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Original Article

Immune predictors of diabetic retinopathy against the background of different glucose tolerance

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Abstract

Pathogenetic mechanisms of diabetic retinopathy are associated with the toxic effects of hyperglycemia and subsequent activation of stress-sensitive systems. The purpose was to determine the features of immune dysfunction in patients with diabetic retinopathy against the background of metabolic syndrome. Clinical and laboratory examinations of 130 patients with diabetic retinopathy have been carried out (70 insulin-dependent patients – group 1 and 60 insulin-independent patients – group 2). In determining the surveyed individuals' contents of the lymphocyte populations and subpopulations, the monoclonal antibodies were used to $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD23^+$, $CD25^+$, $CD56^+$ in the reaction of indirect immunofluorescence with fluorescein-isothiocyanate labeled antibodies and indices were calculated – the ratio of the lymphocyte populations and subpopulations. The population and subpopulation composition of blood lymphocytes were studied, and the ratio of cellular factors of immunity in patients with diabetic retinopathy was calculated. The immune status of patients with diabetic retinopathy is characterized by more pronounced changes in insulin-dependent patients' cellular immunity – the activation of nonspecific killer immunity, suppressor potential and humoral immunity than in insulin-independent patients. The results allow the pathogenetic correction of diabetic retinopathy with the immune imbalance.

Keywords: diabetic retinopathy, insulin-dependent diabetes, insulin-independent diabetes, lymphocyte populations and subpopulations.

Introduction

Metabolic syndrome (MS) with impaired glucose tolerance is one of the topical issues of modern medicine. The basis for MS is insulin resistance, a decrease in the reaction of insulin-sensitive tissues (fat, muscle, liver) to physiological insulin concentrations. It shows that insulin resistance (IR) results from the interaction of genetic and external factors [1–3].

Pathogenetic mechanisms of diabetic retinopathy progression are associated with the hyperglycemia toxic effect on the development of oxidative stress with the subsequent activation of stress-sensitive systems [4–7]. The study aims to determine the features of immune dysfunction in patients with diabetic retinopathy against the background of metabolic syndrome.

Material and methods

Study design and patients

Clinical and laboratory examinations of 130 patients with diabetic retinopathy have been carried out

(70 insulin-dependent patients – group 1 and 60 insulin-independent patients – group 2). The average age of patients is 20–55 years old. The obtained laboratory values were compared with the control group, which included 30 practically healthy individuals.

Laboratory, anthropometric and clinical data collection

In determining the surveyed individuals' contents of the lymphocyte populations and subpopulations, the monoclonal antibodies were used to $CD3^+$ (T-lymphocytes), $CD4^+$ (T-helper cells), $CD8^+$ (T-effectors), $CD19^+$ (B-lymphocytes), $CD23^+$ (activated B-lymphocytes), $CD25^+$ (activated T-lymphocytes), $CD56^+$ (NK-cells) in the reaction of indirect immunofluorescence with fluorescein-isothiocyanate (FITC) labeled antibodies. Phenotyping of peripheral blood lymphocytes was performed using indirect immunofluorescence techniques using monoclonal antibodies produced by R.E. Kavetskyi Institute of Experimental Pathology, Oncology and Radiobiology, Ukraine. The lymphocyte populations and subpopulations were calculated using a luminescent microscope with a phase-contrast attachment (Lumam-8), and so were the indices – the ratio of the lymphocyte populations and subpopulations [8].

Statistical analysis

Parametric data are presented as M±m since the data distribution in the groups was regular; a pairwise posterior comparison of the groups was performed using the Newman-Kales criterion using the STATISTICA 6.0 SOFTWARE package (StatSoft, USA).

Results

Significant changes were found in cellular immunity in the surveyed individuals. Important information was obtained when analyzing the ratios of the lymphocyte populations and subpopulations (Table 1).

The ratio of $CD3^+/CD19^+$ in insulin-dependent and insulin-independent patients with DR was 1.3 times lower than in the control group ($p<0.05$), which shows the activation of humoral immunity in diabetic retinopathy.

The $CD3^+/CD56^+$ ratio is likely to be reduced in patients of groups 1 and 2 compared to the control, respectively, 3.39 and 2.64 times ($p<0.05$). This index is 1.29 times lower in group 1 than in group 2 ($p<0.05$). Such changes indicate a significant activation of the nonspecific immunity killer link, especially pronounced in insulin-dependent patients.

The immunoregulatory index of $CD4^+/CD8^+$ is likely to be reduced in patients of groups 1 and 2 compared to the control, respectively, 1.43 and 1.22 times ($p<0.05$). This index is 1.17 times lower in group 1 than in group 2 ($p<0.05$). The results indicate the activation of the suppressor and more pronounced suppression of the immunity helper link in patients with DR, especially in insulin-dependent patients.

The ratio of $CD3^+/CD25^+$ is probably reduced in patients of groups 1 and 2 compared to the control: twice in group 1 and 2.3 times in group 2 ($p<0.05$), as well as the absence of a statistically significant difference between the groups ($p>0.05$). This index shows the degree of activation of the immunity T-cell link. Thus, in patients with DR, we detected a pronounced activation of the immunity T-cell link.

