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Low-grade inflammation and inflammatory mediators in individuals with prediabetes

Inflamacija niskog stepena i medijatori inflamacije kod osoba sa predijabetesom

Dejan M. Marinković*†, Tamara Dragović*†, Ivan Stanojević†‡, Predrag Djurić†§, Bratislav Dejanović‖, Jelena Rakočević¶, Saša Kiković*, Dragana Malović*, Ivana Stevanović†‡, Petar Ristić*†, Marijana Petrović†, Zoran Hajduković*†**

Military Medical Academy, *Clinic for Endocrinology, ‡Institute for Medical Research, §Clinic for Cardiology, ‖Institute for Medical Biochemistry, **Clinic for Nephrology, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ¶University of Belgrade, Faculty of Medicine, Institute for Histology and Embryology "Aleksandar Đ. Kostić", Belgrade, Serbia

Abstract

Background/Aim. Prediabetes is a condition that refers to the state of hyperglycemia not sufficiently high to reach the diagnostic values for type 2 diabetes mellitus (T2DM). This condition often precedes the appearance of T2DM. The association between the development of early glycoregulation disorders and the state of low-grade chronic inflammation is still not sufficiently well understood. The aim of the study was to assess the values of different inflammatory mediators and biomarkers in individuals with prediabetes. **Methods**. This cross-sectional, observational study included 60 respondents divided into two groups: the prediabetes group (PDG) with 31 patients and the healthy control group (HCG) with 29 respondents. Serum values of seven selected cytokines/biomarkers were compared between the two groups. Examined biomarkers were: interleukin (IL)-1β, IL-6, IL-8, IL-18, tumor necrosis factor (TNF)-α, E-selectin, and vascular endothelial growth factor (VEGF)-A. In addition, the values of body mass index (BMI), waist circumference (WC), blood pressure (BP), serum triglyceride (TG), fasting plasma glucose (FPG), and glycated hemoglobin (HbA1c) were also compared between the two groups. **Results**. PDG patients had statistically significantly higher TNF-α values compared to the HCG patients (73 pg/mL vs. 55 pg/mL, $p = 0.024$). A trend towards higher levels of IL-8 and IL-1β and lower levels of E-selectin, VEGF-A, and IL-18 was registered in PDG patients but without statistical significance. Furthermore, PDG patients had higher values of BMI, WC, systolic BP, serum TG, FPG, and HbA1c when compared to HCG. **Conclusion**. The results of our study suggest the importance of inflammation and some inflammatory mediators in the pathogenesis of early glycoregulation disorder. We believe that the main goal of future studies should focus on anti-inflammatory therapy in prediabetes.

Key words:
biomarkers; blood glucose; diabetes mellitus, type 2; prediabetic state.

Apstrakt

Uvod/Cilj. Predijabetes je stanje povišene vrednosti glukoze u krvi (hiperglikemije) ali nedovoljno visoke da bi se postavila dijagnoza dijabetes melitus tipa 2 (DMT2). Ovo stanje često prethodi pojavi DMT2. Povezanost razvoja ranog poremećaja glikoregulacije sa stanjem hronične upale niskog intenziteta još uvek nije dovoljno dobro shvaćeno. Cilj rada bio je da se ispitaju vrednosti različitih medijatora zapaljenja i biomarkera kod osoba sa predijabetesom. **Metode.** Opservacionom studijom preseka obuhvaćeno je 60 ispitanika, podeljenih u dve grupe: grupu od 31 bolesnika sa predijabetesom (PDG) i kontrolnu grupu (KG) od 29 zdravih osoba. Serumske vrednosti sedam izabranih citokina/biomarkera su upoređivane između dve grupe ispitanika. Ispitivani su biomarkeri: interleukin (IL)-1β, IL-6, IL-8, IL-18, faktor nekroze tumora (*tumor necrosis factor* – TNF)-α, E-selektin i faktor rasta vaskularnog endotela (*vascular endothelial growth factor* – VEGF)-A. Takođe, između ove dve grupe upoređivani su i indeks telesne mase (ITM), obim struka (OS), krvni pritisak (KP), trigliceridi (TG) u serumu, glukoza u plazmi (GP) natašte i glikozilirani hemoglobin (HbA1c). **Rezultati.** Bolesnici iz PDG imali su statistički značajno više vrednosti TNF-α u poređenju sa ispitanicima KG (73 pg/mL vs. 55 pg/mL, $p = 0,024$). Registrovan je trend viših nivoa IL-8 i IL-1β i nižih nivoa E-selektina, VEGF-A i IL-18 kod bolesnika iz PDG, ali bez statističke

