The relevance of serum level of VEGF in type 2 diabetic retinopathy

Dr. Hasanain M. Ahmed / lecturer / department of surgery-ophthalmological unit / medical college / kufa university
Dr. Mahdi Alsihlawi / assistant professor / biochemistry department / medical college / kufa university
Fadhil Abdulameer

ABSTRACT

Background:
Vascular endothelial growth factor plays a major role in angiogenesis under physiologic and pathologic conditions. The main aim of study is to explore the relevance of retinopathy with angiogenic factor VEGF (vascular endothelial growth factor) in type 2 diabetic patients.

Patients and methods:
Thirty five patients of type 2 diabetic retinopathy of the duration of more than five years (group one) (23 male and 12 female) with age range from (41-75) year were enrolled. Thirty patients of diabetic without clinical retinopathy with duration of more than five years (group two) (15 male and 15 female) with the age range from (40-75) year were included in this study.
On the other hand, Thirty five healthy volunteers were included in this study. They were matched in their sex and age with patient groups. 12 of them were female and 13 were male. Their age range from (40–78) years. Human VEGF BioAssay ELISA kit was the chemical and kit that had been used in this study.

Results:
The main results showed significant increase in VEGF (p< 0.01) in patients with diabetic retinopathy (group one) as compared with control group (group three) whereas diabetic without retinopathy (group two) revealed no significant differences as compared with control group (p= N.S.). Furthermore, a statistically significant elevation was observed in the mean VEGF between diabetic retinopathy and diabetic without retinopathy.

Conclusion:
High level of VEGF plays a major role in the pathogenesis of diabetic retinopathy and measurement of VEGF level may be useful in the management and more intensive ophthalmological examination of diabetic retinopathy.

Introduction:
Vascular endothelial growth factor (VEGF) is an angiogenic mitogen secreted from various types of cells and plays a major role in angiogenesis under physiologic and pathologic conditions[1,2]. VEGF has been suggested to play an important role in the progression of DR [1,3]. VEGF is massively unregulated in the eyes of the patients with DR [4]. Several studies have shown that VEGF antagonists can reduce retinal vascular permeability and neovascularization, and inhibit the development of DR [5, 6].
Of all the growth factors, vascular endothelial growth factor (VEGF) is the one most closely related with retinal vascularisation as it participates in the formation of new vessels that appear after retinal ischemia [7].

Several factors are known to play a role in ocular angiogenesis, VEGF plays a central role in this process. VEGF is produced in the eye by retinal pigment epithelium (RPE) cells and is up-regulated by hypoxia. There are four major biologically active human isoforms, of which VEGF165 is predominant in the human eye and appears to be responsible for pathological ocular neovascularization. Besides being a potent and specific mitogen for endothelial cells, VEGF increases vascular permeability, inhibits endothelial cells apoptosis, and is a chemotactant for endothelial cell precursors. Apart from higher intraocular VEGF concentrations, a two-fold increase in VEGF receptor expression has been reported in the retinas from diabetic rats [8].

Retinal ischemia due to capillary occlusion has a crucial role in the development of DR and induces angiogenic factors in order to make up for oxygen deficiency [9,10]. However, uncontrolled up regulation of these angiogenic factors results in pathologic angiogenesis (neovascularization). Since the walls of these new vessels are fragile and easy to break, untreated neovascularization can lead to PDR that causes severe vitreous cavity bleeding. Furthermore, progressive contraction of fibrovascular membranes over large areas of vitreoretinal adhesion can cause tractional retinal detachment and result in blindness. It has been estimated that without treatment for PDR, 50% of all patients will become blind within five years following diagnosis [11].

**The aim of the study:**
To explore the relevance of retinopathy with angiogenic factor VEGF in type 2 diabetic patients.

**Patients and the Control Group:**

**Patients:**
The collection of samples was conducted during the period from December 2010 to March 2011. Thirty five patients of type 2 diabetic retinopathy of the duration of more than five years (group one) (23 male and 12 female) with age range from (41-75) year were enrolled. Thirty patients of diabetic without clinical retinopathy with duration of more than five years (group two) (15 male and 15 female) with the age range from (40-75) year were included in this study. The samples were collected from ophthalmology and laser center in AL- Hakeem general hospital in AL-Najaf city.

