

CLINICAL RESEARCH

Local Immunity Status and Inflammation Markers in Patients with Endogenous Uveitis

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Abstract

In patients with uveitis of infectious etiology, a significant increase in the index of intoxication and acute inflammation (the Leukocyte Intoxication Index -LII), by 3.4 times on average, was observed. At the same time, this increase in the LII index, in our opinion, was due to the decrease in the percentage of eosinophils in the blood, which in turn increased the number of segmented neutrophils and a decrease in the number of lymphocytes. Rheumatic uveitis and systemic disease were accompanied by a significant increase in the IgE levels, not only in the serum, by 2.9 times, on average, but in the LF as well, by 3.5 times, on average. This study revealed that endogenous uveitis of infectious etiology is characterized by high levels of the Leukocyte Intoxication Index and Sensitization Index in the lacrimal fluid.

Keywords: *endogenous uveitis, inflammation markers.*

Introduction

The problem of endogenous uveitis is one of the most real difficulties encountered in ophthalmology, due to the prevalence and severity of the disease, the complexity of the pathogenesis and the variety of etiological forms, including a high frequency of complications leading to disability in 8-50% of recovered patients [1,2].

The socio-economic significance of endogenous uveitis is particularly important because it affects mainly children, young and middle-aged persons, limiting their career choices and the possibility of individual self-fulfillment [3].

The urgency and complexity of the problem is the difficulty with etiologic diagnosis as well as the multiplicity of etiological and pathogenetic factors that induce uveitis, causing a chronic, relapsing course of the disease coupled with the lack of efficacy of treatment.

Recent studies have shown that this pathology is based on the immunopathological changes in the tear fluid as well as a variety of biochemical and hormonal disorders that affect the patient [4-6]. Analysis of the literature suggests the insufficiency

of data on the explored features of the immunopathogenesis, the nature and type of the various immune disorders in the different clinical forms of endogenous uveitis, the clinical significance and the immunological criteria for the diagnosis of endogenous uveitis, in order to elucidate the role of the infectious agents in the development of the disease.

The aim of this study was to improve the diagnosis of the inflammatory diseases of the uveal tract by comprehensive research on the state of the local immunity and inflammatory markers.

Material and Methods

A comprehensive clinical and immunological study was performed on 78 (156 eyes) patients with different clinical forms of endogenous uveitis, between the ages of 17 and 56 years, with disease duration ranging from 2 months to 28 years and who were treated in the Republican Clinical Ophthalmology Hospital from 2011 to 2012. At the time of the first survey, in group 1 uveitis of infectious etiology was diagnosed in 41(52.4%) patients while in group 2 uveitis associated with rheumatic diseases (RD) was diagnosed in 37(47.6%) patients.

The control group consisted of 26 apparently healthy individuals without any physical illness. The research methods included visometry, perimetry, tonometry, tonography, biomicroscopy, ophthalmoscopy, gonioscopy and electrophysiological study.

We used general clinical examination methods to test patients employing specialists in different fields, as well as a thorough elucidation of the epidemiological history and pregnancy history for women.

In all the patients the hematological parameters were analyzed, besides calculating the Leukocyte Intoxication Index (LII) and Sensitization Index (SI), which are based on the ratio of the sum of the lymphocytes and eosinophils to the rest of the white blood cells.

At first, 0.5 ml of Lacrimal Fluid (LF) was collected for research using a micropipette from the lower conjunctival arch of the eye in a dry airtight tube. Investigation of the concentration of the Circulating Immune Complexes (CIC) in the tear fluid was performed using 7.5% polyethylene glycol precipitation in a sodium borate buffer. The analysis data was examined using spectrophotometer SF-46 at a wavelength of 450 nm and expressed in the international units (IU). The concentrations of IL-1 β and IL-4 were performed on the commercial test systems «BioKhimMak» (Russia) using ELISA, at a wavelength of 450 nm. The ELISA results were expressed in ng/ml whereas the concentration of C-reactive protein was determined by the ELISA test system «IBL» («BioKhimMak», Russia) and expressed as IU/ml.

