ORIGINAL ARTICLES

Erythropoietin and Hypoxia-Inducible Factor 1α in Patients with Proliferative Diabetic Retinopathy

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ABSTRACT

To determine the intra-vitreal levels of erythropoietin (EPO), vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (HIF-1α) in patients with proliferative diabetic retinopathy (PDR), and to evaluate the relationship between the level of these parameters and PDR activity. The study included 23 diabetic patients with PDR (13 diabetic patients with early PDR and 10 diabetic patients with active PDR). Sixteen age-matched non-diabetic patients with macular hole served as a control group. EPO, VEGF and HIF-1α were measured by enzyme-linked immunosorbent assay (ELISA). The vitreal levels of EPO, VEGF were significantly higher in diabetic patients with PDR in comparison with the control group. In addition, the vitreous concentrations of EPO, VEGF were higher in patients with active PDR than in those patients with early PDR. We did not observe a significant difference in vitreal levels of HIF-1α between diabetic patients and the control group. Intra-vitreous EPO and VEGF in diabetic patients with PDR are increased and related mutually. VEGF and EPO, especially VEGF, are associated with the angiogenesis of PDR.

Key words: erythropoietin, hypoxia inducible factor, VEGF, diabetic retinopathy.

Introduction

Diabetic retinopathy is the most prevalent cause of blindness world wide (Rossing, 2005). The mechanisms of diabetic retinopathy remain unclear; however, it is believed that retinopathy is an ischemic disorder which leads to the development of neovascularization and blindness (Yam and Kwok, A. K., 2007). One of the molecules responsible for the sight-threatening neovascularization is vascular endothelial growth factor (VEGF), which has been found to be elevated in the retina of diabetic animals and humans (Ayalasomayajula and Kompella, U. B., 2003). The increase in VEGF has been speculated to be the result of ischemic hypoxia (Pe’er et al., 1996).

The transcription factors hypoxia inducible factor-1 and -2 (HIF-1 and HIF-2) can be altered during shifts in oxygen levels (Ke and Costa, M., 2006). There are two isoforms of HIF-1: HIF-1α and HIF-1β (Nangaku et al., 2008). The HIF-1β isoform is constitutively expressed regardless of oxygen tension, while the HIF-1α isoform has a short half-life during normoxia (Ke and Costa, M., 2006).

When oxygen tension drops, the HIF-1α isoform is stabilized and translocates to the nucleus, where it binds to HIF-1β and becomes transcriptionally active. HIF-2α is regulated similarly to HIF-1α, except HIF-2α appears to be stabilized at higher oxygen tensions and is important in adaptations to chronic hypoxia (Arjamaa and Nikkinmaa, M., 2006).

Erythropoietin (EPO) is a growth factor that promotes the differentiation and proliferation of red blood cells from progenitors within the bone marrow in response to hypoxia (Watanabe et al., 2005). EPO is similarly induced by hypoxia-inducible factor (HIF)-1α in a hypoxia-dependent fashion, as is VEGF. In human eyes with proliferative diabetic retinopathy and diabetic macular oedema, elevated VEGF and EPO levels were detected in the vitreous, suggesting that EPO may be produced as an endogenous neuroprotective factor independent of ischaemia (Inomata et al. 2004).

The biological significance of EPO in the diabetic retinopathy is not completely understood; the non-haematopoietic, neuroprotective and neurotrophic functions of EPO have led to proposed therapies for several central nervous system and retinal diseases. (Hernandez et al, 2006). The aim of this study is to determine vitreous EPO, VEGF and HIF-1α levels in patients with PDR.

Materials And Methods

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Twenty three PDR patients of type 2 diabetes were included in the study and medical history was taken. Ophthalmological examination was performed, including: visual acuity and intraocular pressure measurement by applanation tonometry.