Correspondence to: Dejan M. Marinković, Military Medical Academy, Clinic for Endocrinology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: dejmarinkovic@gmail.com

značajnosti. Takođe, bolesnici iz PDG imali su više vrednosti ITM, OS, sistolnog KP, serumskih TG, GP natašte i HbA1c, u poređenju sa ispitanicima KG. **Zaključak.** Rezultati našeg istraživanja ukazuju na značaj inflamacije i pojedinih medijatora inflamacije u patogenezi ranog poremećaja glikoregulacije. Verujemo da bi ključni cilj budućih istraživanja trebalo da bude usmeren na anti-inflamacijsku terapiju u predijabetesu.

Ključne reči:
biomarkeri; glukoza u krvi; dijabetes melitus, tip 2; predijabetes.

Introduction

Type 2 diabetes mellitus (DM) – T2DM, is a heterogeneous group of metabolic diseases characterized by chronic hyperglycemia associated with protein, lipid, and carbohydrate metabolic disorders. It is a direct consequence of a relative or absolute lack of insulin and insulin resistance. Prediabetes is a term for slightly elevated values of blood glycemia, but not high enough to reach the diagnostic criteria for DM [1, 2]. Prediabetes may be defined as either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). It is a state of high risk of developing T2DM. The progression from normoglycemia to prediabetes is thought to be directly caused by insulin resistance and the further progression to T2DM and later complications of the disease by a progressive decline in the secretory capacity of beta cells [3–5]. As a main characteristic of these disorders, chronic hyperglycemia with hyperinsulinism is followed by changes in sera levels of different cytokines [6]. In different ways, the immune system is involved in every single stage of T2DM development [7, 8].

There is a growing body of evidence that both inflammation and pro-inflammatory cytokines play a significant role in the occurrence of T2DM and the development of its complications. This is achieved by different pathophysiological mechanisms and influences on atherogenesis and endothelial dysfunction [9]. The connection between T2DM and chronic inflammation was first suggested more than a century ago when a high dose of sodium salicylate was noticed to reduce glycosuria in patients with a milder form of T2DM [10]. Numerous epidemiological and other studies showed the correlation between the occurrence of T2DM and increased values of certain inflammatory mediators and acute phase reactants [11–14]. It was proved that low-grade chronic inflammation precedes T2DM [15]. Some of the studies were focused on observing the pro-inflammatory markers in prediabetes [7, 8, 16–18]. A few of them were monitoring inflammatory markers during the progression of glycemic status, from normoglycemia through prediabetes to T2DM [8]. The concentration of some of the biomarkers [C-reactive protein (CRP), white blood cell count (WBC), interleukin (IL)-1β, IL-1 receptor antagonist (RA), IL-6, IL-8, IL-18, monocyte chemoattractant protein (MCP)-1, interferon-gamma-inducible protein 10 (IP 10), haptoglobin and fibrinogen] turned out to have been increased for many years before the occurrence of T2DM, which indicates the existence of chronic, subclinical inflammation [12, 19–21]. Increased gene expression of some pro-inflammatory agents has also been proven in people with prediabetes at the level of pancreatic endocrine islets [19].

Therefore, the aim of the study was to investigate the connection between chronic inflammation and glucose homeostasis in the early stages of the disease. Bearing this in mind, the focus of our study was on inflammatory mediators in prediabetes. We intended to determine the structure of inflammatory markers in the prediabetic population compared to healthy individuals.

