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Analysis of Cytokines in Presumed Acute Infectious Endophthalmitis Following Cataract Extraction

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Abstract

Objective: To analyze the involvement of cytokines in acute infectious endophthalmitis following cataract extraction surgery.

Methods: Vitreous humor samples were collected from 18 patients. The multiplex technique was used to measure IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic FGF, eotaxin, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1a, MIP-1b, PDGF-BB, RANTES, TNF-α and VEGF for comparison with levels in 39 patients undergoing vitrectomy for a non-infectious disease (control).

Results: IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-9, IL-10, IL-15, IL-17, eotaxin, bFGF, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1a, MIP-1b, PDGF-BB, RANTES and TNF-α levels were significantly higher ($p < 0.05$) in eyes with endophthalmitis. There were no significant differences between groups for IL-5, IL-12 (p70), IL-13 and VEGF levels. IL-7 levels were higher in the control group ($p=0.0229$). Considering the different diagnosis in the control group, only IL-5 was not significantly different between groups ($p=0.1764$). There was a positive correlation between TNF-α and the initial visual acuity ($r=0.59$, $p=0.0096$). There was no correlation with the final visual acuity for the cytokines measured. The concentration of IL-8 was higher in patients with endophthalmitis and positive cultures ($p=0.0250$).

Conclusions: Infectious endophthalmitis following cataract extraction stimulates the production of cytokines. Levels of TNF-α may be related to the severity of the initial inflammatory processes. IL-8 levels are correlated with positive bacterial cultures, suggesting that IL-8 could be used as a biomarker for bacterial infection.

Keywords: Cytokines; Cataract extraction; Endophthalmitis; Chemokines; TNF-α; Interleukin-8; Eye infection

Introduction

Infectious endophthalmitis is characterized by inflammation of the intraocular tissues as a result of bacterial or fungal proliferation in the vitreous cavity following surgical procedures, trauma or endogenous dissemination [1]. Its incidence after cataract surgery varies between 0.087% and 0.265% [2,3]. The progression of this condition can be serious and includes the risks of vision loss and bulbar atrophy. The treatment consists on the rapid administration of intravitreal antibiotics or posterior vitrectomy procedures [4]. In addition to the tissue injury caused by bacterial growth and the production of toxins, excessive immune responses and inflammation may also be responsible for damage to intraocular tissues, including direct damage to the photoreceptors, injury to the corneal endothelium, pupillary-block glaucoma and retinal detachment [5]. A diagnosis of endophthalmitis is based on clinical criteria, and microbiological studies have variable sensitivities, in addition to requiring extended time periods, between 2 and 12 days, to confirm the results [6-8].

Cytokines are polypeptides that act as inflammatory mediators in the host response and whose release is critical for the elimination of infectious agents and consequent minimization of tissue damage. Cytokines are produced by macrophages, lymphocytes, natural killer cells, retinal pigment epithelial (RPE) cells and other immune cells in response to certain stimuli. They participate in various activities in host defense, including the recognition of infectious agents, leukocyte recruitment, repair of damaged tissue and breakdown of the blood-retinal barrier [9-11].

Cytokine levels can, in certain situations, provide information on the severity of infections. The plasma concentration of TNF-α can be predictive of prognosis in severe septic infections [12]. There is also evidence that the levels of cytokines may serve as biomarkers for infectious processes [13-15].

Experimental studies on infectious endophthalmitis have shown that cytokines, including tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ), are expressed in the vitreous cavity [9,16-18]. The levels of TNF-α and INF-γ can also be correlated with the intensity of the initial inflammatory process [9]. However, little is known about cytokine expression in humans suffering from acute infectious endophthalmitis [1].

The purpose of this study was to analyze the involvement of cytokines in presumed acute post-cataract infectious endophthalmitis, using the multiplex technique. We compared the levels of these molecules in patients with clinical findings consistent with endophthalmitis following cataract extraction with patients undergoing vitrectomy for non-infectious diseases and correlating them with initial and final visual acuity. The cytokine levels in patients with positive and negative cultures were also compared.

