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Original Research

Levels of pro-inflammatory cytokines (IL1β, IL18) and C-reactive protein in patients with diabetic retinopathy

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Abstract

Background and Aims: Diabetic retinopathy is a major complication of diabetes mellitus (DM), which remains a leading cause of visual loss in working-age populations. This study aimed to investigate serum levels of IL1β, IL18, and C-reactive protein (CRP) in patients with diabetic retinopathy. **Material and Methods:** A total of 70 insulin-dependent patients and 60 non-insulin dependent patients with diabetic retinopathy were enrolled in the study. Interleukin levels were assessed using "Vector-Best", Ukraine reagent kits on a STAT FAX 303 plus analyzer. The level of CRP was determined using a CRPLX set of reagents "Roche Diagnostics", Germany on a COBAS INTEGRA 400 plus automatic analyzer. **Results:** In insulin-dependent patients there were no statistically significant differences in IL1β and IL18 levels in the control group. Serum level of IL1β in non-insulin dependent patients was 43.4% higher than in the control group, and 50.0% higher than in insulin-dependent patients. Serum level of IL18 in non-insulin dependent patients was 12.3% higher than the control group and 11.4% higher than the insulin-dependent patients. Also, we have found that the level of CRP in insulin-dependent patients increased by 24.1% in the control group. In non-insulin dependent patients CRP level did not change significantly in the control group. **Conclusions:** The obtained results suggest that insulin-dependent patients with diabetic retinopathy demonstrate predominance of acute inflammation, which is confirmed by acute phase marker increasing, and normal levels of pro-inflammatory cytokines in blood serum. Patients with diabetic retinopathy who do not use insulin were found to have increased serum levels of IL1β and IL18 and normal level of CRP, which suggests the predominance of pro-inflammatory processes (mobilization and activation of cells typically involved in inflammatory process).

Keywords: Diabetic retinopathy, Inflammation, Type 2 diabetes mellitus.

Background and Aims

Diabetes mellitus (DM) is a multifactorial metabolic disorder, characterized by chronic hyperglycemia leading to significant physiological, biochemical, and histological changes in the affected organisms [13]. For about half a century, scientists around the world have been thoroughly engaged in the problem of chronic hyperglycemia, but the causes of its occurrence have not been fully established yet. The most relevant modern theory is the autoimmune inflammation in diabetes pathogenesis [4, 5]. Pathological effects of hyperglycemia are seen in the form of diabetic complications which are equally health-devastating in nature as the disorder of diabetes itself [6, 7]. Despite prolonged studies of the mechanisms behind the development of diabetic retinopathy, as well as thorough investigation into the main stages of its pathogenesis, many aspects of this condition, including the

importance of imbalance in pro- and anti-inflammatory cytokines, remain the subject of ongoing scientific debate.

There are data that confirmed the role of different cytokine classes in diabetic retinopathy [8, 9] pathogenesis. Recent studies have shown that both interleukin 1β (IL1β) and tumor necrosis factor alpha (TNFα) are capable of slowing down retinal endothelial migration and capillary morphogenesis [10]. Other scientific evidence suggests that these interleukins may be playing an important role in disrupting the integrity of the gamma-retinal barrier, the occurrence of retinal leukostasis, and activation of apoptosis in proliferative diabetic retinopathy. Hyperglycemia is viewed as a trigger and retinal endothelium as a source of hyperexpression of IL1β, maintaining its own increased expression by self-stimulation of endothelial cells and macroglia [11].

IL18 is a member of the family of pro-inflammatory cytokines that stimulate the production of IFNγ, TNFα, IL1, IL2, adhesion molecules, and apoptosis factors as well as increase the proliferative activity of T-lymphocytes and activate NK cells. IL18 is involved in cellular and humoral immunity, as well as in innate and acquired immune responses [12]. The above effects of this cytokine allow viewing it as one of the key factors for anti-infective and anti-tumor protection of the body. In addition, there is evidence that in some cases IL18 may act as a pathogenetic factor in some diseases accompanied by acute and chronic inflammation.

C-reactive protein (CRP) is a multifunctional acute phase protein involved in protecting the body from pathogens in a setting of autoimmune and inflammatory processes and it is quite a well-known fact that inflammation is one of the most crucial determining factors in the progression of various diabetic complications [6]. In autoimmune processes, the involvement of CRP is actuated through binding to the ligand, which provides the destruction of autoimmune determinants with loss of antigenic properties. Accordingly, the complement system is activated by stimulating phagocytosis and by eliminating toxic products. CRP is absorbed by neutrophils, where immunoactive peptides are produced during proteolysis in phagosomes; these peptides are capable of modulating various neutrophilic and macrophageal functions. There are data about local CRP synthesis by activated macrophages and endothelial cells [13].

Due to peculiar characteristics of cytokinemia in type 2 diabetes (T2D), as the disease progresses in the patients, there is a growing number of cellular structures with high cytokine production. The research into the role of cytokinemia and the imbalance between pro- and anti-inflammatory cytokines in severe complications of T2D, such as diabetic retinopathy, will provide additional laboratory criteria for assessing the severity and the origins of this disease.

