https://doi.org/10.46389/rjd-2025-1834
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2025; volume 32, issue 1, pages 49–54

Original Article

Peculiarities of the immune status of patients with diabetic retinopathy in the framework of metabolic syndrome

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Received: 13 December 2024 / Accepted: 20 February 2025

Abstract

Diabetic retinopathy (DR) is the most significant and common cause of visual impairment in diabetes patients. The aim of the study was to enhance the understanding of the pathogenesis of DR associated with metabolic syndrome (MS) and elucidate the role of cellular and humoral immunity factors. The study included 130 patients. Group 1 comprised 70 patients diagnosed with DR and insulin-dependent type 2 diabetes against the background of MS. Group 2 included 60 patients diagnosed with DR and non-insulin-dependent type 2 diabetes associated with MS. The immunological analysis focused on evaluating subpopulations of blood lymphocytes using flow cytometry; systemic inflammation markers, such as CRP, specific IgA, IgM, and IgG, cytokines measured by ELISA. Significant changes in immune status were observed in patients with DR associated with MS, depending on diabetes compensation. In Group 1 patients with DR, more pronounced alterations in the T-cell immunity pathway were observed, including T-cell immunodeficiency accompanied by the activation of killer and B-cell immunity, compared to non-insulin-dependent patients. Both groups exhibited type IV hypersensitivity reactions. Elevated CRP level was detected only in insulin-dependent patients with DR. An analysis of the immune parameters indicated predominant activation of the specific humoral immunity pathway, suggesting chronicity of the condition. Non-insulin-dependent patients showed significant activation of mucosal humoral defenses and early humoral protective mechanisms. The data revealed more pronounced changes in specific humoral immunity markers, such as immunoglobulins, compared to systemic inflammation markers like CRP.

Keywords: diabetic retinopathy, insulin dependence, cellular immunity, humoral immunity, pro-inflammatory interleukins, CRP.

Introduction

Recent global statistics indicate a significant increase in the prevalence of type 2 diabetes mellitus, often complicated by metabolic syndrome. Ocular complications of diabetes are among the leading causes of vision impairment and blindness, with the incidence of vision loss continuing to rise. Diabetic retinopathy (DR) is the most significant and common cause of visual impairment in diabetes patients [1].

The pathogenesis of DR involves the toxic effects of hyperglycemia, oxidative stress, and subsequent activation of stress-sensitive systems [2]. The degree of glycemic control and arterial hypertension are critical determinants in the progression of DR. Among adults aged 20 to 75, DR is the most common cause of blindness. Initially, DR is characterized by retinal microaneurysms, progressing to macular edema and neovascularization. Early symptoms are typically absent, but advanced stages involve focal damage, vitreous

detachment, and retinal detachment, leading to partial or total vision loss [3, 4].

The goal is to enhance the understanding of the pathogenesis of DR associated with metabolic syndrome and elucidate the role of cellular and humoral immunity factors. The results obtained are aimed at developing new approaches to early diagnosis, preventing complications, and improving methods of preventing this disease.

Material and methods

The study included 130 patients from the Ophthalmology Department of the Lviv Regional Clinical Hospital and the Transfusion Department. Participants aged 35–65 years (mean age 46.0±2.0 years, male-to-female ratio 1:1) were selected based on clinical data. In 70 patients, diabetic retinopathy and insulin-dependent type 2 diabetes mellitus (T2DM) in the framework of metabolic syndrome were confirmed (Group 1). Another 60 patients were diagnosed with diabetic retinopathy and non-insulin-dependent type 2 diabetes mellitus with metabolic syndrome (Group 2). The control group consisted of 40 practically healthy male and female donors aged 35–65 years, free from diabetes and comorbidities. These participants were recruited from the Transfusiology Department. The immunological analysis in this study focused on evaluating subpopulations of T-lymphocytes (CD3+, CD4+, CD8+, CD25+), B-lymphocytes (CD19+, CD23+), and natural killer (NK) cells marked with CD56+. Additionally, systemic inflammation markers were assessed, such as C-reactive protein (CRP) and specific IgA, IgM, and IgG class immunoglobulins. The study applied laboratory techniques like flow cytometry to analyze cell subpopulations and enzyme-linked immunosorbent assay (ELISA) to quantify cytokine and CRP levels.

Statistical analysis of the obtained results was performed using mathematical statistics methods with the STATISTICA 8.0 software package (StatSoft, USA). The results were presented as mean values with standard deviations, and a p-value of <0.05 was considered statistically significant [5].

Results and discussion

Examination of 130 patients with diabetic retinopathy in the framework of metabolic syndrome revealed complex alterations in hematological parameters and immune status.