Careful slit lamp examination to rule out anterior chamber depth and presence of iris neovascularization. Gonioscopy, if there is suspicion of neovascular glaucoma. Detailed fundus examination using 90-diopter contact lens to rule out neovascularization and macular oedema. Indirect ophthalmoscopy and bio microscopy to evaluate the grade of vitreous proliferation and determine the presence and nature of macular oedema. Fundus fluorescein angiography was done using a 50 field fundus camera, 5 ml of 10% sodium fluorescein was injected in the anti-cubital vein and photography was carried out. Angiography was performed in patients with diabetic retinopathy to differentiate between non proliferative and proliferative retinopathies.

The pre-operative findings were recorded and the clinical disease severity was classified, according to the presence and extent of active fibrovascular tissue, vitreous haemorrhage, tractional retinal detachment (with or without retinal tears). Recent vitreous haemorrhage was excluded to avoid affecting the vitreous samples.

The control group included 16 patients who were undergoing vitrectomy for idiopathic macular hole caused by vitreo-macular traction occurring before posterior vitreous detachment and there are no signs of ischemia, proliferation or inflammation. Therefore, it was believed that vitreous fluid from patients with macular hole is probably similar in constitution to that of normal eyes.

Sample Collection:

The technique used is the standard three port pars plana vitrectomy with one sclerotomy for the infusion canula and other 2 sclerotomies for exchange of instruments. After the sclerotomy ports were placed, the vitreous cutter was introduced into the vitreous body and a sample of undiluted vitreous (0.2-0.5ml) was aspirated manually into a disposable tuberculin syringe before turning on the infusion and completing the surgical procedure.

The vitreous samples were transferred to a sterile tube, placed immediately on ice and centrifuged for 5 min; the samples were rapidly frozen at-80 c until assayed.

Samples of venous blood were collected in a tube on EDTA to estimate HbA1c. Measuring HbA1c with a cation exchange chromatography method assessed recent glycaemic control. The procedure is a micro-chromatographic methodology for the quantitation of glycosylated haemoglobin (non diabetic reference 5.5%-7.7%) Glyco- HbQuick Column procedure (Helana).

EPO, VEGF and HIF-1α were measured by enzyme linked immunosorbent assays (ELISA) using reagents manufactured by (Quantikine).

Statistical analysis:

SPSS software (version 10) was used for data management and analysis. Quantitative data was expressed as mean ± SD. As most of the data were normally distributed continuous variables, Student’s-t test was used to assess whether a statistical significance is present between the studied groups. The degree of association between the variables was assessed using Pearson’s correlation coefficient (r), where values of p < 0.05 were considered significant.

Results:

The main clinical characteristics and the results of vitreous measurements performed in diabetic patients with PDR and non-diabetic control subjects are summarized in Table 1&2.

The vitreal levels of both EPO and VEGF were strikingly higher in diabetic patients with PDR in comparison with the control group (Table 2). The vitreous concentrations of EPO and VEGF were higher in patients with active PDR (n= 10) than in those patients (n= 13) with quiescent PDR [324.5 (80–1670) vs. 183.5 (64–487), p< 0.05 and 3596 (1670–6155) vs. 1143 pg/ml (388–2500), p<0.01, respectively]. A correlation between vitreous levels of EPO and VEGF was observed in diabetic patients with PDR, but not in non-diabetic control subjects. (Fig1). We did not observe significant difference in vitreal levels of HIF-1α between diabetic patients and the control group (Table 2). In addition, no correlation was observed between vitreous levels of HIF-1α and either EPO or VEGF.
Table 1: Demographics of diabetic patients and controls included in this study

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>PDR</th>
<th>Group 1 inactive PDR</th>
<th>Group 2 active PDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>16</td>
<td>23</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/10</td>
<td>8/7</td>
<td>7/8</td>
<td>9/6</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>55.7 ± 7.6</td>
<td>63.6 ± 9.2</td>
<td>63.8 ± 8.3</td>
<td>64.1 ± 8.3</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>-</td>
<td>12.6 ± 8.4</td>
<td>12.6 ± 8.4</td>
<td>12.6 ± 8.4</td>
</tr>
<tr>
<td>HbA1c%</td>
<td>6.9 ± 0.8</td>
<td>9.4 ± 1.2</td>
<td>9.7 ± 1.1</td>
<td>9.7 ± 1.1</td>
</tr>
</tbody>
</table>

