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Pro-inflammatory and Anti-inflammatory Cytokines in Vitreous Fluid of Patients with Proliferative Diabetic Retinopathy

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ABSTRACT

To determine the intra-vitreous levels of two pro-inflammatory cytokines [interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1)] and the anti-inflammatory cytokine interleukin-10 (IL-10) in patients with proliferative diabetic retinopathy (PDR). In addition, the relationship between the level of cytokines and PDR activity had also been evaluated. The study included 23 diabetic patients with PDR (13 diabetic patients with inactive PDR and 10 diabetic patients with active PDR). Sixteen age-matched non-diabetic patients with macular hole served as a control group. IL-8, MCP-1 and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA). The vitreal levels of both IL-8 and MCP-1 were significantly higher in diabetic patients with PDR in comparison with the control group. In addition, the vitreous concentrations of IL-8 and MCP-1 were higher in patients with active PDR than in those patients with inactive PDR However, no significant difference was detected in the vitreal levels of IL-10 between diabetic patients and the control group. The pro-inflammatory cytokines IL-8 and MCP-1 are increased in the vitreous fluid of PDR patients without an increase in the anti-inflammatory cytokine IL-10. In addition, both intra-vitreous IL-8 and MCP-1 levels are asssociated with PDR activity, thus suggesting that these cytokines may be pathogenically related to PDR.

Key words: IL-8, IL-10, MCP-1, vitreous fluid, proliferative diabetic retinopathy

INTRODUCTION

In recent years, growing evidence has emerged indicating that inflammation is an important event in the pathogenesis of proliferative diabetic retinopathy (PDR) [1]. Among the inflammatory cytokines there are the chemotactic cytokines referred to as chemokines [2]. Interleukin-8 (IL-8) is the prototype of C-X-C chemokine which has been recognized as a potent chemoattractant and activator of neutrophils and T lymphocytes but not monocytes [3]. [(]Monocyte chemoattractant protein-1 (MCP-1) is the best-characterized C-C chemokine and exhibits chemoattractant potential for monocytes and lymphocytes but not for neutrophils [4]. Therefore IL-8 and MCP-1 are involved in the recruitment of inflammatory cells. The presence of these inflammatory cells is associated with local secretion of angiogenic factors which are crucial for the development of PDR. During the inflammatory reaction, anti-inflammatory cytokines are also produced and tend to modulate the inflammatory process [5].

However, little information is available regarding the potential role of anti-inflammatory cytokines in PDR.

IL-10 is an anti-inflammatory cytokine with potent deactivating properties on macrophages [6]. In addition, antitumoral effects of IL-10 have been associated with its ability to prevent angiogenesis associated with tumour growth [7]. Furthermore, it has been recently demonstrated that its anti-angiogenic effect is associated with the down-regulation of vascular endothelial growth factor (VEGF) expression [8].

On this basis, it could be postulated that the balance between pro-inflammatory and anti-inflammatory cytokines rather than pro-inflammatory cytokines alone is a primary factor in determining the development of PDR.

In the present study, we have determined the intra-vitreous levels of both pro-inflammatory cytokines (IL-8, MCP-1) and the anti-inflammatory cytokine IL-10 in PDR patients. In addition, the relationship between the profile of cytokines and PDR activity.

MATERIALS AND METHODS

The studied subjects were 23 patients with PDR with type 2 diabetes. Full ophthalmological examination and medical history was taken for each subject including:

Intraocular pressure measurement by applanation tonometry.

Slit lamp examination to determine anterior chamber depth and presence of iris neovascularization . Indirect ophthalmoscopy and biomicroscopy to evaluate the grade of vitreous proliferation and determine the presence and nature of macular oedema.

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

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 control group included 16 patients who were undergoing vitrectomy for idiopathic macular hole, because this disorder is 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 holes is the most similar in constitution to normal eyes.

Sample Collection

The technique used is the standard three port parsplana vitrectomy with one sclerotomy for a 4 mm influsion canula and the other 2 sclerotomies for the exchange of instruments. After the sclerotomy ports were placed , the viteous 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 HbA_1c.

Cytokine assays

Cytokine concentrations were measured by enzyme-linked immuno-sorbent assays (ELISA) using reagents manufactured by (Quantikine) [9].

Principle of the assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-8 or 10 or MCP-1 have been pre-coated onto a micro-plate. Standards and samples are pipetted into the wells and any IL-8 or 10 or MCP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-8 or 10 or MCP-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of IL-8 or 10 or MCP-1 bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

Measuring HbA_1c with a cation exchange chromatography method assessed recent glycaemic control. The procedures is a micro-chromatographic methodology for the quantitation of glycosylated hemoglobin (non diabetic reference 5.5%- 7.7%) Glyco-HbQuick Column procedure (Helana) [10].