Therefore, our aim of study was to investigate serum levels of IL1β, IL18, and CRP in patients with diabetic retinopathy.

Material and Methods

The study included a clinical and laboratory examination performed in 130 patients with diabetic retinopathy (70 insulin-dependent patients constituted Group 1, and 60 non-insulin dependent patients constituted Group 2). The average age of patients ranged from 2055 years. The results of laboratory findings were compared with the control group, which included 30 healthy individuals.

Inclusion criterion: All patients suffering from diabetes, irrespective of the duration of the disease, were included in the study. The pattern of diabetic retinopathy was decided on the basis of ophthalmoscopic findings and fundus photograph.

Exclusion criterion: Patients with media haze obscuring visualization of fundus, and pregnant women were excluded from the study.

Venous blood samples were obtained from all patients, 5 ml per sample. Blood was collected in plastic tubes (BD vacutainer type) with double coagulation activator. All study subjects had their serum levels of IL1β and IL18 assayed. Interleukin levels were assessed using "VECTOR-BEST Ukraine" reagent kits on a STAT FAX 303 plus analyzer.

The levels of CRP were determined using a CRPLX set of reagents by Roche Diagnostics on a COBAS INTEGRA 400 plus automatic analyzer.

Statistical processing of digital data was carried out using the software Excel (Microsoft, USA) and STATISTICA 8.0 (Statsoft, USA). The pattern of the distribution of the studied variables was assessed using the Shapiro-Wilk's criterion. Quantitative characteristics were represented as M ± m (arithmetic mean ± standard error of arithmetic mean). Under the normal distribution, the validity of the differences was estimated using the Student's t-criterion; under the distribution different from normal, Mann-Whitney's non-parametric U-criterion was used. Differences were considered statistically significant at p<0.05.

Ethical Approval

The ethical principles contained in the Declaration of Human Rights adopted in Helsinki, in 1975, and revised in 2008, were fully respected in our study. The subjects enrolled voluntarily, participated in this study, and completed and signed a written informed consent. Protocol of study was approved by the local ethics committee of Danylo Halytskyi Lviv National Medical University.

Results

In the patients of Group 1, there were no statistically significant differences in IL1β and IL18 levels vs control group (Table 1). Serum levels of IL1β in patients of Group 2 were 43.4% higher than those in the control group, and 50.0% higher than those in Group 1. Serum levels of IL18 in Group 2 were 12.3% higher than in the control group and 11.4% higher vs Group 1.

In order to assess the level of systemic inflammation, we tested the level of CRP, a common marker of acute inflammation; the increasing CRP is a known factor in progression of diabetic retinopathy. We have found that the levels of CRP in patients with diabetic retinopathy increased in insulin-dependent patients (Group 1) – by 24.1% compared with the control group (Table 1). In group 2 CRP levels were within normal range.

When calculating the CRP to IL1β ratio, we found a 1.3-fold increase (2.4±0.04 and 1.8 ±0.05, respectively) (p<0.05) in patients of Group 1 vs control group. In patients of Group 2, there was a 1.4-fold decrease in the CRP to IL1β ratio vs control group (1.3±0.05 and 1.8± 0.05, respectively) (p<0.05) and a 1.8-fold decrease relative to patients of Group 1 (1.3 ±0.05 and 2.4 ±0.04, respectively) (p<0.05).

Discussion

Diabetic retinopathy is a major complication of DM, which remains a leading cause of visual loss in working-age populations [14]. Multiple metabolic pathways have been implicated

Table 1: Serum levels of interleukin 1β and interleukin 18 in patients with diabetic retinopathy.

Test parameters	Groups of subjects		
	Control group (n=30)	Group 1 (n=70)	Group 2 (n=60)
IL1β pg/mL	1.59±0.05	1.52±0.05 p>0.05	2.28±0.05 p<0.05 p_1<0.05
IL18 pg/mL	365.0±5.0	368.0±5.0 p>0.05	410.0±5.0 p<0.05 p_1<0.05
CRP mg/L	2.90±0.3	3.60±0.1 p<0.05	2.95±0.1 p>0.05 p_1<0.05

Note: p = the probability of differences compared with the control group; p_1 = the probability of differences compared with the patients of Group 1.

in hyperglycemia-induced vascular damage including advanced glycation end products (AGEs) accumulation. AGEs bind to macrophage receptors, which in turn, secrete cytokines that provoke the development of immune responses [4]. Expression levels of pro-inflammatory cytokines such as TNFα, IL6, IL8, and IL1β were correlated with the severity of diabetic retinopathy [15, 16].

We have found that levels of IL1β and IL18 were significantly increased in patients with diabetic retinopathy who did not use insulin. As for insulin-dependent patients with diabetic retinopathy, the levels of the investigated pro-inflammatory cytokines were within normal range.