Table 2: Comparison of the different studied parameters among all diabetic group

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>PDR</th>
<th>Group 1 inactive PDR</th>
<th>Group 2 active PDR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>16</td>
<td>23</td>
<td>13</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>EPO (pg/ml)</td>
<td>48.9 ± 23a</td>
<td>173 ± 55 b</td>
<td>183.5 ± 64b</td>
<td>324.5 ± 80c</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>438 ± 98a</td>
<td>2170 ± 387b</td>
<td>1143 ± 116 c</td>
<td>3596 ± 430d</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>HIF-1 (pg/ml)</td>
<td>2.46 ± 0.2 a</td>
<td>2.9 ± 0.5 a</td>
<td>1.8 ± 0.58 a</td>
<td>1.82 ± 0.6 a</td>
<td>NS</td>
</tr>
</tbody>
</table>

p< 0.05 is statistically significant. Groups with different letters have a statistically significant difference.

Discussion:

Proliferative diabetic retinopathy is the primary cause of visual loss in diabetic patients and results from accumulation of fluid due to a breakdown of the blood–retinal barrier (Ciulla et al., 2003).

This study demonstrated that vitreous VEGF and EPO were increased in patients with PDR compared to controls without diabetic retinopathy. This suggests that both VEGF and EPO play a role in the pathogenesis of PDR.

Both VEGF and EPO are downstream products of HIF-1α and both are neuroprotective and angiogenic (Nishijima et al., 2007). Up regulation of VEGF is associated with the breakdown of the blood–retinal barrier and increased vascular permeability, stimulation of endothelial cell growth, and pathologic neovascularization (Gora & Josko, 2005).

EPO shares similarities with VEGF, but possibly displays opposing effects. The first fundamental difference between VEGF and EPO is that VEGF increases permeability of the blood–retinal barrier, whereas EPO maintains the integrity of the blood–retinal barrier (Lui et al., 2008). Possibly, EPO protects against the VEGF-induced permeability and acts as an anti-permeability factor (Martinze et al., 2003) Also, there is growing evidence that EPO is a neuroprotective and anti-apoptotic factor and prevents microvascular change by inhibition of oxidative stress in the diabetic retina (Kruegel et al., 2010). In addition, EPO exerts an anti-inflammatory effect on the brain, and this action may be extrapolated to the diabetic retina (Villa et al., 2010). Finally, VEGF plays a major pathological angiogenic role in diabetic retinopathy, whereas EPO does not. Abu El-Asrar et al (2007) reported that retinal neovascularization was correlated with HIF-1α and VEGF expression, but not with immune reactivity for EPO in eyes with proliferative diabetic retinopathy. This suggests that stimulating agents, other than ischaemia, are involved in the increased intraocular EPO in diabetic retinal oedema. Further, retinal neurodegeneration or inflammation might contribute to the increased intraocular EPO levels observed in patients with diabetic retinopathy. Thus, the increased intraocular EPO in diabetic retinopathy may be a self-regulated physiologic mechanism to prevent retinal damage against vascular hyper permeability (Patel et al., 2008).

For all these reasons, it seems that EPO is crucial for retinal homeostasis and its enhancement in diabetic retinopathy could be a physiological consequence rather than a pathogenic contributor. However, further studies are needed to investigate the precise role of EPO in diabetic retinopathy.

Growing evidence suggests that EPO has both neuroprotective and vascular protective functions in diabetic retinopathy (Hong et al., 2007). Theoretically, increased EPO could lead to VEGF inhibition through enhanced HIF-1α feedback or to EPO reduction as a result of less neuronal and glial stress (Zhang et al., 2010).

We did not observe a significant difference in vitreal levels of HIF-1α between diabetic patients and the control group. In addition, no correlation was observed between vitreous levels of HIF-1α and either EPO or VEGF.

Recent studies indicate that intravitreal EPO is a potentially interesting treatment option in retinal disease, although data are limited (Li et al., 2010). Further studies are needed to determine the functional role of EPO in eyes with diabetic retinopathy.
References


