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LABORATORY STUDY

Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy

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Abstract

We measured interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) in the vitreous humour and serum of patients with proliferative diabetic retinopathy (PDR), in order to determine the role of these cytokines in the pathogenesis of the disease. Vitreous and serum samples were collected from 21 patients with PDR who were undergoing pars plana vitrectomy. Control vitreous samples were obtained from cadavers and control serum samples from healthy subjects. The cytokines were measured by enzyme-linked immunosorbent assay. Vitreous IL-1β and TNF-α concentrations in patients with PDR exceeded those of controls ($P<0.05$), as did serum IL-1β and TNF-α. We suggest that increased vitreous IL-1β and TNF-α levels may play a significant role in the pathogenesis of PDR, which features abnormal cell proliferation and neovascularisation.
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Keywords: proliferative diabetic retinopathy; interleukin-1 beta; tumour necrosis factor-alpha; cytokines

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Introduction

Diabetic retinopathy is one of the most serious complication of diabetes mellitus. Neovascularisation is the common final pathway in proliferative diabetic retinopathy (PDR) and often leads to vision loss with vitreous haemorrhage, tractional retinal detachment, and neovascular glaucoma. Vascular, metabolic, endocrine, haematologic, and immunological mechanisms are important in the development of neovascularisation secondary to ischaemia by upregulating the production of growth factors.[1–7] Interleukins play an important role in the regulation of immune mechanisms, and are also thought to be responsible for the development of PDR. Cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon-gama regulate cells and have immune and inflammatory properties.[8–10] When stimulated by human recombinant IL-1β and TNF-α, human RPE cells synthesise and secrete IL-6 and IL-8, the former being a potent mediator of inflammation.[11] In order to determine the relationship between immune mechanisms and diabetic retinopaty, we measured vitreous and serum IL-1β and TNF-α concentrations in patients with PDR.

Materials and methods

The study group consisted of 21 patients (11 female and 10 male) with the diagnosis of vitreoretinal pathology due to advanced PDR, undergoing pars plana vitrectomy. Their ages ranged from 42 to 70 years (median 61). Prior to vitrectomy, 0.5 ml vitreous and 5 cc blood samples were obtained from each patient. Vitreous samples (0.5 ml) obtained from eyes of 21 cadavers with ageing match, within 2 h of death, were used as controls. No specimens were taken from cases with ocular pathology, sepsis, neuroviral pathology, hepatitis A, B, C, or malignancy. Serum was taken from a control group consisting of 21

healthy individuals with ageing match. Serum and vitreous samples were collected in sterile Ependorf tubes and stored at −70°C until the time of assay. We used an enzyme-linked immunosorbent assay (ELISA) kit (Immunotech, catalog no.10755) for IL-1β measurements. TNF-α was quantified with an ELISA kit (The Biosurce International Cytoscreen, catalog no.Dta 50-RD systems). The values were read at 450 nm in an ELISA reader and IL-1β and TNF-α concentrations were calculated from specific calibration curves prepared with known standard solutions.

Statistical analysis

Since vitreous IL-1β, serum IL-1β, vitreous TNF-α and serum TNF-α concentrations were non-normally distributed, the Mann–Whitney *U*-test and Spearman's Rank Correlation test were used in the analysis. *P*-values less than 0.05 were considered significant. Immunological parameters were described by mean, standard deviation (SD), median (M), and range (R).

Results

In PDR patients, the mean levels of vitreous and serum IL-1β were 34.1$\pm$84.3 and 12.8$\pm$21.7 pg/ml; whereas the TNF-α were 160.7$\pm$218.9 and 103.8$\pm$199.9 pg/ml, respectively. In controls, the mean levels of vitreous and serum IL-1β were 5.5$\pm$5.8 and 0.41$\pm$1.91 pg/ml; whereas the TNF-α were 12.3$\pm$6.19 and 5.9$\pm$4.5 pg/ml, respectively. Both vitreous and serum IL-1β and TNF-α levels were significantly greater in the PDR patients than the controls ($P<0.0001$). Vitreous and serum IL-1β and TNF-α concentrations of both groups are shown in Tables 1 and 2.