The ratio of T-helper cells and activated T-lymphocytes ($CD4^+/CD25^+$) is reduced threefold in groups 1 and 2 ($p<0.05$). Changes in this index show the activation of the T-cell link of immunity and the inhibition of the T-helper potential.

The ratio of T-helper to NK-cells ($CD4^+/CD56^+$) in the groups of patients is significantly reduced compared to the control: in group 1 – 5.3 times, in group 2 – 3.9 times ($p<0.05$). In group 1, this index is 1.4 times lower than in group 2 ($p<0.05$). The detected changes indicate a significant activation of the nonspecific immunity killer link

Table 1: The ratio of peripheral blood lymphocyte populations and subpopulations of patients with diabetic retinopathy with different glucose tolerance (M±m).

Indices	Groups of subjects		
	Control group (n=30)	Group 1 (n=70)	Group 2 (n=60)
$CD3^+/CD19^+$	2.95±0.20	2.24±0.21*	2.35±0.22*
$CD3^+/CD56^+$	8.38±0.50	2.47±0.22*	3.18±0.30*#
$CD4^+/CD8^+$	1.73±0.10	1.21±0.10*	1.42±0.10#

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Table 1: Continued.

Indices	Groups of subjects		
	Control group (n=30)	Group 1 (n=70)	Group 2 (n=60)
CD3 +/CD25⁺	6.81±0.50	3.29±0.25*	2.95±0.20
CD4⁺/CD25⁺	5.81±0.35	1.80±0.12*	1.72±0.09*
CD4⁺/CD56⁺	7.15±0.50	1.35±0.10*	1.85±0.15*#
CD8⁺/CD25⁺	3.38±0.15	1.49±0.10*	1.21±0.10*
CD8⁺/CD56⁺	4.15±0.20	1.12±0.08*	1.3±0.10*
CD19⁺/CD23⁺	2.64±0.23	1.16±0.10*	1.38±0.12*#
CD19⁺/CD56⁺	2.85±0.20	1.10±0.10*	1.35±0.10*

Note: * – the probability of difference in comparison with the control group figures ($p<0.05$); # – the probability of difference in comparison with CHD group 1 figures ($p<0.05$).

and suppression of the specific one provided by T-helpers, especially in insulin-dependent patients.

The ratio of T-effectors and activated T-lymphocytes ($CD8^+/CD25^+$) in the groups of patients is significantly reduced compared to the control: in group 1 – 2.27 times, in group 2 – 2.79 times ($p<0.05$). In group 1, this index is 1.23 times higher than in group 2 ($p<0.05$). This index indicates the activation of the immunity T-cell link.

The ratio of T-effectors to NK cells ($CD8^+/CD56^+$) in the groups of patients is significantly reduced compared to the control: in group 1 – 3.71 times, in group 2 – 3.19 times ($p<0.05$), and there is no statistically significant difference between the groups ($p>0.05$). This index indicates the predominant activation of the nonspecific immunity killer link.

The index of humoral immunity activation ($CD19^+/CD23^+$ ratio) is likely to be reduced in the groups of patients with DR compared to the control. In group 1 – the index was reduced 2.28 times, and in group 2 – 1.91 times ($p<0.05$). Between the groups of patients – in the first group, the indicator is 1.2 times lower than in group 2 ($p<0.05$). Such changes in the humoral immunity activation index show an increase in the activated B-lymphocytes population.

The index of the ratio of specific humoral and nonspecific immunity killer links ($CD19^+/CD56^+$) in the groups of patients is likely to be reduced compared to the control: in group 1 – 2.60 times, in group 2 – 2.10 times ($p<0.05$), in the first group the indicator is lower 1.23 times than in group 2 ($p<0.05$). Such changes in the index indicate a predominant activation of the nonspecific killer link, compared with the specific nonspecific immunity humoral link.

Discussion

As a result of our research, we found significant changes in cellular immunity in the surveyed individuals. The patients with diabetic retinopathy reveal the activation of T- and B-cell immunity. Changes in the levels of lymphocyte subpopulations in insulin-dependent patients with DR are more pronounced than in insulin-independent patients with DR. The T-lymphocyte, T-effectors, activated T-lymphocytes, B-lymphocytes, and activated NK cells B-lymphocyte levels grew in both groups. Only the T helper subpopulation was reduced compared to the control.

Significant heterogeneity of diabetes mellitus causes conflicting data on the assessment of the immune status of patients with diabetic retinopathy and explains the unequal effectiveness of immunomodulatory therapy. Immune changes considerably determine the occurrence and severity of vascular complications of diabetes mellitus and the disease itself [7, 9–11]. Nowadays, insulin is determined to have the properties of an immunomodulator. We know that in type 1 diabetes mellitus, absolute insulin deficiency occurs, whereas, in type 2 diabetes mellitus, insulin deficiency is relative [1, 12].

Conclusion

Thus, the immune status of patients with diabetic retinopathy is characterized by more pronounced changes in insulin-dependent patients' cellular immunity – the activation of nonspecific killer immunity, suppressor potential and humoral immunity than in

insulin-independent patients. The results allow the pathogenetic correction of diabetic retinopathy with the immune imbalance.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the Danylo Halytsky Lviv National Medical University (approval ID: No.2/20.02.2023).

Consent to participate

Written informed consent was obtained from the participants.

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