Hemogram changes in diabetic retinopathy patients

The absolute leukocyte count in the group of non-insulin-dependent diabetic retinopathy (DR) patients was at the upper physiological limit (8.7±0.2 G/L). This group exhibited a statistically significant increase in eosinophil levels compared to the normal range and the insulin-dependent DR group (5.5±0.2% *vs*. 3.2±0.2% and 2.7±0.1%, respectively).

Relative levels of basophils, band neutrophils, segmented neutrophils, lymphocytes, and monocytes in DR patients with compensated diabetes remained within physiological norms.

Features of cellular and humoral immunity in diabetic retinopathy

In the insulin-dependent DR group, activation of the T-cell immune pathway (CD3+) was observed, with levels 1.3 times higher than the control group. T-helper cell (CD4+) subpopulations were 1.2 times below the normal range, while the absolute count of T-effectors (CD8+) was doubled compared to the control group. Levels of activated T-lymphocytes (CD25+) were 2.8 times above normal.

In the non-insulin-dependent DR group, T-lymphocyte's absolute count remained within the normal range. T-helper levels were 1.2 times lower than the norm but did not significantly differ from those in the insulin-dependent group. T-effector levels in insulin-dependent patients were 1.5 times higher than normal but 1.3 times lower than those in the non-insulin-dependent group. The levels of activated T-lymphocytes in both DR groups were 2.8 times higher than in the control group.

B-lymphocyte (CD19+) levels in the insulin-dependent DR group exceeded the normal range by 1.8 times, with the activated B-lymphocyte (CD23+) subpopulation increasing fourfold compared to the control group. This group's level of natural killer (NK) cells (CD56+) was 4.6 times higher than the normal range.

The absolute count of B-lymphocytes in the non-insulin-dependent DR group was 1.5 times higher than in the control group but 1.2 times lower than in the insulin-dependent DR group. The levels of activated B-lymphocytes in the non-insulin-dependent DR group were 2.8 times above normal but 1.5 times lower than those in the insulin-dependent group.

The level of natural killer (NK) cells in the non-insulin-dependent DR group was three times higher than normal but 1.5 times lower than in the insulin-dependent

https://doi.org/10.46389/rjd-2025-1834

DR group. Increased levels of NK cells are a marker of a nonspecific immune response that plays an important role in controlling infectious processes and inflammation. Its significant increase in insulin-dependent patients may be a compensatory reaction to the reduced efficiency of the specific immune response.

As demonstrated above, patients with diabetic retinopathy, whether insulin-dependent or non-insulin-dependent, exhibited T-cell and B-cell immunity activation. However, changes in lymphocyte subpopulation levels were more pronounced in the insulin-dependent group. Both groups demonstrated increased levels of T-lymphocytes, T-effectors, activated T-lymphocytes, B-lymphocytes, activated B-lymphocytes, and NK cells. Only the T-helper subpopulation level was significantly lower than in the control group. The immune regulatory index in the insulin-dependent DR group was 1.2, while in the non-insulin-dependent group, it was 1.4.

The significant heterogeneity of diabetes mellitus contributes to conflicting data regarding immune status evaluation in DR patients, which may lead to variable responses to immunomodulatory therapy. Immune alterations largely influence the onset and severity of vascular complications in diabetes, as well as the disease itself. Additionally, insulin exhibits immunomodulatory properties [6–8].

Immunoregulatory indices and ratios

Important information was obtained through the analysis of lymphocyte population and subpopulation ratios.

The CD3+/CD19+ ratio in DR patients from both groups was 1.3 times lower than in the control group ($p<0.05$), indicating activation of the humoral immune pathway in diabetic retinopathy.

The CD3+/CD56+ ratio was significantly reduced in both patient groups compared to controls: in Group 1 by 3.39 times and Group 2 by 2.64 times ($p<0.05$). This index was 1.29 times lower in non-insulin-dependent DR patients compared to insulin-dependent patients ($p<0.05$). These changes indicate significant activation of the nonspecific killer immune pathway, particularly pronounced in patients with decompensated diabetes.

The CD4+/CD8+ ratio (immune regulatory index) was significantly reduced in both patient groups compared to controls: in Group 1 by 1.43 times and in Group 2 by 1.22 times ($p<0.05$). This index was 1.17 times lower in Group 1 than in Group 2 ($p<0.05$). These results suggest activation of the suppressor pathway and more pronounced suppression of the helper pathway in insulin-dependent patients.

The CD3+/CD25+ ratio was significantly reduced in both patient groups compared to controls: by 2.0 times in Group 1 and 2.3 times in Group 2 ($p<0.05$), with no statistically significant differences between the groups ($p>0.05$). This index reflects the degree of T-cell immunity activation. Thus, a pronounced activation of the T-cell immune pathway was identified in DR patients.

The CD4+/CD25+ ratio was threefold lower in both groups of patients ($p<0.05$). Changes in this index indicate activation of the T-cell immune pathway and suppression of helper T-cell potential.

The CD4+/CD56+ ratio was significantly reduced in both patient groups compared to controls: in Group 1 by 5.3 times and in Group 2 by 3.9 times ($p<0.05$). In Group 1, this index was 1.4 times lower than in Group 2 ($p<0.05$). These changes indicate significant activation of the nonspecific killer immune pathway and suppression of the specific pathway mediated by T-helper cells, especially in insulin-dependent patients.

The CD8+/CD25+ ratio was significantly reduced in both groups compared to controls: in Group 1 by 2.27 times and in Group 2 by 2.79 times ($p<0.05$).

The CD8+/CD56+ ratio was also significantly reduced compared to controls: in Group 1 by 3.71 times and in Group 2 by 3.19 times ($p<0.05$), with no statistically significant differences between groups ($p>0.05$). This index indicates dominant activation of the nonspecific killer immune pathway.

The CD19+/CD23+ ratio (humoral immunity activation index) was significantly reduced in DR patients compared to controls by 2.28 times in Group 1 and 1.91 times in Group 2 ($p<0.05$). These changes indicate an increase in the population of activated B-lymphocytes.

The CD19+/CD56+ ratio, which reflects the balance between specific humoral and nonspecific killer immunity pathways, was significantly reduced in both groups compared to controls by 2.60 times in Group 1 and 2.10 times in Group 2 ($p<0.05$). These changes suggest dominant activation of the nonspecific killer immune pathway over the specific humoral pathway.

Thus, the immune status of patients with diabetic retinopathy is characterized by more pronounced changes in cellular immunity in insulin-dependent patients, including activation of the nonspecific killer immune pathway, suppressor potential, and humoral immunity, compared to non-insulin-dependent patients. The obtained results allow for pathogenetic correction of diabetic retinopathy, taking into account the immune imbalance.

Changes in immunoglobulins, C-reactive protein, and cytokines in diabetic retinopathy

As a result of our studies identified a statistically significant increase in IgA levels in the non-insulin-dependent group, which was 2.5 times higher than in the control group and 1.5 times higher compared to the insulin-dependent group ($p<0.05$). The levels of IgM also exceeded the control group by 2.3 times and were 1.7 times higher in the insulin-dependent group ($p<0.05$). IgG levels were also statistically significantly higher compared to the control group, increasing by 1.4 times ($p<0.05$), but did not differ from the levels in the insulin-dependent group ($p>0.05$).

The level of circulating immune complexes (CICs) in the non-insulin-dependent DR group with compensated diabetes was 1.4 times higher than in the control group and 2 times higher than in the insulin-dependent DR group ($p<0.05$), indicating a higher likelihood of Type III hypersensitivity reactions in the non-insulin-dependent DR group.

To assess the activity of systemic inflammation, we conducted a study of C-reactive protein (CRP) levels (mg/L). CRP serves as a marker of acute inflammatory processes, and its elevation is a factor in the progression of diabetic retinopathy [9].Upon studying CRP levels in diabetic retinopathy patients, we found a significant increase in insulin-dependent patients (1.2 times higher compared to the control group). In the non-insulin-dependent group, CRP levels were within normal limits.

The ratio of immunoglobulins and C-reactive protein in diabetic retinopathy

Analysis of the ratios of immunoglobulins and C-reactive protein (CRP) revealed different patterns of immune response in patients with diabetic retinopathy:

- The IgG/IgA ratio in the control group was 1.2 times higher than in the insulin-dependent group and 1.8 times higher than in the non-insulin-dependent group ($p<0.05$). The ratio in the insulin-dependent group was 1.5 times higher than in the non-insulin-dependent group ($p<0.05$). These findings suggest predominant activation of the specific humoral immune pathway and indicate the chronicization of the process. In non-insulin-dependent patients, there is pronounced activation of humoral defense mechanisms in mucosal areas.
- The IgG/IgM ratio in the control and insulin-dependent groups was 1.7 times higher than in the non-insulin-dependent group ($p<0.05$), suggesting predominant activation of early humoral defense mechanisms.
- The IgA/IgM ratio in the insulin-dependent group was 1.23 times higher than in both the control and non-insulin-dependent groups ($p<0.05$), indicating predominant activation of humoral immune responses in the mucosal areas of insulin-dependent patients with decompensated diabetes.
- The CRP/IgA ratio in the control group was 1.38 times higher than in the insulin-dependent group and 2.44 times higher than in the non-insulin-dependent group ($p<0.05$). The ratio in the insulin-dependent group exceeded that in the non-insulin-dependent group by 1.77 times ($p<0.05$), indicating predominant activation of specific humoral immune defense in the mucosal areas in this patient group.
- The CRP/IgM ratio in the control and insulin-dependent groups was 2.3 times higher than in the non-insulin-dependent group ($p<0.05$), suggesting predominant activation of early specific humoral immune defense mechanisms in patients from these groups.
- The CRP/IgG ratio in the control and insulin-dependent groups was 1.4 times higher than in the non-insulin-dependent group ($p<0.05$), indicating predominant activation of specific humoral immune defense in patients in these groups.

