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ORIGINAL PAPER

Effect of pan retinal photocoagulation on the serum levels of vascular endothelial growth factor in diabetic patients

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Abstract This study tests the hypothesis that subjects with proliferative diabetic retinopathy (PDR) have a detectable rise in levels of serum vascular endothelial growth factor (VEGF), which is an important regulator of angiogenesis. Our investigation aims to evaluate plasma VEGF changes after pan-retinal photocoagulation (PRP) in diabetic patients. Twenty-nine type two diabetic patients (17 male, 12 female: mean age 53.13 ± 12.22 years) with PDR secondary to diabetes were studied. Blood samples were obtained before and at 2 months after the last PRP session. Serum VEGF levels were measured by ELISA. After PRP, the mean serum VEGF decreased, but this reduction was not remarkable (88.68 ± 71.09 vs. 77.01 ± 60.33 ng/ml) ($P = 0.18$). There was a statistically significant difference in serum VEGF changes between patients who had regressed PDR with patients who had progressed PDR (−25.98 ± 47.37 vs. 56.44 ± 31.7 ng/ml) ($P = 0.003$). Our results showed a significant reduction in levels of serum VEGF in the patients who had successful laser treatment. Our findings suggest that serum VEGF levels could be used for monitoring diabetic retinopathy outcome.

Keywords Type 2 diabetes · Proliferative diabetic retinopathy · Vascular endothelial growth factor · Pan retinal photocoagulation

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Introduction

Proliferative diabetic retinopathy (PDR), characterized by increased vascular permeability, retinal ischemia, and neovascularization, affects 10–20% of diabetic patients [1]. This process depends on the local production of angiogenic factors and components of the extracellular matrix, which will be substrates for endothelial damage. Vascular endothelial growth factor (VEGF), a potent activator of angiogenesis, enhances collateral vessel formation and increases the permeability of the microvasculature [2, 3]. VEGF

expression is induced by hypoxia and plays an important role in normal and abnormal angiogenesis [4, 5]. Its levels have been found to be markedly increased in the vitreous and aqueous fluids in the patients with PDR [6, 7]. Changes in intraocular VEGF levels have also been related to effective laser treatment [8]. It has been hypothesized that subjects with PDR would have detectable raised levels of systemic VEGF, suggesting angiogenesis [9]. However, the evidence is still lacking about the relation between PDR and systemic VEGF levels [10, 11]. Since diabetic retinopathy is one of the most critical problems in our population [12, 13], a confirmed decline in VEGF levels after successful laser treatment could provide a convenient opportunity for monitoring disease progression or regression via a simple blood sample. Accordingly we meant to assess the relation between serum VEGF levels and PDR outcome after pan-retinal photocoagulation (PRP).

Materials and methods

This study is based on a clinical trial (before and after), which was done at the ophthalmology clinic of Yazd Diabetes Research Center during the years 2007–2008, in Iran. In this study, from patients referred to the clinic, we recruited patients with PDR secondary to diabetes with no previous PRP therapy. The diagnosis of diabetic retinopathy was made with retinal photographs and on slit-lamp biomicroscopy with the aid of fluorescein angiography in doubtful cases. Retinopathy was classified according to Early Treatment Diabetic Retinopathy Study final scale criteria (ETDRS) [14] as absent, NPDR or PDR. The severity of diabetic retinopathy was graded on the basis of the worst eye.

After 2 months of the last PRP session, according to ophthalmologic examination results, the subjects were divided into two groups: patients with improved PDR (regressed PDR) and patients with progressed PDR (progressed PDR).

The institution's Research Ethics Committee approval was obtained prior to study enrollment and was carried out in accordance with the Declaration of Helsinki.

Informed consent was obtained in all cases. At first visit, demographic and clinical information was documented. Blood samples were obtained before and at 2 months after the last PRP session, for serum VEGF, blood glucose and lipids.

Laboratory assay: Blood glucose was determined by glucose oxidase methods. Triglyceride and cholesterol were determined by enzymatic methods (by Pars Azmon kit, Iran). VEGF levels were measured by ELISA with a human VEGF immunoassay kit (BioSource International, Inc., California, USA).

Sample size was calculated by this formula:

$$n = (z_\alpha + z_\beta)^2(\delta 1^2 + \delta 1^2)/(\mu_1 - \mu_2)^2$$

The change in VEGF levels was calculated using the formula: $100 \times$ (VEGF after PRP $-$ VEGF before PRP)/(VEGF before PRP).

Statistical analysis was performed using SPSS for windows, version 13. Data of continuous variables were expressed as mean $\pm$ standard deviation. For comparison of baseline characteristics in the two groups (regressed or progressed PDR), an Independent Sample *t*-test was used. The VEGF levels before and after PRP that were not normally distributed were compared using the Wilcoxon test and the results were presented as median (Interquartile Range).

Results

We studied 29 subjects (17 male, 12 female) with a mean age of 53.13 $\pm$ 12.22 (21–73) years and a mean duration of diabetes for 12.06 $\pm$ 6.28 (1–27) years with PDR secondary to diabetes.

Table 1 shows the baseline characteristics for diabetic patients according the PDR outcome after PRP.