 Patients suffered from the following cases were not included and excluded from the current study:
- Diabetic nephropathy patients
- Diabetic neuropathy patients.
- Type 1 diabetic patient.
- Rheumatoid arthritis patients.
- Patients with cancer.
- I.H.D. (i.e: MI, angina, CHF) patients.
- Pregnancy.

The diagnosis of diabetic retinopathy is carried out by fundus examination using slitlamp after dilatation of the pupil with Topicarmide 5% eye drop.
**The Control Group:**

Thirty five healthy volunteers were included in this study. They were matched in their sex and age with patient groups. They were collected from the same centre in Al-Hakeem general hospital, 12 of them were female and 13 were male. Their age range from (40–78) years.

**Collection of Samples:**

this done by specialized biochemist, Disposable syringes and needles were used for blood collection. Blood samples (5ml) were obtained from diabetic patients and control groups by vein puncture, the blood samples were allowed to clot at room temperature, and then centrifuged at 3000 Xg for 10 minutes. Sera were transferred carefully and stored at -17ºC until analysis time in suitable serum tubes.

The chemical and kit that were used in this study were of the highest purity and are listed in the table below with their supplier.

**Table (1): Chemicals.**

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>US BIOLOGICAL. USA</td>
<td>Human VEGF BioAssay ELISA kit.</td>
</tr>
</tbody>
</table>

**Procedure:**

1. **Standard/ Sample:** Standard was diluted from 1ng/ml to zero in Diluent. Immediately 100 µL of standard or sample was added to each well in triplicate. At RT for at least 2 hours was incubated.
2. **Detection:** The wells were aspirated to remove liquid. The plate was washed 4 times. Detection Antibody was diluted in Diluent to a concentration of 0.25µg/ml. A volume of 100 µL was added per well. At RT was incubated for at least 2 hours.
3. **Avidin Peroxidase:** The wells were aspirated to remove liquid. The plate was washed 4 times. 5.5 µL of Avidin Peroxidase(1 : 2000) was diluted in Diluent for a total of 11 ml. A volume of 100 µL was added per well. For 30 minute was incubated at RT.
4. **ABTS Liquid Substrate:** The wells were aspirated to remove liquid. The plate was washed 4 times. A volume of 100 µL was added to each well. At RT was incubated for color development.
5. **Color development** was monitored with an ELISA plate reader at 405 nm with wavelength correction set at 650nm.

**Calculation:**

1. Reliable standard curve were obtained when either absorbance readings do not exceed 0.2 for the zero standard concentrations, or 1.5 for the highest standard concentration.
2. The plate has been monitored at 5-minute intervals for 45 minutes.
3. A standard curve was created by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit.
Figure (1): The standard curve for determination of VEGF concentration.

**Biostatistical Analysis:**
1. The results were expressed as Mean ± SD.
2. Group’s differences between diabetics (DR and diabetic without retinopathy) and controls were analyzed using one-way ANOVA.
3. Student’s t-test was used to verify the association of inflammatory response markers in the patients relative to the control group.
4. Significant variation was considered when the P value was less than 0.05.

The results were analyzed using Anova and student’s t–test statistical analysis. This analysis was carried out by using the SPSS programme.

**Result.–**
Serum VEGF concentration were calculated from the calibration curve obtained by measuring the absorbance of VEGF, standards supplied with the kit as shown in Figure (1).

Table (2) shows the mean ± SD concentrations of VEGF for three groups. These results showed significant increase (p< 0.01) in patients with diabetic retinopathy (group one) as compared with control group (group three) whereas diabetic without retinopathy ( group two ) revealed no significant differences as compared with control group (p= N.S.).

Table (2): Serum VEGF levels in diabetic patients (DR and DNR) and controls.

<table>
<thead>
<tr>
<th>P- values</th>
<th>VEGF ( pg/ ml)</th>
<th>Number</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ±SD</td>
<td></td>
</tr>
<tr>
<td>P&lt;0.01</td>
<td>355-67</td>
<td>205.9 ±75.4</td>
<td>35</td>
</tr>
<tr>
<td>N.S.</td>
<td>262-34</td>
<td>171.3 ± 51.4</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>305 - 34</td>
<td>160.3 ± 59.5</td>
<td>40</td>
</tr>
</tbody>
</table>

N.S. = Non significant.
The results of serum VEGF in diabetic retinopathy patients in comparison with diabetic without retinopathy group are presented in Table (3).