STATISTICAL ANALYSIS

Data was expressed as mean $\pm$ SD. The groups were compared using the Anova single factor test .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 IL-8 and MCP-1 were strikingly higher in diabetic patients with PDR in comparison with the control group (Table 2). The vitreous concentrations of IL-8 and MCP-1 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 IL-8 and MCP-1

Table 1 Characteristics of diabetic patients and controls included in this study.

	Controls	PDR	Group 1 inactive PDR	Group 2 active PDR
Number (n)	16	23	13	10
Sex (M/F)	6/10	8/7	7/8	9/6
Mean age ± SD	55.7±7.6	63.6±9.2	63± 8.8	64.1 ± 8.3
Duration of diabetes	-	12.6±8.4	12.6 ±8.4	12.6 ± 8.4
HbA1c%	6.9±0.8	9.4±1.2	9.7±1.1	9.7±1.1

Table 2 Comparison of the different studied parameters among all diabetic groups.

	Controls	PDR	Group 1 inactive PDR	Group 2 active PDR	p value
Number (n)	16	23	13	10	
IL- 8 (pg/ml)	48.9 ± 23 a	173 ± 55 b	183.5 ± 64 b	324.5 ± 8 0 c	p<0.001
MCP-1 (pg/ml)	438 ± 98 a	2170 ±387 b	1143 ±116 c	3596 ±430 d	p<0.001
IL- 10 (pg/ml)	2.46 ± 0.2 a	2.9 ± 0.5 a	1.8 ± 0.58 a	1.82 ± 0.6 a	NS

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

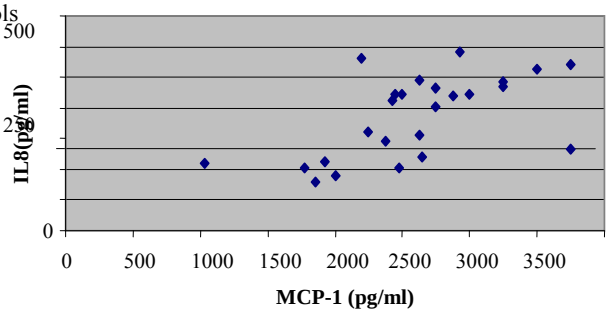

Fig 1 Correlation between vitreal MCP1 and IL8 in PDR patients (r=0.61 p<0.05).

DISCUSSION

The role of cytokines in the pathogenesis of diabetic retinopathy is not completely understood. Diabetic retinopathy is characterized by all microscopic signs of inflammation, i.e., vasodilatation, altered flow, fluid exudation, and leukocyte migration/accumulation [11].

High intra-vitreous levels of IL-8 and MCP-1 have been previously reported in diabetic patients with PDR [12, 13,1 4], these agreed with our results. However, in some reports IL-8 and MCP-1 were undetectable in most patients with PDR [15].

These results mean that inflammation is crucial as a pathogological event that leads to PDR. The source of the high levels of IL-8 and MCP-1 detected within the vitreous fluid of diabetic patients with PDR remains unclear. A more likely possibility is that cells within the vitreous fluid could be the main cause accounting for the high levels of both IL-8 and MCP-1. The vitreous of macular hole patients are largely devoid of of inflammatory cells [16]. Macrophages, monocytes, retinal pigment epithelial (RPE) cells, and glial cells are found in the vitreous of patients with PDR, and the majority of these cells are capable of producing cytokines *in vitro* [17]. Alternatively, cytokines may be expressed by the retina, which is known to be a source of many cytokines and growth factors [18]. So, it is suggested that increased levels of IL8 and MCP-1 are involved in the pathogenesis of inducing neovascularization in PDR.

In the present study, the types of cells expressing IL-8 and MCP-1 were not identified and further studies are required to elucidate the specific source of these chemokines within the vitreous fluid.

In the present study, we provide evidence that the anti-inflammatory cytokine (IL-10) is not increased in the vitreous fluid of diabetic patients with PDR or, in other words, the enhancement of the pro-inflammatory cytokines (IL-8 and MCP-1) is not counterbalanced by an increase of IL- 10.

Although there are no reports on this issue, van Exel *et al* [19], observed a low production capacity of IL-10 in patients with the metabolic syndrome and Type 2 diabetes. Most of the diabetic patients included in our study were Type 2, thus explaining the lower concentrations of IL-10 detected. Our results suggest that low levels of vitreous IL-10 are involved in the pathogenesis of PDR. However, specific and larger studies on this issue are needed to confirm this hypothesis.

CONCLUSION

In summary, the pro-inflammatory cytokines IL-8 and MCP-1 are increased in the vitreous fluid of PDR patients without an increase in the anti-inflammatory cytokine IL-10. In addition, both IL-8 and MCP-1 intra-vitreous levels correlated with PDR activity, thus suggesting that these cytokines may be pathogenically important in PDR. These results might open a new potential therapeutic approach for PDR.

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