Methods

Subjects

Patients were selected with a clinical diagnosis consistent with acute endophthalmitis following cataract surgery based on the EVS criteria [19], from the Emergency Department of the Altino Ventura Foundation [Fundação Altino Ventura] between March 2011 and October 2012 and who were 18 years of age or older.

Patients were excluded if presented with: a history of uveitis, symptoms on the same day of the cataract surgery (clinical presentation consistent with toxic anterior segment syndrome-TASS), a posterior capsule rupture with dislocation of nuclear fragments into the vitreous cavity and a history of ocular surgery or trauma.

For the control group, samples were collected from patients undergoing posterior vitrectomy for non-infectious diseases on the same day that samples were collected from patients with endophthalmitis.

Procedures

The material used to measure cytokine levels was collected from vitreous humor samples during the injection of intravitreal antibiotics or posterior vitrectomy procedures. The procedure was performed under local anesthesia with proximetacaine anesthetic eye drops and lidocaine via peribulbar injection. The sampling technique consisted of peritomy, sclerotomy, aspiration of 0.3 ml of vitreous humor with a 20-gauge vitrector (Accurus®, Alcon, USA) at a low cut rate (600 cuts/min) and only for patients in the case group, intravitreal injection of vancomycin (1.0 mg/0.1 mL), ceftazidime (2.25 mg/0.1 mL) and dexamethasone (0.4 mg/0.1 mL). The initial treatment choice between injection of intravitreal antibiotics or posterior vitrectomy was based on the EVS criteria [19].

The ophthalmological examination consisted of measurements of the best corrected visual acuity (VA) using a Snellen chart with logMAR conversion [20], slit-lamp biomicroscopy of the anterior and posterior segments, measurements of intraocular pressure using Goldmann applanation tonometry, binocular indirect ophthalmoscopy and ocular ultrasonography. The initial VA was considered on the same day of the endophthalmitis diagnosis and the final VA, after six months of follow up.

Multiplex

The samples were centrifuged at 5,000 rpm for 10 minutes and then stored at -80°C until the analysis. The maximum time that the sample was stored was 594 days (371.5 ± 49.5). The Bio-Plex Pro™ Human Cytokine Assaykit (BIO-RAD®, Hercules, VA) was able to identify the following cytokines: interleukin (IL)-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic fibroblast growth factor (bFGF), eotaxin, granulocyte colony-stimulating growth factor (G-CSF), granulocyte-macrophage colony-stimulating growth factor (GM-CSF), interferon-γ (IFN-γ), monocyte chemotactic protein (MCP-1), macrophage inflammatory protein-1α (MIP-1a), macrophage inflammatory protein-1β (MIP-1b), platelet-derived growth factor-BB (PDGF-BB), regulated on activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF).

Statistical analysis

The quantitative variables were expressed by mean and standard error of the mean (SEM). The nonparametric Mann-Whitney U test was used to compare the cytokines data between the study and the control groups. To analyze the correlation between variables, the Spearman test was used. To compare cytokines concentrations in the study group with each control subgroup, the Kruskal-Wallis test and the Duncan *posthoc* test were used. The Shapiro-Wilk test was performed to verify the assumption of normal distribution. A p-value of <0.05 was considered significant to reject the null hypothesis. Statistical calculations were performed with SPSS (Statistical Package for Social Science) version 21.0 and STATA version 11.0.

The study was approved by the Ethics Committee of the Federal University of Pernambuco [Universidade Federal de Pernambuco] and adhered to the tenets of the Declaration of Helsinki. All patients provided written informed consent.

Results

Demographic data

Eighteen patients diagnosed with endophthalmitis were included. Among these patients, nine (50.0%) were male. The age ranged between 48 and 86 years (66.4 ± 2.51). Ten (55.6%) patients presented with involvement of the right eye. Six (33.3%) patients suffered from diabetes mellitus.