According to Mu Z.P. and co-authors, circulating monocytes in diabetic patients exhibit an excessive inflammatory response to gram-negative bacterial lipopolysaccharides, releasing a large number of pro-inflammatory cytokines, such as IL1β and TNFα [17]. On the other hand, the metabolic changes that develop in hyperglycemia can directly affect the lymphocyte immunometabolism [18]. Patients with DM are found to have impaired adhesion and cooperation of immunocompetent cells, which results in their increased activation, effect or functions, and migration to the sites of inflammation in the vascular wall. The expression of adhesive molecules in the endothelium and lymphocytes is the initial stage of their recruitment into the site of inflammation, accompanied by the emergence of a wide range of immunological reactions, the latter being essentially protective in nature, but causing necrotic or dystrophic changes secondary to their long duration or intensity. Constant autoactivation of immunocompetent cells leads to an increase in spontaneous production of pro-inflammatory cytokines, which play a key role in diabetes-associated ocular damage with subsequent progression to more severe diabetic retinopathy.

The role of IL1β in T2D pathogenesis has been a recent topic of interest in the research community. There are data that hyperglycemia induces IL1β production by β-cells, leading to apoptosis of these cells [9]. Studies report the increase in the levels of IL1β receptor antagonist to be associated with the risk of developing T2D in the population [10]. These changes can be found more than 10 years prior to the clinical onset of DM [8] and are associated with insulin resistance. Other studies have shown that hyperproduction of the IL1β receptor antagonists may predict the risk of cardiovascular complications [19].

The studies in recent years [20, 21] suggest a positive effect of IL1 inhibition on the clinical course of DM. The specific effects include decreasing glucose levels and acute markers of inflammation, and improved functioning of pancreatic β-cells. Notably, these positive effects may persist for prolonged periods of time after completion of treatment with IL1 inhibitors.

Also there are data that point out the importance of inhibiting IL1 in relation to controlling microvascular complications of DM, such as nephropathy, neuropathy, and retinopathy, which may not be directly associated with pronounced hyperglycemia [22].

Either directly or via oxidative stress, IL18 may disrupt endothelial function or stimulate proliferation of vascular smooth muscle cells with the resulting characteristic vascular changes [23]. The researchers have found an increase in plasma levels of IL18 in patients with type 2 diabetes and in patients with metabolic syndrome [8]. Their results suggest a close correlation between IL18 activity and the components to the metabolic syndrome [24]. An increase in circulating IL18 was found in patients with T2D and a prospective study has found elevated IL18 levels to precede the development of T2D [22, 23]. Some other studies demonstrate the association between pro-inflammatory cytokines and obesity, dyslipidemia, insulin resistance and hypertension [8, 9].

The principal biological function of CRP, as well as that of any acute phase protein, is to stimulate immune responses. CRP synthesis is triggered and controlled by a number of relevant mediators, including interleukins, such as IL1 and IL6. By enhancing the synthesis of glucocorticoids, activated interleukins cause leukocytosis, have pyrogenic properties and activate complement cascade and coagulation [8, 25]. Low levels of constantly circulating CRP are not viewed as a specific inflammatory marker because, in addition to induction of acute phase protein

synthesis, interleukins have other inherent functions, which are not associated with acute inflammation [26].

Elevated CRP levels have been implicated in the development of T2DM [27]. Emerging laboratory and epidemiological data now link inflammation and CRP to insulin resistance [28]. Insulin has the capacity to reduce the production of CRP and fibrinogen. Thus, in a setting of insulin resistance, the synthesis of acute-phase proteins is enhanced. As chronic inflammation is part of insulin resistance syndrome, an increase in CRP levels suggests systemic inflammation. There are data which have demonstrated a linear increase in CRP levels with a concomitant increase in metabolic alterations [26].

The researchers have found complement activation and stimulation of expression of adhesion molecules on endothelial surface, as well as binding and modification of lipoproteins (occurring with involvement of CRP) to be suggestive of the initial phase of vascular wall damage and endothelial dysfunction. The destruction of the endothelial glycocalyx layer by CRP was proven by evidences found during in-vivo studies such as elevated levels of hyaluronan in the blood circulation (possibly caused by its erosion from the endothelial cell surface) and decreased number of heparin sulphate staining observed on the aortic endothelium of the rats [28]. Moreover, other factors mediated by CRP which lead to endothelial dysfunction include decrease in the activity of endothelial nitric oxide synthase in the endothelial cells which lead to decreased production of nitric oxide in the concerned cells [29]. Since it is a well-known fact that endothelial dysfunction is the precursor of many diabetic complications such as diabetic retinopathy and diabetic nephropathy, CRP can thus be suspected to be involved as one of the mediators leading to the progression of these diabetic complications [30, 31].

Conclusions

The obtained results suggest that insulin-dependent patients with diabetic retinopathy demonstrate predominance of acute inflammation, which is confirmed by acute increase in phase marker i.e., C-reactive protein (CRP), and normal levels of pro-inflammatory cytokines (IL1β and IL18) in blood serum. Patients with diabetic retinopathy who do not use insulin were found to have increased serum levels of IL1β and IL18 and normal levels of CRP, which suggests the predominance of pro-inflammatory processes (mobilization and activation of cells typically involved in inflammatory process).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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