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Quantification of Intraocular IFNγ and IgG in Cataract and Diabetes

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A B S T R A C T

The anterior eye chamber is called the immunologically privileged place. The inflammatory disturbances in the interior of the eye chamber are characterised primarly by the changes of the IFN-gamma, IgG and IL-4 concentration and appearance of the oligoclonal IgG. In type I diabetes, there are antibodies to the beta cells of the pancreas, IgG class (among others such as antiretinal antibodies) and they are also found in preretinal membranes of diabetics. In type II there are IgG antibodies to insulin receptors.

***Key words**: diabetes, retinopathy, cataract, machine learning, C5.0, aqueous humor, serum, IgG, prognosis, barrier*

Introduction

The anterior eye chamber, as also some other body sections, is called the immunologicaly priviledged place. This immunity, i.e. the deviant immunological response--also called ACAID, has its basis in the fact that the APC cells of the anterior eye chamber have a specific order and function, that the chamber is separated from its surrounding by a hemato-ocular barrier, which allows through substances depending on their specific characteristics and that in the ocular water there is a specific cytokinetic micro-enviroment determenated by the cytokines, i.g. TGF-beta, IL-4, IFN-gamma and IL-2. For the mentioned reasons the afferent and the efferent fork of the immunological response hat also been modified. The inflammatory disturbances in the interior of the eye chamber are characterised primarily by the changes of the IFN-gamma, IgG and IL-4 concentration and appearance of the oligoclonal IgG.

Diabetes mellitus type I is considered an autoimmune reaction to the beta-cells of pancreas, where the local and systemic immunological reaction depends on the IL-2 and IFN-gamma. The antibodies, which are of the same class as by type I – IgG, against the insulin receptors are the cause of the type II diabetes. The characteristic changes, in the sense of the aggregation of the inflammatory cells and the increased discharge of the IL-2, IL-4 and IFN-gamma have been observed in the preretinal mambranes of the diabetics. There has also been established, locally and systematically, the existence of the antiretinal antibodies of the IgG class. The mentioned pathological changes are more prominent in the type I diabetes.

Since there are no integral information regarding the fluctuation rate of the IgG, IFN-gamma and about the local IgG production in ocular water in the diabetics of the both types with various complication grades, this study attempts to establish a possible link between the mentioned changes.

Materials and Methods

Immunochemical Parameters Determination

Albumin and IgG in serum were evaluated on Partigen® plates for the standard single radial immunodiffusion technique (Behring, Marburg, Germany). Aqueous humor concetrations of albumin and IgG were determined with LC-Partigen® plates for low protein concetration measurements. (Behring, Marburg, Germany) [1,2,4,6–8].

Intraocular IgG synthesis was evaluated by means of the IgG index, calculated with the following formula [2–4,6–8]:

$$\text{IgG AH index} = (\text{AH/S})\text{IgG} \times (\text{S/AH})\text{albumin}$$

Oligoclonal IgG were detected in diluted serum (1:500) and unconcetrated aqueous humor by isoelectric focus-

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ing of proteins in ultrathin polyacrilamide gel (0.4 mm), followed by direct immunofixatio with monospecific IgG antisera and silver nitrate staining[4,6–8].

Samples with aqueous humor oligoclonal IgG without matched serum bands were considered positive[4,6–8].

IFN-gama contrentration was determined by ELISA test (immunotech, Marseille, France) after apsorbance values have been interpolated in standard curve.

Sample collection

All samples were collected only in the time of the cataract operation of the patients presented for significant visual loss. Aqueous humor (AH) samples were collected at the time of cataract surgery by small cannula threw a standard incision at the beginning of the operation without touching lens or iris. The material was stored at –70 °C and analyzed later. Serum samples were obtained by standard venepunction and stored at –70 °C. While conducting the investigation, the principles of the Helsinki Declaration were observed and informed consent was obtained from the subjects.

The control group consisted of 23 patients with senile uncomplicated cataract without diabetes, retinopathy and other systemic disease that could influence their imunological status. Fifteen of the patients were males (M) and eight were females (F). The mean age of the group was 67.7 years and the mean cataract duration was 2.2 years.

Type I diabetes group had 22 patients (11 M, 11F; 9 without, 13 with retinopathy), and the type II diabetes group had 23 patients with diabetic cataract (11M, 12F; 11 without, 12 with retinopathy). The mean age (type I 69.4 years, type II 71.2) and cataract duration (type I 2.3 years, type II 2.6) were not statistically different from the control group. Patients with retinopathy had suffered from diabetes longer than the ones without retinopathy ($p<0.05$)

Statistical Analysis

Software STATISTICA, version 5.0, was used for data analysis. The values of the IgG index, serum and aqueous humor IgG and albumin were compared by means of two-sided t-test with the correction according Cohran & Cox. For frequency of IFN gama in serum and ocular water $\lambda 2$ test was used.