The time between surgery for cataract extraction and the first patient's visit to the clinic with symptoms of endophthalmitis ranged from 3 to 40 days (13.6 ± 2.61). The initial VA ranged between 20/150 and light perception (2.3 ± 0.087 logMAR). Eight (44.4%) patients had a VA of light perception.

Eight (44.4%) patients were treated with posterior vitrectomy and injection of intravitreal antibiotics. Ten (55.6%) patients were treated with injection of intravitreal antibiotics without performing posterior vitrectomy.

The culture results were positive for 11 (64.7%) patients, in which *Staphylococcus epidermidis* was the agent identified in nine (81.8% of those with positive culture) cases. One culture was positive for *Staphylococcus aureus* and another for *Streptococcus viridans*.

The final VA ranged from no light perception (NLP) to 20/25 (0.92 ± 0.217 logMAR). Sixteen (88.9%) patients presented with improved vision following treatment. Thirteen (72.2%) patients presented with 20/100 or better. One patient progressed to bulbar atrophy and one patient maintained a VA of light perception (Table 1).

For the control group, samples were collected from 39 patients (1:2.17 ratio of endophthalmitis cases to controls). The patients' ages ranged between 23 and 83 years (59.3 ± 2.2). Seventeen (43.6%) patients were male and 20 (51.3%) had involvement of the left eye. Eighteen (46.1%) patients suffered from diabetes mellitus.

The disease that required posterior vitrectomy among patients in the control group included retinal detachment (RD) in 17 (43.6%) patients, maculopathy associated with epiretinal membrane (ERM) or macular holes (BM) in seven (17.9%) patients and proliferative diabetic retinopathy (PDR) in 15 (38.5%) patients.

There were no statistically significant differences between patients with endophthalmitis and the control group regarding to age (p=0.120), gender (p=0.787) or diagnosis of diabetes (p=0.277).

Levels of cytokines

The levels of IL-2, IL-1ra, IFN-γ, IL-4, IL-10, IL-15, IL-17 and TNF-α in the vitreous humor from patients with endophthalmitis were increased compared to those in the control group. In contrast, the levels of IL-7 were significantly higher in the control group. The levels of growth factors G-CSF, GM-CSF and PDGF-BB were found to be significantly higher in patients with endophthalmitis compared to the control group. Similarly, the levels of the chemokines eotaxin, IL-8, MCP-1, MIP-1a and MIP-1b were also significantly higher in patients with endophthalmitis compared to the control group (Table 2).

The Kruskal-Wallis test was used, followed by Duncan's post-hoc test, to evaluate potential statistically significant differences between patients with endophthalmitis and patients in each control group, considering the different diagnoses in this group. Only IL-5 showed no statistically significant difference between groups (Table 3).

Visual acuity

There was a positive correlation between the levels of TNF-α and the initial visual acuity (r=0.59, p=0.0096). For the other cytokines, there was no correlation between their levels and the initial visual acuity. There was also no correlation between the cytokine levels and the final visual acuity (Table 4).

Culture

The concentrations of IL-8 were significantly higher (p=0.0250) in patients with positive cultures (7729.0 ± 1901.0 pg/ml) compared to patients with negative cultures (1869.0 ± 823.5 pg/ml). There were no significant differences in the levels of the other cytokines measured (Table 5). Higher concentration of IL-17(1043.9 pg/ml) was seen in the patient with endophthalmitis who had a positive culture for *Staphylococcus aureus* compared to the other patients (33.52 ± 6.48 pg/ml).