Statistical data processing was performed using the software package Statistica 6.1 for Windows.

Results and Discussion

Uveitis of any etiology is well recognized to be associated with infiltration of the eye tissues by inflammatory and immune cells, the development of a local immune response provoked by a trigger factor (infection, autoantigens, etc.). Violation of the hemato-ophthalmic barrier of the sore eye was followed by the release of tissue-specific antigens into the circulation and the appearance of sensitized lymphocytes, eosinophils, and autoantibodies in the blood.

Endogenous uveitis in its clinical form is known to be either infectious or non-infectious. Considering that RD plays a leading role in the clinical picture of the infectious nature of the eye diseases, we decided, using the integral hematological indicators, to create a differentiated approach to understand certain aspects of the pathogenesis of endogenous uveitis.

As evident from the results of our research (Table 1) in patients with uveitis of infectious etiology, a significant increase in the indicator of intoxication and acute inflammation (LII) was recorded, up to 3.4 times, on average. At the same time, an increase in the index LII, in our opinion, occurred due to the decrease in the eosinophils percentage in the blood, thus increasing the number of segmented neutrophils and decreasing the number of lymphocytes. The SI was reduced by an average of two times.

Activation of the inflammatory process also indicates a more than 30-fold increase in the C-reactive protein (CRP), not only in the blood, but also in the tear fluid ($p < 0.05$).

It is well known that in recent years, in the assessment of the functional status of the various organs and systems under normal conditions and during the development of the pathological process, great importance is given to the study of the various biological fluids, which reveal more than mere testing performed with serum, as the latter reflects the development of the pathological process only at a local level. With respect to the

body, the most accessible biological target for research is the lacrimal fluid (LF).

In light of the information cited above, the LF of patients with endogenous uveitis was selected as a biological substrate.

The concentration levels of the primary proinflammatory cytokine IL-1 β in the LF of patients with endogenous uveitis of inflammatory etiology was found to be 108.4 \pm 8.91 pg/mL, which is six times higher than the initial value in healthy persons. The increasing concentrations of IL-1 β in the LF of the patients under study was apparently associated with the activation of the immune response by the T-helper pathway of the 1st type and was interconnected with the development of the inflammatory processes. In contrast, the concentrations of IL-4 in the LF of the patients examined were lower than the standard indicators of IL-4 in the LF in 43% ($p < 0.05$). This cytokine has anti-inflammatory properties, is an activator of humoral immunity (T-helper cell response of 2nd type) and is involved in the development of an autoimmune response. Low IL-4 levels, i.e. the humoral immunity factor, combined with an increase in the CIK concentration, indicate cell destruction.

Table 1.

Indicators of blood and lacrimal fluid of patients with endogenous uveitis

Parameters	Healthy individuals (control)	Patients with endogenous uveitis	
		Infectious uveitis	Rheumatic uveitis
LII	0.37 \pm 0.04	1.24 \pm 0.12*	0.08 \pm 0.01*
SI	0.92 \pm 0.04	0.44 \pm 0.03*	1.92 \pm 0.21*
CRP in LF, mcg/mL	5.61 \pm 0.33	182.0 \pm 7.81*	58.6 \pm 4.31*
Secretory IgA, IU/mL	0.31 \pm 0.03	0.18 \pm 0.01*	0.27 \pm 0.01
IgE in serum, IU/mL	124.2 \pm 2.16	113.6 \pm 8.71	356.4 \pm 11.4*
IgE in LF, IU/mL	12.9 \pm 2.01	18.6 \pm 2.54	44.9 \pm 3.51*
IL-1 β in LF, pg/mL	17.6 \pm 1.44	108.4 \pm 8.91*	14.3 \pm 0.81*
IL-4 in LF, pg/mL	13.8 \pm 1.33	7.83 \pm 0.9*	18.6 \pm 0.96*
CIC (small), %	13.9 \pm 1.14	28.1 \pm 1.52*	77.8 \pm 5.44*
Lactoferrin in LF, mg/mL	1.34 \pm 0.12	2.11 \pm 0.13*	0.84 \pm 0.07*

Note: * - Significant difference when compared with the control group ($p < 0.05$).