Methods

Study population and design

We conducted a cross-sectional, observational study on 60 participants classified into two groups: 31 patients were in the prediabetes group (PDG) and 29 were in the healthy controls group (HCG). Prediabetes is defined through three clinical entities: IFG, IGT, and the combination of the two (IGT + IFG). Subjects were classified as having a normal glucose tolerance if fasting plasma glucose (FPG) was < 6.1 mmol/L and 2-hour oral glucose tolerance test (OGTT) < 7.8 mmol/L or glycated hemoglobin (HbA1c) < 6.0%. Prediabetes was defined if FPG ≥ 6.1 and IFG < 6.9 mmol/L or 2-hour OGTT ≥ 7.8 and IGT < 11.1 mmol/L. DM was classified with a FPG ≥ 7.0 mmol/L or 2-hour OGTT ≥ 11.1 mmol/L or HbA1c ≥ 6.5%. Body mass index (BMI) was calculated as body weight (in kg) divided by body height (in meters) squared. Participants were selected during regular visits or as part of systematic examinations in ambulances of the Cabinet for Endocrinology of the Military Medical Academy (MMA), Belgrade, Serbia. The study was approved by the Ethics Committee of the Faculty of Medicine MMA (No. 1494-2, from April 11, 2023), and every patient provided a signed consent form.

Exclusion criteria were: diagnosed T2DM, T1DM, ischemic cardiomyopathy, valvular heart disease, prior myocardial infarction, uncontrolled arterial hypertension, existing chronic kidney disease [estimate glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2], acute inflammation, malignant or systemic autoimmune diseases, pregnancy, people younger than 18 years and older than 70 years. Values of detected systolic blood pressure (SBP) > 140 mmHg and diastolic blood pressure (DBP) > 90 mmHg were considered as unregulated arterial hypertension. Demographic and clinical data of the patients were collected by conducting patient interviews or from hospital medical notes and hospital blood test results. The following serum biochemical parameters and inflammatory cytokines were analyzed from the morning venous blood sample: CRP [reference range (RR): 0.0–4.0 mg/L], fibrinogen (RR: 2.1–4.0 g/L), D-dimer (D-D) (RR: < 0.50 mg/LFEU), FPG (RR: 4.1–5.9 mmol/L), HbA1c (RR: < 6.0%), triglyceride (TG) (RR: < 1.7 mmol/L), total cholesterol (TC) (RR: < 5.2 mmol/L), low-density lipoprotein (LDL) (RR: < 3.5 mmol/L), high-density lipoprotein (HDL) (RR: > 1.3 mmol/L), IL-1β, IL-6, IL-8, IL-18, tumor necrosis factor (TNF)-α, E-selectin and vascular endothelial growth factor (VEGF)-A.

Data collection

Anamnesis processing of patients, measurements, and clinical examination [waist circumference (WC), body height and weight, BMI, blood pressure (BP), heart rate (HR), blood sampling for the investigated laboratory parameters (after a minimum of 15 min of rest)] were done at the Clinic for Endocrinology. Two sitting BP and HR measurements were taken for each participant using a mercury sphygmo-manometer according to a standard protocol. The mean of these two BP measurements was used in the data analysis. Following the collection of 24 mL of peripheral blood from consenting fasting study participants between 8:30 a.m. and 10:30 a.m., various biochemical parameters and cytokines were measured. Standard laboratory analyses were performed on the same day at the Institute of Medical Biochemistry of the MMA. HbA1c, CRP, FPG, and lipid profile were measured using an Advia 1,800 automatic biochemical analyzer (Siemens). Coagulation screen, fibrinogen, and D-D were measured using a BCS XP coagulometer (Siemens). The cytokine concentrations were measured at the Institute for Medical Research (IMR), MMA. A peripheral blood sample was submitted to the IMR immunology laboratory within one hour after sampling, where serum was separated and stored at -70 °C until analysis. All collected serum samples were analyzed in the same act. The biomarker/cytokine concentrations (E-selectin, VEGF-A, TNF-α, IL-1β, IL-6, IL-8, and IL-18) were measured in the sera of patients using a Premixed Multiplex Kit-Human Custom 10 Plex (N. Orange Grove Ave., Pomona, CA 91767, USA), performed according to the manufacturer's instructions (flow cytometer Beckman Coulter Navios EX). Detection kits were produced by AimPlex Biosciences, Inc.

Statistical analysis

The differences in demographic, clinical characteristics, and laboratory analyses between patients with prediabetes and the control group were compared using the Chi-square test for categorical variables, the *t*-test for continuous variables with normal distribution, and the Mann-Whitney *U* test for non-normally distributed variables. Association between variables was tested using Pearson's or Spearman's correlation, where appropriate, according to the normality distribution. Statistical analyses were performed using IBM SPSS Statistics version 25 for Windows (IBM Corporation, Armonk, NY, USA). The level of statistical significance was set at $p < 0.05$.

Results

Basic clinical parameters

The average age of the study participants was 47.48 ± 10.21 years, with 53.3% of the study population being male. The median BMI in the study cohort was 28.04 kg/m^2 (25.35–30.45). Patients with prediabetes, compared to the control group, had significantly higher levels of BMI (median: 29.0 kg/m^2 vs. 27.1 kg/m^2, $p = 0.010$), WC (mean: 103.0 cm vs. 93.2 cm, $p = 0.04$), SBP (mean: 126.0 mmHg vs. 119.8 mmHg, $p = 0.035$), FPG (mean: 5.6 mmol/L vs. 5.1 mmol/L, $p = 0.001$), HbA1c (mean: 5.6% vs. 5.1%, $p < 0.001$), and serum TGs (median: 1.75 mmol/L vs. 1.15 mmol/L, $p = 0.007$). There were no significant differences between the two groups for DBP, HR, TC, LDL, CRP, fibrinogen, and D-D. The mean levels of HDL in HCG were significantly higher compared with PDG (mean: 1.56 mmol/L vs. 1.35 mmol/L, $p = 0.046$) (Table 1).

Table 1

Baseline subject characteristics of study participants

Characteristics	HCG (n = 29)	PDG (n = 31)	*p*-value
Age (years)	44.07 ± 9.49	50.68 ± 9.96	**0.011**
Male gender	13 (40.6)	19 (59.4)	0.201
Body mass index (kg/m^2)	27.1 (22.6–29.5)	29.0 (26.6–32.5)	**0.010**
Waist circumference (cm)	93.2 ± 17.9	103.0 ± 16.0	**0.04**
Systolic BP (mmHg)	119.8 ± 11.8	126.0 ± 10.2	**0.035**
Diastolic BP (mmHg)	80 (70–85)	85 (80–85)	0.082
Heart rate (beats/min)	70 (65–85)	75 (70–85)	0.363
C-reactive protein (mg/L)	1.03 (0.40–3.22)	0.71 (0.10–3.15)	0.208
Fibrinogen (g/L)	3.4 ± 0.7	3.3 ± 1.4	0.686
D-dimer (mg/L FEU)	0.31 (0.22–0.41)	0.42 (0.22–0.66)	0.093
Fasting plasma glucose (mmol/L)	5.1 ± 0.5	5.6 ± 0.7	**0.001**
HbA1c (%)	5.1 ± 0.4	5.6 ± 0.4	**< 0.001**
Triglyceride (mmol/L)	1.15 (0.81–1.56)	1.75 (1.06–2.63)	**0.007**
Total cholesterol (mmol/L)	5.0 (4.61–5.73)	5.45 (4.68–6.18)	0.355
Low-density lipoprotein (mmol/L)	3.08 (2.54–3.61)	3.41 (2.61–3.72)	0.261
High-density lipoprotein (mmol/L)	1.56 ± 0.41	1.35 ± 0.41	**0.046**

HCG – healthy control group; PDG – prediabetes group; BP – blood pressure; HbA1c – glycated hemoglobin.
Results are given as mean ± standard deviation or median (interquartile range), except for male gender which is presented as numbers (percentages).
Bold values indicate the significance level of $p < 0.05$.

Cytokines

Baseline levels of cytokines are presented in Table 2. Median TNF-α levels in PDG subjects were significantly higher compared with HCG (73 pg/mL vs. 55 pg/mL, $p = 0.024$) (Figure 1). There was a trend towards elevated serum levels of IL-8 and IL-1β in PDG compared to respondents from HCG, although statistical significance could not be reached. On the contrary, a trend towards lower levels of E-selectin, VEGF-A, and IL-18 among the PDG subjects was seen compared to the HCG. Again, comparing serum levels of E-selectin, VEGF-A, and IL-18 between the groups showed no statistical significance.

Correlation between cardiovascular risk factors, cytokines, and basic clinical parameters

There was a statistically significant correlation between the majority of traditional cardiovascular (CV) risk factors (TC, TG, HDL, LDL, BMI, SBP, DBP) in both groups (Table 3). Furthermore, there was a significant correlation (positive or negative) between most inflammatory cytokines in both groups (Table 4). However, statistical significance in the correlation between traditional CV risk factors and inflammatory cytokines was rarely observed (Table 5). A significant positive correlation was found only between IL-18 and TG in HCG, PDG, and the total monitored population. Likewise, there was a statistically significant cor-

Table 2

Levels of serum biomarkers in healthy control group (HCG) and prediabetes group (PDG).

Biomarkers	HCG (n = 29)	PDG (n = 31)	*p*-value
IL-1β	9 (3.5–17)	11 (9–14)	0.534
IL-6	13 (10–18)	12 (10–18)	0.911
IL-8	336 (157–452)	457 (172–578)	0.258
TNF-α	55 (38.5–64.5)	73 (44–92)	**0.024**
E-selectin	1,665 (1,463–2,065)	1,165 (1,463–2,065)	0.446
VEGF-A	790 (506–1,307)	700 (590–1,391)	0.641
IL-18	170.4 ± 55.9	164.4 ± 54.3	0.672

IL – interleukin; TNF – tumor necrosis factor; VEGF – vascular endothelial growth factor.
Data are expressed as median (interquartile range) or mean ± standard deviation.
Bold value indicates the significance level of $p < 0.05$.
Units of measurement of all presented cytokine concentrations are given in pg/mL.

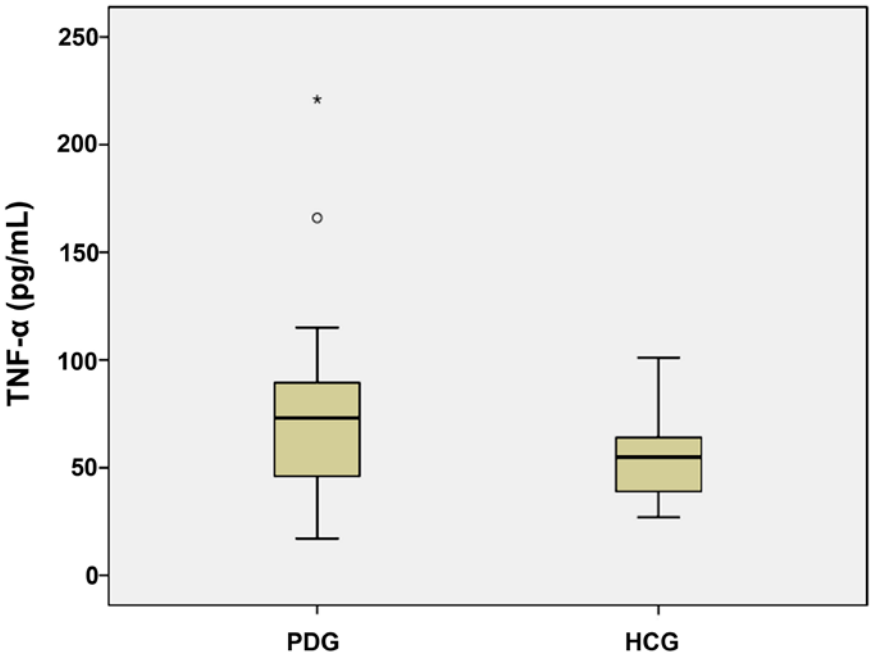

Fig. 1 – Levels of tumor necrosis factor (TNF)-α in the prediabetes group (PDG) and healthy control group (HCG). *$p<0.05$.