Patient #	Sex	Age	Initial VA	Treatment	Final VA	Time–surgery/exam (days)
1	M	82	LP	PPV + IV ATB (02)	20/60	30
2	F	70	LP	PPV + IV ATB (01)	HM	6
3	F	62	HM	IV ATB (01)	20/40	14
4	F	60	LP	PPV + IV ATB (01)	20/40	8
5	F	72	LP	PPV + IV ATB (01)	20/40	6
6	F	62	LP	PPV + IV ATB (02)	20/25	3
7	M	75	LP	PPV	LP	40
8	F	72	HM	IV ATB (01)	20/160	30
9	F	51	20/150	IV ATB (02)	20/100	14
10	M	48	HM	IV ATB (02)	20/100	10
11	M	62	HM	IV ATB (01)	20/50	7
12	M	56	HM	IV ATB (03)	20/100	5
13	M	86	LP	PPV + IV ATB (01)	HM	5
14	M	72	HM	IV ATB (03)	20/25	6
15	F	75	CF	IV ATB (02)	20/100	16
16	M	54	HM	IV ATB (02)	20/32	12
17	F	76	LP	PPV	NLP	29
18	M	61	HM	IV ATB (03)	20/63	4

VA: Visual Acuity; LP: Light Perception; HM: Hand Movement; CF: Count Fingers; PPV: Posterior *Pars plana* Vitrectomy; IV: Intravitreous; ATB: Antibiotics

Table 1: Clinical parameters of the patients.

Cytokines	Groups (mean + SEM)		p Value
	Endophthalmitis (N=18)	Control (N=39)	
IL-1ra	774.0 ± 245.6	9.3 ± 1.4	<0.0001
IL-2	9.7 ± 1.2	1.4 ± 0.2	<0.0001
IL-4	1.3 ± 0.2	0.2 ± 0.1	<0.0001
IL-5	1.0 ± 0.4	0.5 ± 0.1	0.3170
IL-6	3158.3 ± 28.9	95.3 ± 31.8	<0.0001
IL-7	5.1 ± 1.0	9.2 ± 1.2	0.0229
IL-8	5022.5 ± 1305.5	31.5 ± 5.4	<0.0001
IL-9	13.7 ± 1.9	5.4 ± 0.8	<0.0001
IL-10	66.0 ± 17.8	7.0 ± 1.9	<0.0001
IL-12p70	31.9 ± 12.0	49.6 ± 14.9	0.452
Il-13	6.3 ± 1.5	6.0 ± 1.4	0.3283
Il-15	9.9 ± 1.3	5.3 ± 0.6	<0.0001
IL-17	89.7 ± 56.5	3.1 ± 0.2	<0.0001
Eotaxin	34.3 ± 3.9	8.5 ± 1.0	<0.0001
bFGF	26.6 ± 13.8	18.8 ± 6.4	0.005
G-CSF	6906.8 ± 847.6	11.7 ± 4.0	<0.0001
GM-CSF	66.9 ± 9.6	34.7 ± 2.3	<0.0001
IFN-γ	98.3 ± 12.1	25.3 ± 5.1	<0.0001
MCP-1	4498.5 ± 1428.4	850.6 ± 143.2	0.0036
MIP-1a	74.1 ± 27.8	0.9 ± 0.2	<0.0001
MIP-1b	231.9 ± 64.2	11.9 ± 1.4	<0.0001
PDGF	30.8 ± 4.8	8.8 ± 1.8	<0.0001
RANTES	18.8 ± 5.1	14.5 ± 6.0	0.0004
TNF-α	49.1 ± 5.9	3.7 ± 0.4	<0.0001
VEGF	491.7 ± 251.6	523.1 ± 178.5	0.6930

Table 2: Cytokine concentrations (pg/ml) in the endophthalmitis and control groups.