Our study reveal a statistically significant elevation was observed in the mean VEGF between diabetic retinopathy and diabetic without retinopathy.

Table (3): Comparison between DR and DNR in serum levels of VEGF

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR (SD ± mean)</th>
<th>DNR (SD ± mean)</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-value</td>
<td>205.9 ± 75.4</td>
<td>171.3 ± 51.4</td>
<td>(pg/ml)VEGF</td>
</tr>
</tbody>
</table>

N.S. :- No significant.

discussion

In this study, we have confirmed previous observations of increased plasma VEGF levels in diabetic patients, as well as previous observations of higher plasma VEGF levels in patients with more severe retinopathy. [12]

Previous reports have demonstrated that deregulated VEGF expression plays a pivotal role in the induction of diabetic retinopathy in a relevant animal model by triggering inflammatory phenomena, such as adhesion molecule expression, leukocyte adhesion, endothelial dysfunction, and blood-retinal barrier breakdown. [13]

The blood–retinal barrier (BRB) breakdown and retinal neovascularization (NV) are the major retinal vascular dysfunctions leading to vision loss in a variety of retinal diseases such as diabetic retinopathy (DR) [14,15,16]

Another explanation of our results regarding VEGF concentration, that VEGF is a dominant mediator of ischemic retinal neovascularization, [17,18]. It is a potent vascular permeability factor (VPF) and a major angiogenic stimulator with endothelial cell-specific mitogenic activity and plays a crucial role in both normal and pathological angiogenesis [6,19,20]. In diabetic patients with proliferative retinopathy, VEGF levels are significantly increased in the vitreous and the retina, (it must be kept in mind that serum levels could influence the vitreous concentration of a particular protein [21]) and successful laser treatment decreased vitreous VEGF levels by 75% [17,22,23]. In the retina, VEGF is produced by multiple cell types, including the retinal pigment epithelium (RPE), pericytes, endothelial, glial, Muller, and ganglion cells [23,24]. Among them, Müller cells and RPE are believed to be the major source of VEGF in the retina, and endothelial cells to be the primary target of VEGF [18,24]. Our results confirm those of previous reports and suggest that VEGF is a predominant mediator of pathologic angiogenesis in PDR.

These results are in consistence with the hypothesis of elevated VEGF serum levels during diabetic retinopathy [25].

The results of our study are consistence with the study of Faten, et al. (2010) [26], who state that there was significant increase in plasma levels of VEGF in type II diabetics with retinopathy compared to controls and diabetics without complications. This could explain a causative role of VEGF in diabetic proliferative retinopathy. VEGF plays a role in the neovascularization and in the breakdown of the blood–retinal barrier which is characterized by hyperpermeability of retinal vessels [27]. VEGF production is stimulated by hyperglycemia, advanced GEP, IGF-1, angiotensin II, and hypoxia, all of which are present in the retinal microvascular bed. Hypoxia is generally considered to
represent a fundamental stimulus for angiogenesis through VEGF production in diabetic retinopathy [28].

The study of Kondo et al. (2003) [27] who showed that reduction in retinal neovascularization is accompanied by suppression of retinal expression of VEGF, endothelin-1, and nitric oxide synthase, suggesting the importance of these endothelial mediators in retinal neovascularization. Also, Eri et al. (2004) [28] stated that leptin and IL-6 induce angiogenesis by up-regulation of VEGF; this suggests that VEGF antagonism may offer a novel therapeutic strategy to treat diabetic retinopathy.

However, VEGF overexpression can result in retinal neovascularization, and increased retinal vascular permeability, macular edema, bleeding, fibrosis, and loss of vision may follow. Local and systemic VEGF antagonists have been proposed as potential therapeutic interventions for the treatment of diabetic macular edema and proliferative retinopathy [9,29].

Conclusions:
From the results of our study, the following conclusions can be obtained:

• Increased serum levels of angiogenic factor (VEGF) may act as a key regulator of DR when compared with diabetic without complication
• The measurement of serum VEGF may be useful in the management of diabetic retinopathy.

Recommendations:
The following recommendations for the future works are:

• Further studies that evaluate both vitreous and serum levels in various stages of DR are needed to provide a better understanding of the interaction between systemic and local inflammatory and angiogenic factors.
• Patients with increase serum levels of VEGF, need more intensive ophthalmological examination to detect early retinal changes in aim of providing early treatment.

References:


