https://doi.org/10.46389/rjd-2021-1044
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2021; volume 28, issue 3, pages 296-300

Original Research

Serum profiles of proinflammatory cytokines in patients with diabetic retinopathy

Marta Horecha[1], Lyubov Lapovets[1], Viorika Akimova[1,*], Nataliia Lapovets[2], Olha Martianova[1]

[1] Department of Clinical Laboratory Diagnostics, Danylo Halytsky National Medical University, Lviv, Ukraine
[2] The Research Institute of Epidemiology and Hygiene, Danylo Halytsky National Medical University, Lviv, Ukraine

*Correspondence to: Viorika Akimova, Department of Clinical Laboratory Diagnostics, Danylo Halytsky National Medical University, 79034, Lviv, Ugorska str 5/9, Ukraine, E-mail: viorikakimova@gmail.com, Phone: +380931099275

Received: 16 September 2021 / Accepted: 14 October 2021

Abstract

Background and aims: Due to the steadily increasing numbers of patients with diabetes mellitus (DM), the prevalence of diabetic complications is also on the rise. One of the most prognostically adverse complications is diabetic retinopathy (DR), which leads to blindness and vision disability of employable population. Cytokine imbalance is one of the factors in the development of DR. The aim was to study the changes in IL-6, IL-8, and TNF-α levels in biological fluids of patients with DR, as these cytokines are produced by endothelial cells, fibroblasts, monocytes/macrophages and by other cells in response to pathogens. **Material and method:** The objective of this work was to assess serum levels of IL-6, IL-8, and TNF-α in 130 patients with diabetic retinopathy (70 patients with decompensated diabetes mellitus [Group 1] and 60 patients with compensated diabetes mellitus [Group 2]). The age of patients ranged from 20 to 55 years. The laboratory results were compared with respective values in the control group, which included 30 virtually healthy individuals. Interleukin levels were determined using reagent kits manufactured by 'VECTOR-BEST Ukraine' on a 'STAT FAX 303 plus' analyzer. **Results:** The levels of IL-6 in groups of patients with DR with both uncompensated and compensated diabetes were not significantly different from the respective levels in the control group. The level of IL-8 in patients of the first group of study subjects was increased 8-fold compared to the control group and was 1.4 times higher than the respective level in patients of the second group. The level of IL-8 in patients of Group 2 was 5.6 times higher than in the control group. The concentration of TNF-α in both groups of patients with DR was 8 times higher than in the control group. **Conclusions:** As a result of our study, we have found patients with diabetic retinopathy (both with compensated and decompensated DM) to have increased serum levels of TNF-α and IL-8. The level of IL-8 in the blood of patients with DR is a potential diagnostic marker for decompensation of diabetes mellitus.

Keywords: diabetic retinopathy, proinflammatory cytokines, interleukin 6, interleukin 8, TNF-α.

Background and aims

Due to the steadily increasing numbers of patients with diabetes mellitus (DM), the prevalence of diabetic complications is also on the rise. One of the most prognostically adverse complications is diabetic retinopathy (DR), which leads to blindness and vision disability of employable population.

The vascular endothelium is an actively functioning metabolic system. The development of vascular complications during disease occurs at the level of endothelial cells, which are susceptible to the damaging effect of hyperglycemia. A significant number of patients with type 2 DM have characteristic changes in the ocular fundus and other signs of micro- and macro-angiopathy already when diagnosed with diabetes. This suggests a few

years' history of a carbohydrate metabolism disorder prior to the date of diagnosis [1].

The problem of investigating the pathogenetic mechanisms behind the development of angiopathy and retinopathy is especially important given its clinical implications. This is associated with the quality of predictive assessment, determination of the type and scope of medical interventions, treatment efficacy evaluation, and prognostication of further development of the disease process.

The complexity and the multifacetedness of the problem of DR pathogenesis are supported by the fact that the studies in mechanisms behind the development of diabetic angiopathy, and the associated role of the blood-ocular barrier have drawn the attention of not only ophthalmologists but also researchers of other profiles (morphologists and pathophysiologists) in recent years. Such a broad approach provides for the necessary basic science support of the research and opens a prospect of other conceptual add-ons [1, 2].