Cytokines	Endophthalmitis (mean + SEM)	Controls (mean + SEM)			p Value
		RD (N=17)	MH/ERM (N=7)	DR (N=15)	
IL-1ra	774.0 ± 245.6	12.2 ± 2.9 *	3.8 ± 0.8 *	8.6 ± 1.3 *	<0.0001
IL-2	9.7 ± 1.2	1.3 ± 0.1 *	0.6 ± 0.2 *	1.9 ± 0.3 *	<0.0001
IL-4	1.3 ± 0.2	0.3 ± 0.2 *	0.1 ± 0.0 *	0.2 ± 0.0 *	<0.0001
IL-5	1.0 ± 0.4	0.4 ± 0.1	0.3 ± 0.0	0.8 ± 0.2	0.1764
IL-6	3158.3 ± 28.9	151.1 ± 69.1 *	8.0 ± 3.8 *	72.8 ± 20.4 *	<0.0001

IL-7	5.1 ± 1.0	8.2 ± 1.6	3.8 ± 1.0	13.0 ± 2.2 *	0.0013
IL-8	5022.5 ± 1305.5	24.9 ± 5.0 *	8.5 ± 2.1 *	49.8 ± 11.4 *	<0.0001
IL-9	13.7 ± 1.9	4.0 ± 0.5 *	2.5 ± 0.6 *	8.5 ± 1.6	<0.0001
IL-10	66.0 ± 17.8	2.2 ± 0.5 *	0.7 ± 0.1 *	15.7 ± 4.1	<0.0001
IL-12p70	31.9 ± 12.0	12.4 ± 4.8	0.5 ± 0.1 *	112.1 ± 31.7	<0.0001
Il-13	6.3 ± 1.5	3.3 ± 0.9	0.9 ± 0.1 *	11.6 ± 2.9	0.0002
Il-15	9.9 ± 1.3	5.8 ± 1.2	3.0 ± 0.5 *	5.9 ± 0.7	0.0018
IL-17	89.7 ± 56.5	3.4 ± 0.4 *	2.8 ± 0.8 *	2.8 ± 0.3 *	<0.0001
Eotaxin	34.3 ± 3.9	6.5 ± 1.0 *	6.0 ± 0.6 *	11.8 ± 2.2 *	<0.0001
β-FGF	26.6 ± 13.8	39.3 ± 13.2	1.5 ± 0.4 *	3.6 ± 0.6 *	<0.0001
G-CSF	6906.8 ± 847.6	20.5 ± 9.2 *	5.9 ± 1.0 *	11.5 ± 6.3 *	<0.0001
GM-CSF	66.9 ± 9.6	33.5 ± 3.0 *	33.1 ± 6.1 *	36.9 ± 4.0 *	0.0002
IFN-γ	98.3 ± 12.1	16.3 ± 3.5 *	2.7 ± 0.7 *	46.1 ± 10.7 *	<0.0001
MCP-1	4498.5 ± 1428.4	1035.4 ± 291.3	256.5 ± 66.0 *	918.4 ± 138.2	0.0006
MIP-1α	74.1 ± 27.8	1.2 ± 0.3 *	0.3 ± 0.0 *	1.0 ± 0.1 *	<0.0001
MIP-1β	231.9 ± 64.2	15.2 ± 2.4 *	4.0 ± 0.9 *	12.1 ± 1.7 *	<0.0001
PDGF	30.8 ± 4.8	10.1 ± 3.7 *	2.7 ± 0.5 *	10.4 ± 2.1 *	<0.0001
RANTES	18.8 ± 5.1	20.7 ± 12.3	1.0 ± 0.2 *	13.6 ± 7.0	<0.0001
TNF-α	49.1 ± 5.9	3.4 ± 0.6 *	3.3 ± 0.6 *	4.2 ± 0.8 *	<0.0001
VEGF	491.7 ± 251.6	116.5 ± 49.0	3.7 ± 0.9 *	1199.1 ± 395.3 *	<0.0001
* Significantly different compared to the group with endophthalmitis ($p < 0.05$)					

Table 3: Cytokine concentrations (pg/ml) in the endophthalmitis and each control subgroup according to diagnosis.

