant in group 2 ( $p=0.0165$ ) while in group 3, it was not statistically significant ( $p=0.1177$ ). In group 2, Spearman  $r=-0.3596$  with 95% confidence interval: -0.5991 to -0.06117. In group 3, Spearman  $r=-0.269$  with 95% confidence interval of -0.6147 to 0.163.

IL-6 levels in non-diabetic controls varied from 22.2 to 69.05 pg/ml with an average of 41.88 pg/ml; in diabetics without retinopathy it varied from 117.6 to 190 pg/ml with an average of 161.65 pg/ml and in diabetics with retinopathy, values ranged between 278 to 355 pg/ml with an average of 310 pg/ml ( $p$  value <0.0001). VEGF levels in non-diabetic controls varied from 22.2 to 69.05 pg/ml with an average of 42 pg/ml; in diabetics without retinopathy, from 117.6 to 190 pg/ml with an average of 117.59 pg and in diabetics with retinopathy, values were between 278 to 355 pg/ml with an average of 310 pg/ml ( $p$  value <0.0001). ANOVA test revealed that the IL-6 and VEGF levels were higher in diabetic patients especially those with retinopathy. Kruskal-Wallis test was found to be extremely significant ( $p$  -value was <0.0001).

However, the levels showed a negative correlation with ETDRS grades of diabetic retinopathy and were statistically not significant. In group 3, there were fifteen patients with mild retinopathy, six with moderate and four with severe diabetic retinopathy while two patients had proliferative diabetic retinopathy.

## Discussion

Our study showed higher levels of aqueous IL-6 and VEGF especially in those with retinopathy. Prior studies showed similar increase in cases of diabetics with macular edema [2,5-7]. Vascular endothelial growth factors are implicated in the pathogenesis of diabetic retinopathy. The VEGF levels in the aqueous and vitreous are elevated in diabetics [7,11]. The rise in the levels of VEGF may correlate with the onset and progression of diabetic retinopathy. However, we did not find statistically significant change in the VEGF levels in patients with various degrees of diabetic retinopathy. A recent study showed that there is a decline in inflammatory and angiogenic markers namely IL-6 and VEGF with the onset of proliferative diabetic retinopathy changes [12]. The reduction in angiogenic factors with worsening of retinopathy may explain the role of VEGF in initiating the proliferative changes and systemic worsening [13]. However, the final end point is secondary to the sequel arising out of the fibrovascular proliferation. A larger study is required as the sample size in the subsets of diabetic retinopathy group was small to draw conclusions. The increase in VEGF levels may thus be an early

indicator for predicting the patients developing diabetic retinopathy. However, the role of VEGF levels and progression of diabetic retinopathy is not well established.

IL-6 is a cytokine which is implicated in increased vascular permeability and thus considered to be important in pathogenesis of diabetic retinopathy. IL-6 has shown a paracrine effect on VEGF by increasing the vascular permeability. IL-6 levels were increased in diabetic patients especially in those with retinopathy. Blood retinal barrier breakdown is implicated in the pathogenesis of diabetic retinopathy and increased release of inflammatory cytokines is believed to be resulting in macular edema. The rise in VEGF and IL-6 levels may help us in early detection of diabetic retinopathy changes, thereby reducing the incidence of vision threatening complications of diabetic retinopathy.

## Conclusion

There is an increase in the IL-6 and VEGF levels in diabetic patients as compared to non-diabetic controls. Further, the increase in IL-6 and VEGF levels are more in diabetics with retinopathy. This may help us to predict the risk for developing diabetic retinopathy. The levels may reduce with the worsening of retinopathy to proliferative stage.

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