DM is associated with abnormal changes in rheology and aggregation properties of blood and in local hemodynamics. Ocular microcirculation is a functional subsystem; on the one hand, this subsystem is an integral part of the specific biosystem of the eye, and on the other hand, it is a part of the general circulatory system. This allows viewing the problem of pathogenesis from the perspective of the functional status of blood.

Some research papers [2, 3] prioritize osmotic stress as a factor in the development of vascular DM complications; the impact of osmotic stress is primarily considered at the level of intercellular and cellular interactions.

Cytokine imbalance is one of the factors in the development of DR. At present, there is a consensus understanding that cytokines are produced in small quantities under normal conditions. The development of an abnormal condition significantly enhances cytokine production. It is the substantial abnormalities in the cytokine system in inflammatory eye disease of infectious or autoimmune origin that determine the chronic and recurrent course of the disease and the insufficient efficacy of treatment. The specific feature of cytokinemia in type 2 DM is the fact that as the disease unfolds, the patients develop increasing amounts of cellular structures with high production of cytokines. The study of cytokine profile and cytokine imbalance in the course of developing diabetic retinopathy will allow obtaining additional prognostic criteria for the progress of this disease and will improve the understanding of its pathogenesis [4].

Beyond doubt, it is important to study the changes in IL-6, IL-8, and TNF-α levels in biological fluids of patients with DR, as these cytokines are produced by endothelial cells, fibroblasts, monocytes/macrophages and by other cells in response to pathogens. In many studies, the authors have noted increased levels of the aforementioned cytokines in vitreous bodies in patients with diabetic retinopathy.

Material and method

Study design and patients

A clinical and laboratory examination was performed in patients with proliferative diabetic retinopathy [4, 5].

Increased serum IL-8 level has been suggested to have diagnostic value as a potential risk assessment for the progression of diabetic retinopathy [6]. The study of changes in IL-6 levels (high concentrations of IL-6 are found during inflammation of various etiologies and in autoimmune disease) has provided data on the role of this cytokine in the pathogenesis of proliferative DR [7]. However, the authors' conclusions are not unambiguous.

The **objective of this work** was to assess serum levels of IL-6, IL-8, and TNF-α in 130 patients with diabetic retinopathy (70 patients with decompensated diabetes mellitus [Group 1] and 60 patients with compensated diabetes mellitus [Group 2]). The age of patients ranged from 20 to 55 years. The laboratory results were compared with respective values in the control group, which included 30 virtually healthy individuals.

Laboratory, anthropometric and clinical data collection

Venous blood (5 ml) was sampled from all subjects in a fasted state. Blood was sampled into

BD Vacutainer plastic test tubes with a double coagulation activator. Serum levels of IL-6, IL-8, and TNF-α were determined in all test subjects.

Interleukin levels were determined using reagent kits manufactured by 'VECTOR-BEST Ukraine' on a 'STAT FAX 303 plus' analyzer.

Statistical analysis

Statistical analysis of data was performed using the methods of mathematical statistics by means of STATISTICA 8.0 software package (by Statsoft, USA). Core statistical indicators were determined, such as arithmetic mean (M) and standard error of mean (m). In figures and in tables, the results were presented as M±m. The difference between the arithmetic means was considered significant at $p<0.05$. Each indicator was tested for normal distribution using the Shapiro-Wilk test. Depending on the experimental conditions and data distribution, the differences between the groups were assessed using the paired or unpaired t-test and nonparametric Mann-Whitney test [8].

Results

As a result of our research, we have found changes in interleukin levels in both groups of study subjects (Table 1).

The levels of IL-6 in groups of patients with DR with both uncompensated and compensated diabetes were not significantly different from the respective levels in the control group. The level of IL-8 in patients of the first group of study subjects was increased 8-fold compared to the control group and was 1.4 times higher than the respective level in patients of the second group. The level of IL-8 in patients of Group 2 was 5.6 times higher than in the control group. The concentration of TNF-α in both groups of patients with DR was 8 times higher than in the control group.

Discussion

Diabetic retinopathy is one of the most common complications of DM. IL-6 is one of the so-called early cytokines; this emphasizes its significant role in rapid response to pathogenic aggression or tissue damage and the development of appropriate defense reactions [7].