Cytokines	Initial VA	Final VA
	R value (p value)	R value (p value)
IL-1ra	0.24 (0.3268)	-0.10 (0.6811)
IL-2	0.38 (0.1162)	-0.11 (0.6403)
IL-4	0.26 (0.2927)	0.11 (0.6702)
IL-5	0.22 (0.3772)	-0.11 (0.6375)
IL-6	0.10 (0.6801)	-0.09 (0.7178)
IL-7	-0.06 (0.7883)	-0.24 (0.3323)
IL-8	0.22 (0.3650)	-0.18 (0.4516)
IL-9	0.29 (0.2438)	0.21 (0.3881)
IL-10	0.19 (0.4321)	0.09 (0.6963)
IL-12 (p70)	0.13 (0.6023)	0.10 (0.6855)
IL-13	0.11 (0.6636)	0.23 (0.3475)

IL-15	0.16 (0.5081)	-0.24 (0.3372)
IL-17	0.14 (0.5775)	-0.26 (0.2833)
Eotaxin	0.40 (0.0930)	-0.11 (0.6648)
Basic FGF	-0.23 (0.3429)	-0.08 (0.7394)
G-CSF	0.36 (0.1319)	-0.25 (0.3155)
GM-CSF	0.10 (0.6959)	-0.24 (0.3456)
IFN-γ	0.41 (0.0852)	-0.06 (0.8110)
MCP-1	0.26 (0.2825)	-0.05 (0.8311)
MIP-1a	0.17 (0.4907)	0.02 (0.9347)
MIP-1b	0.20 (0.4056)	-0.20 (0.4145)
PDGF-bb	0.29 (0.2364)	0.21 (0.3930)
RANTES	-0.08 (0.7370)	-0.03 (0.8956)
TNF-α	0.59 (0.0096)*	-0.07 (0.7762)
VEGF	0.19 (0.4375)	0.19 (0.4416)
*Significantly different; §Spearman correlation test		

Table 4: Correlation test between cytokines in relation to the final and initial visual acuity.

Cytokines	Culture results (Mean ± SEM)		pValue
	Positive	Negative	
IL-1ra	823.6 ± 324.7	696.1 ± 402.3	0.8563
IL-2	11.13 ± 1.8	7.3 ± 0.6	0.0700
IL-4	1.2 ± 0.1	1.3 ± 0.4	0.5559
IL-6	3167 ± 35.7	3144 ± 51.6	0.5869
IL-8	7729.0 ± 1901.0	1869.0 ± 823.5	0.0250*
IL-9	15.2 ± 2.4	11.4 ± 2.8	0.1889
IL-10	75.9 ± 25.5	50.4 ± 23.4	0.5024
IL-12 (p70)	36.0 ± 18.4	25.4 ± 12.1	0.7511
IL-13	6.0 ± 2.0	6.6 ± 2.1	0.6507
IL-15	11.2 ± 1.8	7.7 ± 1.3	0.1942
IL-17	127.2 ± 92.04	30.66 ± 9.4	0.5261
Eotaxin	39.7 ± 5.4	25.8 ± 3.8	0.0840
Basic FGF	13.2 ± 2.8	47.4 ± 35.2	0.9639
G-CSF	7680.0 ± 1022.0	5692.0 ± 1447.0	0.1743
GM-CSF	73.65 ± 14.4	54.43 ± 3.8	0.3540
IFN-γ	109.8 ± 17.7	80.1 ± 12.5	0.2455
MCP-1	3045.0 ± 974.9	6782.0 ± 3310.0	0.7172

MIP-1a	90.5 ± 41.9	48.3 ± 29.0	0.4687
MIP-1b	279.1 ± 98.6	157.6 ± 55.0	0.7172
PDGF-bb	27.6 ± 5.8	35.7 ± 8.5	0.3651
RANTES	21.3 ± 7.5	14.6 ± 6.3	0.5869
TNF-α	54.7 ± 8.6	40.2 ± 6.0	0.2400
VEGF	609.8 ± 398.4	306.1 ± 190.6	0.5869
*Significantly different			

Table 5: Cytokine concentrations (pg/ml) in the endophthalmitis group according to the culture result.