Table 3

Correlation coefficients between cardiovascular risk factors in healthy control group (HCG) and prediabetes group (PDG)

Parameters	TC	TG	HDL	LDL	BMI	SBP	DBP
HCG (n = 29)							
TC	1						
TG	0.487**	1					
HDL	-0.140	-0.501**	1				
LDL	0.941**	0.391*	-0.248	1			
BMI	0.475	0.538**	0.358	0.438*	1		
SBP	0.403	0.306	-0.479**	0.430*	0.722**	1	
DBP	0.453*	0.305	-0.371*	0.455*	0.695**	0.868**	1
PDG (n = 31)							
TC	1						
TG	0.465**	1					
HDL	0.319	-0.543**	1				
LDL	0.894**	0.377*	0.174	1			
BMI	-0.130	0.152	-0.399*	-0.119	1		
SBP	0.031	0.156	-0.059	-0.037	0.160	1	
DBP	0.243	0.244	-0.055	0.130	0.349	0.648**	1

TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure. *$p < 0.05$. **$p < 0.01$. Units of measurement of all presented parameters are given in Table 1.

Table 4

Correlation coefficients between inflammatory markers in the healthy control group (HCG) and prediabetes group (PDG)

Parameters	IL-1β	IL-6	IL-8	TNF-α	E-selectin	VEGF-A	IL-18
HCG (n = 29)							
IL-1β	1						
IL-6	0.695**	1					
IL-8	0.705**	0.649**	1				
TNF-α	0.571**	0.493**	0.569**	1			
E-selectin	0.362	0.419*	0.560**	0.216	1		
VEGF-A	0.444*	0.646**	0.540**	0.293	0.588**	1	
IL-18	0.658**	0.595**	0.819**	0.440*	0.748**	0.585**	1
PDG (n = 31)							
IL-1β	1						
IL-6	0.680**	1					
IL-8	0.751**	0.757**	1				
TNF-α	0.787**	0.700**	0.816**	1			
E-selectin	0.628**	0.451*	0.492**	0.684**	1		
VEGF-A	0.605**	0.574**	0.696**	0.743**	0.477**	1	
IL-18	0.683**	0.501**	0.656**	0.659**	0.393*	0.653**	1

For abbreviations, see Table 2. *$p < 0.05$. **$p < 0.01$.
Units of measurement of all presented cytokine concentrations are given in pg/mL.

Table 5

Correlation coefficients between inflammatory markers and cardiovascular risk factors in healthy control group (HCG) and prediabetes group (PDG)

Parameters	TC	TG	HDL	LDL	BMI	SBP	DBP
HCG (n = 29)							
IL-1β	-0.224	-0.074	0.203	-0.306	-0.178	-0.264	-0.360
IL-6	-0.154	0.015	0.038	-0.232	0.005	-0.169	-0.340
IL-8	0.069	0.319	0.094	-0.071	0.060	-0.210	-0.276
TNF-α	-0.019	0.072	-0.116	-0.049	-0.080	-0.263	-0.385*
E-selectin	0.151	0.337	0.153	0.020	-0.013	-0.230	-0.240
VEGF-A	-0.126	0.116	0.292	-0.219	0.086	-0.293	-0.330
IL-18	0.068	0.387*	0.036	-0.065	0.166	-0.073	-0.077
PDG (n = 31)							
IL-1β	0.058	0.104	0.114	-0.011	-0.218	0.014	-0.156

Table 5 (continued)

Parameters	TC	TG	HDL	LDL	BMI	SBP	DBP
IL-6	0.037	0.004	0.055	0.008	0.042	0.133	0.112
IL-8	0.176	0.130	0.095	0.095	-0.061	0.022	-0.005
TNF-α	0.078	0.146	0.023	0.020	-0.125	0.115	-0.101
E-selectin	0.302	0.003	0.354	0.227	-0.444*	0.102	-0.149
VEGF-A	-0.031	0.079	-0.071	-0.098	0.059	0.144	-0.107
IL-18	0.210	0.383*	-0.278	0.146	-0.019	0.052	0.077

For abbreviations, see Tables 2 and 3. ∗$p < 0.05$.
Units of measurement of all presented parameters are given in Tables 1 and 2.