Scientific publications [9] highlight the importance of local inflammation in retinal detachment in patients with diabetic macular edema and the associated increase in intraocular IL-6 levels. The presence of correlations between serous retinal detachment and IL-6 levels and the absence of such associations with other cytokines lend support to the above statement. Also, increased levels of IL-6 and of a number of other

Table 1: Serum levels of interleukins 6, 8 and TNF-α in patients with diabetic retinopathy (M±m).

	Groups of subjects		
Study parameters	**Control group (n=30)**	**Group 1 (n=70)**	**Group 2 (n=60)**
IL-6 (pg/ml)	2.0±0.05	1.82±0.05 p >0.05	1.83±0.05 p>0.05 p_1>0.05
IL-8 (pg/ml)	2.1±0.05	16.6±0.05 p <0.05	11.8±0.05 p<0.05 p_1<0.05
TNF-α (pg/ml)	0.5±0.05	3.8±0.1 p <0.05	4.0±0.1 p<0.05 p_1>0.05

Note: p = the significance of differences compared to the values in the control group; p_1 = the significance of differences compared to the values in Group 1.

https://doi.org/10.46389/rjd-2021-1044

proinflammatory cytokines were detected in vitreous bodies of patients with proliferative DR. The results obtained suggest that intraocular inflammation is likely involved in the pathogenesis of proliferative DR. However, intraocular inflammation is not pronounced at the non-proliferative stage, and the systemic increase in pro-inflammatory activity in diabetic patients is related to increased serum TNF-α levels [10]. TNF-α is one of the primary mediators involved in the pathogenesis of various abnormal processes (such as damage and inflammation), as well as in defense reactions of the body and in tissue homeostasis. Depending on interactions with other cytokines and the cellular environment, the biological effects of TNF-α may be either protective or damaging [4, 10].

The increase in IL-8 levels was the most pronounced in DR patients with decompensated diabetes mellitus. Such results are confirmed by research papers [11], which note the production of IL-8 by activated $CD4^+$ T-cells. These results directly demonstrate the involvement of IL-8 in the autocrine regulation of functional activity of T-cells. It is quite obvious that the significance of such regulation may increase in the periphery, beyond lymphoid organs, in the setting of deficiency of helper cells and immunoregulatory cells.

In a series of studies [6, 11, 12], it has been demonstrated that IL-8, the increased levels of which were found in the vitreous bodies of patients with proliferative DR along with other cytokines, which form the cytokine grid, is able to affect the pathogenesis of the disease, including neoangiogenesis [4]. IL-8 has multidirectional effects on the activation and functionality of different T-cell subpopulations. On the one hand, IL-8 hinders the development of excessive T-cell responses in the periphery; on the other hand, it facilitates the development of adaptive T-cell processes that form the immune memory [6].

However, in the work by Gustavsson C. et al. (2013), despite the multidirectional changes in pro-inflammatory, anti-inflammatory and regulatory cytokines, the authors posit that only TNF-α may participate in the mechanisms behind the development and progression of proliferative DR (PDR) and may serve as a therapeutic target in the treatment of the disease [10]. The relationship between serum concentrations of TNF-α and IL-1β may correlate with the severity of the clinical course of PDR [4, 5]. Further studies have demonstrated that the increase in TNF-α and IL-1β levels was seen not only in the blood but also in the vitreous body. This allows the researchers to suggest an important role of these cytokines in the mechanisms behind the development of PDR, namely, the influence on abnormal cell proliferation and neovascularization [5]. The studies conducted in recent years have found that TNF-α and IL-1β were capable of slowing down the migration of retinal endothelium and capillary morphogenesis. These two cytokines may also play an important role in impaired integrity of the blood-retinal barrier, development of retinal leukostasis and activation of apoptosis in DR. It is important to emphasize that hyperglycemia is a trigger, and retinal endothelium is a source of hyperexpression of proinflammatory cytokines [1].

A number of studies have shown the levels of vascular endothelial growth factor (VEGF) and IL-6 in the human intraocular fluid to be substantially higher in patients with proliferative DR compared to patients without DR [7]. A study of the retina in mice with experimentally induced diabetes shows a significant expression only of IL-2 and TNF-α, the cytokines produced by type 1 T helper cells [11].