Given the test results in this study, particularly the significantly higher IL-1 β concentrations, it is of great interest to study lactoferrin in the LF of the patients. This multifunctional protein is involved in the processes of inflammation, thus revealing both anti- and pro-inflammatory properties. It is also capable of regulating the cytokine production. This study showed a significant increase in the lactoferrin levels in the LF, 1.6 times

greater ($p < 0.05$), on average, relative to the level found in healthy individuals.

On analyzing the data obtained from the study of severe inflammatory activity in the LF of patients with endogenous uveitis of infectious etiology, it would likely lead to an increase in the destructive processes and the development of an immune response, when CIC levels were tested. On evaluating the integral index of the activation of the humoral immune system - the CIC level in the LF of patients in the study group showed a two-fold improvement on average of the CIC, relative to the control group ($p < 0.05$).

Thus, endogenous uveitis of infectious etiology is characterized by the activity of local infection, as evidenced by the high relative LII values, C-reactive protein, IL-1 β , acute phase protein lactoferrin and CIC in LF.

The analysis of the results from patients with rheumatic endogenous uveitis (Table 1) revealed a two-fold increase, on average, in the SI ($p < 0.05$). Against this background, the C-reactive protein baseline level exceeded by 10 times, and was 58.6 ± 4.31 mcg/mL in the LF.

Rheumatic uveitis and systemic disease were accompanied by a significant increase in the IgE levels, around 2.9 times, on average, not only in the serum, but also in the LF by 3.5 times, and was 44.9 ± 3.51 IU/mL vs. 12.9 ± 2.01 IU/mL in the control group.

Immediate type of allergic reactions in this group of patients were found associated with the formation of antibodies of the IgE, which are fixed on the mast cells and facilitate the excretion of the biogenic amines. In such reactions, in the patients with rheumatic uveitis examined, antibodies were formed to tissue cells, accompanied by a 5.6-times increase in the CIC level. It enhances proteolysis in the immune complex deposition field resulting in increased tissue damage and an activation of the inflammation reaction, as indicated by the elevated C-reactive protein levels in the LF. This is accompanied by an increased vascular permeability, with the development of edema and a chemotactic effect on the eosinophils, which increases the sensitization index.

It is interesting to note that in patients with uveitis associated with rheumatic diseases, a significant increase in the IL-4 was revealed. The IL-4 has anti-inflammatory properties, which in turn activates humoral immunity.

As evident from the results presented, the IL-4 level in the LF of patients in this group increased by 1.3 times, on average, while the concentration of the CIC went up 5.6-fold. In contrast, this group of patients revealed a significant decrease in the lactoferrin level in the LF, which has both anti- and pro-inflammatory properties.

Thus, in patients with uveitis associated with RD significant increases in the IS and IgE levels in the blood and in the LF were noted, as well as an increase in the IL-4 and CIC levels in the LF against the low indicators of LII, IL-1 β , and lactoferrin.

The results of this study significantly complement the possibilities of the differential diagnosis of endogenous uveitis associated with communicable and allergic changes. At the same time, the parameters of immune reactivity in the LF and the integral indices of blood were defined.

The urgency and complexity of the problem of uveitis is closely associated with the insufficient knowledge regarding the etiology and pathogenesis, the difficulty of etiological diagnosis,

the multiplicity of etiological and pathogenetic factors inducing uveitis and causing a chronic and relapsing course of the disease and the continuing lack of efficacy of treatment.

Based on a comprehensive study of the state of the local immune and inflammatory markers the methods used in the diagnosis of inflammatory diseases of the uveal tract have been improved.

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