Table 6

Correlation coefficients between inflammatory markers and WC, HbA1c, and FPG in the prediabetes group (PDG) and healthy control group (HCG).

Biomarkers	PDG (n = 31)			HCG (n = 29)		
	WC	HbA1c	FPG	WC	HbA1c	FPG
IL-1β	-0.329	-0.566**	0.271	-0.069	-0.313	-0.035
IL-6	-0.196	-0.636**	-0.195	0.132	-0.160	-0.036
IL-8	-0.174	-0.633**	0.175	0.027	-0.208	0.160
TNF-α	-0.276	-0.567**	-0.025	-0.080	-0.113	-0.098
E-selectin	-0.533**	-0.381*	0.089	0.131	-0.127	0.253
VEGF-A	-0.016	-0.429*	0.015	0.151	-0.095	0.095
IL-18	0.053	-0.496**	0.343	0.030	-0.430*	-0.014

IL – interleukin; TNF – tumor necrosis factor; VEGF – vascular endothelial growth factor; WC – waist circumference; HbA1c – glycated hemoglobin; FPG – fasting plasma glucose. ∗$p < 0.05$. ∗∗$p < 0$.
Units of measurement of all presented parameters are given in Tables 1 and 2.

relation between HbA1c, FPG, BMI, and WC (Table 1). There was a significant negative correlation between HbA1c level and all cytokines measured in PDG, which was, at the same time, absent in patients from HCG (Table 6).

Discussion

The inspiring results of epidemiological and other studies connected the occurrence of T2DM with elevated levels of some of the cytokines, mediators of inflammation, and reactants of acute phase [11–14]. Some studies took a step further and focused on elevated pro-inflammatory markers in prediabetes, then proved that low-grade chronic inflammation precedes T2DM [7, 15, 16]. Wang et al. [9] monitored inflammatory markers during the progression of glycemic status, from normoglycemia through prediabetes to T2DM. Numerous markers, both pro- and anti-inflammatory, have been linked to the process of prediabetes progressing to DM [7]. Evidence of a correlation between chronic inflammation and the development of severe, late complications in T2DM has led to the hypothesis that some specific inflammatory factors may serve as screening biomarkers for the early detection of patients with a poor prognosis.

Following the recruitment of the study participants, we comparatively assessed the inflammatory milieu of prediabetes study participants and healthy individuals. The results of our study showed an increased level of only TNF-α in subjects with prediabetes compared to the control group. In addition, our data showed increased BMI, WC, SBP, TG, FPG, and HbA1c levels in prediabetes subjects compared with controls.

TNF-α is a pro-inflammatory cytokine that increases insulin resistance *via* modulation of glucose transporter type 4 and phosphorylation of insulin receptor substrate-1 [22]. Our results regarding TNF-α are in agreement with many similar studies focusing on insulin resistance, obesity, prediabetes, or T2DM. Most studies found that subjects with some glucose impairment had increased levels of TNF-α [23–28]. Marques-Vidal et al. [23] noticed the increased values of TNF-α in people with insulin resistance, metabolic syndrome, and T2DM and CV diseases. In 2016, there was a study that indicated the possibility of using systemic inflammatory cytokines (TNF-α) as a screening tool to detect people with a higher risk of developing T2DM and CV diseases [24]. Guzmán-Flores et al. [25], in a study on the Mexican population with T2DM, explained the importance of ethnicity in relation to TNF-α values and glucose tolerance. Furthermore, certain gene alleles of the *TNF-α* factor promoter (-238A) increase the risk of developing T2DM in Mexican patients. In addition, the frequency of the GA haplotype (created by the -308G and -238A alleles) is significantly increased in T2DM patients compared to the controls [25]. The previous name of TNF-α was cachectin due to its significant role in the pathogenesis of cachexia in various diseases and its influence on lipid metabolism [27]. In 2017, while researching the role of adipose tissue in thrombosis, Vilahur et al. [28] discovered an interesting fact – in diabetic or obese patients, which is reflected by high levels of cytokines such as TNF-α and other inflammatory markers.