As a result of our study, we have found an imbalance of proinflammatory cytokines in the serum of patients with DR. IL-6 levels in patients of both study groups were within normal, which is corroborated by the literature data that report increases in concentrations of this cytokine only at the early stages of progression of diabetic retinopathy. The concentrations of TNF-α in the blood of patients with DR in the two study groups were equally increased relative to the controls. Only IL-8 levels were significantly higher in patients with DR compared to both the control group and across the investigational groups. As noted in works by other scientists, changes in IL-8 levels [6, 12] could be a diagnostic marker of DR progression. As a result of our research, we have noted a significant increase in this cytokine

in the group of patients with DR and decompensated DM. In the group of patients with DR and compensated DM, IL-8 levels were 1.4 times lower than in patients of Group 1.

Conclusions

As a result of our study, we have found patients with diabetic retinopathy (both with compensated and decompensated DM) to have increased serum levels of TNF-α and IL-8. The level of IL-8 in the blood of patients with DR is a potential diagnostic marker for decompensation of diabetes mellitus.

References

1. Memon, W. R., Lal, B., Sahto, A. A. (2017). Diabetic retinopathy. *Prof Med J*. 24(2):234–238.
2. Jan, S., Ahmad, I., Karim, S., Hussain, Z., Rehman, M., Shah, M. A. (2018). Status of diabetic retinopathy and its presentation patterns in diabetics at ophthalomogy clinics. *J Postgraduate Med Inst. (Peshawar-Pakistan)*. 32(1):24–27.
3. Welikala, R. A., Fraz, M. M., Dehmeshki, J., Hoppe, A., Tah, V., Mann, S., Williamson, T. H. (2015). Genetic algorithm based feature selection combined with dual classification for the automated detection of proliferative diabetic retinopathy. *Comput Med Imag Graph*. 43:64–77.
4. Chernykh, D. V., Smirnov, E. V., Gorbenko, O. M., et al. (2015). Disorders of cytokine regulation in the pathogenesis of proliferative diabetic retinopathy. *Ophthalmoll Surg*. 2:50–55.
5. Trunov, A. N., Chernykh, D. V., Eremina, A. V., Chernykh, V. V. (2017). Cytokines and growth factors in the pathogenesis of proliferative diabetic retinopathy. *Ophthal Surg*.1:93–97.
6. Meniailo, M. E., Malashchenco, V. V., Shnarov, V. A., Gazatova, N. D., Todosenko, N. M., Malashchenco, O. B., Goncharov, A. G., Seledsov, V. I. (2016). The direct influence of interleukin-8 on T-cell activation. *Russian J Immunol*. 10(2):174–178 (in Russ.)
7. Yoshida, S., Kubo, Y., Kobayashi, Y., et al. (2015). Increased vitreous concentrations of MCP-1 and IL-6 after vitrectomy in patients with proliferative diabetic retinopathy: possible association with postoperative macular oedema. *Br J Ophthalmol*. 99(7):960–966.
8. Khalafyan, A. (2010). A STATISTICA 6. Mathematical statistics with elements of probability theory for physicians and biologists. M *Binom*, 562.
9. Sonoda, S., Sakamoto, T., Yamashita, T. (2014). Retinal morphologic changes and concentrations of cytokines in eyes with diabetic macular edema. *Retina*. 34(4):741–748.
10. Gustavsson, C., Agardh, C. D., Agardh, E. (2013). Profile of intraocular tumour necrosis factor-α and interleukin-6 in diabetic subjects with different degrees of diabetic retinopathy. *Acta Ophthalmol*. 91(5):445–452.
11. Akhade, A. S., Qadri, A. (2015). T-cell receptor activation of human $CD4^+$ T cells shifts the innate TLR response from CXCL-8hiINFrnull to CXCL8loINF-rhi. *Eur J Immunol*. 45(9):2628–2637.
12. de Oliveira, S., Reyes-Aldasoro, C. C., Candel, S., Renshaw, S. A., Mulero, V., Calado, A. (2013). Cxcl8 (IL-8) mediates neutrophil recruitment and behavior in the zebrafish inflammatory response. *J Immunol*. 190(8):4349–4359.

https://doi.org/10.46389/rjd-2021-1044