Results

Protein Patterns in Control Samples

IgG index in senile cataract controls was 0.49 ± 0.11, *i.e.* within the normal range typical of aqueous humor IgG measurements. Total albumin and IgG values in serum and aqueous humor were also within the normal range. Oligoclonal IgG bands were negative in all samples. Aqueous humor levels of IFNγ in senile cataract controls were below the detection limit of the test and serum IFNγ levels were detectable in only four cases (15.6%). The results are shown in Table 1.

Albumin and IgG in Diabetes and Retinopathy

In the group of patients with type I diabetes without retinopathy a statistically significant difference ($p<0.05$) in aqueous humor albumin was found compared to senile cataract controls. Differences in IgG indexes among groups were also observed. Group of the type I diabetes patients with retinopathy differed from the control group, type I diabetes without retinopathy and type II diabetes with retinopathy. In type II diabetes group, the differences were significant only between its retinopathy group and type I retinopathy.

TABLE 1
AVERAGE DATA FOR CONTROL GROUP

No.	Age	Sex	Cataract	IFN–γ s	Albumin s	IgG s	IFNγ ah	Albumin ah	IgG ah	IgG filtration	IgG synthesis	IgG index
SD	6.3		1.3	0.470	5.1	2.7	0.000	0.065	0.015	0.013	0.010	0.110
SEM	1.3		0.3	0.098	1.1	0.6	0.000	0.014	0.003	0.003	0.002	0.025
M	67.0		2.0	0.000	41.2	11.6	0.000	0.165	0.021	0.033	–0.008	0.507

s – serum; ah – aqueous humor

TABLE 2
AVERAGE DATA FOR PATIENTS WITH DM TYPE I

No	Age	DM	Cataract	IFNγ s	Albumin s	IgG s	IFNγ ah	Albumin ah	IgG ah	IgG filtration	IgG synthesis	IgG index
X	69.4	11.5	2.3	0.917	42.0	13.0	0.070	0.219	0.032	0.039	–0.007	0.588
SD	7.4	8.2	1.1	1.413	2.1	3.8	0.256	0.166	0.017	0.015	0.012	0.229
SEM	1.6	1.7	0.2	0.301	0.4	0.8	0.055	0.035	0.004	0.003	0.003	0.051
M	70.0	9.0	2.3	0.000	41.9	12.3	0.000	0.165	0.033	0.037	–0.008	0.531

s – serum; ah – aqueous humor

Aqueous humor oligoclonal IgG were found in only 5–10% of the cases in different diabetic groups; however, the values were not significantly different when compared to the senile cataract control group that had no aqueous IgG bands ($p>0.05$). The results are shown in Tables 2, 3, 4, 5 and 6.

TABLE 3
AVERAGE DATA FOR PATIENTS WITH DM TYPE II

No	Age	DM	Cataract	IFNγ s	Albumin s	IgG s	IFNγ ah	Albumin ah	IgG ah	IgG filtration	IgG synthesis	IgG index
X	71.2	10.3	2.6	0.318	41.9	12.3	0.001	0.215	0.027	0.039	–0.012	0.484
SD	4.8	7.0	1.4	0.865	2.4	2.3	0.004	0.138	0.019	0.017	0.009	0.130
SEM	1.0	1.5	0.3	0.180	0.5	0.5	0.001	0.029	0.004	0.004	0.002	0.029
M	72.0	10.0	2.0	0.000	42.6	12.1	0.000	0.179	0.021	0.039	–0.010	0.473

s – serum; ah – aqueous humor

TABLE 4
COMPARATION OF AQUEOUS ALBUMIN, IgG AND IgG INDEX BETWEEN DM TYPE I PATIENTS AND OTHERS

Group I in relation to group II		Albumin ah		IgG ah		IgG index	
Group I	Group II	T	P	T	P	T	P
DM type 1 without retinopathy	Controle group	2.09	**0.045**	1.05	0.301	–0.72	0.478
	DM type 1 with retinopathy	1.35	0.192	–0.38	0.711	–2.42	**0.026**
	DM type 2 without retinopathy	0.99	0.333	0.86	0.403	–0.91	0.374
	DM type 2 with retinopathy	0.50	0.621	–0.02	0.981	0.04	0.968
DM type 1 with retinopathy	Controle group	0.51	0.612	1.94	0.061	3.14	**0.004**
	DM type 1 without retinopathy	–1.35	0.192	0.38	0.711	2.42	**0.026**
	DM type 2 without retinopathy	–0.31	0.758	1.63	0.117	1.95	0.065
	DM type 2 with retinopathy	–1.10	0.284	0.37	0.712	3.10	**0.005**
DM type 1 total	Controle group	1.40	0.168	1.83	0.074	1.58	0.122
	DM type 2 total	0.10	0.920	0.95	0.348	1.78	0.083