Discussion

The inflammatory process generated during endophthalmitis is critical for elimination of the infectious agent. Recognition of the microorganism and the subsequent activation of immune cells lead to the secretion of inflammatory factors that are involved in the clearance of the pathogen as well as the modulation of the immune system [3]. This is the first in vivo study to evaluate the inflammatory response through the measurement of cytokine levels in patients with acute endophthalmitis following cataract extraction. The storage of samples at -80°C for up to two years enables cytokines stability for subsequent dosing. IL-2, IL-4 and IL-12 may remain stable for up to three years [19]. As endophthalmitis after cataract surgery is a rare event, storage is important to enable sample size.

Experimental studies have already shown that infectious endophthalmitis can induce the expression of cytokines [9,16-18]. Petropoulos et al. [9] inoculated *Staphylococcus epidermidis* strains into the eyes of lewis rats. They observed that high levels of TNF-α, IL-1βand IFN-γ were associated with intensity of the inflammatory process. The association between the inflammatory process and cytokine levels was also observed in the present study. These cytokines could not be detected in the blood, suggesting that their production is local. Giese et al. [16] used viable colonies of *Staphylococcus aureus* to induce endophthalmitis in the eyes of rats and studied the production of TNF-α, IL-1β, cytokine-induced neutrophil chemoattractant (CINC) and IFN-γ. CINC is the equivalent of IL-8 in humans. The study showed an increase in the four cytokines studied, showing a relationship with the inflammatory process.

We observed high production of cytokines that are involved in several phases of the inflammatory process: recognition of the microorganism, leukocyte recruitment, pathogen clearance and tissue repair. TNF-α is one of the cytokines that emerge early during the inflammatory process, and it has the ability to stimulate other cytokines [1,2]. Fourteen of the 25 measured cytokines (IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-17, eotaxin, G-CSF, GM-CSF, IFN-γ, MIP-1a, MIP-1b, PDGF-BB and TNF-α) had higher levels compared to the control group. With the exceptions of IL-7 (higher in patients with diabetic retinopathy) and IL-5, the cytokines were more abundant in patients with endophthalmitis compared to control patients suffering from maculopathy. Patients with idiopathic macular holes or epiretinal membranes associated with changes in the vitreoretinal interface did not present with significant intraocular inflammatory changes or retinal ischemia [20].

No statistically significant differences in the IL-5 levels were observed between groups, suggesting that this cytokine does not participate in acute infectious endophthalmitis. IL-5 is produced byTH2 lymphocytes in response to stimulation by allergens, *Mycobacterium tuberculosis* and *Toxocara canis*, and it stimulates the differentiation and proliferation of eosinophils, which are characterized as a mediator of allergic processes such as asthma [21]. Because the primary causative agent of acute endophthalmitis is *Staphylococcus epidermidis*, it is not likely that IL-5 has any significant involvement in ocular immune responses.

IL-7 is a hematopoietic growth factor that is produced by stromal cells in lymphoid tissue in the thymus and bone marrow and that participates in the expansion of the immature precursors of T and B lymphocytes [22]. IL-7 participates in the immune response to HIV and neoplastic processes such as lymphoma and acute lymphoblastic leukemia. There was no evidence in this study that IL-7 plays a role in acute infectious endophthalmitis. There was, however, an increase in the vitreous levels of IL-7 in patients with diabetic retinopathy.

G-CSF and GM-CSF are also considered hematopoietic cytokines and act on bone marrow progenitors to stimulate leukocyte production. G-CSF can also be produced at the infection site and acts as an endocrine hormone in the bone marrow [23]. Activated T-cells, macrophages and endothelial cells can express both these cytokines. There were increases in the G-CSF and GM-CSF levels in patients with acute infectious endophthalmitis, suggesting their involvement in the inflammatory process.