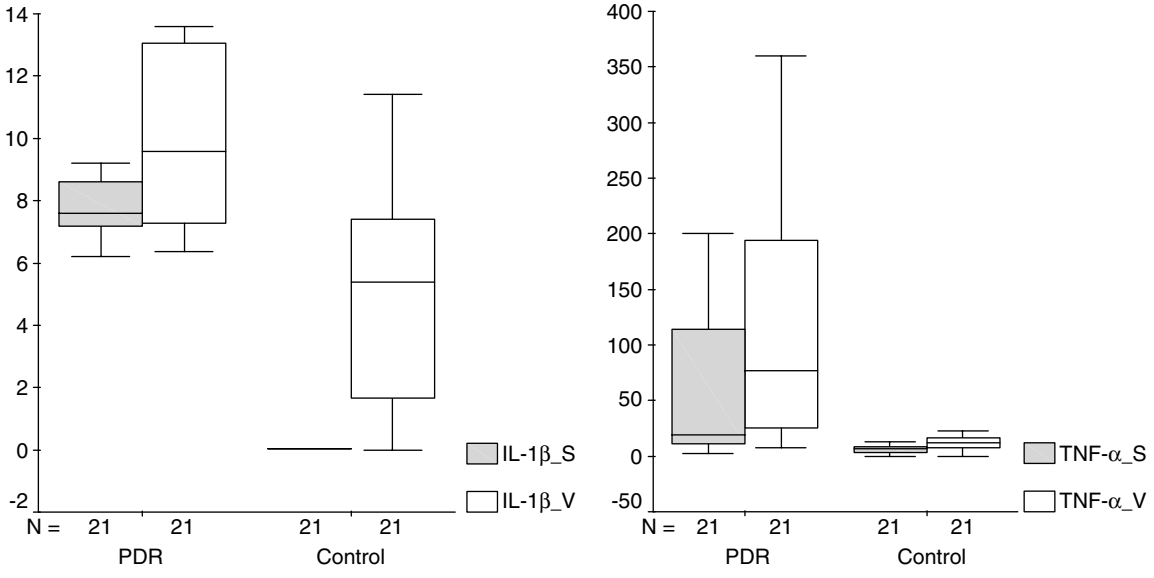
Discussion

Immunological, biochemical, and genetic factors are thought to contribute to the pathogenesis of intraocular changes in PDR. Several studies have addressed the recent hypothesis that the reduced angiogenesis of PDR is due to the release of growth factors and interleukins from the ischaemic retina.[12–16] Abnormal production of

Table 1 The levels of IL-1β and TNF-α in patients with PDR and controls

Cytokines	*PDR* (n = *21*) *Mean*$\pm$*SD (median (min-max))*	*Control* (n = *21*) *Mean*$\pm$*SD (median (min-max))*	P-*value*
IL-1β_Serum	12.8$\pm$21.7 (7.6 (2.6–107.0))	0.41$\pm$1.91 (0 (0–8.7))	0.0001
IL-1β_Vitreous	34.1$\pm$84.3 (9.5 (6.3–388.9))	5.5$\pm$5.8 (5.4 (0–25.0))	0.0001
TNF-α_Serum	103.8$\pm$199.9 (19.2 (2.51–906.2))	5.9$\pm$4.5 (6.5 (0–13.1))	0.0001
TNF-α_Vitreous	160.7$\pm$218.9 (77.1 (7.4–988.2))	12.3$\pm$6.19 (12.1 (0–23))	0.0001

Table 2 Boxplot diagram of IL-1β and TNF-α levels in patients with PDR and controls



cytokines such as IL-1β, TNF-α , IL-6, IL-8 may also be important.[17–20] The biological effects of IL-1β depend on the target cell. Together with TNF-α, IL-1β is responsible for angiogenic activity. It stimulates not only collagen synthesis, but also glial cells and fibroblasts, resulting in proliferation and contraction.

Experimental studies have demonstrated the *in vivo* angiogenic activity of IL-1β, and its influence on the development of ocular neovascularisation, especially in the cornea and iris.[15,21] Studies of vitreous and serum IL-1β concentrations in patients with proliferative vitreoretinopathy (PVR) and PDR have come to different conclusions.[14,22,23] Kaufmann *et al*[23] showed an increased vitreous IL-6 in 29% of patients with PDR, but did not detect any IL-1β.

Limb *et al*[14] found much greater IL-1β and IL-6 concentrations in the vitreous of patients with PVR than in those with uncomplicated retinal detachment. Abu el Asrar *et al*[22] found increased IL-1β in the vitreous of 44% of patients with PDR, but not in their serum or that of controls. Vitreous and serum IL-1β concentrations in PDR were significantly greater than in their controls, an observation that supports the idea of local release of IL-1β from the vitreous or high levels are achieved by a breakdown of the blood retinal barrier. Our PDR patients had significantly greater vitreous and serum IL-1β concentrations than those of Kaufmann *et al*[23] and Abu el Asrar *et al*[22]; IL-1β was present in the serum and vitreous of all the PDR patients, the vitreous of 81% of the controls, and in the serum of 10% of controls.

Experimental studies show the uveitogenic activity of intravitreally injected TNF-α, which also causes increased aqueous humour proteins, corneal neovascularisation and posterior synechia.[24] Intraocular injection of TNF-α and IL-1 induces factors such as IL-8, IL-6, macrophage colony stimulating factor and monocyte chemotactic protein.[11,25] Elner *et al*[11] detected positive IL-1β and TNF-α in the vitreous of patients with PDR and PVR. Limb *et al*[14] found TNF-α in the vitreous of 22% of PVR patients, but only in 8% of uncomplicated retinal detachment patients and controls. However, Abu el Asrar *et al*[22] could detect no TNF-α in the serum, vitreous or aqueous of PDR patients or controls. They suggested that immediate removal of TNF-α from the circulation results in replacement by other cytokines. Kaufmann *et al*[23] observed similar results in patients with PDR, PVR, vitreous haemorrhage, and macular hole.[23] In our study, vitreous TNF-α concentrations in PDR were greater than in the controls; TNF-α was detected in the serum and vitreous of all the PDR patients, in the vitreous of the controls, and in the serum of most controls; IL-1β and TNF-α were present in all samples from the PDR group. Serum TNF-α concentrations in the PDR patients were significantly greater than in the controls. Vitreous haemorrhage, which is usually present, plus the breakdown of the blood-aqueous barrier by locally produced cytokines, result in the leakage of TNF-α to the systemic circulation.[13,15,22] The strong correlation we found between vitreous and serum IL-β and TNF-α concentrations in the PDR patients can be explained by simultaneous synthesis of IL-1β and TNF-α from the same origin, that is, the RPE and macrophages. In PDR, which is characterised by abnormal cell proliferation and neovascularisation, the increased IL-1β and TNF-α vitreous concentrations are attributed to the role of interleukins in the development of this disease. We suggest that the positive correlation between the vitreous and serum concentrations of these two cytokines requires further investigation.

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