ah – aqueous humor

TABLE 5
COMPARATION OF AQUEOUS ALBUMIN, IgG AND IgG INDEX BETWEEN DM TYPE IIPATIENTS AND OTHERS

Group I in relation to group II		Albumin ah		IgG ah		IgG index	
Group I	Group II	T	P	T	P	T	P
DM type 2 without retinopathy	Control group	0.83	0.412	–0.04	0.967	0.60	0.554
	DM type 1 without retinopathy	–0.99	0.333	–0.86	0.403	0.91	0.374
	DM type 1 with retinopathy	0.31	0.758	–1.63	0.117	–1.95	0.065
	DM type 2 with retinopathy	–0.68	0.504	–0.96	0.346	1.30	0.210
DM type 2 with retinopathy	Control group	1.86	0.071	1.20	0.239	–1.01	0.322
	DM type 1 without retinopathy	–0.50	0.621	0.02	0.981	–0.04	0.968
	DM type 1 with retinopathy	1.10	0.284	–0.37	0.712	–3.10	**0.005**
	DM type 2 without retinopathy	0.68	0.504	0.96	0.346	–1.30	0.210
DM type 2 total	Control group	1.51	0.138	0.73	0.468	–0.32	0.752
	DM type 1 total	–0.10	0.920	–0.95	0.348	–1.78	0.083

ah – aqueous humor

TABLE 6
COMPARATION OF AQUEOUS ALBUMIN, IgG AND IgG INDEX BETWEEN DMTYPE I AND II PATIENTS AND OTHERS

Group I in relation to group II		Albumin ah		IgG ah		IgG index	
Group I	Group II	T	P	T	P	T	P
DM type I and II without retinopathy	Control group	1.57	0.123	0.58	0.562	–0.11	0.911
	DM type I and II with retinopathy	0.55	0.587	–1.05	0.299	–1.29	0.204
DM type I and II with retinopathy	Control group	1.36	0.180	1.86	0.069	1.37	0.179
	DM type I and II without retinopathy	–0.55	0.587	1.05	0.229	1.29	0.204
DM type I and II total	Control group	1.52	0.133	1.42	0.162	0.84	0.405

ah – aqueous humor

IFNγ in Diabetes and Retinopathy

Pathological aqueous IFNγ was found in several groups with diabetic complications and in none of the senile cataract controls. However, statistical difference from the senile cataract controls was observed only in type I diabetes without retinopathy (2 pathological IFNγ results out of 9, $p<0.05$)

Serum IFNγ values in type I diabetes without retinopathy were significantly elevated (5 out of 9, $p<0.05$) Consequently, the whole type I diabetes group were significantly different from the cataract controls. ($p<0.05$). The results are shown in Table 7.

Discussion

The integrity of the blood-ocular barrier is a prerequisite for maintaining a stable ratio of individual proteins in the eye chamber. Our data show a change in the equilibrium, which points the fact that the DM and cataract are immunological responses, in part.

The paper found no differences in the aqueous humor albumin concentration between groups. The reason may be that there was no barrier damage that would increase the passage of large proteins. Serum albumin also shows no differences between groups. Its concentration ratio in serum and aqueous humor is an important parameter in evaluating the blood-ocular barrier and is also used to calculate IgG index and the mathematical calculation of IgG synthesis in the Reiber formula guides. Such results have been obtained by other authors.

Total IgG in aqueous humor and serum was not significantly changed in any patient group. In patients with uncomplicated senile cataracts, we found no increase in total IgG, elevated IgG index or oligoclonal IgG, which is confirmed by other authors. Slightly higher intraocular IgG which was found in individual cases does not affect the average results of the entire group. This finding may refer to intraocular IgG production or to increased permeability of blood-ocular barrier.