There was a positive correlation between the initial visual acuity and the vitreous levels of TNF-α. In one patient with infectious endophthalmitis, the visual acuity may reflect the intensity of the inflammatory process. As the inflammatory reaction in the vitreous cavity and the anterior chamber increases, the media opacity also increases, hindering the passage of light to the retina and decreasing visual acuity. In one experimental study, a relationship between the levels of TNF-α in the vitreous humor and the intensity of the inflammatory process was observed [9].

The initial visual acuity is a determining factor in the selection of treatment for these patients. The Endophthalmitis Vitrectomy Study (EVS), developed in the 1990s and still used today, concluded that patients whose visual acuity was hand motions or better should be treated with the injection of intravitreal antibiotics. Patients with light perception only should be treated with vitrectomy combined with the injection of intravitreal antibiotics [24]. If the TNF-α levels are related to the initial visual acuity and the intensity of the inflammatory

process, it may eventually be used as a marker to help to decide between treatment with posterior vitrectomy or intravitreal injection.

The inflammatory process can damage the corneal endothelium through the formation of a severe opacity, which may require corneal transplantation, a fibrin pupillary block and posterior synechiae, including the development of glaucoma, and severe vitreoretinal proliferation in patients with retinal detachment. It is possible to pharmacologically control the inflammatory process. Intravitreal corticosteroids are frequently used in cases of bacterial acute infectious endophthalmitis because the immune reaction itself may damage the ocular structures [5].

Medications that block TNF-α, which consist of monoclonal antibodies including infliximab, adalimumab and certolizumab, are also available. These medications are used in the treatment of autoimmune diseases such as rheumatoid arthritis, and their benefits have already been demonstrated in several studies [25]. Although these medications can reduce the inflammation associated with certain pathologies, their benefits in acute infectious endophthalmitis are not well defined.

In an experimental study, Ramadan et al. [17] evaluated the role of TNF-α in the pathogenesis of *Bacillus cereus*-induced endophthalmitis. In that study, the authors used a TNF-α non-producing mouse strain and compared it to control mice. The authors concluded that the TNF-α non-producing mice had decreased migration of polymorphonuclear leukocytes, despite the increased synthesis of IL-6, keratinocyte chemokine (KC), MIP-1a and IL-1β, resulting in reduced ocular inflammation. However, a histological evaluation showed a significant loss and atrophy of the retinal architecture compared to TNF-α-producing mice. These data suggest that TNF-α-non-producing mice may have a more severe disease course, including greater vision loss due to the increased retinal damage, despite the reduced inflammation. However, modulation of the inflammatory response through the use of TNF-α blockers or blockers for other cytokines, combined with the use of antibiotics, has not yet been evaluated.

In an experimental model, Geiger et al. [18] reported the destruction of photoreceptors and retinal degeneration in mice that received IFN-γ by an intravitreal route. IFN-γ induced glial cells to express class I and II major histocompatibility complex (MHC) antigens and induced retinal pigment epithelial cells to express class II MHC antigens. The activation of retinal pigment epithelial cells could increase the phagocytosis and subsequent loss of photoreceptors.

There are a number of known prognostic factors for endophthalmitis, such as patient's age, the virulence of the microorganism, diabetes, posterior capsule rupture, changes in intraocular pressure, corneal infiltrate and especially the initial VA of light perception [24]. However, there was no correlation between TNF-α, IFN-γ or any other cytokine and final visual acuity. In this study, the cytokine levels could not be used as prognostic markers for infectious endophthalmitis.

There was an increase in the concentration of IL-8 in patients with positive cultures. IL-8 has been identified as a marker for the early detection of bacterial infections [13]. A diagnosis of infectious endophthalmitis following cataract extraction is based on clinical criteria; thus, there's a possibility that some patients who are culture negative, have a sterile endophthalmitis. There was also an increase in the concentrations of IL-17 in the patient with *Staphylococcus aureus* infection. An alpha-toxin produced by strains of *S. aureus* has already been shown to induce IL-17 production [26]. Additional studies with a larger sample size are needed to assess IL-8 as a marker for bacterial infections and IL-17 as a marker for *S. aureus* infections.

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