Thus, the analysis of the ratios of immune parameters indicates predominant activation of the specific humoral immune pathway and points to the chronicization of the process in diabetic retinopathy. In the non-insulin-dependent patient group, there is pronounced activation of humoral defense in mucosal areas and predominant activation of early humoral immune mechanisms. The obtained data indicate more pronounced changes in specific humoral immune parameters—immunoglobulins—in patients with diabetic retinopathy compared to the systemic inflammation marker—CRP.

Analysis of C-reactive protein and cytokine ratios in diabetic retinopathy

A series of mediators, including interleukins, initiate and control the synthesis of CRP. Activated interleukins enhance the synthesis of glucocorticoids, leading to leukocytosis. They also have pyrogenic properties and activate the complement and coagulation

https://doi.org/10.46389/rjd-2025-1834

cascades [10]. CRP contains regulatory elements that interact with cytokines. Like any acute-phase protein, CRP's primary biological function is to stimulate immune responses.

In the presence of an inflammatory process or other damaging factors, CRP is synthesized by hepatocytes under the influence of cytokines such as IL-1 and IL-6, which have pro-inflammatory properties.

When calculating the CRP/IL-1β ratio, we found an increase of 1.3 times (2.4±0.04 and 1.8±0.05, respectively) ($p<0.05$) in the insulin-dependent group relative to the control group due to the activation of the acute-phase peptide. In the non-insulin-dependent group, a decrease in the CRP/IL-1β ratio was observed: 1.4 times lower than the normal value (1.3±0.05 and 1.8±0.05, respectively) ($p<0.05$) And 1.8 times lower compared to the insulin-dependent group (1.3±0.05 and 2.4±0.04, respectively) ($p<0.05$).

An increase in the ratio of the acute-phase marker to the pro-inflammatory factor indicates the predominance of an acute inflammatory process in the insulin-dependent group. The decrease in the CRP/IL-1β ratio in the non-insulin-dependent group suggests a predominance of pro-inflammatory processes (mobilization and activation of cells involved in the inflammatory process).

As a result of our study, we found that in insulin-dependent diabetic retinopathy patients, the acute-phase marker (C-reactive protein) is activated, and pro-inflammatory cytokine levels (IL-1β, IL-18) are normalized. In non-insulin-dependent diabetic retinopathy patients, IL-1β and IL-18 levels were observed, along with normalization of C-reactive protein levels.

The CRP/IL-6 ratio in Group 1 exceeded the control group by 1.37 times and the ratio in Group 2 by 1.23 times ($p<0.05$).

The IL-8/CRP ratio in Group 1 exceeded the control group by 6.4 times and the ratio in Group 2 by 5.6 times ($p<0.05$). There was no statistically significant difference between the two groups ($p>0.05$).

The CRP/TNF-α ratio in the control group exceeded that in Group 1 by 6.1 times and that in Group 2 by 7.8 times ($p<0.05$). The ratio in Group 1 was 1.28 times higher than in Group 2 ($p<0.05$).

In the presence of an inflammatory process or other damaging factors, CRP is synthesized by hepatocytes under the influence of cytokines such as IL-1 and IL-6, which have pro-inflammatory properties.

Researchers have established that the activation of the complement system and the stimulation of adhesion molecule expression on the surface of the endothelium, along with the binding and modification of lipoproteins facilitated by CRP, are indicators of the initial stage of vascular wall damage and endothelial dysfunction. CRP performs several functions: mediator, transport, and immunomodulatory [11, 12].

A small amount of CRP, which continuously circulates in the blood, is not considered a specific marker of an inflammatory process, as interleukins, in addition to inducing acute-phase protein synthesis, have functions that are not directly related to acute inflammation [13].

Conclusions

The study found significant immune changes in diabetic retinopathy with metabolic syndrome, varying by diabetes type. Insulin-dependent patients had more severe immune alterations, including dysgammaglobulinemia, elevated CRP levels, T-cell deficiencies and increased B-lymphocytes, indicating immune dysfunction. Insulin-independent patients showed milder changes, with T- and B-lymphocyte activation, normal CRP levels. Both groups had type IV hypersensitivity reactions, indicating chronic inflammation. These findings highlight the need to monitor both inflammation markers and immunoglobulins for a better understanding of diabetic retinopathy.

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 all the participants.

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