However, there are studies whose results point to a different aspect. Al-Shukaili et al. [29] showed decreased levels of IL-6 and TNF-α in T2DM compared to the healthy controls.

The author of the study mentioned the duration of the diseases, the small sample size, and the differences in age and sex of the studied groups as the possible reasons for this discrepancy. In the study of Wang et al. [4], there was no significant difference between TNF-α and IL-6 in the three monitored groups (the healthy, the prediabetic, and the T2DM one). Gupta et al. [18] reached a similar result in their analysis of inflammatory cytokines in prediabetes. Higher levels of TNF-α, IL-6, and interferon (IFN)-β were detected in PDG but without statistical significance. We do not exclude the possibility of a dual, pro-, and anti-inflammatory role of TNF-α in different stages of the disease. There is also a study hinting at the dual pro- and anti-inflammatory role of TNF or a self-limited inflammatory response in vascular smooth muscle cells [30].

Many studies indicated a connection between elevated values of IL-6 and the progression of glucose impairment. IL-6 is a pro-inflammatory cytokine produced by numerous cells such as activated leukocytes, endothelial cells, and adipocytes [31, 32]. This cytokine proved to induce hyperglycemia and compensatory hyperinsulinemia in murine models and humans [33, 34]. Pradhan et al. [12] study pointed out that elevated levels of IL-6 and CRP are connected to the risk of developing T2DM in the future. According to Spranger et al. [26], plasma IL-6 and TNF-α levels were connected to the forthcoming T2DM. Some studies have shown that HbA1c and IL-6 levels were significantly higher in IFG and IGT patients compared to healthy individuals [35, 36]. Such results are not in accordance with the results of our research, which showed no significant difference in IL-6, and CRP. IL-8 and IL-1β levels were slightly higher, while E-selectin, VEGF-A, and IL-18 levels were lower in PDG compared to HCG. Notably, the differences in cytokine levels among the groups failed to reach statistical significance.

Obtaining significant differences in BMI, WC, SBP, TG, HDL, FPG, and HbA1c values is in agreement with the results of earlier studies [4, 12, 29, 35, 36]. These significant correlations for most traditional CV factors (in both groups), as well as most cytokines (in both groups), were expected. Surprisingly, we found highly significant and inversive correlation values between HbA1c and all the cytokines in the PDG group. Neither the respondents from HCG nor the relations of cytokines with WC, BMI, and FPG (in both groups) contain such correlations. According to available data, one might expect a much higher probability of a positive correlation between HbA1c and various cytokines in T2DM patients. In 2018, a study was published regarding a significant positive correlation of IL-6, TNF-α, and CRP with FPG, HbA1c values, and blood pressure level [37]. In 2019, Sari et al. [38] published the results about the association between HbA1c and serum levels of IL-6 in patients with T2DM. In 2020, a group of scientists pointed out the correlation between the elevated HbA1c and IL-3, IL-4, IL-7, TNF-α, and IFN-α_2 values in obese Afro-American women [39]. However, there is not much evidence on the relation of HbA1c values and cytokines in the state of prediabetes [35, 36]. A question arises: "Is it possible that the same cytokines play a double role during the development of T2DM?" The first role might be anti-inflammatory, with a self-limited inflammatory response in the early stage of prediabetes, and the second, a pro-inflammatory one in the advanced stage of T2DM.

Conclusion

Reducing cardiovascular risk is a major goal in the treatment of patients with prediabetes and diabetes. Early diagnosis of glucose impairment, ideally during the prediabetes phase, as well as promptly starting the intensive therapy, is crucial when it comes to reducing cardiovascular risk. In that regard, the idea of inflammation as a therapeutic target, besides hyperglycemia, is quite interesting. Inflammation during prediabetes could be a useful target for clinicians in the future to prevent, or at least slow down, the progression of prediabetes to T2DM. These results highlight the importance of two items – firstly, the fact that prediabetes, as a precursor of diabetes, is an important clinical entity, and secondly, that inflammation and some inflammatory mediators have their own role (although still not completely understood) in the pathogenesis of prediabetes. Future studies are expected to provide answers to whether certain cytokines have a dual role in different stages of the disease (pro- and anti-inflammatory).

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