Table 1 Baseline characteristic of diabetic patients with regressed or progressed PRP

	Regressed after PRP	Progressed after PRP	*P* value
BMI (kg/m^2)	24.98 $\pm$ 4.53	25.62 $\pm$ 3.09	0.7
Diabetes duration (Year)	12.6 $\pm$ 8.79	11.7 $\pm$ 5.49	0.8
Age (Year)	46.33 $\pm$ 18.51	55.7 $\pm$ 8.89	0.1
FBS (mg/dl)	245 $\pm$ 226.78	254.5 $\pm$ 131.9	0.9
TG (mg/dl)	248 $\pm$ 226.78	208 $\pm$ 62.2	0.7
TC(mg/dl)	201.3 $\pm$ 35.38	251.8 $\pm$ 53.26	0.2

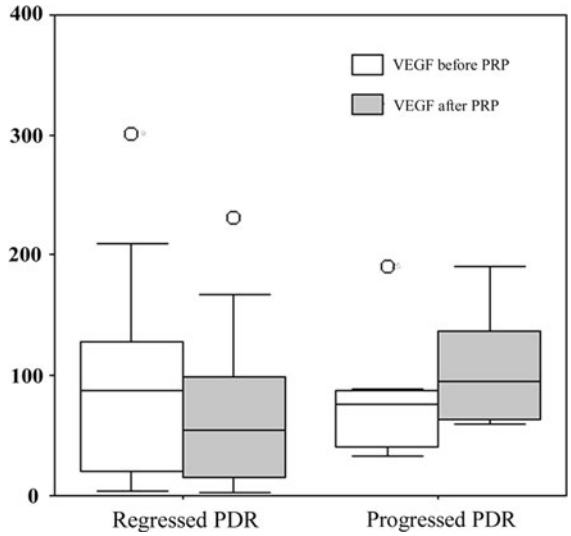

Fig. 1 Serum VEGF (ng/ml) before and after PRP in patients with regressed PDR and progressed PDR

Table 2 Mean VEGF before and after PRP in subjects who had regressed or progressed PDR

	VEGF levels in subjects with regressed PRP	VEGF levels in subjects with progressed PRP
Before (ng/ml)	88.25 (118.6)*	75.65 (50.9)
After (ng/ml)	54.2 (93.2)	95.3 (76.5)
P value**	0.007	0.1

* Median (Interquartile Range)

** Wilcoxon Test

After PRP the overall mean serum VEGF was reduced, but this reduction was not statistically significant (88.68 ± 71.09 vs. 77.01 ± 60.33 ng/ml) ($P = 0.18$). Figure 1 shows serum VEGF levels before and after PRP.

No significant difference was observed between baseline VEGF levels in diabetic patients in whom PDR regressed or progressed ($P = 0.9$). Serum VEGF decreased significantly after PRP in patients with regression of PDR ($P = 0.007$) and increased in patients showing progression of PDR after PRP ($P = 0.1$) (Table 2). When the mean of changes were compared between the two groups, regressed or progressed PDR, the difference was statistically significant (−25.98 ± 47.37 vs. 56.44 ± 31.7 ng/ml) ($P = 0.003$).

We observed no significant correlation between VEGF changes and baseline variables such as fasting blood sugar, serum lipids, age and duration of diabetes ($P < 0.05$).

Discussion

Previously, it has been shown that peripheral markers of angiogenesis may increase in the PDR [10]. If a reduction in VEGF can be considered as a marker for a successful outcome of PRP in DR, it could provide the clinician a convenient opportunity for monitoring disease progression or relapse via a simple blood sample.

An increase in serum levels of VEGF may be a key regulator of DR and provide a potential tool for risk assessment in diabetic patients [15, 16]. However, there are conflicting reports about the association of serum VEGF and DR [15–17]. Ozturk et al. [16] found no difference between serum VEGF levels in non-proliferative DR and PDR. Meleth et al. [17] also found no significant difference in serum VEGF levels among subjects with less severe and severe DR. In contrast, Cavusoglu et al. [15] detected progressively increasing serum levels of VEGF in DR and correlation with the stage of retinopathy.

In our study no significant difference was observed in VEGF levels before and after PRP, but when patients with regressed PDR compared with patients with progressed PDR, a statistically significance was observed in VEGF changes. This was consistent with observations made by the Lip group [10]. Lip et al. found a trend towards lower plasma VEGF levels in the patients with complete improvement of neovascularization when compared with those with incomplete improvement [10].

It has previously been hypothesized that the measurement of plasma VEGF may possibly serve as predictors of effectiveness of laser treatment, as demonstrated by Lip et al. [10]. However, the present study could not find any significant association between serum baseline VEGF and laser treatment outcome.

In our study no significant correlation was observed between blood glucose and VEGF levels. However, Kakizawa et al. [18] and Cavusoglue et al. [15] have shown that plasma VEGF was elevated in

poorly controlled diabetic patients and that glycemic control can reduce levels of plasma VEGF [18].

We used serum to measure VEGF levels, but results of some studies suggested that plasma might be better medium to measure VEGF levels because activated platelets (during blood clotting) release VEGF into serum, and thus, results may be inaccurate [19]. This might be one of the limitations of this investigation. Nevertheless the mean VEGF in our study in diabetic patients with PDR was lower than other studies [10, 11].

Furthermore, many diabetic complications interfere with VEGF systemic values, which may cause confusion in interpretation of plasma VEGF as a marker of only DR [20–22], so the absence of diabetic patients without complications or healthy control group for comparison could be another limitation of this study.

In summary, our results showed a significant reduction in the levels of serum VEGF in patients who had successful laser treatment. These findings suggest that serum VEGF levels can be used for monitoring PDR outcome.

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