IgG index is used for evaluation of IgG synthesis in aqueous humor, considering the pathological elevation of IgG and albumin as the filtered value (and expected IgG filtration). The index was abnormal in the type I DM group with and without retinopathy. In the type II DM group it is very slightly abnormal. These data suggest that there are differences between the smaller, more specific groups of patients, but because of their size, should

TABLE 7
FREQUENCY OF PATHOLOGIC IFNγ VALUES

	n	IFNγ ah pathological		IFNγ s pathological	
		yes	no	yes	no
Control group	23	0	23	2	21
Without retinopathy	9	2	7	**5**	**4**
With retinopathy	13	1	12	3	10
Total type I	22	3	19	8	14
Without retinopathy	11	0	11	2	9
With retinopathy	12	1	11	1	11
Total type II	23	1	22	3	20
Without retinopathy	20	2	18	7	13
With retinopathy	25	2	23	4	21
Total type I and II	45	4	41	11	34

s – serum; ah – aqueous humor

be careful in interpreting the results. It should be noted that none of the patients have had extremely pathological index values.

IgG index proved to be a successful method for the determination of intraocular IgG response, particularly if combined with detecting oligoclonal IgG in the aqueous humor. Both techniques provide reliable information, regardless of factors such as age, sex, hypertension, refractive anomalies, etc.

In this paper, IgG index in the control group, ie those with senile cataract uncomplicated with previous uveitis, is less than the normal 0.71 which is normal result obtained also by other authors. This means that there is no pathological IgG response in the senile cataract.

IgG indexes were elevated in type I diabetes with and without retinopathy. In type II diabetes, indexes were just above the borderline, but there were some cases with high index values.

This confirmed the findings of other authors. The fact that the IgG index is pathological mainly in type I diabetes is not surprising, if we remember the etiology of the disease and the role of IgG in it. There are antibodies to the beta cells of the pancreas, IgG class (among others such as antiretinal antibodies) and they are also found in preretinal membranes of diabetics. In type II there are IgG antibodies to insulin receptors, but the pathology is less prominent than in type I which is in accordance with our results.

According to the Rieber formula, IgG synthesis in aqueous humor is negative in all groups of patients. However, there are few patients with the positive synthesis, but the number of cases is too small to affect the average results.

Differences between the index and the Reiber formula are determined by the insufficient sensitivity of Reiber formula due to excessive filtration, which is fixed at X +2 SD value (to avoid false positive results).

It will require further research to determine the role of intraocular IgG in the development of diabetic complications in different types of diabetes and possibly in the development of cataracts. These results suggest that IgG changes in the aqueous humor in patients with diabetes and cataracts exist, which supplementes the literature.

We have found somewhat higher values of IgG in diabetic patients with retinopathy, which confirms that there are bigger disruptions of blood-ocular barrier, or some other mechanism induces pathological immune responses. There is no intraocular production of IgG in most patients, which means that is not present abnormal humoral immune response.

Values of oligoclonal IgG in aqueous humor are not pathological. This result suggests that the intraocular syntesis in diabetes is mostly polyclonal.

Considering albumin/IgG ratio it is evident that the IgG in type I diabetic patients is less than the control group. This means that IgG is increased in aqueous humor ie there is intraocular production in these patients.

In the group with diabetes type II there is no significant pathology.

There is statistically significant difference in patients with type I diabetes without retinopathy and control group, as well as the entire group with the type I diabetes (regardless to retinopathy) according to the control group. (mainly due to pathology). The same applies to the findings of IFN-gamma in the aqueous humor.

These, as we know, the first available results of IFN--gamma in the aqueous humor and serum of patients with diabetic retinopathy, suggest that there are changes in the level of IFN-gamma in patients with diabetes type I.

Despite the relatively small patient groups, our results confirm the involvement IFN-gamma in ethiopathology of diabetes, primarily type I, as noted in several experimental studies. Changes are found predominantly in serum, but the pathology also exist intraocular.

Regarding these results, there will be needed further research in order to evaluate the impact of IFN-gamma on the development of cataracts and retinopathy, and possibly prognostic value of this indicator.

Additional studies of other immunoglobulins, complement components, cytokines (especially IL-4 which in certain situations promotes proliferation of IgG clones), and lymphocyte subpopulations, should provide a more complete picture of intraocular immune events in diabetics.

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IFNγ i IgG U OČNOJ VODICI KOD PACIJENATA SA KATARAKTOM I DIJABETESOM

S A Ž E T A K

Prednja očna sobica se naziva imunološki privilegirano mjesto. Upalne promjene u prednjoj sobici su karakterizirane promjenama u koncentraciji IFNγ, IgG i IL-4, te prisutnošću oligoklonalnog IgG. Kod dijabetesa tipa I postoje protutijela na beta stanice gušterače, IgG razreda (postoje i druga, kao što su antiretinalna antitijela) i njih također možemo pronaći u preretinalnim membranama kod dijabetičara. Kod dijabetesa tipa II su prisutna antitijela na inzulinske receptore.